

ABSTRACTS

This publication contains the abstracts submitted and accepted for the EUROGIN 2012 Congress, held in Prague, July 8 - 11, 2012.

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.

This section contains summaries provided by the speakers presenting in the Training Courses and the Main Scientific Sessions, followed by those of the Scientific Sessions, Seminars, Workshops and Satellite Sessions, ending with Free Communications abstracts.

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.

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DEFINING NEW STRATEGIES FOR HPV PREVENTION AND CONTROL

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Objectives. We are presently at a crossroads in clinical practice. The traditional cytology model has greatly reduced the burden of cervical cancer worldwide, but a new prevention model based on the natural history of HPV infection is slow to be adopted, especially adolescent vaccination programs and the incorporation of HPV testing into cancer screening. The potential impact of a new public health model for HPV-related cancer prevention and control is substantial, eliminating the need for millions of cancer screening visits. Although the identification and management of HPV-associated diseases is excellent in most developed countries, determinants of viral susceptibility and infectivity are less well-understood.

Methods. Requirements for an evidenced-based transition to a new public health model for HPV prevention and control will be reviewed with a focus on several research gaps. The first gap includes an improved understanding of co-factors in HPV persistence and clearance. While most HPV infections have been shown to clear within a few months or years, the 10-20% of women with a persistent viral infection remain at elevated risk for the development of CIN and cancer. Prolonged HPV infection elicits the production and release of a variety of immune factors which aid recruitment and coordinate the functions of cells essential to pathogen control, but the role of cytokine-mediated mucosal immune response in the clearance of cervical HPV infection is poorly defined. The second gap includes defining the dynamics of HPV transmission, including sexual and non-sexual reservoirs of infection. The third gap includes the development of novel screening modalities in high risk populations, particularly those targeted at difficult to reach groups in low resource settings. Barriers to HPV screening vary between populations, so culturally-sensitive and culturally-aware screening modalities need to be developed. Finally, population-based surveillance programs that monitor prophylactic vaccine uptake and HPV disease incidence need to be developed and supported globally.

Conclusions. The adoption of a new prevention and control model for HPV infection in the post-vaccine era requires evidenced-based changes to health policy. Refocusing our limited research resources during the next decade on understanding ways to reduce the HPV burden is a substantial challenge.

THE BURDEN OF HPV ASSOCIATED DISEASES

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Introduction: High-risk HPV are responsible of virtually all cases of cervical cancer and probably of anal cancer. HPV are also involved in a substantial proportion of cancers of the vagina, vulva, penis and the oropharynx. New data on the HPV involvement in the development of oropharyngeal cancer in some populations is striking. While the incidence of cervical cancer has been decreasing over recent decades, the incidence of anal and oropharyngeal carcinoma for which there are no effective screening programs has been rising over the last couple of decades. Moreover, benign HPV types cause genital warts and recurrent respiratory papillomatosis, a rare but severe disease of the upper airways.

Methods: Recent published reviews will be summarized in order to provide the most updated attributable fraction of HPV and cancer.

Conclusions: Over 600,000 new cancer cases are estimated to be attributable to HPV.

MTC 1-1-3

NATURAL HISTORY OF HPV INFECTION

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Objectives Prospective data comparing the natural history and carcinogenic potential of individual high-risk (HR) types remain limited. However, large cross-sectional comparisons of HPV type distribution can be of practical use for identifying types, or groups of types, that differ in their natural history and carcinogenic potential and hence merit differential management in HPV-based screening programs around the world.

Methods We performed a meta-analysis of cross-sectional HR HPV type distribution in 115,789 HPV-positive women across the complete spectrum of cervical disease from infection to cancer, including 33,154 normal cytology, 6,810 atypical squamous cells of undetermined significance (ASCUS), 13,480 low-grade squamous intraepithelial lesions (LSIL) and 6,616 high-grade SIL (HSIL) diagnosed cytologically, 8,106 cervical intraepithelial neoplasia grade 1 (CIN1), 4,068 CIN2 and 10,753 CIN3 diagnosed histologically, and 36,374 invasive cervical cancers (ICC), from 423 PCR-based studies worldwide.

Conclusions No strong differences in HPV type distribution were apparent between normal cytology, ASCUS, LSIL or CIN1. However, HPV16-positivity increased steeply from normal/ASCUS/LSIL/CIN1 (20-28%), through CIN2/HSIL (40/47%) to CIN3/ICC (58/63%). HPV16, 18 and 45 accounted for a greater or equal proportion of HPV infections in ICC compared to normal cytology (ICC: normal ratios=3.07, 1.87 and 1.10, respectively) and to CIN3 (ICC:CIN3 ratios=1.08, 2.11 and 1.47, respectively). Other HR types accounted for important proportions of HPV positive CIN2 and CIN3, but their contribution dropped in ICC, with ICC: normal ratios ranging from 0.94 for HPV33 down to 0.16 for HPV51. ICC: normal ratios were particularly high for HPV45 in Africa (1.85) and South/Central America (1.79), and for HPV58 in Eastern Asia (1.36). ASCUS and LSIL appear proxies of HPV infection rather than cancer precursors, and even CIN3 is not entirely representative of the types causing ICC. HPV16 in particular, but also HPV18 and 45, warrant special attention in HPV-based screening programs.

MTC 1-1-4

HPV - THE CAUSE OF CERVICAL CANCER MOLECULAR BIOLOGY AND PATHOGENESIS

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Human Papillomaviruses infect basal epithelial cells, and can reprogram them to produce an epithelial lesion able to support the production of virus particles. In such lesions, viral episomes persist at low copy number in the basal layer, but are amplified in the upper epithelial layers as part of the productive life-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.

The 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 the 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.

In general, the development of neoplasia usually requires persistent infection and continuous viral gene expression. In most cases however, lesion regression takes place as a result of a cell-mediated immune response within months or years. The process of lesion-clearance is not well understood, but it appears that clearance or latency can result. Current ideas of lesion formation and regression will be discussed, along with emerging views HPV disease at different epithelial sites, and the mechanistic progress that leads from persistent infection to cancer.

CERVICAL CANCER & CYTOLOGY BASED SCREENING PROGRAMS IN EASTERN AND CENTRAL EUROPEAN COUNTRIES**Poljak M¹, Rogovskaya S², Seme K¹, Maver PJ¹, Kocjan BJ¹, Cuschieri K³, Arbyn M⁴, Syrjanen S⁵***1 Institute of Microbiology and Immunology, Ljubljana, Slovenia**2 Russian Medical Academy of Post-Graduate Education, Moscow, Russia**3 Specialist Virology Centre, Royal Infirmary of Edinburgh, United Kingdom**4 Scientific Institute of Public Health, Brussels, Belgium**5 Institute of Dentistry and Medicine Research Laboratory, University of Turku, Finland*

Objectives: The process of post-socialistic transition, taking place at a different pace in each Central and Eastern European (CEE) country, has significantly affected all health-associated issues and the political attitude towards health problems, including cervical cancer prevention. Here we provide a review of current cervical cancer screening practices in 28 CEE countries.

Methods: Data were collected by a review of published peer-reviewed literature and by detailed survey performed in 16 countries.

Conclusions: Organized screening, mainly based on conventional cytology, is performed only in seven countries in the region, with the estimated coverage ranging from a few percent to over 70%. Slovenia has the national organized screening programme from 2003; the coverage reached 82.1% in the first 5-year period and the incidence of cervical cancer decreased for 40% in the period 2003-2009. Hungary implemented organized screening in 2004, but the country is still struggling with low coverage of target population in organized settings and more than 60% attendance outside the programme. Five countries: Czech Republic, Poland, Estonia, Lithuania and Latvia established at least partially functioning organized screening programs, but are dealing with low coverage. Slovakia is planning to switch to an organized screening programme in 2013. Cervical cancer prevention in non-EU CEE countries, Russia and countries of former Soviet Union rely on opportunistic screening, characterized by relatively high coverage in younger and low coverage in middle-aged and older women. Screening of selected groups of women employed in large companies is performed annually, but this approach has had small effect on morbidity and mortality. Some pilot programs of organized screening were initiated in the region. Redesigning the service and changing attitudes in public, medical profession and at governmental level, will be the main ways to improve current unsatisfactory cervical cancer outcomes in this part of Europe.

OVERVIEW OF HPV ASSAYS AND MOLECULAR MARKERS**Iftner T***University Hospital Tuebingen, Institute for Medical Virology and Epidemiology of Viral Diseases, Division of Experimental Virology, Germany*

Numerous professional organizations recommend testing for the mucosal "high risk" HPV types to increase the efficacy of cervical cancer screening, including The American College of Obstetricians and Gynecologists (ACOG), the American Cancer Society (ACS), the American Society for Colposcopy and Cervical Pathology (ASCCP), and the Association of Reproductive Health Professionals (ARHP). Under the recommended guidelines, high-risk HPV DNA testing is typically conducted in two instances: first as an adjunct to cervical cytology analysis as primary screening tool in all women over 30, and second, for the triage of patients with ASC-US cytology results. Other potential indications for high-risk HPV testing involve the monitoring of women who have been treated for high-grade cervical intraepithelial neoplasia (CIN) lesions and the follow up of women negative in colposcopy but positive for ASCUS, LSIL & ASC-H cytology. The diagnosis of an HPV infection nowadays is almost exclusively made by identification of the viral nucleic acids, mostly DNA, by molecular techniques using complementary probes hybridizing to the DNA/RNA followed by either signal amplification or the nucleic acid is amplified before hybridization to specific complementary probes (target amplification) by methods such as the Polymerase Chain Reaction (PCR) of a subgenomic region. Newer methods include isothermal target amplification of E6/E7 mRNA progression markers by transcription-mediated amplification (TMA) or nucleic acid sequence based amplification (NASBA). As there are numerous commercial tests on the market the decision which test to use for primary cervical screening is difficult. The cellular tumor suppressor protein p16INK4a has been identified as a biomarker for transforming HPV infections and is often used in conjunction with the proliferation marker Ki67 for quality assurance in cases of unclear cytology or histology. It is a cyclin-dependent kinase inhibitor that regulates the cell cycle by inactivating the cyclin-dependent kinases (CDK4/6) involved in the phosphorylation of the retinoblastoma protein (pRb). The published literature indicates especially an improved accuracy of p16INK4a compared to HC2 testing in the triage of ASC-US. In LSIL triage p16INK4a is more specific but less sensitive.

MTC 1-2-3

NEW STRATEGIES OF HPV BASED SCREENING**Cuzick JM,***John Snow Professor of Epidemiology, Centre for Cancer Prevention, Wolfson Institute, Queen Mary University of London*

As the advent of HPV DNA testing for primary screening comes nearer, the need for better triage tests has become stronger. While HPV testing is a highly sensitive approach to screening with much better detection rates than cytology, it also has a higher false positive rate, due to its inability to distinguish persistent infections with progressive potential from transient infections where the poison is capable of dealing with them without any sequelae. Testing for HPV RNA appears to alleviate this problem to some extent, but false positive rates with sensitive RNA tests are still too high. Here we discuss triage newer tests which show promise for dichotomising HPV infections into persistent ones and those which are transient. Among the most promising candidates are HPV typing: HPV16 is clearly a more persistent infection and more often associated with high grade precursor lesions, and HPV 18/45 shows a predilection for endocervical lesions which can be missed by both cytology and colposcopy. P16^{ink} and some cell cycle progression markers such as the mcm proteins also shows promise and new studies have suggested that viral methylation, at least for type 16, and methylation of certain human genes may also be good markers for high grade lesions. These data will be reviewed.

In addition the current status of self sampling for HPV will be evaluated.

MTC 1-2-5

INTEGRATION OF SCREENING AND VACCINATION IN CERVICAL CANCER PREVENTION PROGRAMS**Eduardo Franco***(McGill University, Montreal, Canada)*

Two efficacious prophylactic vaccines against infections with human papillomavirus (HPV) types 16 and 18 have been 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. Triage of HPV-positive women via cytology or HPV genotyping 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.

**HPV DNA TESTING VERSUS CYTOLOGY TO MONITOR RECURRENT OF RESIDUAL CIN
AFTER TREATMENT OF HIGH-GRADE CERVICAL LESION:
AN UPDATED META-ANALYSIS**

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Objectives: A meta-analysis was conducted comparing virological and cytological surveillance to predict the outcome of treatment of cervical precancer.

Methods: A literature search was performed in three electronic databases to identify eligible studies. Studies were selected if 1) women were treated for histologically confirmed CIN2 or CIN3; 2) women had a follow-up cytology and HPV DNA test between 3 and 9 months; 3) follow-up during at least 18 months with colposcopy and biopsy for all women or in case of a positive HPV or cytology test. Treatment failure was defined as recurrent or residual CIN2+.

Results: 15 studies could be included (8 with HC2 and 7 with PCR). The pooled sensitivity was 93% (95 % CI [CI]: 85-97%) for HPV and 72% (CI: 66-78%) for cytology. The pooled specificities were 81% (CI: 74-86%) and 84% (CI: 84% (80-87%)), for HPV and cytology, respectively. HPV testing was significantly more sensitive (ratio=1.25; CI=1.15-1.36) but not less specific (ratio=0.97; CI=0.93-1.02) compared to cytology. There was no heterogeneity by HPV testing system.

Conclusions: Virological surveillance is more accurate in predicting therapeutic failure than cytology.

ASSESSING THE RISK OF CERVICAL CANCER: THE INTERPLAY OF CLINICAL FACTORS AND SCREENING TESTS

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Objectives: Identifying and eradicating CIN3, which is the accepted surrogate end-point for cancer development, effectively manages cervical cancer risk. The risk of prevalent invasive carcinoma at the time of CIN3 diagnosis is ~1%. The cumulative 30-year risk of developing invasive carcinoma in patients with untreated CIN3 is between 30 & 50%. How do readily available screening test results such as cervical cytology, hr-HPV testing and age relate to the prevalence of CIN3?

Methods: Data from multiple clinical trials demonstrate remarkably consistent results. As recommended in 2012 by multiple US-based organizations, a paradigm of co-testing with cervical cytology and clinically validated hr-HPV tests one can promote the assessment of risk as follows: For every cytologic diagnosis there is a defined risk of prevalent or near term CIN3. Each cytologic interpretation can be stratified by whether the concurrent hr-HPV test is positive or negative. Women with positive hr-HPV tests may be further stratified by genotyping results. Age at the time of screening further stratifies risk. Case examples will be presented demonstrating the simplicity of this approach. For instance in women with NILM Pap tests the risk of prevalent CIN3 ranges from <1% to over 10%. In contrast, a women in her 40s with an HSIL Pap may have > an 80% chance of prevalent CIN3. The magnitude of the derived risk can then be used to select women for one of three management strategies: more intensive follow-up than routine screening, colposcopy, or definitive therapy.

Conclusions: Modern approaches to screening produce readily available data that provides excellent estimates of CIN3 risk. A persistent major issue is for the treating community to agree on the magnitude of risk that causes patients to be consistently managed based on these risks. Of course, perfection is not achievable.

MTC 1-3-5

CONSEQUENCES OF THE TREATMENT OF CIN

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Cold knife cone (CKC) biopsy has long been associated with the risk of cervical stenosis which could result in haematometria, infertility and failure to dilate in labour. Conversely CKC also increases the risk of midtrimester miscarriage and preterm labour.

To conserve reproductive function, treatments of CIN moved to more conservative excisional treatments such as LLETZ, laser cone biopsy or NETZ/SWETZ or ablative treatment. Reducing the amount of tissue removed and the need for general anesthetic has reduced the risk of side effects and complications without compromising the success of treatment outcomes.

Pain, bleeding and vaginal discharge are common after-effects following treatment at colposcopy affecting two-thirds of women. The duration and severity are more marked following treatment compared with colposcopy alone or punch biopsy. However for most women these are often reported as mild or moderate. In addition, 71% of women report a change to their normal menstrual cycle.

Longer term, no effect has been reported on fertility. However there are well voiced concerns about the impact on preterm delivery (PTD) and preterm rupture of membranes. Epidemiological studies indicate that treating women with CIN with excisional treatment has an increased relative risk of PTD. Treatment of CIN necessitates removal of the transformation zone to a depth of 7-8mm either by ablation or excision for a type 1 treatment. Excessive depth of treatment increases the risk of PTD but much of the published evidence is limited as either depth of tissue removal is unknown or is related to the height of the tissue specimen and not the depth of the crater. There are wide differences between different countries and these will be discussed.

MTC 2-1

HPV GENITAL INFECTION AND DISEASE IN MEN

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Background and Objectives: Data on the natural history of human papillomavirus (HPV) infection progression to genital warts (GW) in men are sparse, and nearly non-existent for penile cancer. As part of our on-going international prospective study of HPV infection in men we have been documenting the natural history of genital infections and progression to disease. Here we describe the distribution of HPV types in incident GW and estimate GW incidence and time from type-specific incident HPV infections to GW detection in a multinational cohort of men ages 18-70.

Methods: Participants included 2,487 men examined every 6 months and followed for a median of 17.9 months. Samples obtained from 112 incident GW were tested for HPV DNA by PCR. Genotyping tested for the presence of 37 HPV types.

Results and Conclusions: Incidence of GW was 2.35 per 1,000 person-years with the highest incidence rate observed among men ages 18-30 (3.43 per 1,000 person-years). HPV 6 (43.8%), 11 (10.7%), and 16 (9.8%) were the most common types detected in GW. The 24 month cumulative incidence of GW among men with incident HPV 6/11 infections was 14.6% (95% CI: 7.5-21.1). The median time to any GW detection was 17.1 months (95% CI: 12.4-19.3), with the shortest time to detection observed among men with incident infections with HPV 6/11 only (6.2 months; 95% CI: 5.6-24.2). Factors strongly associated with condyloma were incident HPV 6/11 infection (HR=12.42; 95% CI: 3.78-40.77), age (HR=0.43; 95% CI: 0.26-0.77; 45-70 vs. 18-30 years), high lifetime number of female partners (HR=5.69; 95% CI: 1.80-17.97); ?21 vs. 0), and number of male partners (HR=4.53; 95% CI: 1.68-12.20; ?3 vs. none).

Interpretation: HPV 6/11 plays an important role in GW development with the highest incidence and shortest time to GW development observed among men with incident HPV 6/11 infections. The results confirm HPV 6/11 and recent sexual behavior are strongly associated with condyloma.

ANAL NEOPLASIA: BURDEN, EPIDEMIOLOGY AND PREVENTION**Palefsky J,***University of California, San Francisco, San Francisco, CA, USA*

Anal HPV infection is found nearly universally among high risk populations such as HIV-infected men and women, and is highly prevalent among other high risk groups such as men who have sex with men (MSM), men and women with history of solid organ transplant, and women with a history of high-grade CIN, cervical cancer and vulvar cancer. Anal HPV infection is also common in the general population of healthy women, and approaches or exceeds the prevalence of cervical HPV infection in some studies. The prevalence of anal intraepithelial neoplasia, high-grade anal intraepithelial neoplasia (HGAIN) specifically, and anal cancer is also high in these high-risk groups. The incidence of anal cancer has been increasing in the general population by about 2% per year among both men and women since the 1970s. Among the groups at highest risk of anal HPV infection such as HIV-infected MSM, the incidence of anal cancer exceeds 100/100,000 in some studies and does not appear to have been reduced by highly active antiretroviral therapy. Other groups at elevated risk for anal HPV infection and AIN also have elevated risk of anal cancer compared with the general population.

HGAIN is the likely precursor to anal cancer, just as high-grade CIN is the precursor to cervical cancer. Prevention of development of HGAIN (primary prevention) or screening for and treatment of HGAIN (secondary prevention) are two methods that can be used to reduce the risk of anal cancer in at-risk populations. The efficacy of primary prevention has been shown in a study of the quadrivalent HPV vaccine, in which there was a 77% reduction in incident HGAIN among vaccinated HIV-uninfected MSM compared with the placebo group in the per-protocol analysis (Palefsky JM et al. NEJM 2011, 365: 1576-85) and more than 90% reduction in persistent anal HPV infection with vaccine HPV types. Approaches to screening and treating HGAIN to prevent anal cancer as secondary prevention are similar to those used to prevent cervical cancer. Anal cytology is used in many settings as a screening tool, with abnormal cytology followed by high resolution anoscopy (HRA) and HRA-guided biopsies of areas suspicious for HGAIN. Treatment of HGAIN is ablative using infrared coagulation, electrocautery, or topical using treatments such as imiquimod or trichloroacetic acid. Future studies are needed to document the efficacy of primary and secondary prevention methods to reduce the incidence of anal cancer.

HPV ASSOCIATED DISEASES IN THE SKIN – CURRENT KNOWLEDGE AND PERSPECTIVES**Pfister H.,***Uniklinik Köln, Institute of Virology, Cologne, Germany*

HPV, some members of genus alpha and the members of the genera gamma, mu, and nu, are well known to induce benign common, plantar, or flat skin warts mainly in school children up to young adults. Most skin warts regress spontaneously within 2 years but some may persist much longer. Immunosuppressed transplant recipients frequently suffer from multiple, therapy refractory skin warts. HPV from genera beta and gamma are ubiquitous in the general population and frequently establish themselves already during the first weeks of life. Hair follicles are regarded as natural reservoir. These infections are well controlled and usually clinically inapparent. Massive betaPV replication occurs in epidermodysplasia verruciformis patients, associated with the induction of disseminated macular skin lesions with a high risk of malignant conversion to squamous cell carcinoma (SCC). Also solar UV-exposure, keratinocyte hyperproliferation after wounding or in psoriasis patients, and immunosuppression result in increased betaPV activity, which may explain the increased skin cancer risk under these conditions. Immunosuppressed transplant recipients with an about 100-fold increased incidence of cutaneous SCC compared to the general population are significantly more likely to have high betaPV DNA loads in hair follicles. BetaPV DNA can be detected in up to 50% of cutaneous SCC of immunocompetent patients and in more than 90% of skin SCC of immunosuppressed transplant recipients. Some case-control comparisons revealed an increased risk of SCC associated with the presence of betaPV DNA in hair follicles and/or seropositivity for betaPV (OR=1.6-2.8) supporting the hypothesis that betaPV may play a role in the development of cutaneous SCC. This may open perspectives for clinically relevant pre-transplant HPV screening and the development of preventive HPV vaccination.

**2011 COLPOSCOPIC TERMINOLOGY
OF THE INTERNATIONAL FEDERATION FOR CERVICAL PATHOLOGY AND COLPOSCOPY (IFCPC)**

Bornstein J¹, for the IFCPC Nomenclature committee

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The 2011 colposcopic terminology of the International Federation of Cervical Pathology and Colposcopy (IFCPC) includes nomenclature of cervical colposcopic findings (Table A), cervical excision treatment types and specimen dimensions (Table B, Figure 1) and vaginal clinical and colposcopic findings (Table C).

Table A: 2011 IFCPC Colposcopic Terminology of the Cervix

| Pattern | Section |
|--|-------------------------------|
| Adequate or inadequate for the reason ... (eg, cervix obscured by inflammation, bleeding, scar) Squamo-columnar junction visibility: completely visible, partially visible, not visible Transformation zone types 1,2,3 | General assessment |
| Original squamous epithelium: mature, atrophic Columnar epithelium; ectopy Metaplastic squamous epithelium; Nabothian cysts; crypt (gland) openings Deciduous in pregnancy | Normal colposcopic findings |
| General principles Location of the lesion: Inside or outside the transformation zone; Location of the lesion by clock position Size of the lesion: Number of cervical quadrants the lesion covers Size of the lesion as percentage of cervix | Abnormal colposcopic findings |
| Grade 1 (Minor) Fine mosaic; fine punctation; thin aceto-white epithelium; irregular, geographic border | |
| Grade 2 (Major) Sharp border; inner border sign; ridge sign; dense aceto-white epithelium; coarse mosaic; coarse punctuation; rapid appearance of aceto-whitening; cuffed crypt (gland) openings | |
| Nonspecific Leukoplakia (keratosis, hyperkeratosis), erosion Lugol's staining (Schiller's test): stained or nonstained | Suspicious for invasion |
| Atypical vessels Additional signs: Fragile vessels, irregular surface, exophytic lesion, necrosis, ulceration (necrotic), tumor or gross neoplasm | |
| Congenital transformation zone, condyloma, polyp (ectocervical or endocervical), inflammation, stenosis, congenital anomaly, posttreatment consequence, endometriosis | Miscellaneous findings |

Table B: Cervical treatment types and dimensions

| Pattern | Section |
|--|------------------------------|
| Excision type 1,2,3 | Excision treatment types |
| Length – the distance from the distal or external margin to the proximal or internal margin | Excision specimen dimensions |
| Thickness – the distance from the stromal margin to the surface of the excised specimen | |
| Circumference (optional) – the perimeter of the excised specimen | |

Figure 1: Type 3 excision and line drawing of LLETZ specimen with designation of dimensions

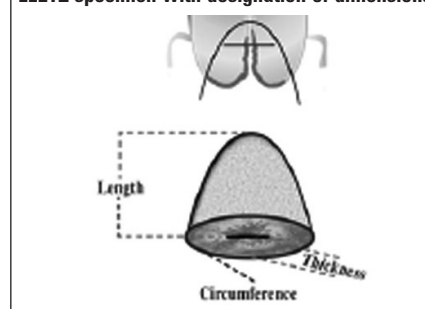


Table C: 2011 IFCPC Clinical and Colposcopic Terminology of the Vagina

| Pattern | Section |
|---|-------------------------------|
| Adequate or inadequate for the reason (eg, inflammation, bleeding, scar) Transformation zone | General assessment |
| Squamous epithelium: mature or atrophic | Normal colposcopic findings |
| General principles Upper third or lower two-thirds Anterior, posterior, or lateral (right or left) | Abnormal colposcopic findings |
| Grade 1 (Minor) Thin acetowhite epithelium, fine punctuation fine mosaic | |
| Grade 2 (Major) Dense acetowhite epithelium coarse punctuation coarse mosaic | |
| Suspicious for invasion Atypical vessels Additional signs: fragile vessels, irregular surface, exophytic lesion, necrosis ulceration (necrotic), tumor or gross neoplasm | Miscellaneous findings |
| Nonspecific Columnar epithelium (adenosis) Lesion staining by Lugol's solution (Schiller's test): Stained or nonstained, leukoplakia | |
| Erosion (traumatic), condyloma, polyp, cyst, endometriosis, inflammation, vaginal stenosis, congenital transformation zone | |

Professor Albert Singer, PhD, DPhil, FRCOG, *Department of Women's Health, Whittington Hospital, London,*

THE NORMAL CERVIX

To understand the abnormal cervix and its premalignant changes knowledge of normal appearances are essential. The cervix consists of 3 epithelial types namely, the original columnar and squamous laid down in fetal life, metaplastic squamous epithelium developing with the original columnar epithelium principally in pregnancy but also at the menarche and in late fetal life. The third type, the abnormal epithelium, has a distinctive colposcopic appearance and contains mainly premalignant changes; they may also be composed of the early stages of metaplasia which can be indistinguishable from early premalignant disease.

The original squamo-columnar junction is a distinct line which separates the two original epithelial types. Medial to this boundary is an area, the transformation zone (TZ); when composed of original columnar and metaplastic epithelium, being referred to as the physiological transformation zone. When abnormal epithelium is present within this zone it is an atypical transformation zone.

The upper extent of the metaplastic process defines the upper extent of any pre malignant squamous change (CIN). This demarcation line is the new squam-columnar junction. If it cannot be seen then there is the possibility of further premalignant or malignant change existing above this line. In this case the colposcopy is defined as unsatisfactory, when seen the colposcopy is satisfactory.

ABNORMAL COLPOSCOPIC FINDINGS: THE ATYPICAL TRANSFORMATION ZONE (ATZ)

Within the boundaries of the ATZ there exists colposcopically recognizable epithelium which contains the stages of cervical pre malignancy (CIN). Also present maybe early stages of squamous metaplasia and more prominently, the results of Human Papilloma Virus infection (HPV).

These abnormal appearing colposcopic changes become pronounced after the application of 5% acetic acid. They consist principally of white epithelium (aceto-white) which can be thin or dense; its white appearance as a result of reflected light blocked from reflecting the underlying vascularity within the stroma. Abnormal vascular patterns within the epithelium may consist of a punctated or mosaic appearance and the distance between the vessels which are viewed "end on" within the aceto-white tissue background, being referred to as of a fine (close spread) or coarse (wide spread) configuration. There may exist atypical vessels defined as irregular vessel with an abrupt or interrupted course appearing as corkscrews, or loops. Staining of the tissue with an Iodine solution will outline the abnormal tissue which will not take up the stain.

Minor grades of CIN are composed of smooth surfaced slight aceto-white changes with fine punctation and regular mosaic vessels; iodine staining may be speckled and mild with this highlighting an irregular outer boundary. More major changes are associated with a generally smooth surfaced epithelium with sharp outer borders with dense aceto-white appearing early after acetic acid application and slow to resolve with coarse punctation and irregular mosaic with iodine negative staining. Accentuation of these changes with an irregularity to the surface with erosion or ulceration with atypical vessels may indicate early malignant change. The appearance of dense aceto-white changes in columnar epithelium with abnormal atypical vessels may indicate the uncommon glandular neoplasia.

INTEGRATION OF HPV KNOWLEDGE IN COLPOSCOPIC PRACTICE

TAN, J.

Royal Women's Hospital, Melbourne, Australia.

Colposcopy has been the recommended management following abnormal cytology (conventional pap smear or liquid-based) in cervical screening program. There are ongoing concerns with the sensitivity of colposcopy for detecting high-grade squamous intra-epithelial lesions (HSIL). We know the sensitivity is enhanced if the cyto-pathology service has a high predictive value with HSIL pap abnormality and colposcopists take more biopsies at colposcopy. The skills of the colposcopist are also important and practice improvement and accreditation programs are increasing being incorporated in colposcopy services. In recent years, high-risk HPV (hr-HPV) tests are becoming incorporated in triage, screening and post-treatment surveillance. The strength of the HPV test is in its negative predictive value. However, there will be an increasing number of colposcopy done for women with HPV + tests with or without accompanying abnormal cytology. ASCUS/LSIL triage and HPV screening with or without genotyping have led to the earlier detection of HSIL compared with cytology screening alone. We will need to assess if the higher rate of detection of HSIL is due to the performance of the HPV test itself or whether it is due to improved sensitivity of colposcopy in these hr-HPV+ women by enhancement of colposcopy features.

MTC 3-1-7

ISSUES RELATED TO FOLLOW UP

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The risk of resistance or recurrence after treatment of preinvasive cervical lesions of 5-15%, being independent of the type of treatment has been employed.

Patients treated for CIN3 have increase risk for developing invasive cancer 5 for more than normal population. We haven't randomized controlled trials comparing different issues. HPV test at 6 or 12 months interval has a sensitivity of 90% and cytology 70%.

The ASCCP, suggests HPV test 6-12 months, if negative annual control for 20 years. If positive, colposcopy and endocervical curettage are recommended. Some facts can increase recurrences: size of the lesion involved margins CIN degree, VIH.... and factors than can reduce the risk as vaccination.

In summary, without definitive protocols it seems quite judicious to recommend cytology and HPV test, at 6 y 24 months, if the results are negative to come back to normal screening (Kocken et al. 2011).

MTC 3-1-8

PSYCHOLOGICAL ASPECTS OF COLPOSCOPY

Harper DM.

University of Missouri-Kansas City School of Medicine, Kansas City, Missouri, USA

Objectives: A spectrum of behavioral responses occurs after being given bad news. When women are told that their initial screening cytology is abnormal, these responses range from not following up for any diagnostic appointments to demanding immediate colposcopic diagnosis as soon as possible.

Methods: The literature is reviewed for preferences towards the documented response behaviors.

Conclusions: There is no one preferred response to an abnormal cytology screen test. Equal proportions of women prefer immediate colposcopy, a triage test or an observational follow up at an extended time interval. Understanding patient behaviors allows multiple appropriate medical choices that serve the patient centered medical perspective.

THE TECHNOLOGY**Professor Albert Singer¹, PhD, DPhil, FRCOG, Dr Quek Swee Chong²***1 Department of Women's Health, Whittington Hospital, London,**2 Parkway Gynaecology Screening & Treatment Centre Gleneagles Hospital, Singapore*

Because colposcopy is the visual examination under magnified illumination of the cervix necessitates that the resulting visual images should be recorded. This has been done over many years with attached cameras to the colposcopy viewing arm, initially with 2 chip and now with sophisticated 3 chip cameras. The static photographs are now supplemented by videos and real time transmission of images which not only aid documentation but also teaching either in the clinic or remotely. The latter methods are valuable in teaching and such platforms as smart phones and tablets are now frequently used by students and teachers

Storage of visual and patient data is facilitated by many computer-assisted systems specifically developed for colposcopy. This aids in teaching and decision making as for instance at regular multidisciplinary team meeting where all data can be collated and reviewed.

Methods to improve the accuracy of the colposcopy examination have been developed. A number of hand held real time electronic devices using optical and electrical feed back from the cervical epithelium have proved potentially valuable but none is yet of proven value. The use of dynamic spectral imaging colposcopy identifies atypical epithelium especially large but also small volume lesions but it is debatable as to how relevant the detection their detection is to clinical management. The recent development of confocal microscopic device to examine the cervix, offering as it does real time visualization of the pathologic epithelium presents an exciting potential in respect of technology and the colposcopic examination

THE BENEFIT OF TAKING MULTIPLE BIOPSIES FOR DETECTION OF CERVICAL PRECANCER**Nicolas Wentzensen,***Division of Cancer Epidemiology and Genetics, National Cancer Institute*

A conventional colposcopic examination detects only 50-60% of prevalent precancers. Several secondary data analyses have demonstrated that taking more biopsies increases the sensitivity of colposcopy. Approaches include taking multiple targeted biopsies from colposcopically abnormal areas and random four-quadrant biopsies.

In the NCI-OUHSC Biopsy Study, we systematically evaluated the benefit of taking multiple targeted biopsies for detecting cervical precancer among 680 women attending a colposcopy clinic. 30.3% women with a low grade or benign colposcopic impression had CIN2+ in one of the biopsies. Conversely, 38.4% women with a high grade colposcopic impression had <CIN2 in their worst biopsy result. In 61.7% of women with CIN2+, the worst lesion was detected in the first biopsy, in 26.1% it was found at the second biopsy and in 12.2% it was detected in the third or fourth biopsies. Similarly, 68.9% of CIN3 were detected with the first biopsy, 21.3% with the second biopsy, and 9.8% with the third and fourth biopsies. Only 1.6% of CIN3s were detected with a random biopsy. In summary, colposcopic impression is unreliable to guide biopsy placement for confirmation of cervical precancer. Current colposcopy-biopsy protocols miss about 30%-50% of prevalent CIN2+. Performing multiple biopsies targeting acetowhite lesions increases the yield of CIN2+ substantially.

MTC 3-2-5

**THE OUTCOME OF WOMEN WITH A NEGATIVE COLPOSCOPY EXAMINATION;
THE PERSPECTIVE FROM THE UK CERVICAL SCREENING PROGRAMME**

Dr M E Cruickshank MB ChB MD FRCOG

Accurate exclusion of CIN or cancer on colposcopic examination alone means that women can be reassured that they do not need unnecessary biopsies or treatment and they can be safely discharge to routine recall without the risk of undetected high grade disease. This is essential in countries such as the UK with a national publicly funded screening programme but all women should expect a high standard of colposcopy including the ability to confirm normality.

The basics of colposcopy training include excluding invasive disease, determining the adequacy of colposcopy and recognising the normal and abnormal cervical transformation zone. Reassurance regarding the future disease is essential to avoid undetected disease and to maintain confidence and compliance with the screening programme. Concerns have been raised from the US on the performance of colposcopy. Yet a number of retrospective and prospective studies in the UK have confirmed that women with low grade smears and normal colposcopy are at low risk of developing high grade CIN before the next screening round. Previous studies have depended on cytological assessment only but data from the TOMBOLA trial also includes an exit colposcopy examination 3 years later. These data confirm the low risk of high grade CIN arising within 3 years of a negative colposcopy examination.

MTC 3-2-7

VAGINA AND HPV

Hampl M¹

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Beside the cervix and vulva, the vagina is one of the organs in the lower female genital tract which is susceptible to HPV infection. Covered by non keratinized squamous cell epithelium, the human papilloma virus may infect, persist and transform epithelial cells.

If the infection is caused by low risk virus, condylomata accuminata may develop. This disease is benign and may be treated locally by immunomodulation (off label use) or surgically, preferentially by laservaporisation after biopsy for histology. In case of HPV high risk infection, premalignant lesions in form of vaginal intraepithelial dysplasia grade 2-3 (VAIN) may develop. VAIN is a rare premalignancy with an incidence of 0,2/100.000 women per year with increasing rates, but by far less then CIN (36.4/100.000).

The mean age of women with VAIN is less then 50 years. HPV 16 is the most common HPV type. About 70% of women with VAIN had undergone hysterectomy, 90% of the lesions are located in the upper third of the vagina.

Diagnosis is made by cytology, vaginoscopy after acid acid or lugol application and biopsy.

Treatment consists of excision or laservaporisation.

VAIN 3 may progress to vaginal cancer. The incidence of vaginal cancer is 0,4/100.000 women per year.

There are two independent pathways in the devolpment of vaginal cancer, HPV induced and independent of HPV infection.

70% of tumors are HPV induced, mostly due to HPV 16 infection.

Diagnosis is made by cytology, vaginoscopy, biopsy, for staging ultrasound, MRI and CT may be helpful.

Treatment of vaginal cancer in lower stage may by surgically (local excision or colpectomy including lymphonodectomy), higher stages are treated by radiation therapy or in rare cases exenteration.

Early HPV positive tumors have a better prognosis then HPV negative.

VULVA AND HPV**Joura E,***Medical University Vienna, Vienna, Austria***Objective:** To review the impact of HPV on 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. The lifetime risk of genital warts is 10%, the prevalence 1%. Women with genital warts are at risk for subsequent disease at the cervix.

Low grade VIN is mainly caused by HPV 6, high grade VIN mainly by HPV 16, 31 and 6. High grade VIN has the potential of progression to vulvar cancer. Data from the quadrivalent HPV vaccine have demonstrated a 100% prevention of VIN caused by the four vaccine types and a 99% protection against genital warts in the prophylactic situation. Population based data have shown that an effective vaccination program with a good coverage can almost eradicate genital warts in young people.

HPV-ASSOCIATED CANCERS – A GROWING PROBLEM?**Margaret Stanley,***Department of Pathology, Tennis Court Road, Cambridge CB2 1QP, UK*

Virus infections cause at least 15% of human cancers and one of the most important oncogenic viruses is human papillomavirus a causal agent it is estimated in 5.2% of all cancers. HPVs are a large group of viruses that infect both cutaneous and mucosal squamous epithelia and have an exclusively intra-epithelial infectious cycle. About 15 mucosal types are high risk or cancer causing HPVs with HPV 16 and 18 being the most important. Infection with one of these oncogenic HPVs is the cause of carcinoma of the cervix in women, the 3rd commonest cancer in women worldwide. Secondary intervention by screening effectively controls this disease in developed societies but not in the developing world which bears 86% of the cervical cancer burden. Projections of population growth indicate that without primary or secondary intervention this disease burden inequality will increase in the coming 3-4 decades.

HPV associated cancers are not confined to the cervix and HPV infection is implicated in the development of a proportion of vaginal, vulval, anal penile and head and neck cancers. Importantly the incidence of HPV related cancers in these sites, particularly anal carcinomas and tonsillar carcinomas is increasing.

MSS 1-3

CURRENT MANAGEMENT OF HEAD & NECK CANCER**Professor Hisham Mehanna**

*PhD, BMedSc (hons), MBChB (hons), FRCS, FRCS (ORL-HNS)
 Director, Institute of Head and Neck Studies and Education, UK
 Consultant Head-Neck and Thyroid Surgeon & Honorary Professor*

Head and neck cancer is managed by a variety of techniques - which mainly consist of radiotherapy, chemotherapy, surgery or a combination of these. Early disease is usually treated with one modality - either surgery or radiotherapy. Advanced disease is usually treated with multimodal therapy which can include all three modalities. The emergence of organ sparing techniques using chemoradiotherapy has given rise to the need for salvage treatment often using complex surgery. In addition there has been a rise in the targeted therapies with EGFR blockers being the first to be approved in HNC.

MSS 1-4

THE ROLE OF PAPILOMAVIRUS INFECTION IN SKIN CANCER DEVELOPMENT**Bouwes Bavinck J.N. and the EPI-HPV-UV-CA group***

Department of Dermatology, Leiden University Medical Center, Leiden, the Netherlands.

Cutaneous squamous cell carcinoma (SCC) is the second most common skin cancer after basal-cell carcinoma. Links between human papillomaviruses from the beta genus (betaPV) and skin cancer were first suspected in the rare genetic disease, epidermodysplasia verruciformis. There is evidence to suggest that infection with betaPV play a synergistic role with ultraviolet radiation in the development of cutaneous SCC.

We evaluated the association between betaPV infection and SCC in conjunction with measures of ultraviolet exposure and susceptibility. We performed case-control studies in the Netherlands, Italy and Australia, countries with profoundly different ultraviolet exposures. The presence of 25 betaPV types in eyebrow hair follicles was determined using a highly sensitive HPV DNA genotyping assay, and antibodies for the 15 most prevalent betaPV types in a total of 689 SCC cases and 845 controls were detected using multiplex serology. Multivariate logistic regression models were used for case-control comparisons.

BetaPV DNA was detected in eyebrow hairs of more than 90% of all participants. The presence of betaPV DNA was associated with an increased risk of SCC in the Netherlands (OR=2.8; 95% CI 1.3;5.8) and Italy (OR=1.7; 95% CI 0.79;3.6), but not in Australia (OR=0.91; 95% CI 0.53;1.6). Seropositivity for betaPV in controls ranged between 52% and 67%. A positive antibody response against 4 or more betaPV types was associated with SCC in Australia (OR=2.2; 95%CI 1.4;3.3), the Netherlands (OR=2.0; 95%CI 1.2;3.4) and fair-skinned Italians (OR=1.6, 95% CI 0.94;2.7). We found an association between SCC and the concordant detection of both antibodies and DNA for at least one betaPV type which was statistically significant in The Netherlands (OR=2.0; 95% CI 1.2;3.3) and Australia (OR=2.1; 95% CI 1.4;3.2) and showed a trend in Italy (OR=1.3; 95% CI 0.81;1.9).

People who had both betaPV DNA and antibodies present for the same HPV type (concordant DNA and antibodies) were at significantly increased risk of SCC, even if individually these measures of HPV infection (DNA or serum antibodies) did not show a significant association with SCC. Although sun exposure and sun sensitivity are the dominant risk factors for SCC, our data provide additional evidence that betaPV infection might play an etiological role.

HPV-ASSOCIATED DISEASES IN NON-ANOGENITAL SITES: ESOPHAGUS AND LUNG

Syrjänen, K.J., MD, PhD, FIAC¹*1 Department of Oncology & Radiotherapy, Turku University Hospital, Turku, Finland.*

Since the original reports of this speaker (in 1979), providing the first evidence to suggest HPV involvement in etiology of a subset of bronchial carcinomas, 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. The literature published until 2002, contains 2.468 bronchial carcinomas subjected to HPV detection and HPV DNA has been reported in 536 (21.7%) of these cases. There is reason to suspect that bronchial carcinogenesis is a multistep process, which is contributed to by the known pathogenetic factors (cigarette smoke, radiation, asbestos exposure). Globally, esophageal squamous cell carcinoma (ESCC) shows a marked geographic variation, with up to 500-fold difference in incidence between low- and high-risk areas, suggesting a dominant role of environmental etiological factors. Similar as BSCC, also the HPV involvement in esophageal carcinogenesis (first suggested by this speaker) has been demonstrated by morphologic studies showing koilocytic changes in benign and malignant 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 among HR-HPV-exposed subjects, and by in vitro studies showing transformation of esophageal epithelial cells by oncogenic HPV types. Of several thousands of ESCC samples analysed, HPV detection rates are 27% by in situ hybridization and 33% by PCR. HPV prevalence in OSCC is highly variable in the high-risk areas than in low-risk regions, suggesting a divergent etiology of this disease in different geographic regions.

THE SIGNIFICANCE OF HPV IN THE COLON & RECTUM

Lorenzon L¹, Borghesi R², Uggeri G², Mazzetta F², Pilozi E², Torrisi MR², Ziparo V¹ and French D².*1 Surgical and Medical Dept. of Clinical Sciences, Biomedical Tech. and Translat. Medicine,**2 Dept of Clinical and Molecular Medicine. Faculty of Medicine and Psychology, University of Rome "La Sapienza"*

Objectives. Over the last few years a possible correlation between HPV and colon 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: a) the presence HPV DNA in colorectal cancers and resection margins, b) the quantification of the E2, E4, E5, E6 and E7 genes and their mRNAs expression in HPV16 positive tissues, c) the integrational pattern of HPV16 in positive tissues.

Methods. 55 consecutive patients (M/F 1.9, mean age 70.8 yrs, SD 11.7, range 46-92) were enrolled in a prospective study from October 2010 to-date. 21 patients had a right colon cancer, 11 patients had a transverse colon location, otherwise 8 patients had a sigmoid carcinoma and 10 patients a rectal cancer. After DNA extraction from frozen tumor tissues, samples were analysed for HPV DNA detection using the Inno-Lipa Genotyping kit. Surgical resection margins were also investigated in HPV positive cancers. Moreover HPV16 positive samples were analysed through Real-Time PCR for the quantification of HPV16 E2, E4, E5, E6 and E7 genes and for calculating the integrational pattern (using the E2/E6 ratio method); samples were also investigated through reverse Real-time PCR for the detection and quantification of the HPV16 E2, E4, E5, E6 and E7 mRNAs.

Conclusion. 16.6% of the patients were HPV16 positive through the Innolipa Genotyping kit. Most of these patients had an episomal HPV infection, presenting the E2, E4, E5, E6 and E7 genes. The evaluation of all the HPV16 early region genes, their mRNAs expression and the study of the integrational pattern of HPV16 positive tissues might help in the understanding the role and the significance of HPV DNA in colorectal positive cancers.

MSS 2-2

CURRENT KNOWLEDGE ON SAFETY PROFILE OF THE CURRENT HPV VACCINES

Lopalco PL,

*Head of Vaccine Preventable Disease Programme, European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden***Key points:**

The HPV vaccines currently in use for girls are safe, well tolerated and highly efficacious in the prevention of persistent infection, cervical cancer and other morbidities related to the vaccine-HPV serotypes.

High safety profile has been recently confirmed also through systematic reviews and meta-analysis that demonstrated no significant difference in occurrence of severe adverse events between vaccinated group and controls.

MSS 2-3

THE IMPACT OF VACCINATION ON HPV-RELATED DISEASES IN MEN & WOMEN: A MODEL-BASED ANALYSIS

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Objectives: The objective was to assess, using an individual-based transmission dynamic model (HPV-ADVISE), the potential impact of HPV vaccination among girls-only on the incidence of HPV-related cancers and genital warts over time in males and females.

Methods: We developed an individual-based transmission-dynamic model of HPV infection and disease in a population stratified by age, gender, sexual activity and screening behaviour. The model was calibrated to highly-stratified (e.g. gender, age, HPV-type) sexual behaviour, HPV epidemiology and cervical screening data from Canada. Population-level vaccine effectiveness is measured by the percentage reduction in the HPV-related outcome of interest (anogenital warts (AGW), or cervical, anal, penile, vaginal/vulvar, anal, or oropharyngeal cancers) in females or males over time post vaccination compared to no vaccination. Base case model predictions assume higher cross-protective efficacy for the bivalent vaccine, vaccination coverage of 80% among girls and that vaccinating girls does not impact HPV-related diseases in men-who-have-sex-with-men (MSM).

Conclusions: Under base case assumptions, vaccinating 12-year-old-girls with the quadrivalent(bivalent) vaccine is predicted to reduce the cumulative incidence of AGW, diagnosed cervical intraepithelial lesions 2 or 3 (CIN2/3), and cancers of the cervix, anus, vulva/vagina and oropharynx by 76%(0%), 55%(60%) and 42%(44%), 23%(23%), 15%(15%), and 12%(12%) among females over 70 years, respectively. Amongst males, a girls-only vaccination strategy is predicted to reduce the cumulative incidence of AGW, and cancers of the anus, oropharynx and penis by 68% (0%), 13%(13%), 10%(10%), and 8%(8%), over 70 years, respectively. In the long term, the overall impact of HPV vaccination is expected to be substantial. However, in the short to medium term HPV vaccination programs are expected to have little impact on cancers of the anus, vulva, vagina, penis and oropharynx, given that these cancers generally occur after 50-60 years of age.

A SECOND-GENERATION BROAD-SPECTRUM HPV VACCINE

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Licensed human papillomavirus (HPV) vaccines are based on virus-like particles (VLP), self-assembled from major capsid protein L1. Both vaccines induce high-titer, yet largely type-restricted antibodies against mucosal high-risk HPV16 and 18, which cause ~70% of all cervical carcinomas. In addition, the quadrivalent vaccine also includes VLP of the mucosal low-risk HPV6 and 11, which induce 90% of genital warts. However, these vaccines do not target about 25 less frequent mucosal or cutaneous HPV.

In contrast, immunizations with peptides of minor capsid protein L2 induce low-titer but cross-protective antibodies. Although L2 contains type-common motives crucially involved in early viral infection, L2 is immunologically subdominant in the context of native virions or co-assembled L1 + L1 VLP.

To improve immunogenicity of L2 we have generated chimeric RG1-VLP by genetic insertion of a broadly cross-neutralizing L2 epitope RG1 within the immunogenic DE-surface loop of HPV16L1, resulting in a 360-fold display of RG1 by the assembled particle.

Immunization with RG1-VLP using human applicable adjuvant alum-MPL induced a strong immune response against HPV16 and broadly cross-neutralizing antibodies against the most prevalent high-risk HPV18/31/45/52/58, low-risk HPV6/11, and cutaneous HPV5 in both rabbits and mice.

Moreover antisera to RG1-VLP cross-neutralized mucosal high-risk

HPV26/33/35/39/68/59/68/73/69/53/34, mucosal low-risk HPV32/40/44/70, and cutaneous HPV2/27/3/76, but not HPV1/4, in both pseudovirion neutralization assays, and newly established infectivity assays using native cutaneous HPV virions isolated from patients' skin.

Using a genital challenge model, passive transfer with RG1-VLP immune serum efficiently protected mice against experimental infection with phylogenetically divergent pseudovirions of mucosal high-risk types HPV16/18/31/33/35/45/52/58/73/59.

Immunization with RG1-VLP induces cross-neutralization against mucosal high-risk, mucosal low-risk, and divergent cutaneous HPV in vitro and in vivo.

THE PROSPECTS FOR AN L2 BASED PROPHYLACTIC VACCINE

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Background: Persistent infection with oncogenic human papillomavirus (HPV) is a necessary but insufficient cause of virtually all cervical cancers. Two licensed preventive HPV vaccines based on L1 VLPs demonstrate the possibility of dramatically reducing rates of cervical (and other HPV-related) cancers worldwide. However, many barriers remain to global reduction in cervical cancer, including type-restricted immunity, vaccine cost and delivery method.

HPV L2 vaccines: Unlike L1 VLP, vaccination with L2 induces broad cross-type neutralizing antibody that provides protection against heterologous type challenge in animal models. Polymeric fusion of L2 peptides derived from different HPV types are being developed for early phase clinical trials. This approach has the potential for low cost manufacture of a single polymeric antigen that might provide broad cross-protection. However, L2 shows weaker immunogenicity as compared to the ordered array structure of L1 VLP, suggesting the need for a potent adjuvant to induce antibody levels providing for long lasting protection. TA-CIN, a vaccine comprising the HPV16 L2, E6 and E7 in a single tandem fusion protein and offers the potential to combine the advantages of broad cross-protection against HPV transmission with therapeutic responses targeting HPV16 early proteins. Preclinical and clinical studies suggest that an appropriately adjuvanted TA-CIN might be able to offer both prophylactic and therapeutic potential.

Conclusion: While the current HPV vaccines based on the capsid proteins are effective for prophylaxis, they have not demonstrated therapeutic activity. Next generation HPV vaccines that include both a broad protection and therapeutic action could provide real competition with the VLP vaccines.

MSS 2-6

THERAPEUTIC HPV VACCINES – UPDATE AND PERSPECTIVES**T.-C. Wu,***M.D., Ph.D, Johns Hopkins University, Baltimore, Maryland, USA*

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.

MSS 3-2

TRIAGE STRATEGIES OF HPV POSITIVE WOMEN**Johannes Berkhof***Department of Epidemiology and Biostatistics, VU University Medical Centre, Amsterdam, The Netherlands*

It has been difficult to come up with a uniform management strategy for HPV positive women. HPV testing has a lower specificity than cytology and may lead to an increase in unnecessary colposcopies. To limit the number of referrals, cytology, genotyping, p16 staining, and repeat HPV testing have been suggested for triage and follow-up testing. An overview of recent studies on the management of HPV positive women will be given. In particular, follow-up data of two Dutch screening trials (POBASCAM and VUSASCREEN) will be presented. In both studies, HPV testing was included as a primary test but different HPV tests were used (GP5+/6+ EIA PCR and hc2). The performance of different triage and follow-up strategies in the two studies will be compared. It will be shown that there is no single, best strategy but there are several feasible strategies that can readily be implemented. The ultimate choice depends on the weights placed on safety, burden, and non-adherence risk.

HPV GENOTYPING BASED SCREENING: CONSIDERATIONS BASED ON CIN 3+ RISK**Stoler M,***Dept. of Pathology, University of Virginia Health System, Charlottesville, VA, USA*

Objectives: Current US cervical cancer screening guidelines recommend a combination of clinically valid HPV testing and cytology. Triage of HPV positive women with genotyping is also recommended, if such testing is available, as a method for focusing attention on the subset of screen positive women that need colposcopic referral as opposed to noninvasive follow-up. Data from recent clinical trials clearly reinforces the concept that there is differential risk of pre-cancer and cancer associated with different HPV types. Here we examine the cross sectional risk of CIN3+ as estimated from the literature with emphasis on data from the cobas HPV test registration trial ATHENA.

Methods and results: What is the risk of CIN3+ in women that are HR HPV positive stratified by genotype? Furthermore, once the genotype is known, does cytology status add further useful discriminating information? Epidemiologic surveillance studies demonstrate a 4-10 fold variation in risk of CIN3+ at 12 years depending on whether a women is persistently HPV16 positive as opposed to screen HPV 16 positive, screen HPV18 positive or other HPV type positive (JNCI 2010 102; 1478-1488). Likewise, in ATHENA, the cross-sectional risk of CIN 3 varies in NILM patients who are 14 type HPV positive almost 5 fold from ~2% for 12 hr-HPV+ to 10% for HPV 16+ women (Am J Clin Pathol 2011; 136: 578–586). Similarly, in women with ASC-US, cross-sectional risk of CIN3 varies in patients who are 14 type HPV positive 5 fold from ~4% for 12 hr-HPV+ to 20% for HPV 16+ women (Am J Clin Pathol 2011; 135:468–475). The consistency of these relative risks stands in contradistinction to the variability in diagnoses used by cytology laboratories on comparable population of patients.

Conclusions. High Risk HPV status defines the majority of a patient's risk for CIN3+. Genotyping helps stratify that risk, perhaps more reliably than cytology. Whether, more detailed genotyping will provide further useful patient stratification based on the genotype specific risk vs. prevalence is debatable and will potentially incur algorithmic complexity as a trade-off with clinical utility.

HPV MRNA IN PRIMARY SCREENING**Iftner T,***University Hospital Tuebingen, Institute for Medical Virology and Epidemiology of Viral Diseases, Division of Experimental Virology, Germany*

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 (AHPV) 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.

A running trial in Germany comparing AHPV and HC2 in a routine screening population of 10,050 women showed that both tests were highly sensitive for CIN2+ and for CIN3+. Specificity of AHPV for CIN2+ was higher than that of HC2: with a corresponding reduction in the false positivity rate of 21% and a rather high positive predictive value.

MSS 3-5

ROLE OF p16 IN SCREENING

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Objectives: p16^{-INK4A}, is a cyclin-dependent kinase inhibitor whose expression is negatively controlled by the RB gene product. Usually p16 is expressed at very low concentration in normal cells while it is strongly over-expressed in cervical cancer cell lines in which RB has been functionally inactivated by the high-risk HPV E7 oncoprotein. Therefore p16 overexpression is considered as an indicator of viral-induced deregulation of the cell cycle. To evaluate the role of p16 immunostaining for triaging HPV positive women we conducted a study nested in NTCC, a large randomized controlled trial comparing HPV DNA testing to cytology. Previously published results showed a cross-sectional sensitivity around 90% and a cross sectional specificity around 50% of p16 immunostaining for CIN2+ among HPV positive women. They also showed that referring to colposcopy only HPV positive women who also show p16 overexpression allows an about 50% increase in cross sectional sensitivity vs. cytology without increasing the referral to colposcopy. However, in order to define how frequently HPV positive but p16 negative women need re-testing, longitudinal data on the subsequent risk of having a CIN detected are needed.

Results: During the second phase of recruitment of the NTCC trial 1137 women had a valid p16 test. Among the women tested for p16, a CIN2+ was detected at recruitment in 95. Of the remaining 1042 women 943 performed further tests. The initial p16 immunostaining result was significantly associated with the risk of CIN3+. This association was particularly strong in women aged 35 to 60 years at recruitment

Conclusions: Our previous results showed that the cross-sectional sensitivity of p16 for CIN2 and 3 among HPV positive women was about 90% and that that immediate colposcopy is not needed in HPV positive women who don't show p16 overexpression. The longitudinal data suggest that HPV positive but p16 negative women don't need new short-interval testing and could be reasonably retested after 3 years. Instead, HPV positive women who also overexpress p16 need re-testing at relatively short intervals if no high-grade lesion is detected at the first colposcopy.

MSS 3-6

METHYLATION AS A SCREENING MARKER

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Objective. Dynamic methylation of DNA and proteins is the main molecular mechanism underpinning epigenetics, it plays a very important role in cancer and may be the predominant way that phenotype interacts with the environment. In diseases such as cervical cancer and precancer human genes and the human papillomavirus (HPV) genome are subject to large secular changes in DNA methylation (DNAm) levels that are accurately measurable using simple assays and show promise as biomarkers for diagnosis and prognosis. Although it appears likely that testing for high-risk (HR) HPV DNA will become the main method for screening sexually active women in the near future there is a possibility that DNAm may be considered an alternative screen or more likely as a companion diagnostic in women who are HPV positive. Thus, the features, benefits and costs of DNAm as a screening biomarker are of interest.

Methods. A literature review and laboratory experimentation employing a quantitatively accurate pyrosequencing (PSQ) DNAm test were conducted on specimens from several major human studies to evaluate the current status and prospects for DNAm as a clinically useful biomarker.

Conclusions. Direct testing of DNAm levels in HPV16 DNA by PSQ, preferably in the L1 open reading frame (ORF), is an option for screening that appears to produce excellent sensitivity and specificity and a corresponding higher area under the curve (AUC > 0.8) than current assays for HPV16 DNA or RNA. Although there are certain CG sites such as 6457 (in the L1 ORF) that appear optimal there are many other sites in E2, L2 and L1 that fall in the same general AUC range and could be incorporated into an assay, possibly configured to avoid the L1 deletions sometimes present in cancers. A technical challenge is to produce a simple consensus assay that can detect DNAm in all relevant HR-HPVs as a cocktail test. Regarding human genes as DNAm diagnostic targets no convincing data has been published to suggest that they could be a replacement for HPV DNA testing. Although there are several human genes (MAL, CADM etc.) that appear to have some potential for triage of HPV or cytology they seem not as good as DNAm assays for the HPV genome and will require large validation studies to be considered serious contenders.

HPV SCREENING IN LOW-RESOURCE SETTINGS**R. Sankaranarayanan MD,***Head, Early Detection & Prevention Group (EDP) and Screening Group (SCR), International Agency for Research on Cancer (WHO-IARC)***Objective:** To describe the role of HPV screening in low-resource countries.**Methods:** Published information from literature is reviewed and findings and conclusions are summarised.

Findings and conclusions: HPV DNA testing is the most objective and reproducible of all cervical screening tests. Its sensitivity and specificity in detecting CIN 2 and 3 lesions varied from 66-100% and 62-96% respectively; it had a higher sensitivity, but lower specificity than cytology in detecting lesions. In low-resource settings, a single HPV testing may provide an objective method of identifying women at risk for disease and investing the limited resources. However, there is still debate over whether HPV DNA testing alone can be used as a primary screening test without cytology or other triaging tests such as VIA/colposcopy. Although, HPV testing is currently an expensive option, a simple, user-friendly, affordable, fast and accurate HPV test (careHPV test) has now been evaluated and was found to have similar accuracy as that of Hybrid Capture II (HC II) test. It is expected to be commercially available in 2012. Evidence from randomized-controlled trials in different settings indicates that the burden of cervical neoplasia can be effectively reduced by HPV screening. Studies in developed countries showed higher detection of CIN 2 or worse lesions in prevalence rounds followed by reduced frequency of such lesions in subsequent screening rounds. In a randomized-trial in South Africa assessing safety and efficacy of HPV screen-and-treat with cryotherapy, the frequency of CIN 2 or worse lesions was reduced by 73% at 36 months as compared to a 'control' group; the prevalence of CIN 2 or worse lesions in HIV-negative women by 69% and among HIV-positive women by 80% in this study. There was a significant 48% reduction in cervical cancer mortality following a single round of HPV testing in a randomized controlled trial in India. Cost-effectiveness studies indicate that strategies incorporating HPV testing are more cost-effective than cytology, especially when widespread HPV vaccination will reduce the prevalence of cervical lesions. Investments in infrastructure and human resources for screening in low-resource countries will facilitate the introduction of affordable HPV testing in due course which will save many precious lives.

NEW HPV SCREENING EXPERIENCES: THE NETHERLANDS**Johannes Berkhof,***Department of Epidemiology and Biostatistics, VU University Medical Centre, Amsterdam, The Netherlands*

HPV Screening is on the brink of implementation in the Netherlands. The POBASCAM randomized-controlled trial has been completed and the CIN3+ and cancer risk in round 2 of the study were markedly lower in the intervention (HPV plus cytology) arm than in the control (cytology only) arm. This is in line with other trials and supports the use of the HPV test as a primary screening test, possibly in combination with an extended interval. An age-dependent strategy with cytology screening in younger women and HPV screening in older women was not supported by the POBASCAM study: the relative CIN3 detection rates in round 1 and in round 2 of the screening trial were similar in women invited for the first time for screening (29-33 years) and in older women. Two independent cost-effective studies further supported HPV testing in combination with cytology triage of HPV-positive women. HPV screening will also involve important changes in laboratory facilities; labs must be efficient as the budget for cervical screening is limited while the quality of the labs must be ensured and controlled. Finally, screening authorities must have a clear plan for evaluating the performance of the HPV screening program.

MSS 4-2

NEW SCREENING EXPERIENCES – HPV VACCINE IMPLEMENTATION: LESSONS LEARNED AND ACTIONS: ITALY**G Ronco***CPO Piemonte, Turin, Italy*

In 2011 some 53,000 women had HPV-based cervical screening, within pilot projects in 9 Italian Regions. Usually HPV testing is the only primary test and 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. 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 (while the other programmes are without control and some start at 25 years). The main purpose is evaluating feasibility and 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. Based on preliminary data, compliance to invitation was similar among women invited for HPV testing and for cytology. Data suggest that knowledge of HPV positivity influences the interpretation of cytology so that strict monitoring of it is needed. With the described protocol, although the estimated cost of a single round per screened woman was higher with HPV than with cytology, when using 5-year intervals with HPV and 3-year intervals with cytology, the lifelong cost per screened woman was lower. Vaccination for HPV16 and 18 will lead to a decreased positive predictive value, strongly for cytology but also, although more mildly, for HPV testing (because non-vaccine types progress less rapidly to pre-cancers). For the latter reason it will plausibly be safe to apply longer screening intervals, which will be essential in order to increase PPV and also to reduce over-diagnosis of regressive lesions.

MSS 4-4

HPV VACCINE IMPLEMENTATION: COUNTRIES DELIBERATION: ENGLAND**Soldan K.,***Department of HIV and STIs, Health Protection Agency, London, UK.*

A national immunisation programme has been implemented in the UK since September 2008. HPV vaccine is offered to girls aged 12 years in September. During the first two years of the programme, a catch-up programme was also implemented to offer vaccine to girls aged upto 18 years. The programme began using Cervarix and will change to use Gardasil in September 2012.

Some analyses of coverage amongst females have been conducted to describe variations that can inform cervical cancer control. The relatively high and broadly equitable uptake of vaccination among pre-sexual females in England, together with high and sustained efficacy of HPV vaccination among women in clinical trials, predicts significant reductions in HPV-related disease in due course. Given the substantial delay to cervical disease endpoints, early measures of the impact of this programme are needed. We are using opportunistic sources of residual clinical specimens to monitor seroprevalence for vaccine-induced antibodies and the prevalence of type-specific genital HPV infections among young women in England.

MSS 4-5

HPV VACCINE IMPLEMENTATION IN FRANCE**Parent du Châtelet I,***French Institute for Public Health Surveillance, France*

HPV vaccination with Gardasil has been introduced in the French immunisation schedule since March 2007 for 14 years old preadolescent girls. A catch-up for older girls and young women up to 23 years of age has also been recommended but restricted to those who have not yet started their sexual life more than one year ago. After licensing of Cervarix, the French advisory board on immunisation recommended in December 2007 a preferential use of Gardasil but this recommendation was discontinued in December 2010 and both vaccines are currently recommended.

There is no school-based vaccination program in France. Targeted girls (or women for the catch up) have to go, on their own initiative, to private practitioners in order to get vaccinated. Very few districts or cities have included HPV vaccines in their public vaccination offer. HPV vaccinations performed in the private sector are reimbursed by the French National Health Insurance at a rate of 65 % (70 % for the clinician's consultation) for the target population for which it is recommended (both for routine and catch up vaccination). More than 90% are covered by an insurance that bears the remaining share.

To monitor vaccines uptake, we used the data from the National Drugs Reimbursement Database. As of June 2011, the proportion of fully vaccinated girls (3 doses) was 27 % among the 15 year-olds and 37%, among the 16 and 17 year-olds respectively. The proportion of partially vaccinated girls (at least one dose) was 44%, 52% and 54% among the same three cohorts.

Different initiatives are being established to monitor the impact of the HPV vaccines including type-specific HPV and disease outcomes (cervical pre-cancerous and cancerous lesions) surveillance.

In conclusion, the HPV vaccines are both geographically and financially available in France. However, the still limited activities of active identification, invitation and follow up of the target population explain the low vaccination coverage.

MSS 4-6

HPV VACCINE IMPLEMENTATION: COUNTRIES DELIBERATION: ITALY**Paolo Bonanni***Department of Public Health – University of Florence, Italy*

Italy has a policy of active and free-of-charge offer of anti-HPV vaccine to 12 year-old females in all regions. Some of them added active offer to supplemental cohorts between 15 and 25 years. Coverage is monitored constantly at the national level, and reaches an average of about 60%. However, progress in coverage is not occurring, being further made difficult by financial constraints of the regional health services. A renovated communication effort is needed in order to increase immunization uptake, and increase population awareness about the benefits of combining vaccination and screening for the prevention of cervical cancer

MSS 4-7

HPV VACCINATION IMPLEMENTATION: AUSTRALIA**Selvey CE,***Senior Director, Communicable Diseases Branch, Queensland Health, Brisbane, Queensland, Australia*

With Professor Ian Frazer awarded "Australian of the Year" in 2006, an industry funded political campaign for GARDASIL funding and a Prime Minister whose wife announced she had cervical cancer ten years earlier, it was not surprising that Australia was the first country to introduce a funded HPV vaccination program. HPV vaccination was considered by the Pharmaceutical Benefits Advisory Committee (PBAC) to be sufficiently cost effective and was recommended for Government funding. The quadrivalent HPV vaccine has been offered to all female students in the first year of high school since 2007. Catch-up was offered to all female high school students in 2007-2008 on the back of an established school based vaccination program. Vaccine was also funded for women aged 18 to 26 years from mid-2007 to end 2009; vaccine was available from general practitioners and other community based vaccination providers. A national vaccination register was established. Coverage of the youngest cohorts eligible in 2007 and vaccinated at school was 83% for dose 1, but dropped to 72% for dose 3. Vaccination coverage is more equitably distributed among socioeconomic groups than cervical screening participation. Adverse events following HPV vaccination seen with the school program include anaphylaxis and syncope; however reporting rates are comparable with those reported from other countries. The PBAC has made a recommendation for funding of HPV vaccine for males in the first year of high school. Achievement of high vaccination coverage will be discussed.

MSS 4-8

HPV VACCINE IMPLEMENTATION: COUNTRIES DELIBERATION**Eastern European countries****Gunta Lazdane, MD, PhD***Programme Manager, Sexual and Reproductive Health, Division of Noncommunicable Diseases and Health Promotion, WHO Regional Office for Europe*

-Relatively low coverage with HPV vaccine in these countries due to low acceptance of vaccine among parents and medical professionals.

Need to develop effective communication strategy targeting teenager girls, their parents and medical workers

-Need to utilize new communication channels and strategies (social media, mobile phones, internet)

-Importance of development of communication plans prior to introduction of HPV vaccine as well as crises communication plans to be prepared for adverse events following immunization and negative publicity

-Importance of monitoring of vaccine coverage and setting the goal to achieve high coverage with three doses of vaccine in target group shortly after introduction

-Need to involve health care professionals including reproductive health and cancer specialists into communication campaign to increase trust to vaccination programme and create demand in the public

HPV VACCINES AND THE BABEL TOWER: THE PEDIATRICIAN POINT OF VIEW**Martinon-Torres, Federico,***Area of Pediatrics, Hospital Clinico Universitario de Santiago, Spain
Vaccine Research Unit, Research Institute of Santiago de Compostela (IDIS), Spain*

Coordinated multidisciplinary approach is key for the success in any vaccine practical implementation and HPV vaccines are not the exception if not more specifically surrogated to this coordination than other vaccines. Many of the limitations in current implementation of HPV vaccines across Europe may be explained by a lack of effective coordination among the different participating actors.

Firm and proactive advice from the healthcare provider is, by far, the principal variable associated with a favorable decision of a patient to receive the vaccine. Pediatricians are the key field players in vaccination, as beyond authorities, public health care providers and scientific societies recommendations, parent will follow their advice in the ultimate decision. As an example, in countries where children are not exclusively cared by pediatricians, vaccination coverage is generally lower, furthermore for those vaccines not reimbursed or included in the national immunization program.

Paediatricians are very active HPV vaccination promoters. They are key in the access to the vaccination target population, but also to inform parents about the additional indications of the vaccine (older age groups, males) and eventually about the maintained need of cervical cancer secondary prevention. Given the importance of applying vaccination and screening as synergic and complementary strategies, pediatricians may play a key role in the inclusion in the secondary prevention program of women not adequately or just not previously screened. Many of the women that have -purposely or not- missed their screening visit, will go to the pediatrician with their children to get them vaccinated, being an excellent opportunity for rescuing them for the cervical screening program.

The view of pediatricians, who have extensive experience administering vaccines to children and adolescents, have to be taken into account in HPV vaccine delivery strategies. On the other hand, guidance for pediatricians as to how to address parental concerns or what and how to inform about secondary prevention of cervical cancer, among other specific educational initiatives, is essential.

HPV vaccines implementation requires the coordinated effort of all players to actually achieve a successful and maintained vaccine coverage. In this sense, the paediatricians participation must be coordinated and integrated with the rest of available resources and players (health authorities, scientific societies, social agents, media...). Coordination of any of these efforts is deemed urgent and necessary, at least at a regional/country level.

CLINICIAN POINT OF VIEW: THE GP**Marc Steben MD,***Unité infections transmissibles sexuellement, Institut national de santé publique du Québec, and director of medical services, of Clinique A, Montréal, Québec, Canada*

Introduction of highly effective prophylactic HPV vaccines in the preventive agenda of GPs cannot be counted as a success. First, with physicians in short supply, much of the preventive agenda is abbreviated to gear the limited time available toward curative care. Second, GPs, as was the case with nurses before, doubt the usefulness of vaccines. In a needs assessment for CME accreditation, the same proportion of physicians doubted the usefulness of vaccines equal to those who thought they were useful. Third, most patients doubt the effectiveness and safety of vaccine due to the mounting importance of web experts and bloggers. Fourth, a fair proportion of physicians are not comfortable discussing sexuality issues especially with parents accompanying their adolescent. Fifth, physicians are not comfortable with the selling of HPV vaccines. If questioned by parents, they frequently refer outside their practice for answers or vaccines. Sixth, most physicians have no immediate access to the vaccine. They refer outside their practice and patients frequently cannot easily get access to the vaccines. Seventh, physicians prefer not being directive and a weak recommendation is not frequently followed by actual vaccination.

Eight, many parents and adults doubt the usefulness of the HPV vaccines. While high completion rate has been seen in the free Québec program, some parents and adults feel the vaccine not a good investment since the government do not pay for it a their age.

Strong personalized recommendations with ready access to the HPV vaccine accompanied by a recall system have been found to be accompanied by high rate of completion of HPV vaccination.

MSS 4-14

PUBLIC HEALTH REPRESENTATIVE POINT OF VIEW: ECDC**Lopalco PL,***Head of Vaccine Preventable Disease Programme, European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden***Key points:**

HPV vaccines cannot replace or modify current routine cervical cancer screening protocols.

It is imperative that national screening programmes are maintained in place and that the impact of vaccines on screening programmes is monitored closely.

MSS 4-15

PUBLIC HEALTH REPRESENTATIVE POINTS OF VIEW: FIGO**Joanna Cain,***International Federation of Obstetrics and Gynecology: (FIGO)***Key Points:**

- Vaccination is part of an overall plan for cervical cancer control, not independent of it. Ignoring the need for concurrent screening of women to find those with disease will not create the rapid disease control in the population that is needed and devalues adult women.
- Education remains the biggest challenge in all resource settings. Engagement of the entire community: religious, social (women's groups, etc.), governmental and medical leaders all need to be educated and active in sharing education with the broad community
- Progress toward coverage of vaccination at a cost point that can be met by low resource settings remains a barrier for many countries
- Linkage of vaccination to a virus and cancer that have been so highly linked to sexual exposure can stimulate the same denial patterns and emergence of false information regarding sexual promiscuity that are seen with other adolescent risks such as prevention of teen pregnancy by offering adequate and consistent sex education. Linking vaccination to the prevention of a major cancer has higher success but does not deal with societal mores that continue to limit beneficial health care for adolescents world wide.

COMBINING HPV VACCINATION AND HPV SCREENING IN OLDER WOMEN: IS IT RATIONAL?**Peto J.***London School of Hygiene and Tropical Medicine, London, UK*

Girls and young women who have not yet been infected have been the primary target of most HPV vaccination programmes. However, most of the cervical cancer deaths in developing countries over the next 20 years will be in older women who have already been infected with HPV. A more urgent public health priority is therefore to develop an effective global intervention strategy for these older women. The polyvalent HPV L1 vaccine currently being tested in large trials includes 7 HR-HPV types that cause about 90% of all cervical cancers in most populations (HPV 16, 18, 31, 33, 45, 52, 58), and alternatives that may have similar efficacy, including HPV L2 vaccines, are being developed. Such vaccines will reduce the cancer risk even in women who have already been infected with one or more HR-HPVs, as they will prevent future infections with other HR-HPVs. The data on long-term cancer risk are still limited, but HPV DNA is probably persistently detectable in most women who will eventually develop cervical cancer. In combination with testing to identify and treat persistent HR-HPV infections, polyvalent HPV vaccination therefore provides a once-in-a-lifetime intervention that is likely to reduce cervical cancer incidence rapidly and effectively at any age.

The lifetime risk of cervical cancer in women aged over 40 who do not already have a persistent HPV infection may be so low that vaccination alone would not be cost-effective irrespective of vaccine prices. The value of polyvalent vaccination followed by HPV testing in these older women is that any infection with an HR HPV type included in the polyvalent vaccine that is detected 2 or more years after vaccination is likely to be a persistent infection, and would therefore justify immediate treatment. The upper age limit for vaccination should be the age beyond which most prevalent HPV infections are persistent and should therefore be treated, not the age at which a new infection is unlikely to cause cancer.

The feasibility and impact of this intervention should be considered irrespective of current costs of polyvalent vaccines and HPV tests, which may fall rapidly in response to demand and competition. The EUROGIN 2010 Roadmap suggested that a programme of mass vaccination followed at least 2 years later by HPV testing and immediate treatment of all HR HPV infections might be cheaper and more feasible than HPV testing with subsequent triage and clinical follow-up of HPV-positive women.

REDUCED / ALTERNATIVE VACCINE DOSE SCHEDULES**Marc Steben MD,***Unité infections transmissibles sexuellement, Institut national de santé publique du Québec, and director of medical services, of Clinique A, Montréal, Québec, Canada*

HPV prophylactic vaccines have been found to be highly immunogenic accompanied a very strong anamnestic response when recipients were challenged with an additional dose of the vaccine more than 4 year after the primary schedule. HPV vaccines have shown higher immunogenicity in younger subjects compared to older. Although immunogenicity was shown in younger age cohorts, protection against incident and persistent infections or lesions was not assessed. Preliminary immunogenicity studies have shown that 2 doses given in 6 months had the same immunogenicity as 3 doses given in 6 months for at least 2 years. These reduced dosing studies are not assessing incident or persistent lesions or lesion protection. Longer term anamnestic response has not been assessed yet. Most of the public health program with 2 doses, are expecting data on longer term immunogenicity and anamnestic response before deciding to give or not a third dose. These studies are not done or assessed the ways the companies' studies were done. Two doses regime have the advantages of requiring less human and financial resources heightening the feasibility of programs in countries with limited resources. Long term evaluations are not yet available to help the adoption or the rejection of such preventive program.

MSS 5-3

THE COMPARATIVE VALUE OF BIVALENT AND QUADRIVALENT VACCINES

Jit M

Health Protection Agency, London, United Kingdom

Objectives: Two vaccines against HPV are widely available. They differ in terms of valency, licensure indications, degree of cross-protection and reported long-term immunogenicity. Vaccine purchasers need to make evidence-based decisions about which of the two vaccines to procure, particularly if they are priced differently.

Methods: Evidence differentiating the two vaccines is reviewed, and its implications on the comparative value of the two vaccines are assessed using epidemiological and economic modelling.

Conclusion: The bivalent vaccine is likely to prevent more deaths because of its likely broader protection against non-vaccine oncogenic HPV types. The quadrivalent vaccine is likely to reduce healthcare costs and improve quality of life by more. However, the remaining uncertainty around long-term protection, range of endpoints prevented and interpretation of trial data needs to be taken into account when comparing the two vaccines.

MSS 5-4

CURRENT BARRIERS TO IMPLEMENT HPV TESTING IN CERVICAL CANCER SCREENING

Eduardo Franco,

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The evidence that has emerged in the last 15 years for the efficacy of HPV testing in primary cervical cancer screening has evolved in sequential steps. Many cross-sectional investigations and a few randomized controlled trials have provided the initial level of evidence that HPV DNA (or RNA) testing is more accurate than Pap cytology in detecting high-grade cervical lesions. The second level of evidence is the verification that the extra detection and treatment of high-grade lesions will reduce the rate of these abnormalities in subsequent screening rounds, thus demonstrating that many HPV-positive lesions may persist or progress and removing them at the outset translates into more safety for the patient. The third level of evidence comes from the claim that, being more sensitive than cytology, HPV testing permits extending screening intervals safely for patients that are initially HPV negative. Yet, despite the coherent and overwhelming body of evidence favouring the adoption of molecular HPV testing into cervical cancer screening programs changes have been slow. Thus, conventional or liquid-based cytology continues to dominate in most high-resource settings. Several barriers exist for widespread implementation of HPV testing in cervical cancer screening. Foremost is the issue of cost. Most cost-effectiveness analyses indicate that affordability is a key deterrent. However, projected costs of molecular testing have not yet factored in the reductions that will inevitably come from an economy of scale and competition among manufacturers of HPV tests. A second obstacle comes from the erroneous perception that cytology-based cervical cancer screening programmes only need better coverage and sustained quality control to have an impact on incidence and mortality rates. Much of that perception is related to concerns about loss of control by some professional groups of the core activity of cytology screening in cancer prevention. A third obstacle is the lack of proper knowledge by providers on how to communicate the significance of HPV positive results to their patients. A related concern stems from the ever increasing diversity of commercial HPV assays and their claims, which has led to confusion among clinicians. As new professional guidelines have gradually emphasized the role of HPV testing and minimal standards for assay performance the latter concerns are likely to become moot in the future. Essential in permitting widespread rollout of HPV testing in cervical cancer screening will be the example of early adopters, such as The Netherlands. As success stories accumulate it is likely that more countries will take the courage to change practices and embrace HPV testing as a primary screening tool.

PERSPECTIVES OF AN ORGANIZED PROGRAM ON THE INCLUSION OF HPV TESTING IN CERVICAL CANCER SCREENING

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Background: Organised screening programs are responsible to report to the health care authorities on the beneficial and adverse effects and costs of the programs. Compared to cytology, HPV testing has been shown to have greater sensitivity and longer duration of protective effect against CIN3+ among women testing negative, which may permit lengthening screening intervals for HPV-negative women. However, as the specificity of HPV testing is lower, an overuse of HPV testing could result in increased adverse effects and costs. The EU guidelines have therefore recommended only a controlled implementation in organised programs.

Objective: We wished to pilot the implementation of controlled HPV testing in an organised screening program (Greater Stockholm area, Sweden).

Methods: The organized program issued a public tender for an HPV-based screening test and implemented organized use starting on the 1/1 2012. Use of the HPV screening is controlled, as the test is offered only within the organized program. Screening invitations, sampling, administration and economy is the same for all cervical screening, regardless of whether cytology or HPV is the primary test. Implementation is done in a stepwise fashion (with defined evaluation Stop/Go points), successively enlargening the age groups and the proportion of the population that receives the HPV test. When the samples arrive at the laboratory, they are sorted to either cytology or HPV testing based on i) age of the woman and ii) randomization. If HPV testing results are favourable in terms of effect, safety, acceptability and cost-efficiency at the evaluation points, full implementation will have occurred after 3 years.

Conclusions: Organised implementation of a switch of screening test is feasible and allows for stringent follow-up whether the policy switch did represent an improvement.

HPV SCREENING CONTROVERSIES: CONTROVERSIES FROM THE UPDATED GUIDELINES ON CERVICAL CANCER SCREENING

Joanna Cain,

International Federation of Obstetrics and Gynecology (FIGO):

1. Should we offer 2 rather than 3 doses? The financial impact and potential ability to immunize more is weighed against the present strength of evidence.
2. Should we consider the role that women's health rights plays in LACK of access to vaccination/ screening/ or treatment of cervical cancer and how might that allow us to address the problems?
3. Women die of cervical cancer—too often without adequate access to pain control and end of life care. In our passion to assure the access to vaccines, how do we acknowledge and care for women throughout the course of disease in low resource settings?

MSS 5-9

MOVING FORWARD IN COMPREHENSIVE SCREENING AND VACCINATION PROGRAMS: CONCEPTS FOR A EUROPEAN IMPLEMENTATION TRIAL

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Program Chief, Cancer epidemiology research program, Institut Catala Oncologia, Barcelona, Spain

Phase III vaccination trials have clearly shown the potential of HPV vaccines to reduce the burden of cervical cancer by at least 70% in HPV-naïve vaccinated cohorts. Likewise, HPV DNA screening trials have provided robust evidence of an increased sensitivity of 30–35% over conventional cytology, and a large trial in India has produced evidence of a reduction in cervical cancer mortality related to just one HPV test at ages 30–59. Current proposals are now aimed at promoting the use of HPV testing as the primary screening option, especially in areas where screening needs to be organized de novo. When used in adolescent and young girls, vaccination will take several decades to display its full potential, whereas increasing the efficacy of screening programs will lead to a rapid reduction in cervical cancer amongst sexually active adult women. These two highly desirable options now need to be harmonized and strategic trials combining HPV vaccination and advanced screening protocols need to be considered and eventually implemented. These trials could take advantage of the literally tens of thousands of women who have entered screening trials and are known to be HPV negative and cytology normal, and could be randomly allocated to either HPV vaccination or conventional follow-up. Likewise, women who have participated in the large Phase III vaccination trials could be randomized to conventional screening or to 3-5-7-year interval HPV screening.

MSS 6-1

INTRA-LABORATORY VARIATION IN THE PERFORMANCE OF LIQUID-BASED CYTOLOGY; INSIGHTS FROM ATHENA

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OBJECTIVE: Although it is well recognized that cervical cytology is highly subjective and that there is considerable intra-laboratory variation, relatively little is known as to how this impacts the performance of cytology. In the ATHENA trial, cytology specimens from women undergoing routine cytological screening were evaluated at 4 large regional U.S. cytology laboratories. This provides a unique opportunity to evaluate the impact of intra-laboratory variations on the performance of cervical cytology.

METHODS: Liquid-based cytology specimens from 40,901 women ≥25 years of age were routinely processed at 4 U.S. laboratories. All women with abnormal cytology (atypical squamous cells of undetermined significance, ASC-US, or higher) were referred to colposcopy, as were all high-risk HPV positive women and a random subset of those who were negative by both high-risk HPV testing and cytology. All HPV testing, colposcopy, and review of biopsies and cytology specimens were done in a blinded fashion.

RESULTS: Sociodemographics, risk factors for cervical disease, and prevalence of CIN were similar across the laboratories. The prevalence of high-risk HPV (pooled 14 types) varied only from 9.1% to 11.2% and HPV 16 positivity from 1.8% to 2.3%. In contrast there were considerable differences between the laboratories in the cytological abnormal rates and these differences had a pronounced impact on the prevalence of CIN2+ in women with different cytology results.

| | Lab A | Lab B | Lab C | Lab D |
|------------------------|-------|-------|-------|-------|
| ASC-US rate | 2.3% | 2.4% | 4.3% | 6.5% |
| CIN2+ in ASC-US | 8.1% | 5.0% | 2.6% | 4.1% |
| Abnormal cytology rate | 3.8% | 5.2% | 8.1% | 9.9% |
| CIN2+ in NILM | 5.2% | 4.0% | 3.6% | 2.1% |

CONCLUSION: Considerable variations were observed in both the overall abnormal cytology rates as well as the ASC-US rates. As might be expected, the laboratory with the lowest ASC-US rate had the highest prevalence of CIN2+ in women with ASC-US. Somewhat unexpectedly, the prevalence of CIN2+ in women with NILM cytology showed a greater than 2 fold variation and was significantly lower in the laboratory with the highest overall abnormal rate.

MONITORING OF HPV VACCINATION EFFECTS IN SWEDEN

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Background: Sweden mandated population-based HPV vaccination in 2010, but owing to delays with public tender for HPV vaccines the vaccination program was not launched until January 2012. The program targets 10-11 year old girls in school and included organised, population-based catch-up up to 18 years of age.

Objective: To design and implement a state-of-the art monitoring program of HPV vaccination in Sweden

Methods: The national HPV vaccination monitoring program was designed in a collaboration between the Swedish Institute for Infectious Disease Control, the Swedish National Quality Registry for Cervical Cancer Screening and the WHO HPV LabNet Global Reference Laboratory in Sweden. The major components are: i) an HPV vaccination registry, that includes consent for linkage with health data registries and biobanks ii) a sentinel HPV prevalence monitoring program targeting sexually active teenagers, that is nested in the Chlamydia trachomatis screening program in 3 regions of Sweden. iii) systematic HPV typing of formalin-fixed paraffin-embedded specimens of invasive cancers of the cervix, vulva, vagina, penis, anus and tonsils. iv) mandatory HPV typing of cytology specimen with HSIL or worse v) sentinel HPV typing of condylomas vi) registry linkages of the HPV vaccination registry with patient registries, pharmaceutical registries and the National Quality Registry for Cervical Cancer Screening to determine safety and effectiveness of the vaccination program.

Conclusions: The Swedish HPV vaccination monitoring system has accumulated a substantial amount of baseline data and data from non-organised HPV vaccinations. Organised HPV vaccination has, however, just recently started.

SURVEILLANCE OF EFFECTS OF HUMAN PAPILLOMAVIRUS IMMUNISATION IN BELGIUM (SEHIB)

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Objectives: To generate baseline data at the introduction of prophylactic HPV vaccination and to provide a surveillance framework for measuring the impact of HPV vaccination in Belgium.

Methods: From 10 cytopathology laboratories (5 universities, 5 other), 10x600 residual liquid cervical cell samples, collected from women aged 18-64 years participating in screening are currently being included, as well as 5x240 (80 ASC-US, 80 LSIL, 80 HSIL) additional abnormal samples from the 5 university laboratories. Included samples are tested for presence of DNA from individual HPV genotypes using a sensitive rtPCR targeting E6/E7 genes. Only the university centers recorded the HPV vaccination status, which was validated by contacting the vaccinating doctors. However, for women declaring being non-vaccinated, the status was not verified.

Results: the 3592 first samples, among which 2501 were genotyped by December 2011, are the object of the current analysis. Vaccination status was recorded for 954 women. The prevalence of cytological abnormalities was: ASC-US (3.3%), AGC (0.2%), LSIL (3.8%), ASC-H (0.5%), HSIL (0.7%). Substantial inter-laboratory differences were noted. The overall prevalence of high-risk HPV typically varied by age: >15% among women younger than 29, decreasing progressively with higher age, reaching a prevalence of 4% among women aged 60-64 years and was strongly correlated with cytological findings. HPV16 was the most common infection in women with normal cytology (2.5%), followed by HPV31 (1.5%) and HPV52 (1.2%). In HSIL, the most common types were: HPV16 (63%), HPV18 (21%), HPV31 and HPV51 (both 16%). Considering women with missing vaccination status as non vaccinated, the estimated 3-dose vaccination coverage was 43% for women < 20 years, 19% in age group 20-24, 4% in age group 25-29 and zero in women of 30 and older. The prevalence of HPV16/18 in women younger than 30 years (N=235) was lower in vaccinated (2.6 %) than non-vaccinated women (6.1 %).

Conclusions: The SEHIB study provides crucial baseline information indicating that the cyto-virological correlation could be used in quality control of cervical cytology. Even though a high proportion of vaccinated women <30 years were most likely exposed to HPV before vaccination a reduction of HPV 16/18 infections was observed suggesting an early effect of HPV vaccination. More results are awaited to assess the impact of vaccination on the prevalence of cytological and histological cervical lesions, related to vaccine HPV types, related to non-vaccine high-risk types and irrespective of HPV types.

DECISION BY GAVI ALLIANCE TO INCLUDE HPV VACCINE IN ROSTER OF VACCINES SUBSIDIZED FOR WORLD'S POOREST COUNTRIES

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In November 2011, the GAVI Alliance Board approved the opening of a funding window for human papilloma virus (HPV) vaccines paving the way for GAVI-eligible countries to apply for HPV programmes in 2012. The Board opened the funding window provided that: the Secretariat secures acceptable price commitments from industry for HPV vaccines; HPV proposals demonstrate the ability of the country to deliver HPV vaccines to the new target population including through successful demonstration projects and that a communication strategy is in place in that country; and requested the Secretariat to work with technical partners to develop HPV demonstration projects following the Board Meeting.

Background: GAVI Alliance is a global public-private partnership organisation formed in 2000, and has a mission to 'save children's lives and protect people's health by increasing access to immunisation in poor countries'. Partners include developing country and donor governments, WHO, UNICEF, the World Bank, the vaccine industry in both industrialised and developing countries, research and technical agencies, civil society organisations, the Bill & Melinda Gates Foundation and other private philanthropists. As such, it 'pulls the specialist skills of all players in immunization' into 'one decision making body'. By the end of 2011, GAVI had supported the immunisation of 326 million additional children, who might not otherwise have had access to vaccines, and prevented over five million future deaths.

In June 2008, the GAVI Board approved a Vaccine Investment Strategy (VIS) objective to "reduce the overall disease burden". Later in 2008, the Board endorsed HPV, Japanese encephalitis, rubella and typhoid as key vaccines that can contribute to this objective. The GAVI Board selected these 4 diseases from a selection of 18 provided by the WHO in 2007. The decision was made on the basis of potential health impact as well as the costs and operational feasibility of introducing each vaccine in developing countries.

The GAVI Secretariat was also encouraged by the GAVI Board to develop the vaccine portfolio taking into account technical advice and developments related to discussions of the WHO Strategic Advisory Group of Experts (SAGE). The Board at that time did not make a financial commitment for this vaccine investment strategy however given the financial climate.

In May 2011, the Programme and Policy Committee of GAVI, in anticipation of a successful pledging conference, endorsed a process of developing implementation strategies and guidelines for the four vaccines such that new windows of funding could be opened in the next round of applications.

Methodology: In collaboration with technical partners from eight organizations, GAVI's Accelerated Vaccine Introduction (AVI) initiative coordinated a process to refine the 2008 implementation strategies for each vaccine. Sub-teams were formed for each vaccine to review WHO guidelines and SAGE recommendations and availability of vaccines. Strategic demand forecasts were also revised. Recommendations for GAVI support were aligned with the most recent WHO and SAGE guidance on the vaccines.

GAVI Board's endorsement to open the funding window for HPV means that working with countries will require a new way of coordinating with multiple stakeholders and new partnerships at country and global levels. More specifically, it will require active engagement of reproductive health and cancer prevention and control programmes. The vaccine is targeted at adolescents and prevents a sexually transmitted infection and cervical cancer. Thus, groups from the fields of reproductive health, adolescent health, school health, women's health and cancer who have not traditionally played a role in childhood immunisation should be engaged to coordinate efforts on vaccine implementation and to promote relevant aspects of health relevant to this new target population. The HPV vaccine needs to be administered to 9-13 year old girls, a population that has not been routinely served by infant immunisation services. A GAVI-supported HPV vaccination programmes may require the establishment of new systems to reach this target age with the required three doses. Vaccination strategy using a school based programme will also require active engagement of leadership and staff within the Ministry of Education. GAVI will need to include criteria which will differ from those for other vaccines to ensure that countries are sufficiently prepared to introduce the vaccine into national programmes. The criteria will include for instance, a description of educational systems for girls for school based delivery. All GAVI-eligible countries may apply for national introduction of HPV vaccines and would need to address requirements specific to HPV vaccine in their application, especially those specified by the GAVI Board. For the introduction year and each subsequent GAVI-supported year, countries would select and vaccinate the same single-year cohort selected from the WHO-recommended target population of girls aged 9-13 years old. GAVI will fund the cost of the vaccine in line with the current GAVI co-financing policy, and will also provide routine introduction grant for countries to introduce the vaccine into their routine systems. Countries without experience of delivering HPV vaccines may not be ready to apply for support of a national roll out. In order to help them make an informed decision and gather appropriate information to support a national plan, countries would have an option of applying for support for a demonstration project. GAVI is working through an AVI HPV sub-team to define what the GAVI HPV demonstration programme will entail and to develop a detailed protocol. The HPV sub-team is composed of experts from relevant departments of WHO, UNICEF and PATH. The sub-team has continuing discussions on issues related to the HPV programme including demonstration projects.

Conclusion: In introducing HPV vaccine, GAVI would place a focus on women's reproductive health for the first time in its history. The GAVI Alliance will play a crucial role in encouraging support for comprehensive cervical cancer strategies including appropriate screening and treatment. In addition, introduction of HPV vaccines will set a new public health precedent in establishing primary preventive care for girls and could be used to improve access to other health services for this population. Where HPV is introduced through a school based delivery system, GAVI has the opportunity to have a wider impact on other health issues among young girls.

OPTIMIZATION OF SCREENING AND VACCINATION BY USING MODELING

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Background: Many countries have implemented routine HPV vaccination programmes, often with catch-up campaigns to late adolescence or early adulthood. In some settings, the first vaccinated cohorts are reaching the age of routine cervical screening. At the same time, the development of HPV testing and discovery of other biomarkers of cervical cancer risk offer new possibilities for screening modalities.

Methods: The optimal method of screening in a population depends on a number of factors including (i) the effect of vaccination on the prevalence of different oncogenic HPV types in vaccinated and unvaccinated women, (ii) the test characteristics of the screening test or combination of tests used, (iii) the cost of different combinations of vaccine and screening strategies, (iv) the interaction between vaccination coverage, screening uptake and risky behaviour in different population subgroups. To address all these factors at the same time requires mathematical models that can combine features used to model vaccination (such as sexual transmission and herd protection) and screening (such as test characteristics and differentiation of populations by risk).

Conclusion: Using modelling to find optimal combinations of screening and vaccination offers the potential to develop combined strategies that are cost-effective and that can decrease the burden of cervical cancer.

VACCINE IMPACT IN AUSTRALIA AND THE NEED TO REVIEW SCREENING

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Australia was the first country to introduce a funded HPV vaccination program. The quadrivalent HPV vaccine has been offered to all female students in the first year of high school since 2007 with catch-up offered to all female high school students in 2007-2008 and to women aged 18 to 26 years from mid-2007 to end 2009. A national vaccination register was established. Coverage of the youngest cohorts eligible in 2007 and vaccinated at school was 83% for dose 1, but dropped to 72% for dose 3. Cervical screening register data has shown a decrease in high grade cervical abnormalities in young women following the introduction of vaccination. Data from sexual health clinics shows a reduction in the proportion of attendances for genital warts in vaccine eligible female cohorts; attendances for warts in heterosexual men of the same age also decreased suggesting a herd immunity effect. Two studies are underway to examine HPV type prevalence women with normal and abnormal cervical smears. Reductions in cervical abnormalities due to vaccination will change the cost-benefit ratio of Australia's successful cervical screening program. Current Australian cervical screening guidelines recommend screening with Papanicolaou smear every two years, beginning two years after sexual debut or at age 18 years whichever is later, and continuing until aged 70 years. Australia's process for renewal of its cervical screening program will be discussed.

MSS 7-2

SIMILARITIES AND DIFFERENCES IN THE EPIDEMIOLOGY OF CANCER (CERVICAL VS. ORAL)**Xavier Castellsagué, Laia Alemany, Silvia de Sanjosé, F. Xavier Bosch***Unit of infections and Cancer, Cancer Epidemiology Research Program, Institut Català d'Oncologia (ICO), L'Hospitalet de Llobregat, Catalonia, Spain*

Objectives: A subset of head and neck cancers, in particular oropharyngeal cancers, and anogenital cancers, in particular cervical cancer, share a common etiology, namely persistent infection with oncogenic HPV types. Despite this, HPV-related anogenital versus head and neck cancers do not behave equally. This communication analyses similarities and differences in the epidemiology of these cancers.

Methods: Comparative epidemiology analyses from a comprehensive literature review were performed to identify common and contrasting traits in the epidemiology of head and neck cancers versus cervical cancers. Comparative analyses focus on descriptive epidemiology, detection of HPV markers, and risk factors for both cancer groups.

Results: Key similarities include: an overall consistent pattern in the relative burden of both cancers in developed versus developing countries, the sharing of HPV as a common etiological factor, the top frequency of HPV16 detection in both cancers, the role of smoking as a factor or co-factor of carcinogenesis in both cancers, a similar pattern of risk factors for HPV positive cancers for both sites, and a potential similar preventative value of HPV vaccination. Concerning key differences, as compared to cervical cancer, head and neck cancers show an increasing incidence trend and improved survival of HPV-positive cancers, a different epidemiological pattern in terms of age and gender, a wider multifactorial etiology, a differential role of smoking, a dual distinctive entity, a more predominant role of HPV16 with regard to other types, a less clear pattern of pre-cancerous lesions, and the unavailability of validated screening assays for their early detection.

Conclusions: Our knowledge on the epidemiology, etiology and natural history of head and neck cancers has dramatically increased in the last decade. Even though several important gaps in our understanding of the true etiological role of HPV in head and neck cancers still remain, HPV vaccination have the potential to prevent a yet unknown fraction of head and neck cancers in men and women.

MSS 7-3

SIMILARITIES AND DIFFERENCES BETWEEN CANCER OF THE CERVIX AND THE UPPER AERODIGESTIVE TRACT**Franceschi S,***International Agency for Research on Cancer, Lyon, France*

Background HPV carcinogenesis in the upper aerodigestive tract and in the ano-genital tract have in common that, although viral infection can be found in different anatomic sites, it mainly causes carcinomas in certain highly specialized epithelia (the epithelium of the tonsil, and the squamous-columnar junction in the cervix). The natural history of cervical HPV infection is well-studied but it is not clear to what extent extrapolation of cervical findings to HPV carcinogenesis in the tonsil is appropriate.

State of the art Important differences between cancer of the cervix and cancer of the oropharynx include: (i) cervical HPV infection is mainly transmitted through sexual intercourse and is very common and on the rise in nearly all world populations. Conversely, information on the prevalence of and the age at first acquisition of HPV infection to the oropharynx is limited in quantity and quality (mainly deriving from mouth rinse/gargle-based studies of adult individuals); (ii) HPV16 accounts for approximately 50% of cervical carcinoma but nearly 90% of HPV-positive carcinomas of the oropharynx; (iii) the precancerous HPV-associated lesions that precede cervical carcinoma (cervical intra-epithelial neoplasia grades 1-3) are well defined and widely employed in screening programs. On the contrary, precancerous lesions in the oropharynx are ill-understood and not consistently classified. The very existence and scope for early diagnosis of these lesions are controversial; (iv) contrary to cervical carcinoma, very strong risk factors other than HPV (notably tobacco use and heavy alcohol intake) exist for oropharyngeal carcinoma implying that it can be induced by different causes.

Conclusions The etiological heterogeneity of cancer of the oropharynx has many implications: 1) the fraction of cancers attributable to HPV varies substantially from one population to another, on account of methodological differences between studies but also of large variation in the relative burden of HPV infection and tobacco/alcohol use; 2) the presence of HPV DNA in tissue biopsies is not sufficient to attribute a cancer of the oropharynx to HPV, notably in patients who also report tobacco/alcohol exposure; 3) the type and extension of precancerous alterations are different in HPV-associated carcinomas from those caused by tobacco (for which "field cancerization" has been well documented for decades).

MOLECULAR MECHANISMS

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Approximately 50% of Head and Neck Cancers (HNC) are in the oral cavity, followed by 30% in the larynx and 10% in the oropharynx. Alcohol consumption, smoking, poor oral hygiene and genetic features are key risk factors for HNC development. In addition, in the last decade it has become clear that a sub-set of HNC covering approximately 25% of the worldwide cases is associated with certain HPV types. More than 120 different HPV types have been isolated so far, and they can be sub-grouped into cutaneous or mucosal types according to their tissue tropism. Twelve mucosal HPV types, referred to as high-risk (HR) HPV types, have been recognized by IARC as class I carcinogens causing cervical cancer. HPV16 and HPV18 are the most oncogenic types within the HR group, and are responsible for approximately 55% and 16%, respectively, of cervical cancers worldwide. In contrast, the majority of the HPV-associated HNC (between 86-95%) are positive for HPV16, while the remaining HPV types appear to play only a marginal role. In addition, the different types of HNC, oropharyngeal carcinomas are most frequently HPV-associated, while HPV prevalence in cancers of the oral cavity, larynx and hypopharynx appears to be considerably lower. The reason why HPV16 is preferentially linked to oropharyngeal cancers is still unclear, but a plausible hypothesis is that specific features of the oropharyngeal tissue render this anatomic region more susceptible to HPV infection. Similarly to cervical cancer, the main oncoproteins E6 and E7 play a key role in the development of oropharyngeal cancers by deregulating crucial cellular events, such as cell cycle, apoptosis, DNA repairing senescence and differentiation. However, based on the fact that the oral cavity may be exposed to higher levels of carcinogens in comparison to genital track, it is likely that different mechanisms are implicated in cervical and oropharyngeal carcinogenesis.

HPV TYPE SPECIFIC PREVALENCE IN CANCERS FROM US CANCER REGISTRIES

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Objectives: To determine the baseline prevalence of HPV types in cancers commonly associated with HPV using population-based cancer registry tissue samples from regions of the United States with high cervical cancer rates or with unique racial/ethnic distributions: Hawaii, Louisiana, Michigan, Florida, and Kentucky.

Methods: Central cancer registries identified all cases of invasive cancer from eligible primary sites [cervix, vagina, vulva, penis, anus, tongue, tonsil, oropharynx, other head and neck] diagnosed in 2000-2005. Archived tissue was retrieved from a representative sample of eligible cases, and one diagnostic block per case was serially sectioned for DNA extraction with confirmation of histology in sections immediately preceding and following. Histology review, extraction and testing were performed at CDC. All samples were tested using the Linear Array HPV Genotyping Test (Roche Diagnostics), and those negative for HPV or failing to amplify endogenous control were re-tested with INNO-LiPA HPV Genotyping Assay (Innogenetics). Samples failing to amplify control sequences in both assays were considered inadequate and excluded from analysis. HPV testing has been performed on 1846 cancers; 1808 (98%) yielded adequate results on eligible samples. HPV was detected in 81% of adequate samples; HPV 16 or 18 in 60%. HPV detection stratified by anatomic site: Cervix (n=531) 91% [67% 16/18]; Anus (n= 94) 89% [80% 16/18]; Vulva (n=137) 72% [53% 16/18]; tongue / tonsil / oropharynx (n=422) 72% [61% 16/18]; Vagina (n=55) 73% [55% 16/18]; Other head and neck (n=132) 33% [22% 16/18]; Penis (n= 64) 64% [47% 16/18]. Results will be updated to include 2 additional cancer registries (Los Angeles and Iowa). Type specific results will be presented by anatomic site.

Conclusions: If vaccine coverage were high and reached those at highest risk, an efficacious HPV16/18 vaccine could prevent the occurrence of a large proportion of HPV-associated cancers in the United States. Periodic measurement of HPV distribution will be an important monitoring activity.

MSS 7-6

CLINICAL VALUE OF TESTING FOR HPV INFECTION AND ADDITIONAL MARKERS**Peter J.F. Snijders¹, Daniëlle A.M. Heideman¹, Bart Hesselink¹, Ruud H. Brakenhoff², Chris J.L.M. Meijer²***Department of Pathology¹ and Otolaryngology/Head and Neck Surgery², VU University Medical Center, VU University Medical Center, Amsterdam, the Netherlands*

Many efforts have been made to distinguish disease-related ('clinically relevant') from irrelevant, transient hrHPV infections. For cervical screening purposes an optimal hrHPV test should display a good balance between clinical sensitivity and clinical specificity for CIN2+/3+. The clinical sensitivity should be sufficiently high to ensure a high, long-term reassurance of low risk of cervical cancer among test negative women, whereas the specificity should be high enough to minimize detection of clinically irrelevant hrHPV infections and reduce unnecessary follow-up procedures. It is known that various HPV detection methods differ in their clinical performance of detecting HPV-related premalignant disease and that detection of very low viral copy numbers in cervical scrapings does not reflect (risk of) high-grade cervical disease, but the presence of clinically irrelevant infections instead. Therefore, it is important that for cervical screening purposes HPV tests are clinically validated according to validation strategies as outlined in guidelines, such as recently have been proposed (Meijer et al., 2009).

Also for tissue specimens the discussion on distinguishing clinically relevant from irrelevant HPV infections has become important. This particularly holds true for HNSCCs, particularly oropharyngeal SCCs, which are known to be etiologically heterogeneous. Reported HPV prevalence rates in these tumors are highly variable, and it has become clear that HPV DNA detection does not per se indicate a causal association. Only subsets of tumors in which HPV DNA can be detected display E6/E7 mRNA expression. Many assays have been analysed for their capability to detect biologically relevant HPV infections in HNSCC. Thus far, no single method has reached a sufficient clinical sensitivity and specificity. However, sequential or combined testing for HPV DNA and p16ink4a overexpression has emerged as a promising algorithm for detecting clinically meaningful HPV infections. Further validation studies are underway.

In conclusion, when aiming at the detection of clinically relevant HPV infections, it is of utmost importance to clinically validate the assay or assay combinations.

MSS 8-1

CLINICAL VALIDATION OF HPV DNA TESTS IN CERVICAL SCREENING**Daniëlle A.M. Heideman, Johannes T. Berkhof*, Albertus T. Hesselink, Peter J.F. Snijders, Chris J.L.M. Meijer***Departments of Pathology and Clinical Epidemiology and Biostatistics*, VU University Medical Center, 1007 MB Amsterdam, The Netherlands*

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 for 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.

HPV PRIMARY SCREENING COMPARED TO CO-TESTING: ESTIMATES OF EFFICACY VS. EFFICIENCY USING THE COBAS HPV TEST

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Objectives: The 2012 guidelines for cervical cancer screening in the US, state that co-testing with cytology and a clinically valid HPV test is the preferred screening method for women over 30. Given the relative sensitivity and specificity of the two components of this screening approach one must question the advantages compared to the potential disadvantages of doing just primary HPV testing first and followed by cytology as a reflex test. The cobas HPV test or any test that provides integrated as opposed to sequential genotyping data may well further impact the balance between considerations of efficacy vs. efficiency.

Methods and Results: Data from the ATHENA registration trial for the cobas HPV test were analyzed and modeled in women ≥ 25 for CIN 3 outcomes. The primary outcome was the relative contribution of each test to CIN3 diagnosis. The cobas HPV test was more sensitive than liquid-based cytology for detection of CIN3 or worse [92.0%, 95% CI 88.1–94.6] vs. [53.3%, 95% CI 47.4–59.1]; difference 38.7%, 95% CI 31.9–45.5; $p < 0.0001$. Addition of liquid-based cytology to HPV testing increased sensitivity for CIN3 or worse just 4.7% to 96.7% (95% CI 93.9–98.3), but increased the number of screen positives by 35.2% compared with HPV testing alone. As a triage test to identify CIN3 or worse in HPV-positive women, detection of HPV16, HPV18, or both alone was equivalent to detection of ASC-US or worse alone in terms of sensitivity [59.5% vs. 52.8%; $p = 0.11$] and positive predictive value (PPV) (15.5% vs. 14.1%; $p = 0.20$). Among HPV-positive women, detection of HPV16, HPV18, or both or low-grade squamous intraepithelial lesion or worse cytology had better sensitivity 72.2%; $p < 0.0001$ and similar PPV 13.9% $p = 0.70$ for detection of CIN3 or worse than ASC-US or worse cytology alone. Trading off more specific and predictive cut-points for cytology as a reflex triage to cobas testing leads to up to a 3-fold improvement in efficiency with minimal loss of CIN3 sensitivity

Conclusions: Data from ATHENA clearly demonstrate how primary HPV testing with integrated HPV16 and HPV18 detection can provide an alternative, more sensitive, and efficient strategy for cervical cancer screening than do methods based solely on co-testing or cytology alone.

HPV FOCAL: FIRST ROUND SCREEN RESULTS FROM A CANADIAN POPULATION BASED PROGRAM

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Objectives: HPV FOCAL is the first North American population based trial to compare the efficacy of primary hr-HPV DNA with cytology (Liquid Based LBC) triage of hr-HPV-positive women compared to LBC followed by hr-HPV triage of ASCUS with \geq CIN3 as the outcome. Results of round 1 baseline and follow-up screening are presented.

Methods: 25,000 British Columbia women aged 25 to 65 were assigned to one of two study arms- Control: LBC primary testing with reflex hr-HPV testing for ASCUS results; Negatives screen again at 2 and 4 years. Colposcopy referral at \geq LSIL or ASCUS/HPV positive. HPV Arm: hr-HPV testing with reflex cytology in HPV positive women. Colposcopy referral if HPV positive and \geq ASCUS, rescreen in 12 months if HPV positive/cytology negative. Exit screen at 2 or 4 years. Exit colposcopy referral at ASC-US threshold and or HPV-positive. Outcome measures: Confirmed \geq CIN3 detected at 4 year exit screen in control and HPV arms.

Conclusions: Data available for 22,072 of 25,000 participants who have completed round 1 screening. Overall referral to colposcopy was 40.2/1000 women screened in control and 59.1/1000 in the HPV arm. To date, overall CIN2+ and CIN3+ rates are 10.6/1000 and 4.9/1000 in the control and 16.2/1000 and 7.8/1000 in the HPV arm. In the control arm, ASCUS/HPV negative women with repeat cytology in 12 months had no cases of CIN2+ detected (PPV=0%). In comparison, women in the HPV arm who were baseline HPV positive /cytology negative and remained HPV positive at follow-up screen 12 months later, the CIN2+ rate in follow-up was 230.8/1000 (PPV=23.1%).

At round 1 screening, preliminary results show overall referral rates to colposcopy are higher in the HPV than in the control arm. Comparing results for the follow-up screening round to date, the PPV in HPV arm was substantially higher than in the control arm (0% vs 23.1%), indicating the greater risk associated with persistent hr-HPV infection for precancerous lesions. Trial remains ongoing, with women attending second round/exit screening.

MSS 8-5

THE APTIMA HPV ASSAY VERSUS THE HYBRID CAPTURE II TEST IN TRIAGE OF WOMEN WITH ASC-US OR LSIL CERVICAL CYTOLOGY: A META-ANALYSIS OF THE DIAGNOSTIC ACCURACY

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Objectives: Testing for presence of DNA of 13 high-risk HPV types with the Hybrid Capture-2 (HC2) test (Qiagen, GmbH, Hilden, Germany) has consistently been shown to perform better in triage of women with equivocal but often not in triage of low-grade cytological lesions detected in cervical cancer screening. In the current meta-analysis, we compared the accuracy of the APTIMA HPV test (Gen-Probe Incorp, San Diego, CA, USA), which identifies RNA of 14 high-risk HPV types, with that of HC2 for the triage of women with ASC-US (atypical squamous cells of undetermined significance) or LSIL (low-grade squamous intraepithelial lesions).

Methods: Literature search targeted studies where the accuracy of APTIMA HPV and HC2 for detection of underlying CIN2/3+ was assessed concomitantly including verification of all cases of ASC-US and LSIL. Authors were contacted to provide data separately for ASC-US or LSIL. HSROC curve regression was used to compute the pooled absolute and relative sensitivity and specificity.

Results: Eight studies, comprising 1839 ASC-US and 1887 LSIL cases, were retrieved. The pooled sensitivity and specificity of APTIMA to triage ASC-US was 95.1% (95% CI [CI]: 91.5-97.2%) and 56.4% (CI: 44.7-67.5%), respectively, for CIN2+ and 96.2% (CI: 91.7-98.3%) and 54.9% (CI: 43.5-65.9), respectively, for CIN3+. APTIMA and HC2 showed similar pooled sensitivity the specificity of the former was significantly higher (ratio: 1.19 (CI: 1.08-1.31 for CIN2+)). The pooled sensitivity and specificity of APTIMA to triage LSIL were 91.0% (CI: 85.2-94.7%) and 42.5% (CI: 33.3-52.3%), respectively, for CIN2+ and 96.7% (91.4-98.9%) and 38.7% (CI: 30.5-47.6%), respectively, for CIN3+. APTIMA was as sensitive as HC2 but more specific for CIN2+ (ratio of 1.37; CI: 1.22-1.54) and CIN3+ (ratio: 1.35; CI: 1.11-1.66).

Conclusions: In both, triage of ASC-US and LSIL, APTIMA is as sensitive but more specific than HC2 for detecting cervical precancer.

MSS 9-1

HUMAN PAPILLOMAVIRUS TESTING FOR TRIAGE OF WOMEN WITH LOW-GRADE SQUAMOUS INTRAEPITHELIAL LESIONS

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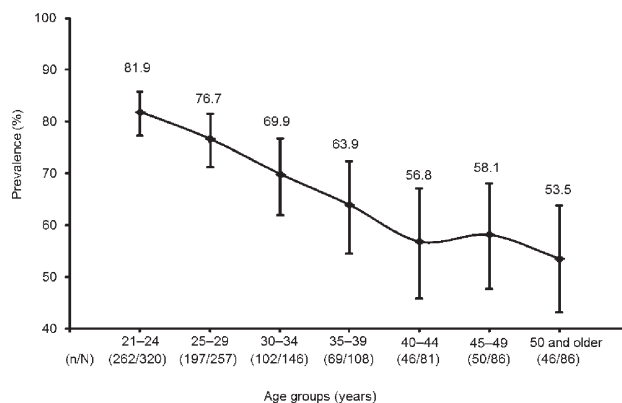
Objective: Low-grade squamous intraepithelial lesion (LSIL) is a common cytologic finding in cervical screening, yet only about 10–20% have significant histologic abnormalities and these are almost always positive for high-risk human papillomavirus (hrHPV). This analysis aims to clarify the role of HPV DNA testing in the triage of women with LSIL cytology.

Methods: In the ATHENA screening trial we examined the 1,084 cases of LSIL, of which 925 had an evaluable biopsy, to determine the extent to which HPV testing can identify which patients have precursor lesions in need of immediate clinical referral and which have changes more likely to regress spontaneously.

Results: Overall, 71.2% of LSIL cases were HPV positive, but the prevalence was age-dependent, being only 56.1% in women 40 years or older (Figure 1). Among women with LSIL, 11.6% (107/925) had a cervical intraepithelial neoplasia grade 2 or worse (CIN2+) histologic diagnosis and, of these, only nine were HPV negative. For CIN3+, 91.7% (44/48) of women with LSIL were HPV positive. The negative predictive value of HPV testing for CIN3+ in LSIL was 100% for women aged 40 years or older. Women who were HPV16 positive had a higher positive predictive value for CIN2+ (25.4%) than those who were positive for 12 other pooled hrHPV types (11.5%) (Table).

Conclusion: HPV testing in women with LSIL cytology is effective in identifying high-grade cervical lesions, thereby avoiding referral and potential overtreatment of lesions likely to regress, and is likely to be cost-effective, especially for women aged 40 years or older.

Figure 1. hrHPV positivity by age group in women with LSIL cytology



Predictive values for CIN2+ and CIN3+ based on cobas® HPV Test genotyping result

| Age group years | N | HPV16 positive | | HPV18 positive | | 12 other high-risk HPV positive | | | HPV negative | | | |
|-----------------|-----|----------------|------------|----------------|------------|---------------------------------|-------|------------|--------------|-----|--------------|--------------|
| | | CIN2+ | CIN3+ | CIN2+ | CIN3+ | CIN2+ | CIN3+ | CIN2+ | CIN3+ | | | |
| | | n (PPV, %) | n (PPV, %) | N | n (PPV, %) | n (PPV, %) | N | n (PPV, %) | n (PPV, %) | N | n (1-NPV, %) | n (1-NPV, %) |
| 21-24 | 70 | 16 (22.9) | 8 (11.4) | 16 | 1 (6.3) | 1 (6.3) | 135 | 9 (6.7) | 0 (0.0) | 48 | 2 (4.2) | 1 (2.1) |
| 25-29 | 51 | 10 (19.6) | 5 (9.8) | 11 | 2 (18.2) | 1 (9.1) | 106 | 17 (16.0) | 4 (3.8) | 50 | 2 (4.0) | 1 (2.0) |
| 30-39 | 28 | 13 (46.4) | 9 (32.1) | 12 | 0 (0.0) | 0 (0.0) | 104 | 12 (11.5) | 6 (5.8) | 74 | 4 (5.4) | 2 (2.7) |
| 40-49 | 13 | 2 (15.4) | 0 (0.0) | 7 | 2 (28.6) | 2 (28.6) | 65 | 11 (16.9) | 6 (9.2) | 65 | 1 (1.5) | 0 (0.0) |
| 50 and older | 7 | 2 (28.6) | 1 (14.3) | 3 | 0 (0.0) | 0 (0.0) | 26 | 1 (3.8) | 1 (3.8) | 34 | 0 (0.0) | 0 (0.0) |
| Overall | 169 | 43 (25.4) | 23 (13.6) | 49 | 5 (10.2) | 4 (8.2) | 436 | 50 (11.5) | 17 (3.9) | 271 | 9 (3.3) | 4 (1.5) |
| [95% CI] | | [19.5-32.5] | [9.2-19.6] | | [4.4-21.8] | [3.2-19.2] | | [8.8-14.8] | [2.4-6.2] | | [1.8-6.2] | [0.6-3.7] |

Confidence interval (CI) was calculated using Wilson's Score. CIN, cervical intraepithelial neoplasia grade; HPV, human papillomavirus; NPV, negative predictive value; PPV, positive predictive value.

MSS 9-2

GENOTYPING FOR HPV 16 AND 18 TO TRIAGE WOMEN WITH ASC-US OR LSIL CERVICAL CYTOLOGY: A SYSTEMATIC REVIEW AND META-ANALYSIS

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Objectives: The best method to identify women with minor cervical lesions that require diagnostic work-up remains unclear. High-risk HPV testing suffers from low specificity in particular when women have LSIL cytology. Genotyping for the most carcinogenic HPV types (HPV16 and 18) could identify those women at high risk requiring intensified follow-up.

Methods: A literature search was performed in three electronic databases to identify eligible studies. Authors were contacted to request non-published data.

Results: Up hereto, nine studies evaluating 6 assays could be included in the meta-analysis. The pooled sensitivity of HPV16/18 genotyping to detect CIN2+ was 60% (95%CI: 55-65%) and 64% (95%CI: 49-73%) in ASC-US and LSIL, respectively. The pooled specificities were 84% (95%CI: 79-88%), in women with ASC-US, and 76% (95%CI: 68-83%), in women with LSIL. The sensitivity was 3-4% higher and the specificity was 3-5% lower when the outcome of CIN3+ was considered. Heterogeneity by assay was noted.

Conclusions: Testing for HPV16 or HPV18 is substantially more specific but less sensitive than general HPV tests. It allows the identification of a subgroup of women requiring intensive follow-up. Women with ASC-US or LSIL testing negative for HPV16/18 can not be released for routine screening but can be offered repeat testing, for instance 12 months later.

MSS 9-3

ANALYTICAL AND CLINICAL PERFORMANCE OF THE APTIMA® HPV 16 18/45 GENOTYPE ASSAY FOR DETECTION OF HPV 16, 18 AND 45 MRNA

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Background: The APTIMA HPV 16, 18/45 Genotype Assay (AHPV-GT, Gen-Probe Incorporated) is a multiplex assay that specifically detects high-risk HPV genotypes 16, 18, and 45. The AHPV-GT assay is intended for testing APTIMA HPV Assay (AHPV, Gen-Probe Incorporated) positive samples to determine if types 16, 18, and/or 45 are present.

Objectives: The performance of the AHPV-GT assay was evaluated using analytical and clinical specimens.

Methods: Dilution panels were tested to determine the 95% detection limit for each genotype using Probit analysis. The analytical sensitivity and specificity in samples containing various microorganisms or potentially interfering substances was also evaluated. AHPV-GT assay clinical performance was evaluated using specimens (n=378) collected from women in Canada, Mexico and Germany at routine screening or follow-up visits. Agreement of the AHPV-GT assay, for detection of high-risk HPV 16, 18 and 45, was determined using the Linear Array assay (Roche Diagnostics) and an HPV Reverse Transcription PCR genotyping assay (Gen-Probe Incorporated) as the reference.

Conclusions: The 95% detection limit of the AHPV-GT assay for HPV genotypes 16, 18 and 45 ranged from 57 to 85 copies per reaction. There was no cross-reactivity or interference of the AHPV-GT assay with 15 low-risk HPV genotypes tested (6, 11, 26, 30, 34, 42, 43, 44, 53, 67, 69, 70, 73, 82, 85), or the other 11 high-risk HPV genotypes (31, 33, 35, 39, 51, 52, 56, 58, 59, 66, 68) or with 43 common vaginal flora and pathogens associated with sexually-transmitted diseases. No interference was observed with the 13 gynecological, feminine hygiene and other substances evaluated. Overall clinical agreement between the AHPV-GT assay and the reference DNA and RNA assays was 98.7%, with a positive agreement of 98.3% and a negative agreement of 99.0%. These results show that the AHPV-GT assay is a sensitive and specific test for the detection of HPV types 16, 18, and 45.

MSS 9-4

CLINICAL SIGNIFICANCE OF THE DYNAMIC PROFILE OF TYPE-SPECIFIC VIRAL LOAD

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Objectives: Persistent high-risk human papillomavirus (HPV) infection is strongly associated with the development of high-grade cervical intraepithelial neoplasia or cancer (CIN3+). However, HPV infection is common and usually transient. Viral load measured at a single time-point is a poor predictor of the natural history of HPV infection. The profile of viral load evolution over time could distinguish HPV infections with carcinogenic potential from infections that will regress.

Methods: A case-cohort natural history study was set up using a Belgian laboratory database processing more than 100 000 BD-SurePath liquid cytology specimens annually. All liquid cytology leftovers were submitted to real-time PCR testing identifying E6/E7 genes of 17 HPV types, with viral load expressed as HPV copies/cell. Samples from untreated women who developed CIN3+ (n=138) and women with transient HPV infection (n=601) who contributed at least three viral load measurements were studied. Only single-type HPV infections were selected. The changes in viral load over time were assessed by the linear regression slope for the productive and/or clearing phase of infection in women developing CIN3+ and women with transient infection, respectively.

Results: Transient HPV infections generated similar increasing (0.21 copies/cell/day) and decreasing (-0.28 copies/cell/day) viral load slopes. In HPV infections leading to CIN3+, the viral load increased almost linearly with a slope of 0.0028 copies/cell/day. Difference in slopes between transient infections and infections leading to CIN3+ was highly significant (P<.0001).

Conclusions: Serial type-specific viral load measurements predict the natural history of HPV infections and could be used to triage women in HPV-based cervical cancer screening.

DIAGNOSTIC ACCURACY OF TESTING FOR HIGH-RISK (HR) HUMAN PAPILLOMAVIRUS (HPV) TYPES 16, 18 AND 45 IN PRECANCEROUS CERVICAL LESIONS: RESULTS OF A 3-YEAR PROSPECTIVE STUDY

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Aims: Incidence rates of CIN2+ lesions in women infected with HPV 16 and 18 are higher [Wright et al. *Gynecol. Oncol.* 2005, 98:84-91] and HR-HPV infections with subtypes 16, 18 and 45 seem to have a higher risk of developing cervical cancer [Bulk S, et al. *Br J Cancer* 2006;94:171-5]. In Germany, cervical cancer screening is available for all women from the age of 20 on the basis of the Papanicolaou (PAP) smear. HPV-DNA testing with hybrid capture® 2 HPV-DNA test (Qiagen) is reimbursed in cervical cancer screening in case of LSIL (PAP 3d) or HSIL (PAP 3d) and ASCUS-H (PAP 3) lesions.

Methods: Between 2007 and 2008 a total of 619 women were recruited: a group of 510 women with a history of known cervical lesions and/or hpv infections (eligibility criterion: HR-HPVDNA positive test result with HC2T) and a group of 109 women who were examined as part of their routine cervical cancer screening. Baseline HR-HPV status was measured at enrollment with the FDA-approved hybrid capture® 2 HPV-DNA test and the HR-HPV 16/18/45 Probe Set Test (HC2T, PST; QIAGEN, Hilden, Germany). Both tests use hybrid capture hybridization genotyping technology. Cervical smears were classified according to the Second Munich Nomenclature (1989). The results were converted to the nearest equivalent in the Bethesda system.

Results: Data from 281 women of the high-risk cohort (HRK, drop-out-rate: 44.9%) and 77 women of the control cohort (KK, drop-out-rate: 18.1%) were evaluable. Regardless of the initial Pap-status the odds ratio for CIN2 or worse within the high-risk cohort was 3.74 (95% CI: 2.07-6.78) for hpv 16/18/45-positive women whereas the odds ratio for CIN3+ lesion within the high-risk cohort was 4.16 (95% CI: 2.16-8.02) for hpv 16/18/45-positive women. The Cox regression model returned significant hazard ratios for CIN2+ lesions (2.25 (95% CI: 1.36-3.71; p = 0.002)) and for CIN3+ lesions (2.67 (95% CI: 1.49-4.78; p = 0.001)). Besides, hazard ratios demonstrated an increased risk for hpv-16/18/45-positive women for the establishment of CIN2+ lesions (1.47 (95% CI: 0.87-2.46, p = 0.147) respectively for the establishment of CIN3+ lesions (1.69 (95% CI: 0.93-3.08, p = 0.086) while taking into consideration influencing factors (HPV, Pap-Status and age). But these findings were not significant at a nominal significance level of $\alpha = 5\%$. The positive likelihood ratio of the hpv-16/18/45 Probe Set Test was 1.4 for the development of a CIN2 lesion or worse. The negative likelihood ratio for the development of a CIN2+ lesion was 0.37 and 0.34 for the development of a CIN3+ lesion. We neither detected a CIN2+ nor a CIN3+ lesion in the control cohort during our follow up. The prevalence of the hr-hpv-infection in the control cohort was 13.8% and 8.3% for hpv-16/18/45-infections. The hr-hpv-incidence was 6.5% (approximately 5.2% for hpv-16/18/45-infections).

Conclusions: The hazard ratios for hpv-16/18/45-infections demonstrated an increased risk of progression to CIN2+ and CIN3+ lesions. Furthermore, the combined analysis of the Cox regression model with respect to age and Pap for hpv-status demonstrated an increased risk for CIN2+ and CIN3+ lesions for women with hpv-16/18/45-infections. But due to small sample size, the results were not significant.

METHYLATION MARKERS IN TRIAGE & SCREENING

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Objective. Testing for high risk (HR) human papillomavirus (HPV) DNA is gaining momentum and is expected to become the predominant method for screening populations at risk of cervical cancer in the coming decade. One of the bigger barriers to routine adoption of HR-HPV testing is the relatively large proportion of women (5 to 10%) who are infected by HPV but who do not have any morphological abnormalities. Thus, the features, benefits and costs of DNAm as triage and screening biomarkers are of interest.

Methods. A literature review and laboratory experimentation employing a quantitatively accurate pyrosequencing (PSQ) DNAm test were conducted on specimens from several major human studies to evaluate the current status and prospects for DNAm as a clinically useful triage biomarker for women infected by HPV DNA or with abnormal cytology.

Conclusions. Quantitative measurement of DNAm by PSQ shows strong promise as a next generation biomarker with a potentially profound impact on the diagnosis and prognosis of many cancers. In women infected by HPV16 the quantitative levels of HPV-genomic DNAm increase with time of persistence and jump dramatically with the development of cervical cancer. These observations open up the possibility of accurately predicting which women may develop cancer many years in advance. In several studies DNAm in the L1 region was able to detect cervical intraepithelial neoplasia grade 2 or higher (CIN 2+) among all HPV16 infected women with a sensitivity of 90% and a specificity of 60%, while in women over age 28 at the same sensitivity the specificity increased to more than 75%. DNAm patterns of several other HPV types are similar to HPV16 suggesting the possibility of a HPV cocktail DNAm triage assay. With respect to DNAm in human genes, promising targets include MAL and CADM measured by quantitative methylation-specific PCR. This assay also shows promise as a triage test in HR-HPV screen positive women giving a sensitivity of 90% and specificity of 40%, in the same women the corresponding values for cytology triage were 65% and 80% respectively.

MSS 10-2

HOST GENE METHYLATION MARKERS FOR TRIAGE OF HPV POSITIVE WOMEN

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Objectives: Screening women for high-grade cervical intraepithelial neoplasia or cervical cancer (CIN3+) by hrHPV testing has as side-effect the detection of hrHPV-positive women without clinically relevant lesions. Here, we developed an objective assay assessing the methylation status of the promoter regions of two genes that are functionally involved in cervical carcinogenesis (i.e. CADM1 and MAL) to triage hrHPV-positive women for CIN3+.

Methods: In a training set of cervical scrapes of women participating in cervical screening (51 women with CIN3+ and 224 without CIN2+) panels consisting of one to four quantitative methylation-specific PCR (qMSP) assays (two for CADM1 and MAL each) were analyzed. Cross-validated receiver-operating characteristics (ROC) curves were constructed and amongst the panels containing only two markers the combination of one CADM1 (i.e. CADM1-m18) and one MAL (i.e. MAL-m1) qMSP marker had the highest partial area-under-the-curve (AUC 0.719). This two marker panel was subsequently used for validation in an independent set of cervical scrapes of 236 consecutive hrHPV-positive women from a screening cohort. Here, this panel revealed CIN3+ sensitivities ranging from 100% (95%CI:92.4-100) to 60.5% (95%CI:47.1-74.6), with corresponding specificities ranging from 22.7% (95%CI:20.2-25.2) to 83.3% (95%CI:78.4-87.4). For cytology these percentages were 65.8% (95%CI:52.3-79.0) and 78.8% (95%CI:73.7-83.1) and for cytology/HPV16/18 genotype combination these were 84.2% (95%CI:72.0-92.7) and 54.0% (95%CI:49.2-58.7), respectively. The point-estimates of both cytology and cytology/HPV16/18 were equal to the values of the ROC-curve of CADM1-m18/MAL-m1.

Conclusions: The CADM1/MAL methylation marker panel was equally discriminatory for CIN3+ as cytology or cytology with HPV16/18 genotyping in hrHPV-positive women. This opens the possibility for complete cervical screening by objective, non-morphological molecular methods.

MSS 10-3

ALTERED miRNA EXPRESSION AND CHROMOSOMAL CHANGES IN CERVICAL CANCER

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Objectives: This study aimed to determine alterations in microRNA (miRNA) expression patterns during the consecutive stages of cervical cancer development in association with the concurrent chromosomal changes.

Methods: miRNA expression profiles were determined using microarrays in normal cervical squamous epithelium, high-grade precancerous lesions (CIN2-3), squamous cell carcinomas (SCC) and adenocarcinomas (AdCAs). miRNA profiles were integrated with chromosomal profiles of the same samples.

Significantly differential expression during the consecutive stages of cervical SCC development was observed for 106 miRNAs. Of these differentially expressed miRNAs 27 showed early transiently altered expression in CIN2-3 lesions only, 46 miRNAs showed late altered expression in SCCs only and 33 showed continuously altered expression in both CIN2-3 and SCCs. Altered expression of 5 significantly differentially expressed miRNAs, hsa-miR-9 (1q23.2), hsa-miR-15b (3q25.32), hsa-miR-28-5p (3q27.3), hsa-miR-100 and hsa-miR-125b (both 11q24.1) was directly linked to frequent chromosomal alterations. Functional analyses were performed for hsa-miR-9, representing a potential oncogene with increased expression linked to a chromosomal gain of 1q. Hsa-miR-9 overexpression was found to increase cell viability, anchorage independent growth and migration in vitro. Upon organic raft culturing hsa-miR-9 hampered differentiation and induced proliferation in all strata of the epithelial layer. These findings support a potential oncogenic function of hsa-miR-9 in cervical cancer.

Conclusions: Differential expression of 106 miRNAs, partly associated with chromosomal alterations, was observed during cervical SCC development. Altered expression of hsa-miR-9 associated with a chromosomal gain of chromosome 1q was shown to be functionally relevant, underlining the importance of deregulated miRNA expression in cervical carcinogenesis.

MOLECULAR MARKERS IN TRIAGE OF PRIMARY HPV SCREENING ROLE OF p16 AND GENOTYPING

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Objectives: Methods for triaging HPV positive women are warranted.

Methods: In the NTCC trial women on DNA samples from women who were HC2 positive we performed PCR by GP5+/GP6+ and typing by RLB, viral load physical status of HPV 16 and 18 infections by real time and p16 by ICC. We computed the sensitivity and specificity for CIN2 or more severe histology and we estimated the relative sensitivity and relative referral rate vs. conventional cytology.

Results: We studied 2921 samples. If only women with infection by HPV 16 or 18 were considered as positive (group A) sensitivity was 59.5% (95%CI 52.4-66.4) and specificity 64.9% (63.1-66.7). When also including types 33, 35 and 52 (group B) sensitivity increased to 74.5% (67.9-80.4) and specificity decreased to 58.4% (56.5-60.2). When adding HPV 31 (group C) sensitivity was 85.5% (79.8-90.1) and specificity was 47.9% (46.1-49.9). PPVs for groups A, B and C, was 11.1% (9.3-13.1), 11.6% (9.9-13.5) and 10.8% (9.3-12.4) respectively. Among women aged 35-60 yrs the relative sensitivity and relative referral rate vs. conventional cytology was 0.96 and 8.83 respectively for group A, 1.18 and 0.99 respectively for group B, 1.33 and 1.21 respectively for group C. By comparison these values were 1.53 and 1.08 respectively with triage by p16^{INK4a} immunostaining. When excluding women with infection from HPV16 or 18 the positive predictive value (PPV) of HC2 positivity decreased from 6.9% (5.9-7.8) to 4.4% (3.5-5.3) and the PPV of LBC cytology ASCUS+ decreased from 8.8% (7.4-10.1) to 4.9% (3.7-6.0).

Conclusions: Genotyping is a little more efficient than p16^{INK4a} immunostaining for triaging HPV positive women aged 25-34 years while genotyping is less efficient than p16^{INK4a} immunostaining for triaging HPV positive women aged 35-60 years.

MOLECULAR MARKERS IN REFERRAL POPULATION

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High risk HPV DNA/RNA testing provides higher sensitivity but lower specificity than cytology for the identification of high grade cervical intraepithelial neoplasia (CIN). Several new HPV tests are now available for this purpose and a direct comparison of their properties is needed. Seven tests were evaluated on liquid PreservCyt® samples from 1099 women referred for colposcopy: Hybrid Capture II (Qiagen), Cobas (Roche), PreTect HPV-Proofer (NorChip), APTIMA HPV assay (Gen-Probe), Abbott RealTime, BD HPV test and CINtec p16^{INK4a} Cytology (mtm) immunocytochemistry. Sensitivity, specificity and positive predictive value (PPV) were based on the worst histology found on either the biopsy or the treatment specimen after central review. 359 women (32.7%) had (CIN2+), with 224 (20.4%) having CIN3+. For detection of CIN 2+, Hybrid Capture II had 96.3% sensitivity, 19.5% specificity and 37.4% PPV. Cobas had 95.2% sensitivity, 24.0% specificity and 37.6% PPV. The BD HPV test had 95.0% sensitivity, 24.2% specificity and 37.8% PPV. Abbott RealTime had 93.3% sensitivity, 27.3% specificity and 38.2% PPV. APTIMA had 95.3% sensitivity, 28.8% specificity and 39.3% PPV. PreTect HPV-Proofer had 74.1% sensitivity, 70.8% specificity and 55.4% PPV. CINtec p16^{INK4a} Cytology had 85.7% sensitivity, 54.7% specificity and 49.1% PPV. Cytology taken at colposcopy (mild dyskaryosis or worse) had 88.9% sensitivity, 58.1% specificity and 50.7% PPV. This study confirms that, in a referral setting, HPV testing, by a number of different tests, provides high sensitivity for high grade disease. Algorithms for using these tests to improve specificity but uncertain high sensitivity will be discussed.

MSS 11-1

HPV TYPE-SPECIFIC NATURAL HISTORY OF CIN3

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Objectives: Prospective data comparing the potential of individual high-risk (HR) types to progress from CIN3 to cancer tend not to exist. However, large cross-sectional comparisons can be a useful proxy in this regard, and can help interpret how the results of screening and vaccination studies that rely upon CIN3 as a main outcome are relevant to invasive cancer prevention.

Methods: A meta-analysis of cross-sectional HR HPV type distribution in 115,789 HPV-positive women was performed including 10,753 histologically diagnosed CIN3 and 36,374 invasive cervical cancers (ICC) worldwide. Type-specific positivity was compared between HPV-positive CIN3 with that in HPV-positive ICC by ratios. ICC:CIN3 ratios were also assessed separately by the source of HPV DNA from CIN3 (i.e. exfoliated cells versus biopsies/tissue).

Conclusions: HPV16, 18 and 45 accounted for a greater proportion of HPV infections in ICC compared to CIN3 (ICC:CIN3 ratios=1.08, 2.11 and 1.47, respectively). Other HR types accounted for important proportions of HPV-positive CIN3, but their contribution dropped in ICC, with ICC:CIN3 ratios ranging from 0.49 for HPV33 and HPV58 down to 0.20 for HPV51. These effects were robust in sensitivity analyses calculated separately for CIN3 tested for HPV DNA from cells and from biopsies/tissue. ICC:CIN3 ratios from biopsies/tissue were also consistent when additionally restricted to comparison with squamous cell carcinoma (SCC) only (e.g. SCC:CIN3 ratios=2.10 for HPV45, 0.36 for HPV31). These data suggest that the HPV type distribution in CIN3 is not entirely representative of the types that cause ICC, and that studies using CIN3 as the principal outcome under-estimate the carcinogenic potential of HPV18 and 45 relative to other HR types. Potential explanations for these findings will be discussed.

MSS 11-2

THE HETEROGENEITY OF CIN3: UNDERSTANDING WHY NOT ALL CIN3 PROGRESSES TO CANCER

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Objectives: Part of the essential dogma of cervical cancer screening is that we must treat precancer to prevent cancer. The risk of prevalent invasive carcinoma at the time of CIN3 diagnosis is ~1%. The 30-year risk of developing invasive carcinoma in untreated women with CIN3 is between 30 & 50%. Our perceptions of the natural history of CIN3 are confounded by many variables in the workup of a patient during screening. While CIN 3 is often felt to be a reliable diagnosis, issues of criteria application, interpretive variability and pathologic differential diagnosis may mask the true natural history of this important diagnosis.

Methods: Review of the literature on interpretive variability demonstrates in multiple databases that individual pathologist diagnoses of CIN3 are downgraded to less than CIN3 in 25-30% of cases. As much as half of these are interpretations are less than CIN2. However, the differential diagnosis of CIN3 also includes pathologic processes that are not related to HPV and that confer little if any cancer risk. These include, atrophy, immature squamous metaplasia, reparative processes, transitional metaplasia and issues with specimen orientation. All of these can be mistaken for CIN3 (as will be illustrated) and collectively they may account for up to 10% or more of CIN3 diagnoses. Can biomarkers help clarify these problematic differential diagnoses? The answer seems to be yes! Adjudicated CIN3 is HR-HPV positive and p16 positive in >99% of cases. Essentially none of the mimics of CIN3 are related to HPV or demonstrate precancer biomarker associated over expression.

Conclusions: There is a significant fraction of diagnosed CIN3 that is not precancer. This issue is confounded further by the other variables in the screening and diagnostic workup. For problematic cases on histology, readily available biomarkers can help refine the diagnostic reliability of CIN3. The consequence of this is the natural history may change as diagnosis becomes more reliable. True CIN3 may be actually be of somewhat higher risk than perceived.

MSS 11-3

VIRAL AND HOST MARKERS OF CIN3 HETEROGENEITY

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Cervical intraepithelial neoplasia grade 3 (CIN3) is the most proximal precursor of invasive squamous cell cervical carcinoma. However, only 30-50% of large CIN3s will progress to invasive cancer. The determinants of progression from CIN3 to cancer are not well understood. Since it is not possible to follow CIN3 prospectively, we studied molecular features of and risk factors associated with CIN3 and cervical cancer in a large cross-sectional molecular epidemiology study, the Study to Understand Cervical Cancer Early Endpoints and Determinants (SUCCEED). We evaluated histology results, HPV genotypes detected in cytology specimens, and host gene expression profiles in frozen histology specimens among women with CIN3 and cancer. We observed differences in HPV genotype attribution between CIN3 and cancer, with a higher proportion of HPV16 and fewer multiple infections in women with cervical cancer compared to women with CIN3. We identified specific expression profiles for CIN3 lesions found in women with cancer that were distinct from CIN3s in women with CIN3 as the worst lesion. These genes are likely associated with early invasion events and may serve as specific progression markers. We are now validating these markers in a large series of CIN3s.

MSS 11-4

MANAGEMENT FOLLOW UP

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Objective: CIN 3 is a heterogeneous histologic diagnosis where less than 50% will progress to invasive cervical cancer within 30 years. CIN 2 is less reproducible histologically, and less likely to progress to invasive cervical cancer than CIN 3.

Methods: The literature is reviewed to provide perspective for management of women with high grade cervical abnormalities.

Conclusions: Until therapeutic vaccinations against HPV attributed CIN 2/3 lesions is successful in clinical trials, surgical excision remains the only method of removing cells whose true malignant conversion is highly probable.

MSS 11-5

THE ROLE OF NATURALLY-ACQUIRED ANTIBODIES AND HPV TYPE IN THE NATURAL HISTORY OF CIN

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Background: Only a fraction of cervical HPV infections will induce antibody responses. However, it is not well known whether the elicited antibodies will reduce the risk of subsequent infection and cervical abnormalities by a homologous HPV type. Also limited information exists about the differential role of certain HPV genotypes in the progression to CIN using prospective designs.

Objectives: To provide prospective data on the role of naturally-acquired antibodies and HPV genotype in the natural history of CIN.

Methods: Prospective data from HPV DNA-negative women enrolled in the control arm of two selected clinical trials of the bivalent HPV vaccine were reviewed. The Costa Rica trial included 2813 HPV16 DNA negative and 2950 HPV18 DNA negative women at enrolment. The PATRICIA trial included 8193 and 8463 women that were DNA negative for HPV16 and HPV18 respectively. Serum samples taken at enrolment were tested for total HPV16/18 antibodies, by ELISA and cervical specimens were obtained for type-specific HPV DNA detection over 4 years of follow-up. The two analyses compared the risk of new infection and cervical abnormalities associated with HPV seropositivity versus seronegativity at enrolment, and by level of antibody titre. The PATRICIA study also assessed the risk of developing CIN lesions associated with a previous 6-month persistent infection with the same HPV type.

Results: In the Costa Rica study having high HPV16 or 18 antibody titer at enrolment was associated with a reduced risk of subsequent HPV16 or 18 infection, respectively. In the PATRICIA study both, HPV16 seropositivity and HPV16 higher antibody titre, were significantly associated with lower risk of incident and persistent infection, ASC-US+, and CIN1+. Statistically significant inverse linear associations were found additionally for all outcomes including CIN2+. For HPV-18, while seropositivity status was associated with lower risk of ASCUS+ and CIN1+, a relationship with infection was not demonstrated. The HPV type was the most important risk factor for progression to a lesion (CIN1+, CIN2+). Compared with non-oncogenic HPV types, a 6MPI infection with HPV-16 and HPV-33 had approximately a 10-fold higher risk for progression to a CIN2+ lesion, HPV-31 and HPV-45 had approximately a 6-fold higher risk, HPV-18 a 4-fold higher risk and other oncogenic types had approximately a 3-fold higher risk.

Conclusions: Naturally-acquired antibodies to HPV-16 and, to a lesser extent, HPV-18 are associated with some reduction in the risk of infection and cervical abnormalities with the same HPV type. The risk of type-specific CIN2+ is strongly associated with previous persistent infection with the same type, and the risk is statistically significantly higher for oncogenic than non-oncogenic HPV types.

MSS 12-1

HUMAN PAPILLOMAVIRUS (HPV) TYPE DISTRIBUTION BY SEVERITY OF LESION

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Objectives Genotyping may improve risk stratification of high-risk (HR) HPV-positive women in cervical screening programs, but prospective data comparing the natural history and carcinogenic potential of individual HR types remain limited.

Methods A meta-analysis of cross-sectional HR HPV type distribution in 115,789 HPV-positive women was performed, including 33,154 normal cytology, 6,810 atypical squamous cells of undetermined significance (ASCUS), 13,480 low-grade squamous intraepithelial lesions (LSIL) and 6,616 high-grade SIL (HSIL) diagnosed cytologically, 8,106 cervical intraepithelial neoplasia grade 1 (CIN1), 4,068 CIN2 and 10,753 CIN3 diagnosed histologically, and 36,374 invasive cervical cancers (ICC), from 423 PCR-based studies worldwide.

Conclusions No strong differences in HPV type distribution were apparent between normal cytology, ASCUS, LSIL or CIN1. However, HPV16-positivity increased steeply from normal/ASCUS/LSIL/CIN1 (20-28%), through CIN2/HSIL (40/47%) to CIN3/ICC (58/63%). HPV16, 18 and 45 accounted for a greater or equal proportion of HPV infections in ICC compared to normal cytology (ICC:normal ratios=3.07, 1.87 and 1.10, respectively) and to CIN3 (ICC:CIN3 ratios=1.08, 2.11 and 1.47, respectively). Other HR types accounted for important proportions of HPV positive CIN2 and CIN3, but their contribution dropped in ICC, with ICC:normal ratios ranging from 0.94 for HPV33 down to 0.16 for HPV51. ICC:normal ratios were particularly high for HPV45 in Africa (1.85) and South/Central America (1.79), and for HPV58 in Eastern Asia (1.36). ASCUS and LSIL appear proxies of HPV infection rather than cancer precursors, and even CIN3 is not entirely representative of the types causing ICC. HPV16 in particular, but also HPV18 and 45, warrant special attention in HPV-based screening programs.

HPV16 VS. OTHER TYPES: NATURAL HISTORY AND DISEASE HETEROGENEITY**Nicolas Wentzensen,***Division of Cancer Epidemiology and Genetics, National Cancer Institute*

Human Papillomavirus type 16 (HPV16) has been identified as the most carcinogenic HPV genotype. HPV16 is associated with a high percentage of cervical cancers and with almost all extracervical HPV-related cancers. We studied the natural history and disease heterogeneity related to HPV16 in several NCI-based studies. Overall, we found an increasing proportion of HPV16-related disease with increasing disease stage. In SUCCEED, we observed that HPV16-related CIN2 and CIN3 are diagnosed at younger age and have worse colposcopic appearance than lesions caused by other carcinogenic types. CIN3 had the same lesion size at detection irrespective of HPV type, suggesting that HPV16-related CIN3s grow faster and reach the size of detectability earlier. Our findings support earlier follow-up of women with HPV16 infections, but indicate that colposcopy may be challenged to find early precancers independent of HPV genotype.

**HPV 16 VS OTHER HR HPV CARCINOGENIC TYPES:
CROSS SECTIONAL AND LONGITUDINAL ASSOCIATION WITH HIGH-GRADE CIN****Guglielmo Ronco***CPO Piemonte, Turin, Italy.*

HPV16 is clearly more strongly cross-sectionally associated, to high-grade (hg) CIN and SCC than the other high-risk HPV types. In a recent meta analysis, the prevalence of HPV16 among HPV infected-women with normal cytology with CIN2, CIN3 and ICC was 1.89, 2.85 and 3.07 times respectively the prevalence of HPV16 in HPV infected with normal cytology (22.9%). In principle, such cross-sectional association could allow strongly reducing the referral to colposcopy by restricting it to the HPV positive women at highest probability to carry a colposcopically detectable hgCIN (those with HPV16). It must, however, be kept in mind that in the above meta-analysis just 39.2% and 58.2% of HPV-infected women with CIN2 and CIN3 respectively carried HPV 16. Similar sensitivities of HPV16 among HPV positive women were observed in the ATHENA study (52.8% for CIN3+) and in preliminary data from the experimental arm of the NTCC (53.9% for CIN2+). The resulting proportion of prevalent CIN3 left undetected seems too high (even when considering the presence of either HPV16 or 18) to retest the women with other hgHPV types after a long interval. Indeed it has been proposed to refer immediately to colposcopy the women with HPV16 or 18 instead of or in addition to those with abnormal (at different levels) cytology and re-testing the remaining after 1 year to verify HPV persistence. Such triage strategy needs to be compared with others.

It is well known that the longitudinal risk of having a hgCIN detected is much higher in HPV positive than in HPV negative women, suggesting that the former should be re-screened at shorter intervals even if no hgCIN was initially detected. Many studies also found that such longitudinal risk of hgCIN is much higher with HPV16 than with other hrHPV types (although the latter is still about double that in hrHPV negative women and some specific non-16/18 types also show high risk). This property could be exploited to define the screening interval in HPV positive women who had no hgCIN previously detected. However, it must be kept in mind that in the NTCC study (preliminary data) 44% of the HPV positive women who had a CIN3 detected during 3 years of follow-up after recruitment had an initial non-16/18 infection.

MSS 12-4

**HPV 16 VERSUS NON-HPV16:
COMBINATION OF FINDINGS FROM CLINICAL SAMPLES AND IN VITRO TRANSFORMATION DATA**

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Objectives: Previous studies have revealed that, compared to non-HPV16, HPV16 infections confer an increased risk of CIN2+. The mechanisms underlying this different biological behaviour are largely unknown. In order to study this phenomenon we investigated whether CIN2/3 with different hrHPV types vary with respect to their chromosomal profiles. In addition, we compared the in vitro transforming capacities of seven hrHPV-types in keratinocytes of two independent donors.

Methods: Chromosomal profiles were determined of 43 p16^{INK4a}-immunopositive CIN2/3 of women with long-term hrHPV infection (≥5 years) participating in the POBASCAM trial. Sixteen of the lesions harboured HPV16, and 14 contained HPV31. Unsupervised hierarchical clustering analysis of the chromosomal profiles revealed two major clusters, characterized by different numbers of chromosomal aberrations. The majority (i.e. 87.5%) of lesions with HPV16 were in the cluster with relatively few aberrations, whereas no such unbalanced distribution was seen for lesions harbouring other hrHPV types. Analysis of the two most prevalent types (HPV16 and HPV31) in this data set revealed a three-fold increase in the number of losses in lesions with HPV31 compared to HPV16-positive lesions.

Retroviral constructs containing the respective E6/E7 coding sequences of hrHPV types HPV16/18/31/33/45/66/70 were transduced to primary human keratinocytes. Low-risk HPV11E6/E7 and empty vector were included as controls. All transductants are monitored for their growth behaviour and other phenotypical and genotypical characteristics. Cells transduced with HPV16, HPV31 and HPV33E6/E7 showed the highest growth rate without any signs of senescence or crisis. Further characteristics are being evaluated and will be presented.

Conclusions: HPV16, HPV31 and HPV33 display the highest growth promoting activities early during the transformation process. However, the relatively low number of chromosomal aberrations observed in HPV16-positive CIN2/3 suggests that the development of these lesions is less dependent on genetic insult than those caused by other types like HPV31.

MSS 12-5

HPV TYPES IN THE CONTEXT OF VACCINATION

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Background: Evaluation of the disease burden caused by different specific HPV types is of importance for assessing the benefits of cross-protection and multivalent vaccines. While this is well known for cervical cancer, the disease burden of early lesions caused by different HPV types is more difficult to assess as many such lesions contains multiple HPV types that are also common in control samples from age-matched women.

Objective: To assess the disease burden caused by different HPV types targeted by vaccines.

Methods: Attributable proportions were estimated using the IARC global metaanalyses as well as using population-based cohorts from Sweden.

Conclusions: Reliable, quantitative and global estimates of the morbidity caused by each HPV type are essential for assessing the benefits of their prevention. Other oncogenic HPV types than HPV16/18 are particularly important as causes of precursor lesions.

PERFORMANCE OF BD™ HPV GT ASSAY ON BD VIPER™ LT SYSTEM

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Objectives: The objective of the BD HPV GT Assay is to establish a test for the BD Viper LT System capable of extracting and detecting human papillomavirus (HPV) DNA from BD SurePath™ and ThinPrep® (Hologic) specimens (prequot and residual) and a proprietary collection device. The design goals include the ability to detect selected individual genotypes, tolerance to the presence of interferents and competing genomic DNA, and no cross-reactivity with non-target HPV genotypes and other microorganisms. The BD HPV GT Assay must also have a high sensitivity and specificity for CIN2+ detection utilizing a simple, unified workflow for all specimen types collected within manufacturer's claims for stability.

Methods: The sensitivity of the BD HPV GT Assay was verified with analytical detection studies using cloned HPV DNA and HPV cell lines spiked into simulated clinical specimen matrix. Titrations of DNA were performed to establish preliminary limit of detection (LOD / C95) values for each HPV genotype used in the analytical studies. The BD HPV GT Assay's specificity was addressed by extracting potential cross-reactant organisms (bacteria, viruses, and low and high risk HPV types) at approximately 1×10^7 /mL. Stability of HPV DNA in liquid based cytology (LBC) specimens and the impact of interferents were determined by challenging the assay with target DNA at 3x LOD. The impact of competing targets on genotyping in mixed infections was evaluated with dual spikes of low (3x LOD) and high (1×10^7 /mL) levels of target DNA.

Conclusions: The BD HPV GT Assay is analytically sensitive and capable of detecting less than 11,000 HPV DNA copies per mL (250 copies per reaction) of specimen using a clinically relevant cutoff. It exhibits zero cross-reactivity with non HPV genotypes and other organisms. The assay design allows the detection of six individual and three HPV genotype groups, and thus, it can identify HPV targets in mixed HPV infections. The combination of analytical performance, specimen stability, compact instrument footprint, and unified workflow offer a number of advantages for the detection of HPV DNA.

A LARGE UK HOSPITAL'S EXPERIENCE WITH THE HOLOGIC CERVISTA® MEDIUM THROUGHPUT AUTOMATION (MTA)

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Objectives: North Bristol NHS trust hosts a large cytology laboratory in the UK which has been a leader in implementing change in the NHS Cervical Screening Programme (CSP). The laboratory was one of the "Sentinel sites" piloting HPV triage and test of cure. It is also involved in the project to assess the suitability of various HPV testing platforms for use in the NHS CSP.

Methods: The fully automated Hologic Cervista® system has been installed in the department since September 2011.

Conclusions: We will describe our day-to-day experience of using the platform, including workflow, staff training and feedback, ensuring quality, laboratory requirements and LEAN working practices.

MSS 13-3

COMPARISON OF APTIMA AND HC2 IN ROUTINE SCREENING IN GERMANY

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Background: E6/E7 mRNA testing of high-risk HPV might improve the specificity for cervical precancer compared to HPV DNA by reducing the false positive rate.

Objectives: As the APTIMA HPV Assay (AHPV) has been compared to HPV DNA tests mostly in triage settings we compared both tests with liquid-based cytology (LBC) in women aged ≥ 30 attending routine cervical screening.

Methods: 10,043 Women aged 30–65 were screened at office based gynecologists in three different areas: Tübingen, Saarbrücken and Freiburg. All specimens were collected with a cytobrush and tested centrally by LBC in Saarbrücken, and by AHPV and HC2 in Tuebingen. Women were referred to colposcopy if they had an abnormal cytology result and/or tested positive on either HPV assay. Not all of the primary histology has been reviewed so far. Sensitivity, specificity and positive predictive values were therefore calculated based on all available test results.

Results: Preliminary screening results are available for 7649 women so far. Currently we found 68 women with CIN2+ detected on histology of which 31 were CIN3+. AHPV and HC2 were highly sensitive for CIN2+: 92.6% (95% CI 83.7-97.6) and 95.6% (87.6-99.1), respectively; and for CIN3+: 93.5% (78.6-99.2) and 100% (88.8-100), respectively. Out of the two CIN3 missed by AHPV one was 0.2mm in size and the other had HPV62. LBC had a sensitivity of 37% (25.4-49.3) for CIN2+ and 48.4% (30.2-66.9) for CIN3+. Specificity of AHPV for CIN2+ was higher than that of HC2: 96.1% (95.7-96.6) versus 95.2% (94.7-95.6), with a corresponding reduction in the false positivity rate of 21%. The positive predictive value for CIN2+ amongst those who have attended colposcopy were 19%, 18.3% and 23.0%, for AHPV, HC2 and LBC respectively. The final data on the whole cohort will be presented.

Conclusions: The AHPV assay is both specific and sensitive for the detection of high-grade lesions and can be used as an option for routine cervical screening for women ≥ 30 years old.

MSS 13-4

HPV TESTING IN THE DIAGNOSIS AND FOLLOW-UP OF CIN PATIENTS: COMPARISON OF DIFFERENT METHODS

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Objectives. Many methods are available today for HPV testing: they differ for technology, targets, and information on the genotypes detected. A key issue is represented by the differences in analytical and clinical sensitivity. At the European Institute of Oncology of Milan a study was planned aimed at comparing the performance of different methods both at diagnosis and during follow-up of patients with cervical lesions.

Methods. Two hundred and forty-five women scheduled to be conservatively treated for a CIN lesion will be enrolled. For all the patients a cervical sample will be taken before treatment and at first follow-up visit. Qiagen Hybrid Capture 2 and Roche Linear Array Test will be performed and cytology and histology will be available. The following methods will be evaluated: Qiagen HPV Genotyping Probe Set Test and Lichip + Pyrosequencing Test, Roche Cobas 4800 HPV Test, Abbott ms2000 RealTime HR HPV Test, ProDect CHIP HPV Typing Test, Medical Systems Inifiniti HPV Genotyping Test and GenProbe Aptima HPV Test.

Conclusions. To date 120 women (median age 37 years) have been enrolled. Qiagen HC2, Roche Linear Array and Cobas 4800, Abbott ms2000 and GenProbe Aptima HPV Test were performed. Histology was the following: 29 \leq CIN1, 18 CIN2, 7 ?CIN3. Preliminary data showed that 86% were HC2 positive; the genotypes identified with Roche Linear Array method were 60 HPV16 (50%), 7 HPV18 (6%), 40 other High Risk HPV genotypes (34%), and 12 negative (10%).

This preliminary analysis showed a concordance at baseline between HC2 and the methods tested ranging between 89% and 92%. Regarding genotyping methods, HPV16 concordance between Linear Array and ms2000 was 95% while between Linear Array and Cobas was 97%; for genotype 18 the concordance was $>95\%$ with both ms2000 and Cobas 4800. The study is still ongoing.

THE HR-HPV TESTING IN FOLLOW-UP OF CYTOLOGICAL ABNORMALITIES WITH NEGATIVE COLPOSCOPY

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Objective: The causal role of HPV-hr in the development of cervical intraepithelial lesions and cancer, however, has suggested new and interesting applications of molecular testing in screening programs, such as in the follow-up of women with abnormal cytology and colposcopy negative. The Florence screening program introduced in 2006 the HPV-hr for the follow up of women with abnormal cytology and subsequent negative colposcopy. The rationale of this protocol is based on evidence that women with HPV-hr persistent infection have a much higher risk of disease than the general population.

Methods: The present study shows the preliminary data on 1027 women with negative colposcopy for abnormal cytology who performed HPV-hr test and followed from January 1, 2006 to May 05, 2011.

Incident CIN2 or worse (CIN2+) lesions were identified through linkage with regional flows of hospital discharge records and neoplastic pathology reports and the archive of the screening program for the years 2006-2011. We found 110 women with CIN2+ lesions with a median follow up time of about 3 years after negative colposcopy, of them 109 CIN2+ lesions were diagnosed in women with HPV-hr positive (109/593 = 18.4%) and one in the group HPV-hr negative (1/434 = 0.2%).

The risk of developing CIN2+ lesions during the follow-up is about 80 times higher in women with HPV-positive hr and negative colposcopy than women with HPV-negative hr. Women with HPV-negative hr in the follow-up, regardless of cytology that determined the first shipment in colposcopy, have a very low risk, virtually zero, to develop a lesion CIN2+ in the next 3 years.

We analysed some predictors of risk for the development of CIN2+ lesions, such as age (<35years, ≥35 years), the result of the Pap test index and viral load measure by HC2. Age not showed any statistical significance, while the result of the index Pap test was significant for ASC-H class (p = 0.03) and especially for the AGC class (p = 0.001). Regarding the semi quantitative viral load measure, has been the evaluation of the difference, in logarithmic scale, of the viral load present in the samples of 155 subjects with at least 2 hr HPV test-positive, ie with two successive calls with HPV-hr. The relative increase in viral load between the two tests was highly significant regardless of the level of viral load to the first test.

Conclusion: Our data confirm that HPV testing performed within one year after the first negative colposcopy in the follow-up of women with cytology ASC-US or worse was able to select the real group of women at a risk of developing CIN2+. Therefore, women with HPV-negative test could return to screening and only hr-HPV positive women underwent follow-up.

PERFORMANCE OF HPV DNA, E6/E7 MRNA AND P16INK4A/KI-67 PROTEIN CO-EXPRESSION AS A TRIAGE TOOL FOR LSIL/ASCUS

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Introduction: HPV DNA testing has proven useful in the management of low grade abnormalities however it is limited in regards to specificity. Biomarkers such as E6/E7 mRNA and p16INK4A/Ki-67 dual expression show potential to offer increased specificity for the detection of CIN 2+ in women presenting with LSIL/ASCUS. The aim of this study is to investigate the utility of these markers for detecting women at risk of developing high grade disease presenting at colposcopy with LSIL/ASCUS. This study is carried out under CERVIVA the Irish Cervical Screening Research Consortium funded by the Health Research Board and is supported by The Irish Cancer Society.

Methods: Cervical smears for HPV testing and immunocytochemical analysis were collected from 1073 women presenting with LSIL/ASCUS at their first visit to colposcopy at the National Maternity Hospital, Dublin. HPV DNA was detected by Hybrid Capture II (Qiagen, UK), HPV E6/E7 mRNA expression by the PreTect™ HPV Proufer (NorChip AS, Norway) and p16^{INK4a}/Ki-67 expression was assessed using CINtec PLUS (Roche). All women are currently under a two year follow up.

Results: 1073 patient samples referred with LSIL/ASCUS have been HPV tested from patients with a mean age of 33 years (range 18-65). To date a subset of 443 has undergone a two year follow up, within this group 139 (31%) developed CIN 2+ over the two year period. Detection of HPV E6/E7 mRNA appears to be more specific 70% (95% CI 0.7277-0.8495) than HPV DNA testing 45% (CI 0.4849-0.6327) for detection of CIN 2+. p16^{INK4a}/Ki-67 dual expression analysis is currently underway to investigate combined testing for optimal clinical sensitivity and specificity.

Conclusion: This offers prospective evidence that HPV testing in the management of women presenting with low grade abnormalities could be useful in detecting those at risk of developing high grade disease.

MSS 13-7

SCOTTISH TEST OF CURE STUDY - HPV TEST COMPARISONS (STOCS-H)

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Objective: To compare the technical and clinical performance of 5 HPV screening assays approved by the NHS Cervical Screening Programme in a post-treatment population, in which the majority of samples would be expected to be negative.

Methods and Results: The Test of Cure (TOC) Early Implementers programme in Scotland involved combined cytology and HPV testing using Hybrid Capture 2 (HC2) on women treated for high grade cervical disease at 6 months and 12 months post-treatment. This phase involved one-third of the Scottish screening population from three Health Boards: Lothian, Grampian and Highland to pilot the logistics of HPV testing before national roll-out from May 2012. Residual material from over 1000 ThinPrep samples was available after testing by HC2 for testing/comparison with other HPV assays, namely - rtHPV assay (Abbott) on m2000 platform, the APTIMA HPV Test (Genprobe) using the Panther, the CerVista test (Hologic) using the HTA platform and the cobas 4800 HPV test (Roche).

Technical analysis of the first 500 samples showed that 21.3% were HC2 HPV positive at a cut-off of 1 and 18.4% at a cut-off of 2. Preliminary analysis showed positivity of other assays ranged from 20-24%, depending on assay. According to the McNemars test, we noted some differences in the distribution of discordant results for some test-comparison combinations. Full comparisons and the clinical performance of each assay for the detection of residual/recurrent disease will be presented on the first 500 samples at 6 months post-treatment.

Conclusions: As expected, we observed variation in positivity according to assay, but only some differences between tests reached significance. Tests approved for HPV triage may also be suitable for the test of cure setting, but recognition of differences is important to estimate the number of women who will need to be returned to colposcopy for further assessment.

MSS 14-1

COMPARISON OF 7 TESTS FOR HIGH GRADE CIN IN WOMEN WITH ABNORMAL SMEARS:
THE PREDICTORS 2 STUDY

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Objectives: High risk HPV DNA/RNA testing provides higher sensitivity but lower specificity than cytology for the identification of high grade cervical intraepithelial neoplasia (CIN). Several new HPV tests are now available for this purpose and a direct comparison of their properties is needed.

Methods and results: Seven tests were evaluated on liquid PreservCyt[®] samples from 1099 women referred for colposcopy: Hybrid Capture II (Qiagen), Cobas (Roche), PreTect HPV-Proofer (NorChip), APTIMA HPV assay (Gen-Probe), Abbott RealTime, BD HPV test and CINtec p16^{INK4a} Cytology (mtm) immunocytochemistry. Sensitivity, specificity and positive predictive value (PPV) were based on the worst histology found on either the biopsy or the treatment specimen after central review. 359 women (32.7%) had (CIN2+), with 224 (20.4%) having CIN3+. For detection of CIN 2+, Hybrid Capture II had 96.3% sensitivity, 19.5% specificity and 37.4% PPV. Cobas had 95.2% sensitivity, 24.0% specificity and 37.6% PPV. The BD HPV test had 95.0% sensitivity, 24.2% specificity and 37.8% PPV. Abbott RealTime had 93.3% sensitivity, 27.3% specificity and 38.2% PPV. APTIMA had 95.3% sensitivity, 28.8% specificity and 39.3% PPV. PreTect HPV-Proofer had 74.1% sensitivity, 70.8% specificity and 55.4% PPV. CINtec p16^{INK4a} Cytology had 85.7% sensitivity, 54.7% specificity and 49.1% PPV. Cytology taken at colposcopy (mild dyskaryosis or worse) had 88.9% sensitivity, 58.1% specificity and 50.7% PPV.

Conclusions: Our study confirms that, in a referral setting, HPV testing, by a number of different tests, provides high sensitivity for high grade disease. Further work is needed to confirm these findings in a routine screening setting.

VALGENT 1: FINDINGS FROM BELGIUM**Depuydt CE¹ on behalf of the VALGENT study group***1 RIATOL, Department of Molecular Diagnostics, Sonic Healthcare Benelux, Antwerp, Belgium*

Objectives: From case-control studies it was derived that the carcinogenic potential of human papillomaviruses (HPV) is very similar for about 5 to 8 types which are most prevalent in cervical cancer (HPV 16, 31, 33, 35, 45, 52, 58) with Odds Ratios >100 up to 500. Recent cohort studies indicate different carcinogenic potential of certain types such as HPV 16,18, or even HPV 45. This generates the hypothesis that genotyping could be used in clinical practice. Also the surveillance of the effects of vaccination will require genotyping to answer questions of efficacy against vaccine type associated lesions, cross protection and type replacement.

Although different HPV typing systems exist they are poorly validated.

Methods: Using a Belgian laboratory database processing more than 100 000 BD-SurePath liquid cytology specimens annually a validation study for 5 different HPV genotyping systems was set up. Thousand consecutive BD-SurePath liquid based cytology samples were collected during routine gynecological health checks from women in Flanders (Belgium) in October 2006. The study was supplemented with 100 consecutive ASC-US, LSIL and HSIL samples.

DNA isolation from liquid-based cytology leftover was performed by Proteinase K digestion (n = 1300), divided in 5 aliquots and shipped to the different centers performing the genotyping assays.

Conclusions: During the 60 month follow up period 84 histologically verified CIN2+ cases were detected.

An overview of the quantitative data for the whole spectrum of HPV genotypes is given for the Belgian screening population using the real time quantitative E6/E7 HPV genotyping PCR assay.

VALGENT 3**Poljak M¹, Arbyn M²***1 Institute of Microbiology and Immunology, Ljubljana, Slovenia**2 Scientific Institute of Public Health, Brussels, Belgium*

Objectives: The VALGENT (clinical VALidation of human papillomavirus GENotyping tests) protocol provides a comprehensive design to validate and compare general HPV tests (identifying hr-HPV infection) and HPV genotyping assays (identifying hr-HPV separately) using residual archived cervical cell samples from 1,000 screened women enriched with 300 pathological samples (100 ASC-US, 100 LSIL, 100 HSIL). Follow-up from the 1,300 women according to local guidelines will identify 70–120 CIN2+ cases and about 800 subjects without CIN, allowing computation of sensitivity and specificity. One general HPV assay and four genotyping tests were already evaluated using samples derived from a Belgian biobank in VALGENT 1 study. A VALGENT 2 study including six HPV assays has been set up using the samples from the Scottish HPV archive.

Methods: The VALGENT 3 panel will be set up using samples from Slovenian HPV Prevalence Study involving women attending cervical cancer screening enriched with pathological samples from triage of ASC-US/LSIL from a colposcopy clinic. In the Slovenian HPV Prevalence Study, we consecutively enrolled 4,431 women in 16 outpatient gynecological services among 20–64 years old Slovenian women routinely screened for cervical cancer. All women were screened by traditional cytology and by HC2 and Abbott Real Time Test. All specimens with HC2/RealTime concordant positive results and HC2/RealTime discordant results were tested using Roche Linear Array, HPV-52 type-specific real-time PCR assay, INNO-LiPA HPV Genotyping Extra Test and GP5+/GP6+ PCR assay with additional HPV-68 specific primers. Women were called for colposcopy using a cytology threshold of ASC-H/AGC or worse. Irrespective of cytology result, women positive for HPV-16 or HPV-18 were also invited for colposcopy. An expert histopathology system was used for histopathological assessment. Second round screening of the women is planned for December 2012–May 2013 using similar approach as in the first round.

Conclusions: The VALGENT 3 panel will be set up using the available 0.5 ml aliquots of ThinPrep samples stored at -800C derived from Slovenian HPV Prevalence Study enriched with samples from main colposcopy clinic in the country.

MSS 14-4

EXCELLENT AGREEMENT WAS SEEN BETWEEN THE DIGENE® HPV GENOTYPING PS TEST AND QUANTITATIVE PCR**Thai H., Chen G., Chen W., Pfister D., Shrestha A., Xuezhou H., Rothmann T.***QIAGEN Gaithersburg Inc., 1201 Clopper Road, Gaithersburg, MD 20878 USA*

Objectives: The digene HPV Genotyping PS Test (PS Test) currently under development is to be used as a triage for positive digene HC2 HR HPV DNA Test (HC2) results. In this study we demonstrate excellent overall agreement of the PS Test to an in-house validated type specific quantitative-PCR (qPCR) test. In addition, the study also shows the improved specificity and overall performance of the PS Test with an increase of the positive cutoff from 1.3 to 2.0.

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. Performance of the PS Test was compared to an in-house validated type specific qPCR test to evaluate concordance. The results were generated by testing cervical clinical specimens collected in both QIAGEN Specimen Transport Media (STM) and Hologic PreservCyt® media (PC). Accuracy of the PS Test was determined by comparing the results to a QIAGEN in-house validated type specific qPCR method for the detection of HPV 16, 18, and 45 using a threshold of 5000 copies per assay as the positive cutoff.

Conclusions: The overall agreement between the PS Test and qPCR was demonstrated to be greater than 90%, as was the positive and negative agreement respectively. An improvement was seen in the specificity of the PS Test using a 2.0 RLU/CO as the positive cutoff in comparison to a 1.3 positive cutoff. This improvement in specificity was realized with only a small loss in sensitivity. The overall agreement also improved with the cutoff adjustment to 2.0. The applications presented here are for research use only and are not to be used for diagnostic procedures.

MSS 14-5

COMPARISON OF COBAS PCR AND HC2 HPV TESTING IN A GERMAN ROUTINE LABORATORY**Ikenberg H¹, Iftner T², Börsch C¹, Pittel P¹, Khaja A¹, Britz F³***1 Cytomol, Laboratory for Cytology and Molecular Diagnostics, Frankfurt, Germany, 2 Section for Experimental Virology, Institute for Virology, University of Tübingen, 3 Roche Diagnostics, Mannheim, Germany*

Objectives: Since nearly a decade the Digene HC2 test (Qiagen, Hilden, Germany) has been regarded the gold standard for routine human papillomavirus (HPV) DNA testing. This rating was recently confirmed by an international expert panel. However, meanwhile several new test systems for HPV are available, among them the cobas HPV assay (Roche Diagnostics, Mannheim, Germany) which allows simultaneous genotyping for HPV 16 and 18. We therefore compared the performance of the cobas HPV with the HC2 assay in cervical samples collected with the PreservCyt collection medium (Hologic, Frankfurt, Germany) stored at room temperature for up to two years.

Methods: 1781 anonymized routine specimens pretested with the HC2 test were available for analysis with the cobas HPV test. Cases with discrepant results between the two tests were retested with the Linear Array HPV genotyping test (LA) (Roche). Partially, histologic diagnoses were available.

Results: In 1566 (87.9%) of the cases the HPV results were concordant. Of the 215 specimens (12.1%) with discrepancies, LA results are available in 214 cases. 94 cases were LA-negative: 13 of 105 cobas-pos/HC2-neg and 81 of 99 cobas-neg/HC2-pos cases. 110 cases were LA-positive: 92 of 105 cobas-pos/HC2-neg and 18 of 99 cobas-neg/HC2-pos cases. In 325 cases CIN2+ had been histologically confirmed, of which in 293 a HC2 result was available. In 261 out of these 293 cases (89.1%) this was positive, while 298 out of 318 of the cases (93.7%) tested with cobas HPV, were positive. The rate of HPV positivity in cytologically normal cases and in HSIL was slightly higher with cobas HPV, while it was in the same range in ASC-US and in LSIL cases. With increasing severity of the cytologic findings the rate of HPV 16 and 18 positivity increased proportionally.

Conclusions: In routine specimens from a German commercial laboratory the cobas HPV test showed similar performance compared to the HC2 test. The preliminary data point to a potentially higher sensitivity and specificity with cobas HPV while adding information by HPV 16 and 18 genotyping.

SCREENING INTERVAL: COTESTING VS. CYTOLOGY, A 5 YEAR ANALYSIS**Cox JT***UC Santa Barbara, CA, US (Retired)*

Cervical cancer screening of women age 30 and over with both a Pap and a HPV test (cotesting) has been approved in the U.S. since 2002. Women testing negative on both tests (cyto-/HPV-) were advised to return for their next cervical screen no sooner than 3 years. As of March, 2012, the U.S. Preventative Services Task Force (USPSTF) and the American Cancer Society (ACS) now recommend that these women be screened only every 5 years on the basis of several large studies that demonstrated safety for at least 5 years after a double negative screen. One of these studies is an evaluation of the safety and efficacy of cotesting with 3 year screening intervals in a large HMO (Kaiser Permanente Northern California) that adopted cotesting soon after approval. The 5-year or greater follow-up of the 331,818 women aged 30 years and older cotested from 2003-2005 demonstrated that: 1) In 315,061 women negative by HPV testing (94.9% of women cotested), the 5-year cumulative incidence of cancer was 3.8 per 100 000 women per year, similar to the 5 year risk of age-matched women for vulvar cancer for which there is no screening recommended, 2). This was slightly higher than the 3.2 per 100,000 rate for the 92.5% who were cyto-/HPV-, and half the cancer risk of the 96% who had a normal Pap without consideration of HPV results (7.5 per 100,000). Almost all (99.5%) of women negative by HPV testing had either normal cytology or ASC-US. In fact, a single negative test for HPV was sufficient to reassure a woman of extremely low risk of CIN3+ for 5 years, and negative cytology provided no extra reassurance against cancer beyond that conferred by a negative HPV test result. In contrast to the low risk of women cyto-/HPV-, or even just HPV-, women cyto+/HPV+ had the highest risk of CIN 2,3+, but even women cyto-/HPV+ also accrued substantial risk of CIN3+ over time. Although cytological abnormalities suggested prevalent disease, testing for HPV predicted future disease much better than cytology. The findings suggested that 5-year screening intervals for women negative by both HPV and Pap testing might be safe and that HPV testing without adjunctive cytology might be sufficiently sensitive for primary cervical cancer screening. These results will be further discussed in the global context of optimal intervals for cervical cancer screening.

WILL CYTOLOGY WITH ADJUNCTIVE STAINING FOR P16/KI67 MAKE HPV TESTING SUPERFLUOUS IN CERVICAL CANCER SCREENING?**Guglielmo Ronco***CPO Piemonte, Turin, Italy*

Cervical screening based on HPV DNA as primary test has been shown to allow, compared to cytology, earlier diagnosis of high grade cervical intraepithelial neoplasia (CIN) and increased protection from invasive cervical cancer. This earlier detection of hgCIN allows longer intervals between screens in HPV negative women. Indeed, the 5-year cumulative incidence of CIN3 after a negative test is lower than the 5-year cumulative incidence after a negative cytology. This knowledge from longitudinal randomised and non-randomised studies allows determining the intervals between screens with HPV DNA. A limitation of HPV DNA testing is its low specificity. This led to studying triage methods for HPV positive women, including the overexpression of p16. A study nested in the NTCC randomised controlled trial showed that referring immediately to colposcopy only the HPV positive women who also over-expressed p16 would have allowed a sensitivity close to that of direct referral of all HPV positive women, but referral to colposcopy similar to that resulting from cytology-based screening. The specificity of p16 immunostaining can be increased by adding Ki67 immunostaining. In a multicentre European study (PALMS), double staining with p16 and Ki67 showed slightly lower cross-sectional sensitivity but higher cross-sectional specificity than HPV DNA testing by Hybrid Capture 2. Little is however known about the duration of the low-risk period after a negative p16 test. In the NTCC study, HPV positive women were followed up at yearly intervals by HPV and cytology until HPV become negative and referred to colposcopy if cytology was ASC-US or more. The 3-year cumulative incidence of CIN2 and CIN3 among the women who at baseline were HPV-positive p16+ HPV positive p16 negative was computed. The detection rate of CIN3 at round 2 in the women who were HC2 negative at baseline was considered as an estimate of the 3-year cumulative incidence of CIN3 in these women. Preliminary data show that the 3-year cumulative incidence in HPV-positive p16 negative women was much lower than in HPV positive p16 positive women but much higher than in HPV-negative women. Therefore HPV DNA as primary screening test allows longer screening intervals than p16, which should better be applied as a triage test for HPV DNA positive women.

SS 1-3

RANDOMISED IMPLEMENTATION OF PRIMARY HPV SCREENING IN SWEDEN**Eklund C¹, Persson S², Elfgrén K³, Törnberg S⁴, Dillner J¹***1; Karolinska Institutet, Dept of Laboratory Medicine, Stockholm, Sweden, 2; Karolinska University Hospital, Dept of Clinical Microbiology, Stockholm, Sweden, 3; Karolinska University Hospital, Dept of Obstetrics and Gynecology, Stockholm, Sweden, 4; Regional Cancer Center, Cancer Screening Unit, Stockholm, Sweden*

Objectives: To evaluate the effectiveness of primary HPV screening when introduced into an organised, population-based cervical screening program in Sweden.

Methods: HPV based primary screening was implemented in a randomized age-specific fashion in the population-based organised screening program for cervical cancer in the greater Stockholm County, Sweden. Phase Ia started in January 2012 and has switched the screening policy for 50% of the women in the population who are aged 56-60 years. In the HPV screening arm (new policy), HPV-positive women are triaged by cytology. Among the 50% of women who are randomised to the old policy, there is primary cytology with HPV triaging for ASCUS and LSIL smears. Phase Ib is anticipated in 3Q 2012 and will extend the primary HPV screening in the New Policy Arm also to women in the ages 35 and 49. HPV+/Cyt- women in the screening ages are referred to the next round of organised screening, whereas HPV+/Cyt- women aged 60 (who otherwise would have been acquitted from the programme) will continue to be screened. The primary evaluation is the cost-effectiveness of the new policy in relation to the previously used policy.

Conclusions: Whether primary HPV screening within an organized program can improve the cost-effectiveness of screening is being evaluated by a randomized implementation strategy.

SS 1-4

SCREENING INTERVAL AMONG HPV NEGATIVE WOMEN: A LONGTERM FOLLOW-UP OF THE SWEDSCREEN STUDY**Elfström M¹ and Dillner J¹ on behalf of the Swedescreen study group***1 Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden*

Background: The duration of the "protective effect" of being screen-test negative is a determinant of the length of the screening interval. Previously, a joint analysis of 7 primary HPV screening studies in 6 European countries that had enrolled 24,295 women found that the cumulative incidence rate (CIR) of CIN3+ after 6 years was considerably lower among HPV-negative women (0.27%, 95% CI 0.12% to 0.45%) than among cytology-negative women (0.97%, 0.53% to 1.34%). As the CIR for cytology-negative women at the most commonly recommended screening interval in Europe (3 years) was 0.51% (0.23% to 0.77%)[1], a 6-year screening interval for HPV-negative women seemed appropriate.

Objective: We now wished to establish the duration of the protective effect against CIN2+ for screen-test negative women, using an extended follow-up of up to 14 years.

Methods: Swedescreen is a population-based randomized controlled trial of primary HPV screening that started in 1997. Women aged 32 - 38 attending organized screening in 5 different regions of Sweden were invited to participate. In total, 12,527 women were enrolled and randomized to HPV and cytology double testing (intervention arm, n=6257) or cytology only (control arm, n=6270). All women were followed through comprehensive mass screening registry-based follow-up since 1997. For this long-term follow-up study, the analysis focuses on 14 years of screening data to assess risk for development of CIN2+ among women who were cytology-negative or HPV-negative at baseline. Women are censored at first histologically confirmed diagnosis of a CIN2+ lesion (CIN2, CIN NOS, CIN3, or invasive cancer including squamous and adenocarcinoma) or the last registered testing date. The CIR for CIN2+ is estimated by original baseline group (cytology-/HPV-, cytology+/HPV-, and cytology+/HPV+) using Kaplan Meier curves. The CIR of CIN2+ remains low during extended follow-up among women negative for HPV at baseline.

Conclusions: Compared to 2008 study that had only 6 years of follow-up data, this study contributes considerably more follow-up time on the duration of the protective effect of HPV-negativity.

VIA VS. HPV BASED SCREENING IN DEVELOPING COUNTRIES**R. Sankaranarayanan MD***Head, Early Detection & Prevention Group (EDP) and Screening Group (SCR)
International Agency for Research on Cancer (WHO-IARC)***Objective:** to describe the relative merits of visual and HPV screening in developing countries.**Methods:** Findings from studies evaluating visual and HPV screening in the early detection and prevention are reviewed and findings described.**Findings and conclusions:** Although cytology has substantially reduced cervical cancer burden in developed countries, the complex inputs required and the limited success of cytology screening in Latin America have encouraged evaluation of alternative screening methods such as HPV testing and visual inspection with acetic acid (VIA) as well as new paradigms such as a single life time screening and single visit approach to maximise treatment of women with lesions. HPV testing has a higher accuracy and reproducibility than VIA in detecting cervical neoplasia. In a randomized trial in South Africa, cryotherapy for HPV-positive women resulted in 77% decline in CIN 2-3 lesions, while VIA followed by cryotherapy resulted in a 37% lower prevalence of such lesions. A single round of VIA screening resulted in 35% reduction in cervical cancer mortality in a randomized trial in South India. A 50% reduction in cervical cancer mortality following a single round of HPV testing was demonstrated in a randomized trial in Western India. Randomized trials in developed countries have demonstrated lower incidence of high-grade precursor lesions and invasive cancers in subsequent screening rounds following the prevalence round of screening. Although HPV testing has been found to be a more objective, reproducible and accurate and effective screening approach than visual screening, VIA is a more feasible screening approach than HPV screening in low-resource settings, since HPV testing requires sophisticated equipment and a range of consumables, which may not be affordable or accessible. A simple, affordable and rapid testing platform is needed to introduce HPV screening in a mass scale in low-resource settings and the eventual availability of a simple, affordable, faster (results within 3 hours) and accurate HPV test (careHPV test) may facilitate wider HPV screening in low-resource settings. However, introduction of VIA based screening will facilitate the development of resources and infrastructure which may support future introduction of HPV screening when affordable tests become available.**METHYLATION MARKERS FOR THE TRIAGE OF HPV POSITIVE WOMEN****Nicolas Wentzensen***Division of Cancer Epidemiology and Genetics, National Cancer Institute*

Primary HPV testing, either alone or as co-testing with cytology, is increasingly being introduced into clinical practice. While HPV testing is very sensitive and provides a good negative predictive value and great long-term reassurance against developing disease, it lacks specificity, and thus positive predictive value. Several biomarkers with higher specificity for cervical precancer have been proposed, among them host and viral methylation markers. We evaluated both viral and host methylation markers in ALTS, NHS, and PaP, and in a systematic review of the literature. We observed strong HPV methylation in the E2, L2, and L1 regions for 4 different carcinogenic HPV genotypes (HPV16, 18, 31, 45) in CIN3 compared to normal controls. Few host methylation markers looked promising in the systematic review. We evaluated several additional candidate host methylation markers not previously evaluated in cervix, and we conducted methylation profiling in cervical tissues to expand the set of current candidates. Validation of these novel host methylation candidates is currently ongoing.

SS 2-1

SELF-SAMPLING AND REPEAT HPV TESTING IN SCREENING FOR CERVICAL CANCER**Gyllensten U (1), Gustavsson I (1), Sanner K (2), Wikström I (2), Olovsson M (2), Wilander E (1).***1. Department of Immunology, Genetics and Pathology, Rudbeck Laboratory, Uppsala University, Uppsala, Sweden. 2. Department of Women's and Children's Health Uppsala University and Uppsala University Hospital, Sweden.*

Objects: In many countries with organised gynaecological screening based on Pap-smears, the incidence of cervical cancer has been reduced by about 50%. A further reduction could be achieved by using HPV typing as a primary test and employing a system where women can take the samples at home at their own convenience and thereby increase the population coverage.

Methods and conclusions: To explore this possibility we have made use of a filter-paper device for sampling of cervical cells, based on the micro elute (FTA) card, and subsequent HPV typing using real-time PCR. Cervical cells are collected by a brush (Viba-brush) and applied to the FTA card. The dried cards can be sent by regular mail and stored at room temperature for many years. The HPV typing of samples applied to the FTA card yield similar results as using liquid collection media. Testing for high-risk human papillomavirus (HPV) in primary screening for cervical cancer is considered more sensitive but less specific than using Pap-smear cytology. We recently evaluated the specificity for detection of histologically confirmed CIN2+ lesions using short-time repeat testing, with the primary sample for HPV analysis taken by self-sampling. A total of 8000 women aged 30-65 years were offered self-sampling of vaginal fluid at home and the samples sent for HPV typing. Women positive for high-risk HPV in the self-sampling test were invited for a follow-up HPV test and a cervical biopsy on average 3 months after the initial HPV test. 39% of invited women performed self-sampling at home and high-risk HPV infection was found in 6.6% of the participating women. Short-time repeat HPV testing increased the specificity for detection of CIN2+ lesions from about 94.2% to 97.8%. At presently we are determining the efficiency of using self-collected cervicovaginal samples and repeat HPV testing as the primary screening test for identification of women at risk of developing cervical cancer in comparison to cytology. A strategy based on self-collected samples and repeat HPV typing can result in a higher population coverage and provide very significant economic savings to the health care system.

SS 2-2

A NEW PARADIGM FOR CERVICAL CANCER SCREENING BASED ON SELF-SAMPLING.**Belinson JL.***Preventive Oncology International and the Women's Health Institute of the Cleveland Clinic, USA.*

Recent findings based on our self-sampling work over the past 15 years, recent developments in solid transport media, and our enlightening experience with a low cost, high throughput, high tech HPV testing system, have lead us to re-design our concepts of reaching the medically underserved. With the majority of the worlds' medically underserved now living in middle income countries, it is no longer necessary to think simple, low-tech, low sensitivity, with poor quality control. We can now think high-tech, high sensitivity, specimen transport to centralized labs for processing with good quality control. Using many of the principles founded in community based participatory research; we have developed a new preventive health-care model and applied it to a cervical cancer prevention program. The model, based on primary self-collection for HPV is totally community based until the positives have been identified, then the clinical healthcare system becomes involved. With deeper community involvement in the design and implementation of the screening program, the result is more complete community coverage followed by high return rates for those testing positive.

HPV TESTING IN NON-ATTENDEES OF CERVICAL SCREENING

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Various studies report on hrHPV testing on self-samples to be at least as, if not more, sensitive for CIN2+ as cytology on clinician-obtained cervical samples, and hrHPV testing on self- and clinician-sampled specimens is generally similarly accurate with respect to CIN2+ detection. Pooled data from two consecutive self-sampling studies in the Netherlands (PROHTECT) show that HPV self-sampling increases the efficacy of the screening programme by targeting a substantial portion of non-attendees of all ethnic groups who have not regularly been screened and are at highest risk of CIN2+. HPV self-sampling is accepted by up to 30% of non-attendees of the regular cervical screening programme. There was no age trend in the response rate to HPV self-sampling. CIN2+/CIN3+ yields of HPV self-sampling responders were higher than those of screening participants. Native Dutch non-attendees responded better than immigrants, and those screened in the previous round revealed a higher response than underscreened (i.e. previous smear taken >7years ago) or never screened women. But, interestingly, amongst women ≥ 39 years (i.e. who had been invited at least to two prior screening rounds), women who had never have been screened before responded better than underscreened women. CIN2+ rates were higher amongst responding native Dutch women than immigrants, and higher in under-/never screened women than in women screened in the previous round. These data indicate that HPV self-sampling reaches the women with the highest risk of cervical cancer.

Altogether, the stage has been reached for the implementation of HPV self-sampling into screening practice. Together with the introduction of guidelines for clinical validation of hrHPV tests in combination with self-sampling devices, this screening tool will allow an improved screening coverage and acceptability, especially in "hard to screen" women, with also potential as an alternative for hrHPV testing on physician-collected samples.

MOLECULAR TRIAGE OF HPV-POSITIVE WOMEN IN SELF-SAMPLED MATERIAL

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Objectives: Previously, we demonstrated that quantitative methylation-specific PCR (qMSP) assays that assess the methylation status of two human genes have a high potential value as triage marker for hrHPV-positive women. A combination of two qMSPs yielded a methylation marker panel that, when applied to cervical scrapes, was equally discriminatory for CIN3+ as cytology or cytology/HPV16/18 genotyping combination in hrHPV-positive women (Hesselink et al., Clin Cancer Res 2011).

In this study we analyzed if promoter methylation analysis can also serve as triage tool for hrHPV positive women when applied to cervico-vaginal lavage samples self-collected with a Delphi-screener (Delphi-bioscience).

Methods: hrHPV-positive self-sampled lavage specimens of 355 screening non-attendees who participated in the PROHTECT trial (Gok et al., BMJ, 2011) were tested by qMSP for three promoter regions. The series included 74 specimens of women who were subsequently diagnosed with CIN3+. Cross-validated ROC curves for panels consisting of one to three qMSP markers (total 7 combinations) were constructed. The most discriminating panel for CIN3+ was considered the panel with the highest cross-validated partial area-under-the-curve (partial AUC).

Conclusions: After cross-validation a two marker panel displayed the highest partial AUC (0.740) for detection of CIN3+. Depending on the qMSP threshold settings the CIN3+ sensitivities ranged from 97.3% (95%CI:91.7-99.3) to 63.5% (95%CI:53.9-73.6), with corresponding specificities ranging from 20.6% (95%CI:18.8-22.6) to 80.1% (95%CI:75.9-83.7). A trade-off point that appeared optimal on the ROC curve was achieved with thresholds yielding a CIN3+ sensitivity of 85.1% (63/74; 95%CI:77.0-93.2) at a specificity of 53.7% (151/281; 95%CI:48.1-59.4) amongst these hrHPV positive women.

The two marker methylation panel is an attractive triage tool for self-sampled cervico-vaginal lavage specimens of hrHPV positive women. Its application could obviate the need for an extra visit to the general practitioner for a cervical scrape when a woman has a hrHPV positive self-sample. This will save costs and reduce loss to follow-up.

SS 2-5

ACCEPTABILITY AND PERFORMANCE OF CAREHPV IN SELF-COLLECTED VAGINAL SAMPLES**Jeronimo J¹, Lim J¹, Sequeira M², Rani Poli U³, Byamugisha J⁴, Labani S⁵.***1 PATH, Seattle, USA. 2 PATH, Managua, Nicaragua.**3 MNJ Institute of Oncology & Regional Cancer Center, Hyderabad, India.**4 Makerere University, Kampala, Uganda. 5 ICPO, Noida, India.*

Background: Despite multiple efforts to implement screening programs, cervical cancer is still one of the leading causes of cancer-related mortality in developing countries. PATH is conducting a multi-country demonstration project to compare the detection rate of CareHPV with other screening options. Self-collected vaginal sampling is one of the options evaluated since that strategy could potentially increase the coverage of screening in high-risk populations.

Methods: Approximately 20,000 women were enrolled in India, Uganda and Nicaragua. After completion of informed consent, participants were instructed to self-collect a vaginal sample for careHPV testing in a private setting. Health providers then conducted a pelvic evaluation and collected cervical samples for careHPV and cytology, followed by visual inspection with acetic acid (VIA). Participants with any positive screening test underwent colposcopy and biopsy for disease confirmation. Cases are defined as any woman with histological diagnosis of CIN2+. Twenty percent of randomly selected participants were invited to complete a questionnaire to evaluate their acceptability of the self-collection process.

Results: More than 90% of women agreed to self-collect a vaginal sample, with lower acceptability in Nicaragua (81.1%), followed by Hyderabad, India (90.7%) and the highest in Uttar Pradesh and Uganda where more than 99% of women accepting. The sensitivity of the self-collected vaginal sample for detecting CIN2+ cases was 65.9% in Nicaragua, 76.5% in India, and 85.4% in Uganda. Sensitivity of Pap smear was 37.1%, 72.5% and 58.5% respectively.

Conclusions: Self-collection of vaginal samples was highly accepted in India, Uganda and Nicaragua. The sensitivity of careHPV using those samples was better than Pap smear or VIA. Our results open the possibility for creating new strategies for cervical cancer screening, even at the community level where sample collection could be done at women's homes without the need for pelvic evaluation.

SS 2-6

SELF-COLLECTION OF VAGINAL SPECIMENS FOR HPV TESTING IN CERVICAL CANCER PREVENTION (MARCH): A RANDOMIZED CLINICAL TRIAL**Lorincz A and Lazcano-Ponce E.***Centre for Cancer Prevention, Wolfson Institute of Preventive Medicine, Queen Mary University of London, London UK. Centro de Investigación en Salud Poblacional. Instituto Nacional de Salud Pública, Cuernavaca, Morelos, Mexico.*

Objective. Vaginal self-sampling for HPV DNA testing could increase screening participation rates. In clinic-based settings vaginal HPV testing is at least as sensitive as cytology for detecting cervical intraepithelial neoplasia grade 2 or greater (CIN 2+); however, performance characteristics in a home setting are unknown. A community-based randomized equivalency trial was conducted to assess the relative sensitivity and positive predictive value (PPV) of HPV screening of vaginal specimens self-collected at home as compared to clinic-based cervical cytology for CIN 2+ in Mexican women of low socio-economic level aged 25 to 65.

Methods. Allocation to HPV screening (n=9,202) or cervical cytology (n=11,054) was by a central computer random sequence. Eight masked community nurses received daily lists and carried out the assigned home visits. HPV testing for high-risk HPV types was by Hybrid Capture 2 and cytology was the routine Papanicolaou smear test. Women with positive results to either test were referred to colposcopy for diagnosis.

Conclusions. HPV prevalence was 9.8% (95% CI 9.1-10.4) and the abnormal cytology rate was 0.38% (95% CI 0.23-0.45). HPV testing identified 117.4/10,000 (95% CI 95.2-139.5) CIN 2+ versus 34.4/10,000 (95% CI 23.4-45.3) identified by cytology, a 3.4-fold (95% CI 2.4-4.9) greater relative sensitivity. Similarly, HPV testing detected 4.2 (95% CI 1.9-9.2) times more invasive cancers. The PPV of HPV testing for CIN 2+ was 12.2% (95% CI 9.9-14.5) compared to 90.5% (95% CI 61.7-100) for cytology. Despite its much lower PPV, HPV testing on self-collected vaginal specimens may be preferred for detecting CIN 2+ in low resource settings. Since women in these sites will be screened at most a few times in their lives, the high sensitivity of a single HPV screen is of paramount importance.

BURDEN OF CERVICAL CANCER IN EUROPE – WESTERN VERSUS EASTERN EUROPEAN COUNTRIES**Arbyn M (1), Weiderpass E (2,3,4,5)***(1) Unit of Cancer Epidemiology/Belgian Cancer Centre, Scientific Institute of Public Health, Brussels, Belgium**(2) The Cancer Registry of Norway, Oslo, Norway. (3) Karolinska Institutet, Stockholm, Sweden.**(4) Department of Community Medicine, Tromsø, Norway. (5) Samfundet Folkhälsan, Helsinki, Finland*

Objectives: We analyse cervical cancer mortality trends in the 27 member states since 1970. We describe trends in incidence of and mortality from this cancer in the two most affected areas: the Baltic countries (Estonia, Latvia and Lithuania) and Southeast Europe (Bulgaria and Romania).

Methods: Data on number of deaths from uterine cancers and overall female populations from EU member states were extracted from the World Health Organisation mortality database. Incidence data were obtained from the national cancer registries. Three different reallocation rules were applied to correct cervical cancer for inaccuracies in certification of cause of death or incidence of not otherwise specified uterine cancer. Joinpoint regression was used to study annual variation of corrected cervical cancer mortality and incidence. Changes were assessed by calendar period and age group, whereas the evolution by birth cohort was synthesized by computing standardized cohort incidence/mortality ratios.

We distinguished the 15 old from the 12 new member states, which acceded to the EU in 2004 or later. For Finland, France and Romania, age-specific trends by calendar period and the standardised cohort mortality ratios by birth cohort were analysed.

Conclusions: Corrected age-standardised cervical cancer mortality rates have decreased significantly over the past decades in the old member states. Member states in Eastern Europe and also the Baltic states showed mortality rates that decreased at a lower intensity (Czech Republic, Poland), remained constant at a high rate (Estonia, Slovakia) or increased (Bulgaria, Latvia, Lithuania, Romania). The standardised cohort mortality ratio indicated that mortality does not decrease further or even increase among women born after 1940. Joinpoint regression revealed rising trends of incidence (in Lithuania, Bulgaria and Romania) and of mortality (in Latvia, Lithuania, Bulgaria and Romania). In Estonia, rates were rather stable. Women born between 1940 and 1960 were at continuously increasing risk of both incidence of and mortality from cervical cancer.

SURVIVAL AND CURE FROM SCREEN-DETECTED VS SYMPTOMATIC CERVICAL CANCER**Andrae B (1,2), Andersson T (2), Lambert P (2), Kemetli L (2), Silfverdal L (3), Strander B (4), Ryd W4, Dillner J (2), Törnberg S (2) and Sparén P (2)***1 Centre for Research and Development Uppsala University/Gävle, 2 Karolinska Institutet Stockholm, 3 Umeå University and 4 Sahlgrenska Academy Göteborg, Sweden*

Cervical screening reduces the incidence of cervical cancer but also detects invasive disease at early stages. It is not known to what extent this also implies better chances of cure.

Methods: A cohort of all cervical cancer cases in Sweden diagnosed 1999-2001 was followed prospectively until 2008, and the cure fraction, a measure not affected by lead time bias, was estimated for different screening histories, mode of detection, and further stratified by age at cancer diagnosis, histopathological type and FIGO stage.

Results: In the screening ages, the cure proportion for women with screen detected invasive cancer was 92% (95% confidence interval 75% to 98%) and for symptomatic women was 66% (62% to 70%), a statistically significant difference in cure of 26% (16% to 36%). Among symptomatic women, the cure proportion was significantly higher for those who had been screened according to recommendations (interval cancers) than among those overdue for screening: difference in cure 14% (95% confidence interval 6% to 23%). Cure proportions were similar for all histopathological types except small cell carcinomas and were closely related to FIGO stage. A significantly higher cure proportion for screen detected cancers remained after adjustment for stage at diagnosis (difference 15%, 7% to 22%).

Conclusion: Early stage detection of invasive cervical cancer in screening implies improved cure for squamous cancer as well as for adenocarcinoma and was unaffected by lead time or length bias. Few deaths remain to be prevented among regularly screened women. Any further reduction of death in cervical cancer will require excellent organization to cover more under-screened women. Evaluations of screening programmes should consider the assessment of cure proportions.

SS 3-3

HPV TYPES AMONG HIV-POSITIVE WOMEN

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HIV-positive women are at increased risk for HPV infection and progression to cervical intraepithelial neoplasia grade 3 (CIN3), and have higher invasive cervical carcinoma (ICC) incidence. A meta-analysis of HPV prevalence in HIV-positive women worldwide showed a relative underrepresentation of HPV16 and overrepresentation of the other high-risk HPV types in HIV-positive compared with HIV-negative women with or without cervical abnormalities¹. A lower relative prevalence of HPV16 even in high-grade lesions, raised the fear that HPV16/18 vaccination may prevent a smaller proportion of ICC in HIV-positive than HIV-negative women. At that time, information on HPV prevalence in HIV-positive women with ICC was scarce. However, in the past few years, a lot of new data on HPV types in HIV-positive women has been published. In particular, a number of studies in sub-Saharan Africa have described HPV type-specific prevalence among HIV-positive ICC cases². In general, although HPV16 appears slightly under-represented, and HPV18 slightly over-represented, in HIV-positive ICC, the combined prevalence of HPV16 and/or 18 appears similar in HIV-positive and HIV-negative ICC cases. This suggests that current prophylactic HPV vaccines against HPV16 and 18 may prevent similar proportions of ICC regardless of HIV status, provided that vaccine-related protection is sustained after HIV infection. Nevertheless, the evolution of the HIV epidemic (i.e., the increase in the proportion of women who will have acquired HIV infection early in life but survived long time because of combination antiretroviral therapy will provide the ultimate confirmation of the similarity in the importance of different HPV types in the causation of ICC in HIV-positive and HIV-negative women.

1 Clifford GM, Gonçalves MAG, Franceschi S, for the HPV and HIV Study Group. Human papillomavirus types among women infected with HIV: a meta-analysis. *AIDS* 20: 2337-2344 (2006)

2 De Vuyst H, Ndirangu G, Moodley M, Tenet V, Estambale B, Meijer CJLM, Snijders PJF, Clifford G, Franceschi S. Prevalence of human papillomavirus in women with invasive cervical carcinoma by HIV status in Kenya and South Africa. *Int J Cancer* (in press)

SS 3-4

CERVICAL CYTOKINES AND HPV CLEARANCE

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Objectives. While most HPV infections have been shown to clear within a few months or years, the 10-20% of women with a persistent viral infection remain at elevated risk for the development of CIN and cancer. It is thought that HPV persistence requires a tolerogenic local immune environment involving avoidance or repression of both the innate and adaptive immune responses. Prolonged HPV infection presumably elicits the production and release of a variety of cytokines which aid recruitment and coordinate the functions of cells essential to pathogen control, but the role of cytokine-mediated mucosal immune response in the clearance of cervical HPV infection is poorly defined.

Methods. In 2005, we initiated a multiethnic cohort study of women in Hawaii for long-term follow-up to test the hypothesis that the mucosal expression of several candidate antiviral (IFN- α 2), type-1 (IFN- γ , IL-12), regulatory (IL-10) and pro-inflammatory (IL-1 α , IL-1 β , IL-6, IL-8, MIP-1 α , TNF) cytokines is induced with the establishment of HPV infection. In addition to measuring cervical HPV DNA at each 4-month study visit, repeated measures of mucosal cytokines were obtained by multiplex immunoassay. Laboratory analyses included 154 women, ~21 yrs, with incident cervical HPV infection. Participants completed ~7.5 visits and ~2 yrs of follow-up. Levels of candidate cervical cytokines were modeled from pre-infection to viral clearance or censoring through Cox regression. We observed significantly higher local expression of MIP-1 α , TNF, IL-8, IL-10, and IL-12 from baseline in association with reduced clearance times in our cohort.

Conclusions. Our findings are consistent with the hypothesis that following initial exposure to HPV, rapid immune activation and cytokine induction occurs. Sustained elevated levels of several pro-inflammatory and, perhaps, type-1 and regulatory cytokines were indicators of cervical HPV persistence in our study population, underscoring the notion that avoiding, rather than inhibiting, the immune response is key to viral success. Further observation on our cohort may assist in determining whether a prolonged inflammatory response contributes to the progression of HPV-associated lesions.

REDETECTION OF HPV: REINFECTION VS. LATENCY**Moscicki AB***Division of Adolescent Medicine, Department of Pediatrics, University of California San Francisco, 3333 California Street, Suite 245, San Francisco, CA 94118, USA*

In the advent of repeated HPV DNA testing in routine cervical cancer screening, redetection of HPV after clearance is likely to bring up questions regarding its meaning: both clinically and in respect to partner's fidelity. Several studies have shown that in North American and Europe there is a slight rise in prevalence after the age 55 years suggesting aging is associated with reactivation. On the other hand, this age period is a time when many women are divorced or widowed. In a recent study by Trottier et al, redetection of HPV 16 after known clearance was associated with women reporting new sexual partners supporting the likelihood that the second peak found in adult women was reinfection not reactivation. Understanding the clinical outcome associated with redetection is critical for clinical triage. In a population based study, Rodriquez et al showed that the risk for CIN 2/3 in women with redetection was low suggesting these redetections do not reflect reactivation of persistent infections. In addition, detection of HPV DNA does not always reflect established infections. In a group of recent sexually active women, redetection of the same type occurred in 19.5% of women within 1 year. It is plausible that these HPV DNA detections reflect partner's residua from a recent sexual encounter-rapid clearance and redetection would be expected if the relationship continues. In contrast, studies in HIV infected women have shown redetection occurring in women reporting abstinence. The difficulty in interpreting this data is that autoinoculation has been shown to occur from hand to genital area and anus to cervical.

In 1,500 young women, we examined patterns of re-detection of HPV. WE examined two cohorts; Group 1 are women who had HPV 16 DNA detected but showed clearance and Group 2 are those HPV 16 negative at entry but had serologic evidence of HPV 16 infection. Of the women in group 1 who showed clearance of HPV 16 as defined by at least two consecutive negative tests 4.2% acquired HPV 16 by 2 years, 9.1% by 3 years and 18.1% by 8.5 years. Of the 33 women with redetection, 68% cleared by 1 year. Risks for redetection in Group 1 group included having a recent new sex partner or having more than 1 sex partner, having an STI and use of medroxyprogesterone. In Group 2, detection of HPV 16 DNA was similar with 5.8% having HPV 16 DNA detected by year 2 and 15.3% by year 5. Risks for HPV 16 detection were similar as Group 1. In conclusion, these data suggest that the majority of redetections of HPV 16 DNA are not latent but due to new sexual exposure. Only a minority appear to reflect latent reactivation.

COMPARATIVE EPIDEMIOLOGY OF HPV INFECTION IN MEN & WOMEN**Giuliano AR.***Moffitt Cancer Center, Tampa, FL, USA*

Background and Objectives: Until recently most studies of HPV natural history have been conducted among women and at one anatomic site, the cervical epithelium. As male HPV natural history cohort studies mature it is now possible to document the natural history of HPV infections at multiple anatomic sites in men and compare this to what is known regarding HPV natural history in females. The purpose of this presentation is to briefly present a male to female comparison of HPV prevalence, incidence, and duration of infections.

Methods: Using published data from prospective female and male HPV natural history studies genital, anal, and oral HPV prevalence among males and females will be presented. In addition HPV incidence and duration of infection at external genital sites in men and cervix in women will be compared.

Results and Conclusions: Overall male genital HPV prevalence is higher than cervical HPV prevalence, and does not vary with age, as has been observed among women. While there are mixed reports of HPV antibodies to natural infection conferring protection against a subsequent infection with the same type in females, we see no evidence of such protection in males. In females, HPV incidence is highest at younger ages and decreases steadily with advancing age. Among males, we see no difference in rates of genital HPV acquisition by age. While duration of cervical HPV infections is higher in older compared to younger women, duration of external genital infections appears shorter in older compared to younger men. However, duration of external genital HPV 16 infection does not vary with age in men. Anal HPV infection prevalence differs by sexual orientation in men and is different than prevalence observed in females. Anal canal HPV prevalence is lowest in men who have sex with women, higher in women, and highest among men who have sex with men. Oral HPV prevalence is significantly lower than either anal or external genital HPV infections, and is higher in males compared to females. The paucity of natural history studies of anal and oral HPV infections prohibits a male to female comparison. The differences in prevalence, incidence and infection duration when comparing males to females can be attributed to differences by sex (e.g., oral) as well as differences in the epithelial site (e.g., cervix and anal vs. keratinized penile skin) of infection. More research is needed to fully understand the interaction of sex differences and tissue specific viral interactions.

SS 4-2

MALE VACCINATION: IS IT COST-EFFECTIVE AND CAN IT REDUCE INEQUITIES?**Bogaards J^{1,2}, Coupé V¹, Meijer C¹, Berkhof J¹***1 VU University Medical Centre, Amsterdam, The Netherlands**2 National Institute for Public Health and the Environment, Bilthoven, The Netherlands*

Objectives: The question of whether or not HPV16/18 vaccination should be recommended for boys depends on a multitude of factors, including the age-specific incidence and mortality of HPV-related malignancies, the proportion of cases preventable by vaccination, cancer-specific survival rates, utilities of life-years since diagnosis, extent of herd immunity from female vaccination, the cost of vaccination and the medical savings from preventing cancers. Models are an indispensable tool in synthesizing all the available epidemiologic and economic data and can be useful to guide policy decisions. Our aim is to provide a transparent probabilistic framework for estimating the net benefit of male HPV16/18 vaccination that allows for a quantification of decision uncertainty.

Methods: We derived simple formulae for the calculation of the number of quality-adjusted life-years (QALYs) gained by, and the medical savings due to, vaccinating boys against the high-risk HPV types 16 and 18. We included cancers of the penis, anus, oral cavity, oropharynx and larynx. Parameter uncertainty was derived from the published literature. Calculations were done given the cost of the vaccine and immunization coverage among women. We included herd immunity from female vaccination but did not consider the herd immunity from male vaccination.

Conclusions: The cost-effectiveness of male vaccination strongly depends on the vaccine uptake among women. In countries where HPV vaccine uptake among pre-adolescent girls exceeds 50%, vaccinating boys has a small impact on health and is unlikely to be cost-effective. From a budget allocation point of view, male vaccination does not seem a strong candidate for reimbursement when it has to compete with other candidate disease prevention measures.

SS 4-3

CROSS-PROTECTION: HOW DO WE INTERPRET TRIAL DATA, AND HOW IMPORTANT IS IT AT A POPULATION-LEVEL?**Malagon T¹, Van de Velde N^{1,2}, Drolet M¹, Laprise JF¹, Boily MC², Brisson M¹***1. Laval University, Faculty of Medicine, Canada**2. Imperial College, Department of Infectious Diseases, UK*

Objectives: The bivalent and quadrivalent vaccines have demonstrated differential efficacies against non-vaccine oncogenic types, such as HPV-31, 33, 45, 52 and 58. We aim to better understand 1) the factors underlying differences in vaccine cross-protection estimates observed in clinical trials, and 2) how differences in clinical efficacies translate into population-level effectiveness and cost-effectiveness.

Methods: We conducted a systematic review of clinical trials measuring HPV vaccine efficacy (VE) against outcomes associated with non-vaccine HPV types for the quadrivalent and bivalent vaccines, and analyzed factors influencing differences in VE estimates. We used HPV-ADVISE, the first calibrated individual-based transmission-dynamic model of sequential partnership formation/dissolution and natural history of multi-type HPV infection and diseases, to estimate the population-level effectiveness and cost-effectiveness of each vaccine taking into account their respective cross-protection.

Conclusions: Our analysis suggests that VE measured in naive populations produce the least biased cross-protection estimates. VE estimates against persistent infections with non-vaccine types are less subject to bias than VE estimates against CIN lesions with non-vaccine types, as many lesions are co-infected with HPV-16/18. Excluding the cases of CIN2+ co-infected with HPV-16/18 reduces overall cross-protective efficacy by 28-39%. VE estimates against individual types have more external validity than VE estimates against grouped HPV types, which depend on the distribution of these types in the trial population and are less applicable to other populations. The bivalent vaccine generally demonstrates higher efficacy against non-vaccine types than the quadrivalent vaccine across infection and lesion outcomes in naive populations. Based on type- and vaccine-specific VE estimates, our model predicts slightly higher population-level effectiveness for the bivalent than the quadrivalent in the reduction of diagnosed CIN2/3 and SCC cases over 70 years post-vaccination. Conversely, even with a higher bivalent cross-protection, the quadrivalent was estimated to be more cost-effective at equal price due to its efficacy against genital warts.

RE-INFECTION: HOW CAN IT AFFECT THE SUCCESS OF HPV VACCINE PROGRAMS?**Vink A^{1,2}, Bogaards J^{1,2}, Vriend R², de Melker H², Meijer C¹, Berkhof J¹***1 VU University Medical Centre, Amsterdam, The Netherlands**2 National Institute of Public Health and the Environment, Bilthoven, The Netherlands*

Objectives: The impact of a vaccine program is partly determined by the potential to provide herd immunity to non-vaccinated individuals. An assessment of the herd immunity effect requires quantitative knowledge of HPV transmissibility: the lower the transmissibility, the higher the effect.

Estimating transmissibility from pre-vaccine data is complicated because there is a trade-off with natural immunity: a model that allows for re-infection with the same HPV genotype generally yields a lower estimate of transmissibility and predicts a larger benefit from vaccination than a model that assumes long-lasting natural immunity.

Using data from a young and sexually active population, we attempted to estimate sex-specific parameters of transmissibility and immunity and investigated how these affect the impact of HPV vaccine programs.

Methods: We obtained data on type-specific HPV infection from sexual health service clinics in the Netherlands for the years 2009 and 2011. A total of 941 heterosexual men and 2358 heterosexual women aged 16–24 years were tested for the presence of high-risk HPV DNA. We calibrated a deterministic HPV transmission model to match the prevalence of the most common high-risk types. The baseline parameterization (obtained by fitting the model to pre-vaccine data on HPV infection in cervical screening) did not give a satisfactory fit to the prevalence of HPV infection in this younger age group. We established new ranges of sex-specific transmissibility and immunity that were compatible with the data and evaluated their effect on the predicted impact of vaccination.

Conclusions: Re-infection may affect the success of HPV vaccine programs via its relation with transmissibility. Our analysis suggests a different degree of natural immunity and an increased transmissibility in men compared to women. The implications for vaccination are relevant: girls' vaccination may be more effective in reducing overall transmission levels relative to boys' vaccination.

2-DOSE VS. 3-DOSE HPV VACCINATION: CAN A 2-DOSE STRATEGY BE THE BEST USE OF RESOURCES?**Jit M¹, Choi YH, Brisson M²***1 Health Protection Agency, London, United Kingdom**2 Universite Laval*

Objectives: Recent studies suggest that a two-dose HPV vaccination schedule provides a similar level of immunological response and clinical protection in the short-term as a three-dose schedule. However, the long-term protection afforded by such a schedule is unknown. Several health authorities (including Quebec, British Columbia and Mexico) have implemented vaccination programmes in which the third dose of vaccine is delayed by a number of years. Reduced and delayed schedules offer the potential to reduce the cost and logistical challenges of HPV vaccination.

Methods: In the UK and Canada, economic models were used to inform the design of population-wide HPV vaccination programmes. We adapted these models to investigate the effect of a two-dose schedule in the same settings, in terms of reduction of cervical disease and anogenital warts. We explored a range of assumptions about the comparative long-term protection against both vaccine and non-vaccine type disease afforded by two and three-dose schedules.

Conclusion: Our results can be used to inform the potential impact and cost-effectiveness of two-dose schedules when evidence from studies and populations emerges on their long term protection. Such evidence is important for evidence-based decision making about the best use of resources for prevention and control of HPV-related diseases in different settings.

SS 4-7

VACCINE EFFICACY, TRANSIENT INFECTION AND HERD IMMUNITY: IMPLICATIONS FOR THE IMPACT OF VACCINATION

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OBJECTIVES: HPV vaccine clinical trials have been designed to measure efficacy against clinical endpoints, specifically cervical and anogenital lesions, persistent infection and transient infection as indicated by the detection of HPV DNA. They are neither designed to nor capable of measuring efficacy against transmission. Results so far have demonstrated differential efficacy against the three endpoints. In particular, efficacy against transient infection has been shown to be less than for the other two endpoints. This raises some important and related questions. 1) Does transient infection in vaccinated individuals, as indicated by the detection of HPV DNA, represent transmissible infection? 2) Does this provide insight into the mechanism of action of the vaccines and their efficacy in preventing transmission? 3) What are the implications for the potential impact of vaccination at the population level?

METHODS: We have developed mathematical models of HPV transmission to explore the potential population-level impact of vaccination on HPV incidence and prevalence, and to address the questions outlined above. Our work has focused on the quadrivalent vaccine because it is used in the National HPV Immunisation Program in Australia. We have used the models to compare the predicted long-term reductions in HPV incidence and prevalence, and herd immunity benefit to the unvaccinated population, under different assumptions regarding vaccine efficacy against transmission.

CONCLUSIONS: Long-term relative reductions in HPV incidence are predicted to be as much as 30-40% lower if it is assumed that vaccine efficacy against transmission is equivalent to the trial-measured efficacy against DNA detection rather than the higher efficacies measured against persistent infection and disease. These results vary by HPV type (6, 11, 16 or 18) and by whether vaccination is implemented as a female only program or one that covers males and females. The models also predict reductions to be considerably lower for a scenario whereby the vaccine is assumed to have a high efficacy against transmission (~90%) but has little impact on duration of breakthrough infection, than for a scenario whereby the vaccine does not protect against infection but considerably reduces the duration of infection. These results and those of on-going investigations will be presented in detail.

SS 5-1

INCIDENCE AND RISK FACTORS FOR HEAD AND NECK CANCER AND CERVICAL CANCER AMONG HIV-INFECTED INDIVIDUALS IN NORTH AMERICA

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Background: Human papillomavirus (HPV) is an important risk factor for both invasive cervical cancer (ICC) and a subset of head and neck cancer (HNC). Prior evidence suggests HIV infection and low CD4+ T cell count are associated with increased HPV prevalence and persistence. However, there are limited cohort data characterizing the incidence of HPV-related cancers among HIV-infected individuals.

Methods: 13,707 HIV-infected women and 41,352 men who participated in the North American AIDS Cohort Collaboration on Research and Design (NA-ACCORD), from January 1996-December 2010, and were cancer-free at baseline, contributed to one or both cancer-specific analyses. All cases were confirmed through cancer registry match and/or pathology report. Median follow-up was 5.3 and 3.9 years in the ICC and HNC analyses respectively. Incidence rates of each cancer were calculated, and directly age standardized to SEER, a US national cancer surveillance system. Risk factors for HNC and ICC were assessed using age-adjusted Poisson regression models. History of Pap screening and cervical treatment was abstracted from medical records for all ICC cases.

Results: There were 37 incident HNC cases observed in HIV-infected participants in NA-ACCORD, an incidence rate (IR) of 12.6 per 100,000 person-years (pys). After age-standardization, the rates in HIV-infected individuals were higher than in the general US population [SEER] (17.5 vs 10.6 per 100,000 pys, $p=0.2$). Incident ICC was diagnosed in 17 women (IR=26 per 100,000 pys). As with HNC cancer, HIV-infected women had a higher age-standardized ICC IR (16.4 vs. 8.1 per 100,000 pys, $p=0.07$) than the general population. Compared with individuals with baseline CD4 ≥ 350 , those with baseline CD4 < 350 cells/mL had significantly higher HNC IR (30.1 vs. 16.3 per 100,000 pys, $p=0.04$) and ICC IR (22.5 vs. 10.9 per 100,000 pys, $p=0.04$). Inadequate screening (35%), false-negative Pap results (29%) and untreated pre-cancerous lesions (35%) were equal contributors to the observed ICC incidence among these HIV-infected women.

Conclusion: In this large collaboration of North American HIV cohorts, the incidence of both HNC and ICC was elevated among people with HIV compared with the general population. Incidence was significantly associated with lower CD4 at baseline, suggesting immunosuppression may contribute to the increased HNC and ICC risk among HIV-infected individuals.

DIFFERENCES BETWEEN HPV+VE AND HPV-VE HEAD AND NECK CANCERS AT THE MOLECULAR LEVEL**Brakenhoff RH, Nagel CR, de Kemp SR, Buijze M, Snijders PJF, Braakhuis BJM, Leemans CR***Dept. Otolaryngology/Head-Neck Surgery and Pathology, VU University Medical Center, Amsterdam, The Netherlands*

Squamous cell carcinomas in the head and neck (HNSCC) develop in the mucosal linings of the upper respiratory and digestive tracts. HNSCCs are caused by tobacco smoke exposure and alcohol consumption as well as by human papillomavirus infection. HPV+ve and HPV-ve tumors are different entities at the clinical level. HPV+ve tumors are generally of low T-stage but a higher N-stage. Despite the frequent tumor-positive lymph node status, the most important prognostic factor in head and neck cancer, they have a favorable prognosis when compared to HPV-ve tumors.

Previously it was shown that HPV+ve and HPV-ve tumors are also very different at the molecular level, both using genetic analysis and expression profiling. HPV+ve tumors are p53 wild type and show considerably less genetic changes than HPV-ve tumors. The question arises whether these molecular differences are reflected in phenotypic characteristics. Recently we tested a large panel of head and neck cancer cell lines that were either HPV+ve or HPV-ve and evaluated their phenotypic characteristics such as proliferation rate, migratory capacity, cisplatin sensitivity, radiation sensitivity, and EGFR inhibitor-sensitivity. HPV-ve cell lines typically showed a reduced proliferation rate, but not an increased sensitivity to cisplatin, radiation or EGFR-inhibitors. The available data on the molecular characteristics of HPV+ve and HPV-ve tumors will be summarized in this presentation.

ARE WE ENCOUNTERING AN HPV EPIDEMIC OF OSCC – THE STOCKHOLM –DATA**Anders Näsman¹, Per Attner², Lalle Hammarstedt², Juan Du¹, Linda Marklund², David Lindquist¹ Mircea Romanitan¹, Torbjörn Ramqvist¹, Eva Munck-Wikland² and Tina Dalianis¹,***1 Dept. of Oncology Pathology, Karolinska Institutet, Karolinska University Hospital, R8:01,**2 Dept of Oto-Rhino Laryngology, Head and Neck Surgery, Karolinska University Hospital, 171 76 Stockholm, Sweden*

Objective: In 2007, HPV was accepted as a risk factor, in addition to smoking and alcohol, for oropharyngeal squamous cell carcinoma (OSCC) by the IARC. Moreover, during the past decade clinicians in some Western countries were suspecting that the incidence of OSCC was increasing. For this purpose we first followed the incidence of tonsillar and base of tongue cancer over the past decades as well as the proportion of HPV positive tonsillar and base of tongue cancer. Subsequently we also examined for the presence of HPV in oral samples of young men and in oral and genital samples in women attending a youth clinic in the center of Stockholm to obtain a base line for oral and genital HPV before the introduction of public HPV vaccination in Sweden.

Methods: In tonsillar and base of cancer the presence of HPV was analyzed by PCR with HPV general primers GP5+/GP6+, and CPI/GPII and with S14 as a household gene for positivity for PCR amplifiability. In HPV positive samples HPV typing was performed with HPV type specific primers and sequencing. In oral and genital samples from the youth clinic a Luminex based multiplex assay was used for the analysis of 24 mucosal HPV types and betaglobulin used as the control household gene.

Conclusion: From the Swedish Cancer Registry it was possible to identify that between 1970-2009 the incidence of tonsillar and base of tongue cancer had increased in Stockholm and Sweden. Furthermore, when examining for HPV in available pretreatment biopsies from tonsillar cancer (n=301) from patients diagnosed for tonsillar cancer between 1970-2006 in the Stockholm area, we found that the proportion of HPV-positive tonsillar cancer increased from 23% in the 1970s to 93%, 2006-2007 (p<0.0001). Likewise, when examining available pretreatment biopsies from base of tongue cancer (n=95) the proportion of HPV positive cancer increased from 55% to 84% from 1998 to 2007. In oral mouth wash samples (n=483) from a youth clinic almost 10% were positive in both men and women. However in women, where both genital and oral samples were available (n=174), the proportion of HPV positive genital infections was (74%) and in women with a genital HPV infection the proportion of HPV positive oral samples was significantly higher (17%) as compared to those without a genital HPV infection (4%) p<0.043. In summary, the data suggest that we are dealing with an epidemic of HPV induced OSCC and that the proportion of HPV positive genital and oral samples is relatively high at a youth clinic in central Stockholm.

SS 6-1

QUADRIVALENT VACCINE - LONG-TERM FOLLOW-UP STUDIES

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End of study results have confirmed high safety and high immunogenicity of the quadrivalent HPV prophylactic vaccines in 9- to 15-year-old adolescents. Results from a long-term extension study in these adolescents have confirmed high safety and effectiveness. In subjects who have at least one effectiveness follow-up visit after month 42 no HPV 6/11/16/18 persistent infection was detected in the early vaccination group. In the catch-up group vaccinated end of study compared to early vaccination group, the rates of HPV positivity were for HPV 6/11/16/16, 16% compared to 2%, for HPV-6, 9 vs 0.7%, for HPV-11, 6% vs 0.1%, for HPV-16, 11% vs 0.7% and for HPV-18, 6 vs 0.3%. One SAE, a fatal road accident was reported in the context of the long-term follow-up study. The follow-up study finding support a favorable long-term safety profile for the quad HPV vaccine.

End of study results have confirmed high safety and efficacy of the quadrivalent HPV prophylactic vaccines. Extension study of long-term follow-up study in Nordic countries has reported no HPV 16-18 related CIN 2+. Subject new medical history conditions during the long-term follow-up study has reported 2 deaths in the cohort immunized at onset of the trial compared to none for the end of study vaccinated cohort. Surgical and medical procedures were respectively 12.1 vs 11.4% and vascular diseases 0.1 vs 0.3%. The quad HPV vaccine shows a trend of continued protection 6 years in adolescent vaccinated prior to sexual début and up to 7 years in women and continues to be generally safe and well tolerated up to 7 years following vaccination.

Long term extension study, men's study as well as the adolescent studies will be extended to at least 10 years end of study. More results will be presented as they become available.

SS 6-2

EVALUATION OF HPV TYPE COMPETITION IN WOMEN: AN EPIDEMIOLOGICAL APPROACH TO ADDRESS THE POTENTIAL FOR TYPE REPLACEMENT POST-VACCINATION

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Objectives: Two vaccines prevent the HPV genotypes that cause the majority of cervical cancers. It is possible that the distribution of other high-risk types will change following the elimination of these vaccine types, a phenomenon referred to as type replacement (TR). Using HPV DNA typing data, we addressed the potential for TR by evaluation of HPV type competition.

Methods: Data came from the Ludwig-McGill cohort study (n=2462), HPV Infection and Transmission among Couples through Heterosexual activity cohort study (n=1038), McGill-Concordia cohort study (n=636), Biomarkers of Cervical Cancer Risk case-control study (n=1687), and the Canadian Cervical Cancer Screening Trial (n=10154). PGMY or MY09/11 PCR protocols with linear array or line blot assays allowed genotyping of 37 genital HPV types. For each HPV pair combination we calculated the expected frequency (E) of coinfection assuming infections occur independently, and compared this with the observed frequency (O). We also constructed hierarchical logistic regression models for each HPV vaccine type by including other HPV types and relevant confounders as covariates. Kaplan-Meier curves and Cox proportional hazard models were used to evaluate sequential acquisition/clearance of HPV types according to presence/absence of vaccine-preventable types.

Conclusions: Multiple-type coinfection was common in all studies (range=26-65%). The O/E and odds ratio for most pair combinations was above one and not statistically significant, suggesting no interaction between HPV type-specific infections. For HPV 16, only type 52 was flagged as a candidate for replacement (O/E and odds ratio < 1.0). Survival analysis provided similar inference. HPV types generally do not seem to compete during natural infection. Our analyses provide some reassurance that TR is not expected post-vaccination.

MAIN CHALLENGES IN TRYING TO COMPARE BOTH VACCINES**Paolo Bonanni***Department of Public Health – University of Florence, Italy*

The two prophylactic anti-HPV vaccines are among the most effective vaccines available today. Their ability to prevent nearly 100% of pre-cancerous lesions of the cervix due to vaccine serotypes make them almost invariably protective in the pre-adolescent female population.

However, they were developed starting from non-overlapping aims (prevention of both malignant and benign genital lesions for the quadrivalent, prevention of pre-cancerous lesions only for the bivalent vaccine), their composition is different both for the VLP sequence and for the adjuvant used, and also different tests were utilized to verify the antibody response to immunization.

Direct comparison between the two vaccines is therefore very hard to perform. A study based on a head-to-head detection of antibody titres achieved after a full course of immunization using the pseudovirion-based neutralization assay showed a significantly higher titre for the bivalent compared to the quadrivalent vaccine for both HPV 16 and 18. However, although the importance of antibodies is clear in the prevention of HPV infection, the clinical relevance of different antibody levels is still debated. As a matter of fact, no correlate of protection has been identified to date, and the exact mechanism by which vaccines protect from acquiring persistent infection has yet to be exactly elucidated. Only studies on the possibility to obtain an anamnestic response at the mucosal level after natural infection, together with follow-up of immunized women whose antibody titres reverted to those detected after natural infection will hopefully clarify the importance of antibody titres for long-term protection. Also the full understanding of cross protection and related mechanisms is needed in order to evaluate the overall impact of the two available vaccines.

HPV VACCINATION: UNANSWERED QUESTIONS FROM THE AVAILABLE CLINICAL STUDIES**Eduardo Franco***(McGill University, Montreal, Canada)*

Phase II and phase III randomized controlled trials by pharmaceutical companies have provided the essential answers concerning efficacy and safety of the available HPV vaccines. Results from these trials have been used for regulatory purposes in all countries in which the vaccines have been licensed. However, several questions remain that could be answered via post-hoc analyses of the existing trial data or via epidemiologic surveillance in countries that have adopted HPV vaccination. Perhaps the most important question, which would have an impact of the cost of vaccination programs, is whether the efficacy against clinically relevant endpoints is maintained when the course of vaccination is incomplete or when the regimens are not followed? Evidence has begun to emerge that the protective effect from an immunological standpoint may be adequate but more evidence is required with respect to lesion endpoints. Is there any effect on serological titers by natural exposure to HPV? In other words, among women with new partners acquired after one year post vaccination is there an increase in titers (via anamnestic response) relative to those who remained monogamous or did not acquire new partners? There are variations in this question that could take into account age-related differences as well. Does vaccination protect against infection by target HPV types in sites other than the cervix and anus? Could the companies entertain substudies that would sample the oral cavity of the trials' original subjects to look for differences in rates between vaccinated and placebo recipients? Among those enrolled who had HPV 16/18 and/or associated lesions at enrolment (and were thus removed from the ATP or PP cohorts) is there any vaccine protection against the vaccine-target HPVs in other anatomical sites, such as vagina and vulva? We know that the vaccine will not clear existing infections but will it prevent infection in adjacent or distant mucosal sites? Another important question is whether correlates of protection exist. We know that there are very few outcomes defined as persistent infections or lesions among the vaccinated even on long term follow-up. What insights could be gained by relaxing the definition of outcome to include incident infections? The number of outcomes would increase and possibly permit an analysis of the effect of titers post-vaccination on the incidence of vaccine-target types, thus potentially revealing a threshold for a correlate of protection. An additional question of great public health interest is the potential for type-replacement. Would it be possible to use the existing trial data to assess whether there is any hint of HPV type replacement post-vaccination via a negative VE effect for some of the HPV types that are phylogenetically related to the vaccine types? What are the methodological caveats in such an analysis? These and additional questions will be discussed during the presentation.

SS 6-5

EPIDEMIOLOGY AND DISEASE BURDEN CONSIDERATIONS IN FUTURE HEALTH TECHNOLOGY ASSESSMENTS FOR HPV VACCINATION IN EUROPE

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Objectives: Health technology assessments (HTAs) for human papillomavirus (HPV) vaccination have been conducted by health authorities or HTA agencies in most western European countries and played an important role in the decision-making for implementation of HPV vaccination in girls. Our research aims to point out lessons learnt from previous HTAs for HPV vaccination and to suggest general best practices for future HTAs of HPV vaccination.

Methods: A comprehensive research was conducted into the current HTA landscape in Western Europe. A multidisciplinary and pan European group of experts has been established to discuss the development of best practices to optimise future HTAs for HPV vaccination.

Conclusions: The research shows that different approaches and methods have been used in previous HTAs for HPV vaccination and their conclusions influenced differently the decision-making.

Expert consensus was reached that such HTAs should follow a comprehensive approach, including all key HTA domains (epidemiology and disease burden, medical strategy, vaccine efficacy and safety, health economics, ethical/legal/social implications, advice for implementation, monitoring). All evidence available when HTA is conducted should be integrated, taking into account part of existing uncertainty mainly regarding trend in incidence/prevalence of HPV-infection and related diseases, vaccine duration of protection, relative effectiveness, population impact, vaccination coverage. Natural history, epidemiology and burden of all HPV-related diseases in both sexes, as well as distribution in high-risk populations, should be assessed in HTAs, using reliable country-specific data. An important issue to improve future HTA for HPV vaccination is to take into account epidemiological trends and projections for all HPV diseases, stratified by gender and age group, and also changes in sexual behavior. An accurate methodology based on modeling and existing epidemiological predictors should be used to estimate the future HPV burden. Trend and severity of the burden of all HPV-related diseases may be major drivers in future policy decisions, as well as associated unmet medical and public health needs. Changes in epidemiological knowledge should be taken into account in future HTAs in order to help the policy makers addressing new public health issues arising since latest HTA

SS 7-1

HPV, SKIN & SEX

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Human papillomavirus (HPV)-associated pathology represents one of the significant problems in Dermatovenereological clinical practice. The HPV "specificity" among sexually transmitted infections (STIs) consists of the high recurrence rate, difficult eradication and oncogenic potential. Besides, the young, sexually active population in the generative period is mostly affected which gives the special burden to the societies which tend to be modern and highly developed. Very careful and friendly-orientated manner of taking the medical history and clinical examination is rather important in order to obtain the exact data. Otherwise, we might "miss the point". Most of the epidemiological studies regarding the sexual debut, for example, take only the age of the first "full sexual intercourse" into the consideration, whereas the mutual physical touching of the genitalia only (without any further activity in this context), very often being practised much earlier than the full intercourse, might be much more relevant in the terms of the HPV transmission. The clinical variations might range from clinically invisible or poorly visible, "asymptomatic" lesions to the bizarre forms of giant condyloma of Buschke-Löwenstein type. Thus, it is sometimes necessary to identify the HPV DNA type in the clinically associated lesions. According to our results, HPV 16 and 18 have been isolated from "benign" HPV-associated genital lesions in 20% of patients, i.e. more than it is usually expected, so not all "benign" clinical forms of the HPV-infection are necessarily always benign. Therefore, the diagnostic approach to HPV genital infections needs to be complex including HPV DNA typing whenever it seems appropriate. Ultimately, within the spectrum of therapeutic options for condylomata, no method is really superior to others; recurrences occurred in 30-70% of cases. Since HPV is mostly sexually transmitted, managing both partners is necessary in order to eliminate the virus in the population. Approaches to this include prophylactic vaccines for both men and women. This should be the only way to significantly decrease the numbers of infected persons. Besides, a proper dermatological training is required as the clinical criterion is still very important and the HPV-induced lesions get quite often misdiagnosed unless managed by the skilled professional.

NEW APPROACHES TO THE TREATMENT OF AIN**Palefsky J***University of California, San Francisco
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High-grade anal intraepithelial neoplasia (HGAIN) is the likely precursor to anal cancer. Just as high-grade CIN is treated to reduce the risk of cervical cancer, treatment of HGAIN may reduce the risk of anal cancer. Treatment of HGAIN is challenging for several reasons: 1) The lesions may be extensive in size in number; 2) Parts of lesions may be obscured because of anal anatomy, e.g; sitting in folds or at the base of crypts, and directing therapy to remove the entire lesion may be difficult; 3) many individuals at highest risk for HGAIN are also immunocompromised and therapeutic results are often less in this population; and 4) it is difficult to remove large portions of affect anal tissue without causing unacceptable morbidity. Several studies have been done to document the clearance rates of HGAIN using different methods. Most have not been randomized, placebo-controlled trials, and many have small patient numbers. Overall, these studies show that many methods, either ablative or topical therapies, can clear a high proportion of HGAIN lesions. However, care must be taken to choose the best method, depending on several factors such as HIV status, size, number and location of lesions. Many studies also document a high recurrence rate or high rate of metachronous disease, highlighting the need for close follow-up and frequent need for re-treatment using the same method or switching to an alternative method.

Treatment of HGAIN is ablative including infrared coagulation or electrocautery, or topical including treatments such as imiquimod, 5-fluorouracil or trichloroacetic acid. Given the high frequency of multifocal lesions and large lesion size in many patients, newer therapies that can treat large areas of anal mucosa while minimizing morbidity are needed. These may include therapeutic vaccination against HPV, photodynamic therapy and topical cidofovir. Novel agents that interfere with molecular pathways thought to be important in the pathogenesis of anal cancer are under consideration, and the quadrivalent vaccine is being explored as a method to reduce lesion recurrence post-therapy. Importantly, studies are needed to document the efficacy of treating HGAIN to reduce the incidence of anal cancer.

PENILE HPV INFECTION & DISEASE IN HIV-INFECTED MEN**Lacey, C.***Centre for Immunology & Infection, Hull York Medical School, University of York, UK*

Although clinicians frequently see penile HPV disease in HIV infected males, there is not a wealth of published literature. Prevalent and incident asymptomatic penile high risk (HR) HPV infections and multiple penile HPV infections are more frequent in HIV infected versus un-infected men. No studies were located which address the incidence & prevalence of penile warts in HIV infection.

Penile warts & HPV disease in HIV infected men more frequently display dysplasia / PIN 2/3 than in HIV negative men. Imiquimod 5% cream has been shown to be effective for treatment of penile warts in HIV infection, although response rates are lower than in HIV negative men. Relapse after treatment / clearance of penile warts is more frequent in HIV infection. Relapse is less frequent in those on anti-retroviral therapy (ART) compared to those not on ART.

Rates of penile cancer are elevated in HIV infected men, but those risks do not appear to vary by CD4 count. Circumcision in HIV positive men reduces the prevalence and incidence of multiple HR HPV infections

SS 7-5

HPV AT NON CERVICAL SITES IN WOMEN

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It is clear that human papilloma virus is causal in cervical cancer and we have seen the introduction of safe and effective HPV vaccination programmes in countries around the world. Over the last decades we have also seen an increase in knowledge about HPV and its effects at sites other than the cervix.

There are assumptions about transmission of the HPV to these other sites such as the anus and the oro-pharynx. I shall present data on the presence of HPV at sites other than the cervix and review the literature on its significance. The value of the vaccination cannot be underestimated if the presence of HPV at these other sites carries any risk for future carcinogenesis.

SS 7-6

ECONOMIC BURDEN OF NON-CERVICAL HUMAN PAPILLOMAVIRUS-RELATED CANCERS IN WESTERN EUROPE:
A SCOPING REVIEWMoro L¹, Largeron N¹*1 Health Economics Department, Sanofi Pasteur MSD, Lyon, France*

OBJECTIVES: Human papillomavirus (HPV) is a common sexually transmitted infection. Historically, cervical cancer was the first disease recognised as a consequence of HPV. However, the burden of HPV-related diseases extends beyond cervical cancer such as cancers in the anogenital region (i.e., vulva, vagina, penis, and anus) and head & neck (H&N) area (i.e., oral cavity, oropharynx, and larynx) in women and men. The main objectives of this review were (1) to synthesize the best available cost evidence on non-cervical HPV-related cancers; (2) to estimate the associated societal economic burden and/or lifetime cost per case; and (3) to identify and highlight methodological challenges to be considered for further research.

METHODS: A literature search (1999-2011) was conducted using PubMed database. All articles addressing the economics of non-cervical HPV-related cancers in Western Europe were selected. Articles that met selection criteria were reviewed, and systematic evaluation was performed considering characteristics of the study (country, cancer type, population group, objective, study design, results, and data sources), type of economic evaluation (e.g., cost-of-illness study, cost-effectiveness analysis), analytical method (primary and/or secondary researches), and cost analysis (societal and/or per patient economic burden).

CONCLUSIONS: 17 studies from 6 countries were identified. Literature search showed a paucity of available studies regarding non-cervical anogenital cancers (1 or 2 references per anatomical site) whereas H&N cancers were relatively better documented (9 references). From disease perspective, the economic burden of non-cervical HPV-related cancers is substantial, representing the main part of the overall economic burden of HPV-related cancers (from 50% to 70%). The economic burden of non-cervical HPV-related cancers is mainly driven by men (around 70%). H&N and anal cancers reported the highest cost per patient as compared to other non-cervical genital cancers. From methodological perspective, a range of cost approaches was applied for measuring resource consumption, from macro-costing to micro-costing. Further research priorities should focus on indirect costs as well as time horizon which should be long enough to capture the relevant period of time for cost estimation.

USE OF HPV PROPHYLACTIC VACCINES IN INFECTED PERSONS

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HPV prophylactic vaccines have been evaluated in patients with infections or lesions in the FUTURE 1 and 2 studies. In patients that were seropositive and HPV positive to the respective type pre-vaccination prior to immunization, there were higher rates of HPV16/18-related CIN2-3/AIS. These data suggest that the observed higher incidence of CIN2-3/AIS in the quad HPV vaccine arm compared to the Placebo arm of this non-randomized subpopulation of protocol 013 was likely related to differences in the subjects' baseline characteristics. Ultimately, population-based surveillance of vaccinated individuals beyond these clinical trials will be required to further address questions of the impact of vaccination in women exposed to vaccine HPV types prior to vaccination.

Seropositive and DNA negative subjects were followed for an average of 44 months. Seven subjects in the placebo group developed cervical disease, and eight subjects developed external genital disease related to a vaccine HPV type they had previously encountered, mounted a serological response yet cleared type specific DNA. No subject receiving HPV 6/11/16/18 vaccine developed disease to a vaccine HPV type to which they were seropositive and DNA negative at enrolment.

Protection against non-exposed vaccine types has been shown to be as good as for patients not exposed to any vaccine types.

For women who developed vulvar lesions such as genital warts, protection against recurrent vulvar disease and cervical definite therapy was shown for vaccine types as well as for all types. For women with cervical lesions that needed definite therapy, protection against recurrent vulvar disease and cervical definite therapy was shown for vaccine types as well as for all types.

Having genital warts is associated with a heightened risk for HPV related cancers and having had pre cancer or cancer is associated with an heightened risk for a second pre cancer or cancer. Patients who have develop HPV related infections and pre cancerous and cancerous lesions may benefit from receiving vaccines.

HPV VACCINATION WITH CERVARIX IN ADULT WOMEN

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Objectives: Four-year follow-up results are available from the Human PapillomaVirus: Vaccine Immunogenicity and Efficacy study in women 26 years or older (VIVIANE; NCT00294047), a double-blind, randomised, placebo-controlled, multinational efficacy study.

Methods: 5,752 women were randomised (1:1) to receive 3 doses of HPV-16/18 vaccine (n=2881) or Al(OH)₃ control (n=2871) at Months 0, 1, 6. Efficacy analyses were performed in the according-to-protocol cohort (ATP; 3 doses per protocol; vaccine, n=2264; control, n=2241) and in the total vaccinated cohort (TVC; ≥ 1 dose), including 15% of subjects with history of HPV disease/infection. Safety was assessed in TVC.

Results: Vaccine efficacy (VE) against HPV-16/18 cervical intraepithelial neoplasia grade 1 or greater (CIN1+) and/or 6-month persistent infection (PI) was 81.1% (97.7% CI: 52.1; 94.0) in ATP and 43.9% (23.9; 59.0) in TVC. In the same cohorts, VE against HPV-16/18 6-month PI was 82.9% (53.8; 95.1) and 47.0% (25.4; 62.7); VE against HPV-16/18 CIN1+ was 86.1% (-35.4; 99.9) in HPV-16/18 seronegative women and 37.8% (-3.2; 63.1), respectively and was 91.1% (25.4; 99.9), irrespective of serostatus in ATP. VE against 6-month PI associated with any oncogenic HPV types was 23.8% (3.4; 40.0) in ATP and 16.0% (2.4; 27.7) in TVC. Corresponding VE for HPV-31/45 was 77.6% (45.4; 92.3) and 43.6% (16.7; 62.2). VE was shown against HPV-16/18 atypical squamous cells of undetermined significance or greater (ATP: 93.7% [71.5; 99.5]; TVC: 57.2% [32.9; 73.3]). Incidences of adverse events, serious adverse events and medically significant conditions were generally similar between groups.

Conclusions: The HPV-16/18 AS04-adjuvanted vaccine showed high efficacy against HPV-16/18 CIN1+ and/or 6-month PI, evidence of cross-protection and a clinically acceptable safety profile, in women aged ≥26 years.

SS 8-3

NO ADVERSE SIGNALS OBSERVED AFTER EXPOSURE TO HUMAN PAPILLOMAVIRUS TYPE 6/11/16/18 VACCINE DURING PREGNANCY: 5 YEAR PREGNANCY REGISTRY DATA

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Objective: To describe the safety profile of pregnancy exposures to the quadrivalent HPV type 6/11/16/18 vaccine (qHPV vaccine) by acquiring and analyzing five years of pregnancy registry data on pregnancy outcomes (live births, abortions, fetal deaths, and congenital anomalies).

Methods: To collect further data regarding the safety profile of the vaccine, Merck and Co., Inc. established the Pregnancy Registry for qHPV vaccine. Enrollment criteria include an identifiable patient and health care provider from the US, France, or Canada and exposure within 1 month before the onset date of the last menstrual period or at any time during pregnancy. Outcomes of interest were pregnancy outcomes and birth defects. Prospectively reported cases (exposures reported before the outcome of the pregnancy was known) were used for rate calculations.

Conclusion: For prospective reports, rates of spontaneous abortions and major birth defects were not greater than the unexposed population rates. For the 1591 prospective reports with known outcome, 1383 (87%) were live births (1391 infants). Of 1391 neonates, 1314 (94%) were born without birth defects; 1359 (97.6%) without major birth defects. The prevalence of major birth defects was 2.4 per 100 live born neonates (95% CI 1.7– 3.3) as compared to the background rate of 2.7 per 100 live born neonates (as per Metropolitan Atlanta Congenital Defects Program [MACDP]). The rate of spontaneous abortion was 6.4 per 100 outcomes (95% confidence interval [CI] 5.2 –7.8). There were seven fetal deaths for a rate of 1.5 per 100 outcomes (95% CI 0.60 –3.09) not clustered around a specific abnormality. Although no adverse signals have been identified to date and despite the robustness of the Registry data, the qHPV vaccine is not recommended for use in pregnant women.

SS 8-4

HOW DO WE BEST MEASURE HPV VACCINE EFFECTIVENESS?

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Objectives and Methods: Utilising Australia as an example of where a population based HPV vaccination program in young school-aged girls has been well established since April 2007 and resulting in continued high population coverage to illustrate a multi faceted approach to measuring the impact of vaccination in a real world situation. Some of the parameters monitored as outcomes of an effective program include: pre and post vaccination era new genital wart diagnoses, recurrent respiratory papillomatosis incidence, type-specific high grade cervical dysplasia, as well as type specific HPV prevalence especially vaccine-type HPV carriage in a representative cohort of young women who were offered vaccination and comparing to that described in the pre-vaccination era. Other aspects of relevance include monitoring of vaccine coverage, vaccine safety, as well as knowledge, attitudes and beliefs about HPV, HPV vaccination, as well as compliance with cervical cytology screening.

Conclusion: Where vaccine coverage for three doses in the young female population has resulted in coverage of around 73%¹ and with a catch up to 26 years of age in the first two years of the program of at least 40%, already there is a reduction in diagnoses of genital warts (around 73% of vaccine eligible age women (for those less than 27 years in 2007) and falling, with a herd immunity effect in similar aged males of 44%)², histologically confirmed high grade lesions in young women (<18years),³ and now a marked reduction in vaccine-related HPV carriage amongst vaccine eligible age⁴.

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2. Donovan B. et al *Lancet Infect Dis* 2011 Jan;11(1):39-44
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48-MONTH EFFICACY OF THE HPV-16/18 AS04-ADJUVANTED VACCINE IN YOUNG JAPANESE WOMEN

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Objectives: We present results from a long-term extension study of the human papillomavirus (HPV)-16/18 AS04-adjuvanted vaccine (GlaxoSmithKline Biologicals) in healthy Japanese women aged 20–25 years (NCT00929526).

Methods: In the primary study (NCT00316693), women aged 20–25 years were randomised (1:1) to HPV-16/18 vaccine or control at Month (M) 0, 1 and 6. Women receiving ≥ 1 dose were invited for follow-up to M48. Vaccine efficacy (VE) against 12-month persistent infection (12MPI) and histopathologically-confirmed cervical intraepithelial neoplasia (CIN)1+ and CIN2+ associated with HPV-16/18 and high risk HPV types (HR-HPV, HPV-16/18/31/33/35/39/45/51/52/56/58/59/66/68) was evaluated in the according-to-protocol cohort for efficacy (ATP-E: 3 doses; HPV DNA-negative at M0 and M6 for corresponding HPV type; normal/low-grade cytology at M0). VE against CIN irrespective of HPV types in the lesions was evaluated in the total vaccinated cohort (TVC, ≥ 1 dose) and TVC-naïve (≥ 1 dose; seronegative for HPV-16/18; HPV DNA-negative for HR-HPV at M0; normal cytology at M0).

Conclusions: The table shows VE against HPV-16/18 and HR-HPV associated 12MPI and CIN lesions in the ATP-E. VE against CIN1+ and CIN2+ irrespective of HPV type was 56.7% [32.8–72.6] and 54.9% [20.5–75.3] in the TVC of both studies combined, respectively, and 61.0% [11.8–84.2] and 73.9% [1.1–95.3] in the TVC-naïve of both studies combined, respectively.

| | ATP-E extension | | | ATP-E primary/extension studies combined | | | |
|---|-----------------|---------|-------------------|--|---------|-------------------|---|
| | n/N | | VE [95%CI] | n/N | | VE [95%CI] | |
| | Vaccine | Control | | Vaccine | Control | | VE [95%CI] |
| HPV-16/18 associated clinical endpoints* | | | | | | | |
| 12MPI | 0/241 | 9/257 | 100% (54.3–100) | 0/382 | 16/383 | 100% (74.8–100) | n/N=no. cases/no. subjects; |
| CIN1+ | 0/332 | 5/335 | 100% (-3.7–100) | 0/406 | 8/404 | 100% (42.2–100) | |
| CIN2+ | 0/332 | 4/335 | 100% (-44.0–100) | 0/406 | 5/404 | 100% (-8.0–100) | |
| HR-HPV associated clinical endpoints | | | | | | | |
| 12MPI | 19/280 | 31/261 | 46.1% (1.6–71.3) | 34/419 | 53/413 | 39.4% (5.0–61.8) | *for subjects baseline seronegative for HPV-16/18 |
| CIN1+ | 8/363 | 22/359 | 66.4% (21.6–81.7) | 13/444 | 34/435 | 63.4% (28.8–82.3) | |
| CIN2+ | 2/363 | 11/359 | 83.0% (22.1–98.2) | 4/444 | 17/435 | 77.3% (30.4–94.4) | |

The HPV-16/18 AS04-adjuvanted vaccine showed high VE against persistent infection and cervical lesions associated with vaccine types and also overall protection against cervical lesions irrespective of HPV types in the lesion in healthy young Japanese women.

GARDASIL® AND AUTOIMMUNE DISORDERS: SAFETY ASSESSMENT USING THE PGRX INFORMATION SYSTEM

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Objectives: This study investigated whether the human papillomavirus vaccine Gardasil® is associated with a modified risk of autoimmune disorders (ADs). It used the PGRx system to monitor specialist centers for changes in the occurrence of ADs and to carry out a case-control study, within the population targeted for Gardasil® vaccination.

Methods: The following ADs were studied: central demyelination, Guillain-Barré syndrome, connective tissue disorders (lupus, rheumatoid arthritis, myositis, dermatomyositis, undifferentiated), type 1 diabetes, autoimmune thyroid disorders (Grave-Basedow, Hashimoto thyroiditis) and immune thrombocytopenia. A network of specialist centers (internal medicine, neurology, rheumatology, paediatrics, endocrinology, dermatology) throughout France recruited incident cases in females 14 - 26 years-old. Cases were diagnosed according to standardized criteria. Controls were recruited from general practice settings and matched to cases on date of recruitment, sex, age and region of residence. Vaccinations and other potential risk factors for ADs were assessed in a standardized telephone interview of patients or their parents. The interviewer was blind to case/control status. 97.4% of reported Gardasil® vaccinations were confirmed using medical records from the patient or her general practitioner.

Conclusions: Between December 2007 and April 2011, 113 specialist centers recruited 248 definite cases of ADs which were matched to 1001 controls. The proportion of vaccinated cases and controls with Gardasil® was 10.5% and 23.2% respectively, within predefined time windows up to 24 months before the index date (odds ratio adjusted for potential confounders 0.72, 95% confidence interval 0.45, 1.18). We also found no evidence of an increase in AD with vaccination when the ADs were considered separately, although these analyses were based on small numbers of cases. Similar conclusions were drawn from analyses investigating various time windows before the index date, the inclusion of uncertain exposures to Gardasil® and possible cases. No significant increase in incidence was observed for any of the ADs in the population eligible for Gardasil® vaccination. This was the largest clinical pharmacoepidemiology study of incident ADs to be conducted in young females. We found no evidence that Gardasil® vaccination was associated with an increase in the incidence of ADs.

SS 9-1

ANTIVIRAL AGENTS AGAINST HPV: RECENT ADVANCES**Fisher C.***NanoVir, 4717 Campus Dr., Kalamazoo, MI 49008, U.S.A.*

Infections by human papillomaviruses (HPVs) are common and associated with the development of benign warts or malignant lesions of the skin and mucosa. Infection by a high-risk HPV type is the starting point of virtually all cases of cervical cancers, the majority of anal cancers and a subset of head-and-neck cancers. Although prophylactic vaccines are now available to protect against the four most prevalent types found in anogenital cancers (HPV16 and HPV18) and anogenital warts (HPV6 and HPV11), these neither protect against all genital HPVs nor are of therapeutic value to already infected patients. Thus, the need for antiviral agents to treat HPV-associated diseases remains great, but none currently exist. Furthermore, the small HPV genome encodes few clear antiviral targets making the challenge of developing an effective treatment all the more pronounced. Objective: in this presentation recent progress made towards the development of antiviral drugs to treat HPV infections will be reviewed. Method: recent findings on the identification of lead compounds that antagonize key viral proteins and processes have been gathered from the scientific and patent literature. Conclusion: potential antiviral agents that target key pathways and protein-protein interactions have been reported. These and other potential antiviral agents that target viral DNA or relevant cell pathways usurped by HPVs will be presented..

SS 9-2

LOCAL IMMUNE RESPONSE IN HPV INDUCED CANCER**Sjoerd H. van der Burg***Department of Clinical Oncology, Leiden University Medical Center, Leiden, the Netherlands*

Objectives: To analyze the local immune response to HPV in HPV-induced cancers.

Methods: We analyzed the total immune infiltrate in situ as well as the presence of cervix-infiltrating and tumor draining lymph node (TDLN) resident HPV-specific T-cells in a large group of patients and studied their interactions in vitro.

Conclusions: We detected the presence of HPVE6/E7-specific T-cells in at least 40% of these patients. A systematic analyses of the proportion, breadth, and polarization of HPVE6/E7-specific T-cells within the total population of TIL and TDLN-cells revealed that the relative contribution of HPVE6/E7-specific CD4+ and CD8+ T-cells varied between <1% and 66% of all T-cells and that these HPV-specific responses were surprisingly broad, aimed at multiple E6 and E7 epitopes and involved multiple T-cell receptor families. However, in most patients either the number of IFN γ -producing T-cells or the amount of IFN γ produced was low, suggesting that these T-cells were rendered functionally inactive within the tumor environment. In depth study of the function of these tumor-associated T-cells revealed in addition to Th1 cells, the presence of HPVE6/E7-specific regulatory T-cells. In a prospective study we showed that if HPV-immunity is strong and more in favor of a Th1/CTL response, this is associated with an improved prognosis. To gain more insight in the different roles HPV-specific Th1 cells may play locally we investigated their interaction with tumor-promoting M2-macrophages, as our in situ analyses of cervical cancers showed that they are infiltrated by different types of macrophages. Interestingly, cognate interaction with Th1 cells switched M2-macrophages to classical pro-inflammatory activated M1 macrophages in an IFN γ -dependent fashion. Overall, our data argues that it is relevant to boost HPV-specific CD4+ Th1-immunity.

INVESTIGATION OF THE ACTIVITY OF THE HIV PROTEASE LOPINAVIR AGAINST HPV

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Objectives: To define the activity and mode of action of the HIV protease inhibitor lopinavir against HPV16 immortalised and transformed cells.

Methods: Colorimetric cell growth assays, antibody microarrays, siRNA silencing, RTPCR western blotting and co-immunoprecipitation were used in this investigation.

Conclusions: Lopinavir stabilises the expression of p53 and ribonuclease L (RNAse L) in HPV16 expressing tumour cells and shows selective toxicity against keratinocytes immortalised with HPV16 E6/E7. Moreover the toxicity of lopinavir against HPV16 expressing cells was shown to be dependent on expression of the RNase L protein. It is significant that these effects were produced at a tenfold higher dose of lopinavir than is achieved in the cervical vaginal fluid by standard oral dosing with this drug as part of a HAART regimen for HIV therapy. Thus we propose that local application of this compound to the cervix may provide the means to achieve a therapeutic effect against HPV related cervical lesions.

CIDOFOVIR/IMIQUIMOD FOR THE TREATMENT OF VIN

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Background: Historically Vulval Intraepithelial Neoplasia (VIN) has been managed with surgery. However, this is often associated with significant physical and psychosocial morbidity, particularly in extensive disease and a high risk of recurrence. Several small studies have evaluated medical treatments and small, separate trials of two topical treatments (cidofovir and imiquimod) showed similar promising results, with complete response rates in the order of 40%. In order to assess these treatments in the same setting, a phase 2 randomised controlled trial was designed.

Objectives: The aim of the trial is not to make a direct comparison between arms but to assess each arm for complete response rate by 30 weeks, feasibility of use and toxicity. A Fleming's single stage design was assigned to each of the treatment arms and assuming a dropout rate of 15%, a total of 204 patients were required.

The primary outcome measure is complete response. Secondary outcome measures are: toxicities, treatment compliance, HPV presence, type and integration status, and recurrence rate at two years.

Methods: Women with biopsy proven VIN 3 are randomised to have topical treatment with either imiquimod or cidofovir. Treatment is self applied 3 times per week for a maximum of 24 weeks. Women are reviewed every 6 weeks to assess the disease and the need for further treatment. Repeat biopsies for histology and HPV testing are taken 6 weeks post treatment. Women with complete response are followed up for a further 2 years.

Results: Recruitment is ongoing. There are currently 32 open centres throughout the UK and 140 patients have been recruited. The study is part of the UK National Cancer Research Network portfolio, sponsored by Cancer Research UK and run by the Wales Cancer Trials Unit.

Conclusion: Alternatives to surgery need assessment. Randomised trials in uncommon conditions require multicentre studies that are challenging to set up, but can be achieved.

SS 9-6

NEW APPROACHES FOR SECOND GENERATION HPV VACCINES: CHIMERIC P16^{INK4a}-HPV16 L1 PARTICLES

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Objectives: In HPV-transformed precursor lesions and invasive carcinomas the cellular tumor suppressor p16^{INK4a} is strongly overexpressed, whereas in normal tissues barely any p16^{INK4a} expression is detectable. Therefore, p16^{INK4a} is considered to be an interesting target for immunotherapy in patients with HPV-associated cancers. We designed chimeric particles consisting of HPV16 L1 and p16^{INK4a} as a vaccine candidate with combined prophylactic and therapeutic properties.

Methods: Two constructs were generated and compared. The complete p16^{INK4a} encoding cDNA sequence was cloned a) upstream and b) downstream of the HPV16 L1 sequence into the pGEX4T² expression vector. The GST-fusion proteins were expressed in *E. coli*, purified using GSTrap glutathione columns and cleaved from the GST-tag with thrombin. Further purification was achieved by size exclusion chromatography. Expression was evaluated by Coomassie staining and Western blot analysis and particle assembly by electron microscopy.

Conclusions: Chimeric constructs of HPV16L1 and p16^{INK4a} can be expressed in and purified from *E. coli*. The protein is capable of forming stable virus capsomeres. These particles will be further evaluated for their capacity to induce neutralizing antibodies and p16^{INK4a}-specific cytotoxic T cells.

SS 10-1

P16^{INK4a} IMMUNOCYTOCHEMISTRY VERSUS HPV TESTING FOR TRIAGE OF WOMEN WITH ASC-US OR LSIL CERVICAL CYTOLOGY: A SYSTEMATIC REVIEW AND META-ANALYSIS.

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Objectives: The best method to identify women with minor cervical lesions that require diagnostic work-up remains unclear. We performed a meta-analysis to assess the accuracy of p16^{INK4a} immunocytochemistry compared to hrHPV DNA testing with hybrid capture II (HC2) to detect cervical intraepithelial neoplasia (CIN2+ and CIN3+) in women with a cervical cytology showing atypical squamous cells of undetermined significance (ASC-US) or low-grade cervical lesions (LSIL).

Methods: A literature search was performed in three electronic databases to identify studies eligible for this meta-analysis.

Results: Seventeen studies were included in the meta-analysis. The pooled sensitivity of p16^{INK4a} to detect CIN2+ was 83.2% (95%CI: 76.8-88.2%) and 83.8% (95%CI: 73.5-90.6%) in ASC-US and LSIL cervical cytology respectively; pooled specificities were 71.0% (95%CI: 65.0-76.4%) and 65.7% (95%CI: 54.2-75.6%). Eight studies provided both HC2 and p16^{INK4a} triage data. p16^{INK4a} and HC2 have a similar sensitivity and p16^{INK4a} has significantly higher specificity in the triage of women with ASC-US (relative sensitivity: 0.95 (95%CI: 0.89-1.01); relative specificity: 1.82 (95%CI: 1.57-2.12)). In the triage of LSIL, p16^{INK4a} has a significantly lower sensitivity but higher specificity compared to HC2 (relative sensitivity: 0.87 (95%CI: 0.81-0.94); relative specificity: 2.74 (1.99-3.76)).

Conclusions: The published literature indicates an improved accuracy of p16^{INK4a} compared to HC2 testing in the triage of ASC-US. In LSIL triage p16^{INK4a} is more specific but less sensitive.

**p16/Ki-67 DUAL-STAINED CYTOLOGY USING THINPREP LIQUID-BASED CYTOLOGY
– SUB-ANALYSES OF MORE THAN 9,000 PALMS STUDY PARTICIPANTS**

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Background: The PALMS trial (Primary ASC-US LSIL Marker Study) evaluated the diagnostic performance of the novel p16/Ki-67 Dual-stained cytology testing in cervical cancer screening as well as in the triage of ASC-US or LSIL Pap cytology results. The outcomes were compared to Pap cytology and HPV testing in the screening setting, and to HPV testing in the triage of ASC-US or LSIL. For both Pap cytology and Dual-stained cytology testing, liquid-based cytology and conventional cytology methods were included in the PALMS trial.

Objectives and Methods: We performed an analysis of the diagnostic performance of Dual-stained cytology, Pap cytology and HPV testing (hc2; Qiagen) limited to the sub-cohort of 9,231 women enrolled to the PALMS trial who had ThinPrep (Hologic) liquid-based cytology testing for both Pap and Dual-stain.

Results: Test positivity rates for Dual-stained cytology, Pap, and HPV testing over all ages were 5.7%, 5.6%, and 11.2%, respectively. Sensitivity of Dual-stained cytology for CIN2+ (n=67 cases) was found at 95.6% (95%CI 87.1-98.6%), significantly higher (p=0.008) than Pap cytology (80.2%; 95%CI 68.5-88.3%). Specificity levels were identical for both methods (95.0% for Dual-stained cytology vs. 95.1% for Pap testing; p=0.564). In women aged 30 and older, sensitivity (specificity) of Dual-stained cytology for CIN2+ was 91.2% (96.0%), compared to 77.9% (95.9%) for Pap testing and 96.1% (92.1%) for HPV testing.

For the triage of abnormal Pap cytology results, dual-stained cytology showed identical (ASC-US triage: 100% for both tests) or similar (LSIL triage: 94.5 vs. 100%) sensitivity as HPV testing for the detection of underlying CIN2+, at significantly higher specificity rates.

Conclusions: p16/Ki-67 Dual-stained cytology performed on ThinPrep liquid-based cytology may achieve a sensitivity level as high as 95% for underlying CIN2+ in primary screening of women of all ages, combined with a 95% specificity. Furthermore, dual-stained cytology was shown to be a highly efficient tool for the triage of abnormal Pap cytology results when performed as a reflex test out of ThinPrep liquid-based cytology specimens.

**COMBINED MORPHOMETRIC, E6/E7 MRNA, DNA CELL CYCLE BIOMARKERS (HPV ONCOTECT 3DX)
DECONVOLUTE ASCUS INTO INFLAMMATORY OR TRANSFORMED DIAGNOSTIC CATEGORIES**

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Background: Atypical squamous cells of undetermined significance (ASCUS) is a poorly reproducible diagnosis occurring in 4%-6% of the cytologic diagnoses in the US on a yearly basis. Given recent advances in cervical cancer screening technologies that incorporate combined cell biology and molecular biomarkers, the ASCUS diagnosis should give way to more informative characterization.

Methods: In a 100 ASCUS cytology cells were analyzed on the iCyt Eclipse Cell Analyzer (Sony Co). Electronic volume (mean corpuscular volume), E6, E7 mRNA, nuclear DNA staining and PMN quantification were all performed on intact cells from these samples.

Results: The increasing volume of the differing ectocervical cell populations creates a virtual N/C ratio, and as expected this population exhibited an increased proliferative rate matching a dysplastic cytology. In a one category of ASCUS samples (inflammatory, n=52), the samples have a normal DNA proliferation rate (8%), are E6/E7 mRNA negative and have a mean corpuscular volume of 178. In the other category of ASCUS samples (transformed, n=48) the proliferation rate is increased to 12%, E6, E7 mRNA is overexpressed, and the mean corpuscular volume is significantly increased in the abnormal cells to 241 (p<0.001). In addition, the number of PMNs in the transformed samples are significantly increased (p<0.01) in the transformed group compared to the inflammatory group.

Conclusions: The combination of the HPV OncoTect 3Dx™ technology collected on the iCyt Eclipse Analyzer revealed the ability to distinguish at least two major categories of ASCUS samples, inflammatory and transformed. Additional studies are underway to determine whether the ASCUS cytologic diagnosis can be eliminated.

SS 10-4

DETECTION AND QUANTIFICATION OF HPV E7 ONCOPROTEINS IN CERVICAL SMEARS**Barbara Lener, Pidder Jansen-Dürr***Institute for Biomedical Aging Research of the Austrian Academy of Sciences, Innsbruck, Austria AND Tyrolean Cancer Research Institute, Innsbruck, Austria*

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 cervical smears. Results of a clinical study will be presented and discussed.

SS 10-5

VALIDATION OF THE TRIAGE VALUE OF HPV16 DNA METHYLATION IN THE CRISP COHORT.**Brentnall A, Sasieni P, Vasiljevic N, Castanon A, Cuzick J, Lorincz A***Centre for Cancer Prevention, Wolfson Institute of Preventive Medicine, Queen Mary University of London, London UK.*

Objective: DNA methylation (DNAm) has an important role in cancer but most studies have focused on CpG islands in human genes. Using pyrosequencing (PSQ) on specimens from the Costa Rica cohort(1) we previously quantified levels of DNAm in the HPV16 genome and showed some CG sites in the L1, L2 and E2 open reading frames (ORF) as promising for triage. In the present CRISP study our aim was to validate DNAm using a pre-defined statistical analysis plan with a primary hypothesis that DNAm can differentiate between cervical intraepithelial neoplasia grade 2 or higher (CIN 2+) and benign HPV infection.

Methods: CRISP(2) is a longitudinal randomized study of women with mild cytology taking diindolylmethane to determine if the drug can promote HPV clearance. 91-paired SurePath™ exfoliated cervical cell specimens of women with HPV16 infection at baseline were available from both baseline and followup colposcopy 4 to 8 months later. Twenty five women had a final diagnosis of CIN 2+ based on histopathology at followup. There was full masking between existing clinical variables and the DNAm data generated by the testing laboratory. Purified DNA was bisulfite converted, amplified by PCR and sequenced by PSQ using primers for various CG sites in URR (CG: 31, 37, 43, 52, 58), E6 (218, 220, 245), L2 (4238, 4247, 4259, 4268, 4275) and L1 (6367, 6389). Levels of DNAm determined by the PSQ96MA software were analyzed in STATA and compared to outcomes.

Conclusions: DNAm was substantially higher (median approximately 20%) in the L1 and L2 ORFs than in other genomic regions, as previously observed in the Costa Rica study. Low levels of DNAm were seen in the E6 ORF (median < 10%) with little to no DNAm in the URR. Increasing levels of DNAm in L1 and L2 were significantly associated with outcome CIN2+ and also with HPV16 persistence. A model based on earlier data from Costa Rica combining a weighted DNAm score had a ROC operating point with sensitivity 92%, specificity 40%, PPV 44% and NPV of 90% for identifying women with CIN 2+. Quantitative DNAm measurement of HPV16 L1 and L2 may provide an improved triage for immediate colposcopy in HPV16 positive women.

(1)Mirabello et al. Methylation of the HPV16 genome is associated with disease progression in a prospective population-based cohort. JNCI In Press. (2)Castanon et al. Effect of diindolylmethane supplementation on low grade cervical cytological abnormalities: double-blind, randomized, controlled trial. Brit J Cancer 2012, 106:45.)

THE METHYLOME OF EPISOMAL AND INTEGRATED HPV 16 GENOMES IN PRE-INVASIVE AND INVASIVE CERVICAL LESIONS

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Objectives: Upregulation of the E6-E7 oncogenes of high-risk human papillomaviruses in basal squamous epithelial cells marks the shift from permissive to transforming HR-HPV-infections and is highlighted by substantial overexpression of p16^{INK4a} in the respective cells. The HPV E2 protein is a key regulator of the E6-E7 genes. Its loss of function appears to play a predominant role in HPV-triggered cellular transformation. It was hypothesized that integration of the HPV genome into host cell chromosomes and disruption of the viral E2 gene cause inactivation of E2. However, in majority of preneoplastic lesions and even a substantial part of cervical carcinomas the HPV genomes remain in an episomal state. We recently reported that methylation of 2 CpGs in the distal E2BS1 results in strong activation of the URR indicating that epigenetic modification of the HPV 16 URR contributes to the enhanced expression of E6/E7. Here we tested whether methylation of the proximal E2BSs 2, 3 and 4 further contributes to the transformation associated activation of episomal HPV genomes.

Methods: To test this, we first determined the physical status of HPV genomes in 85 HPV 16 positive lesions including 2 NILM, 17 CIN 1, 19 CIN 2-3, and 47 invasive cervical cancers. The methylation pattern of all E2 binding sites within the HPV 16 URR were subsequently compared between transformed p16^{INK4a}-positive lesions encompassing only episomal and/ or integrated HPV 16 genomes.

Conclusions: CpGs within the E2BS1, 3 and 4 were consistently methylated in all HPV 16-transformed, p16^{INK4a} positive lesions with only episomal HPV16 genomes. The same sites were not methylated in lesions displaying single chromosomally integrated copies of the HPV 16 genome. These data support the hypothesis that methylation of the E2BSs 3 and 4 in addition to methylation of E2BS1 may further interfere with the regulatory function of the HPV-E2 protein thereby enhancing overexpression of the HPV oncogenes that essentially contribute to neoplastic transformation and carcinogenesis of HPV-induced squamous epithelial lesions.

RIGHTS, ETHICS AND CERVICAL CANCER: BARRIERS TO PRIORITIZING CERVICAL CANCER CONTROL IN LOW RESOURCE SETTINGS

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The rights of women to cervical cancer control derive from the affirmation of the right to the highest attainable standard of health recognized by the United Nations and legislated through international and regional human rights treaties. The achievement of MDG3 to promote gender equality and empower women is equally essential to assuring that cervical cancer control. Prioritizing women's health means prioritizing access to care for women by health ministries, empowering women enhances income from jobs and the ability to afford care, it prioritized use of vaccination and screening funds, as well as assuring adequate care for women with cervical cancer. The lack of education for women in many settings leads to lack of knowledge about disease and the overall risk as well as the need to seek screening or care. The lack of control over sexual exposure (rape, age of marriage, inability to require use of condoms) leads to increased exposure to both HPV and HIV which are both related to the development of cervical cancer. Finally, the cumulative effect of the lack of gender equality leads to reduced advocacy for women's health within country and globally.

Cervical cancer control and choices for care directly involve benefit and harm and autonomy—all ethical infrastructures for any health care system. Unique issues such as those raised by research in international settings, lack of coordination of care between programs in country, and lack of a benefit back to the population carrying the burden of research are also woven in the complex mix of rights and ethics issues that influence cervical cancer control in low resource settings.

As health providers, we have an ethical and rights based obligation to provide the evidence for best practices including low resource options, to work with in country education for women and health professionals, to support cancer registries to provide surveillance of the impact of interventions, to strengthen the collaboration of in country researchers and health systems, and to advocate for rights and ethical health care that will improve the health of women, particularly in the effort to control cervical cancer.

SS 11-2

PROGRESS IN IMPROVEMENT OF CERVICAL CANCER PREVENTION IN COUNTRIES OF EASTERN EUROPE AND CENTRAL ASIA – TRIGGERS AND BARRIERS

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Despite the fact that in the 21st century technologies are available to avoid deaths from cervical cancer, in many countries of the WHO European Region mortality rates from this disease have not decreased and vary from 13 to less than 1 death per 100 000 women. On 11–12 October 2011 more than 100 experts and policy-makers from 42 countries and 7 partner organizations gathered in Istanbul, Turkey to participate in a WHO European regional meeting on cervical cancer prevention, where they analysed progress in primary and secondary cervical cancer prevention and defined priorities for the future in line with the action plan for implementation of the European Strategy for the Prevention and Control of Noncommunicable Diseases 2012–2016 approved by the sixty-first session of the WHO Regional Committee for Europe. Countries of Eastern Europe and central Asia are at a different stage regarding screening and vaccination and face challenges in relation to choosing target groups, opportunistic screening and vaccination, registration, and gaining political leverage. Some of the key points presented were (1) socioeconomic inequities; (2) transparent communication of the strong evidence for HPV vaccine safety; (3) data regarding the target groups that are not vaccinated and screened; (4) an integrative primary health care approach in cervical cancer prevention; and (5) synergy between cervical cancer prevention strategies. Essential triggers for comprehensive cervical cancer prevention and management include development of a strategy and a comprehensive action plan with a communications plan, available human and financial resources, monitoring and evaluation and improvement of quality of services. WHO in collaboration with other international partners is assisting many countries of the European Region to develop long-term national strategies on different prevention options including organized screening and vaccination, to establish population-based cancer registries and to improve the quality of health services involved in primary and secondary prevention of cervical cancer.

SS 11-3

COST-EFFECTIVENESS OF CERVICAL CANCER PREVENTION IN THE EASTERN EUROPEAN REGION

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Background: Within Europe, cervical cancer incidence and mortality rates are much higher in the eastern part than in the western part. We studied the cost-effectiveness of cervical cancer prevention strategies in the Eastern European region.

Methods: The cost-effectiveness of HPV16/18 vaccination of 12 year-old girls was calculated for 21 countries, under the assumption that vaccination prevents 70 percent of all cervical cancer cases and that cervical cancer and all cause mortality rates are stable without vaccination. The cost-effectiveness of integrated screening and vaccination was evaluated for Slovenia, Poland, and Georgia using a published simulation model that was calibrated to country-specific HPV genotype data. The screening interval was set at 3, 6, and 10 years and both cytologic and HPV (DNA) screening were considered.

Results: According to the World Health Organization, vaccination is deemed very cost-effective if the costs of saving one life year are less than the country's gross domestic product per capita. If the cost of vaccination was set at 100 international dollars, then vaccination was very cost-effective in all 21 countries. This cost level has been observed in European immunization programs and indicates that HPV vaccination can be implemented in a cost-effective way. The model-based analyses for Slovenia, Poland, and Georgia indicated that in Slovenia and Poland, combined vaccination and screening was very cost-effective. Six-yearly HPV screening was cost-effective in particular when screening coverage was low or gynecological work-up was imperfect. In Georgia, combined vaccination and screening was cost-effective at vaccination cost of 25 international dollars.

Interpretation: Vaccination can be implemented in a cost-effective way under current cervical cancer mortality rates. Cervical cancer prevention strategies by both vaccination and HPV screening are likely to be very cost-effective in countries with sufficient resources.

PERFORMANCE OF HPV-mRNA TESTING IN PHYSICIAN- AND SELF-COLLECTED SPECIMENS FOR CERVICAL LESION DETECTION IN HIGH-RISK WOMEN

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Objectives: A primary cervical cancer screening approach based on high-risk human papillomavirus (hr-HPV) testing of self-collected specimens may help extend screening coverage to unscreened or under-screened populations in resource-poor areas. However, little is known about the comparative performance of physician- versus self-collected specimens for hr-HPV mRNA testing, or the risk factors for hr-HPV mRNA detection in physician- and self-collected specimens. This study compared the performance and risk factors of hr-HPV mRNA testing of physician- and self-collected specimens for high-grade squamous intraepithelial lesions (HSIL) in female sex workers (FSW) in Kenya.

Methods: A cross-sectional analysis was conducted on 344 FSW in Nairobi. Women self-collected a cervico-vaginal specimen according to standardized instructions. A physician conducted a pelvic examination to obtain a cervical specimen. Physician- and self-collected specimens were tested for hr-HPV mRNA by the APTIMA HPV assay (Gen-Probe Inc.), and for sexually transmitted infections (STIs). Cervical cytology was conducted using physician-collected specimens. Women with HSIL underwent biopsy.

Conclusion: Overall hr-HPV mRNA prevalence in our population (median age 28 years) was similar in physician- and self-collected specimens (30% versus 29%). Prevalence of \geq HSIL in the population was 4% (N=15). Overall, hr-HPV mRNA testing of physician-collected specimens detected one \geq HSIL case more than that of self-collected specimens (sensitivity: 87%; 95% CI= 68-98% versus 80%; 95% CI=51-96%). Overall specificity for \geq HSIL appeared similar in both hr-HPV mRNA testing of physician- and self-collected specimens (73%; 95% CI: 67-77% versus 74%; 95% CI: 69-78). Sensitivity and specificity of hr-HPV mRNA testing of both physician- and self-collected specimens for \geq HSIL appeared higher in women \geq 30 years than in women <30 years. Hr-HPV mRNA positivity in both physician- and self-collected specimens appeared higher in women of younger ages or those with trichomonas infection. The performance of hr-mRNA testing of self-collected specimens was comparable to that of physician-collected specimens in detecting women with \geq HSIL. In resource-poor settings, hr-HPV mRNA testing of self-collected specimens can help identify women at high risk of cervical disease. Limited resources may then be channeled into clinical follow-up of women with hr-HPV mRNA positivity.

PERSPECTIVES ON TREATMENT OF CERVICAL NEOPLASIA IN LOW-RESOURCE SETTINGS

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Objective: To describe the methods of treatment of cervical neoplasia and their outcome in the context of developing countries.

Methods: Findings and conclusions are described based on review of published studies.

Findings and conclusions: Effective treatment of screen-positive women diagnosed with high-grade cervical intraepithelial neoplasia (CIN 2-3) is critical for the success of screening in preventing cervical cancer. Inadequate coverage of screen-positive women with diagnosis and treatment is a major reason for the sub-optimal performance of screening programmes in developing countries. CIN 2-3 lesions may be effectively treated by excision treatment methods such as loop electrosurgical excision procedure (LEEP), laser excision or cold knife conization or by ablative methods such as cryotherapy, cold coagulation or laser ablation. Excisional treatment methods are indicated for large ectocervical lesions or lesions extending into the endocervical canal. Ablative methods are indicated for lesions involving three fourths or less of the ectocervix. Excisional methods are more resource intensive and require skilled medical providers whereas ablative procedures such as cryotherapy and cold coagulation are more feasible and affordable methods that can be provided by trained nurses, midwives and paramedical health workers in field conditions. Recent results from studies in developing countries indicate that more than 90% of women with ectocervical CIN 2-3 lesions can be cured with a single application of cryotherapy and ablative treatments are safe and acceptable to women in field settings and can be the major work horses for outpatient treatment of CIN in resource poor settings. In recent years, new paradigms such as single visit approach have been evaluated to maximise participation of women in screening and treatment. However, it is important to develop resources and infrastructure for LEEP to manage large lesions and treatment facilities for invasive cancer to provide comprehensive treatment care in the context of screening programmes.

SS 11-6

HOW MOLECULAR TESTING WILL CHANGE THE WAY WE SCREEN IN DEVELOPING COUNTRIES.**Jeronimo J¹, Qiao YL², Chen W², Zhang X², Kang LN², Roger P¹, Chen F², Zhao FH², Castle PE³***1. PATH, Seattle, USA; 2. Cancer Institute/Hospital, Chinese Academy of Medical Sciences/Peking Union Medical College, Beijing, 3. American Society for Clinical Pathology Institute, Washington DC, USA*

Background: In the last decade multiple new screening techniques have become available for clinical use. There are more technologies in development and expected to be commercially available in the few next years, some of which could be suitable for developing countries. Even though having more screening options could help to decrease the cervical cancer burden, there is the need for evaluating how they will be implemented in population-based programs.

Methods: A total of approximately 25,000 women have been included in multiple studies in China, India, Uganda and Nicaragua for screening using different screening options: visual inspection with acetic acid (VIA); careHPV test for 14 oncogenic genotypes; careHPV for genotypes 16, 18 and 45 (careHPV 16-18-46); Hybrid Capture 2 (HC2); a Test for HPV16, 18, and 45 E6 oncoprotein; and Pap smear. Some of the molecular tests were used with self-collected vaginal samples as well as provider-collected cervical samples. Colposcopy and biopsy were done for any positive screening result and the histological result was used as the gold standard for evaluation of test performance for cervical intraepithelial neoplasia grade 3 or more severe (CIN3+).

Results: Results from approximately 20,000 women with complete screening and diagnostic procedures were evaluated. Self-collection of vaginal samples was highly acceptable in all the study sites ranging from 85% to 99%. careHPV testing of self-collected samples had a sensitivity that ranged from 65% to 98%. careHPV testing of provider-collected samples had a sensitivity that ranged from 75% to 98%. The HPV E6 Test had a sensitivity of 67.4% and the percentage of women with positive results was 1.6%, approximately 10-fold less than for HPV DNA, thereby reducing significantly the number of women requiring colposcopy. If careHPV-16/18/45 was used as triage for careHPV positives, the sensitivity is 70% with 3% referral rate.

Conclusions: There are multiple options for cervical cancer screening in low-resource setting. The decision on what test or combination of test should be used in a given population will depend on the resources available and the algorithm adopted by local decision makers. A detailed analysis of different tests or combination of tests for primary screening as well as triage will be presented.

SS 12-1

POPULATION IMPACT AFTER OPPORTUNISTIC QUADRIVALENT HPV VACCINATION IN SWEDEN 2007-2011**Wikström A¹, Cassel T², Young C²***1 Dept. of Dermatology & Venereology, Karolinska Hospital, Stockholm, Sweden
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Objectives: In Sweden, HPV vaccines are partly reimbursed for women aged 13-17 since 2007. Approximately 400 000 doses of the quadrivalent HPV vaccine (qHPV) against HPV types 6, 11, 16 and 18 have been distributed in Sweden. As there has been no organized vaccination program in place, the vaccination has been opportunistic. The purpose of this ecological analysis was to assess the population impact after vaccination with the qHPV in Sweden.

Methods: One of the earliest markers of population impact after qHPV vaccination is the decline of genital warts (GW) disease. Podophyllotoxin is used only to treat GW and is the first-choice treatment for GW in Sweden. In this analysis, national prescription data for podophyllotoxin through the Swedish drug prescription register during the years 2007-2011 has been used as a proxy to identify patients with GW in the age group 15-24. In addition, the cumulative vaccination coverage of qHPV has been identified in women and men aged 15-19, and 20-24 as of 31 October 2011.

Conclusions: The vaccination coverage of qHPV was highest in the age group 15-19 reaching 32 % at the end of 2011, most probably as a result of the reimbursement of the vaccine in these cohorts. The coverage rate in the age group 20-24 was 10 %, but the coverage rate in 20-year old women was higher, 28 %. The coverage rate in men was nearly 0%. When using podophyllotoxin prescription as a proxy for GW cases, there was a marked decline of 41 % in female GW cases between the years 2007-2011 in the age-group 15-19, while the decline in male GW cases was about 12 %. For the age group 15-19, the trend downward is significant for women but not for men, corresponding to an average yearly decrease of 0.5 cases per 100,000 population. In the age group 20-24, the decline in GW cases was 29 %. The decline in GW cases in men in the age group 20-24 was 10 %. The trend downward is significant for both women and men in the age group 20-24, corresponding to an average yearly decrease of 0.7 and 0.2 cases per 100,000 population respectively. In summary, this ecological study demonstrates a decline in GW cases in 15-19 and 20-24 year old women and 20-24 year old men. The decline in GW cases among young women aged 15-24 could be due to qHPV vaccination. However, it cannot be excluded that other factors are influencing GW occurrence in Sweden during this time period.

EFFICACY OF THE HPV-16/18 AS04-ADJUVANTED VACCINE AGAINST HPV TYPES ASSOCIATED WITH GENITAL WARTS

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Objectives: The AS04-adjuvanted human papillomavirus (HPV)-16/18 vaccine (GlaxoSmithKline Biologicals) shows high prophylactic vaccine efficacy (VE) against cervical intraepithelial neoplasia (CIN)2+ associated with HPV-16/18, with cross-protection against some non-vaccine oncogenic types. HPV types 6 and 11 are rarely associated with CIN2+, but are commonly found in subjects with genital warts. We evaluated VE against these HPV types in women from the PATRICIA study (NCT00122681) in the dataset from the end-of-study analysis.

Methods: Women aged 15–25 years were randomised (1:1) to receive HPV-16/18 vaccine or control. Cervical samples were collected every 6 months for HPV DNA typing by SPF₁₀-LiPA₂₅*; gynaecological and cytopathological examinations were performed every 12 months. Women were referred to colposcopy and treatment according to pre-defined algorithms, but were not screened for genital warts. In this post-hoc analysis, VE against HPV-6/11 was evaluated in the total vaccinated cohort (TVC, women who received ≥1 vaccine dose, regardless of baseline HPV-DNA/serostatus, n = 18,644) and TVC-naïve for all HPV types (women seronegative for HPV-16/18 and DNA-negative for all high and low-risk HPV types by SPF₁₀-LiPA₂₅, with normal cytology at baseline, n = 11,644). Serological testing for HPV-6/11 was not performed.

Results: Median follow-up time was 47.4 months post-dose 1 in the TVC and TVC-naïve. VE against 6-month persistent infection in the TVC and TVC-naïve is shown in the table.

| | TVC-naïve for all HPV types | | | TVC | | |
|----------|-----------------------------|---------|---------------------|--------------|---------|--------------------|
| | No. of cases | | | No. of cases | | |
| | HPV | Control | VE, % [95% CI] | HPV | Control | VE, % [95% CI] |
| HPV-6/11 | 74 | 112 | 34.5% [11.3, 51.8] | 232 | 260 | 10.9% [-6.8, 25.6] |
| HPV-6 | 61 | 93 | 34.9% [9.1, 53.7] | 182 | 208 | 12.6% [-7.2, 28.8] |
| HPV-11 | 14 | 20 | 30.3% [-45.0, 67.5] | 53 | 56 | 5.3% [-40.4, 36.2] |

Conclusions: These results suggest that the HPV-16/18 vaccine may have some efficacy against persistent infection with HPV-6/11.

*SPF₁₀ HPV-LiPA₂₅ version 1 is manufactured by Labo Biomedical Products (Rijswijk, The Netherlands), based on licensed INNOGENETICS SPF₁₀ technology.

THE INCIDENCE OF ANOGENITAL WARTS IN GERMANY AFTER INTRODUCTION OF HPV VACCINATION

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Background: Since March 2007, Human Papilloma Virus (HPV) vaccination is recommended for 12- to 17-year-old females in Germany and is covered by the health insurance companies. Predominantly the quadrivalent vaccine is used which also targets HPV subtypes 6 and 11 being responsible for >90% of anogenital warts (AGW). We assessed whether an effect of introducing HPV vaccination can already be seen for AGW at the population level in Germany by looking at the incidence of AGW between 2005 and 2008.

Methods: In this retrospective cohort study we used data from one large statutory health insurance with >6 million members (~8% of the German population). We estimated the incidence of AGW based on ICD-10 code A63.0 for females and males and applied piecewise segmented Poisson regression model to investigate changes in incidence after the introduction of HPV vaccination.

Results: The overall incidence of AGW among 10-79-years-old increased from 168 per 100,000 person-years in 2005 to 196 in 2008. However, among females aged 15 to 19 years the incidence decreased from 316 per 100,000 in 2007 to 242 in 2008 (a 23% reduction, p = 0.0001), starting in the 2nd quarter of 2007.

Conclusions: Already within few months of the introduction of HPV vaccination, incidence of AGW decreased among the vaccinated age group. These data show a possible vaccination effect on the incidence of AGW. Further follow-up studies are needed to underline this trend.

SS 12-4

INCIDENCE OF GENITAL WARTS IN SWEDEN PRE AND POST QUADRIVALENT HPV VACCINE AVAILABILITY**Leval A¹, Herweijer E¹, Arnheim-Dahlström L¹, Walum H¹, Frans E¹, Sparén P¹, Fridman Simard J²***1 Dept. of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm 171 77, Sweden**2 Dept. of Medicine, Clinical Epidemiology Unit, Karolinska Institutet, Stockholm 171 77, Sweden*

Objectives: To estimate annual incidence proportions of genital warts in the Swedish population immediately prior to and following the commercial availability of the quadrivalent vaccine.

Methods: We established a national population-based cohort of over 4 million women and men aged 10-44 living in Sweden between 2006 and 2010, which were identified through Swedish health care registers. The primary endpoint was genital warts (GW) either identified through the Prescription Drug Register (prescription of Podofyllotoxin or Imiquimod) or the Patient Registers (ICD10). Annual age-standardized incidence proportions were estimated from men and women through 2006 and 2010 using Poisson regression.

Findings: In 2010, age-stratified incidence proportions of GW were highest for 20-year old women (957 per 100 000) while men peaked slightly later at age 24 (1137 per 100 000). Crude rates were marginally higher among men than women during 2006-2007 and appeared to later diverge. Among men overall incidence appeared to increase while incidence in women declined between 2008 and 2010. Women ages 17 and 18 years had over a 25% decline in GW rates when comparing 2006 with 2010, with significant decreases through age 25.

In conclusion: This study provides a reasonable estimation of the incidence of GW in the Swedish population using register data with results comparable to previous smaller studies. There was a downward trend of GW incidence among younger women between 2006 and 2010 but not among men in any age group.

SS 12-5

OCCURRENCE OF ADVERSE EVENTS AFTER QUADRIVALENT HPV VACCINATION IN DENMARK AND SWEDEN**Arnheim-Dahlström L¹, Pasternak B², Svanström H², Sparén P¹, Hviid AP²***1 Dept. of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm 171 77, Sweden**2 Statens Seruminstitut, Copenhagen DK-2300, Denmark*

Objectives: To assess the risk of adverse events after quadrivalent human papillomavirus (qHPV) vaccination.

Methods: A cohort of 954,182 Danish and Swedish women aged 10-18 years, of which 301,366 were vaccinated with qHPV vaccine, were followed for adverse events during October 2006 to December 2011 (Denmark) or December 2009 (Sweden). Primary endpoints of autoimmune, neurologic and thromboembolic events were assessed from Patient Registers (ICD10) in Denmark and Sweden, 3 months after the first dose of qHPV vaccine. Women had to be disease free from the event under study 3 years prior to entry and only events with at least 5 exposed cases were considered as outcomes. Women were censored at receipt of bivalent HPV vaccine, emigration, death, event under study, or end of follow-up, whichever came first. Age-standardized incidence rate ratios were estimated as relative risks (RR) with 95% confidence intervals (CI), using log-linear Poisson regression, comparing vaccinated and unvaccinated women.

Findings: Mean age at entry was 12.9 years, and mean age at qHPV vaccination 14.3 years. For autoimmune events there was no sign of increased risk for qHPV vaccinated women, but a significantly decreased risk for coeliac disease (RR 0.52, CI 0.35-0.79). For neurologic events no increased risks were observed, but decreased risks for Bell's palsy (RR 0.47, CI 0.22-0.98) and epilepsy (0.65, CI 0.47-0.89). For venous thromboembolic events no deviance from unity was observed.

In conclusion: This is to date the largest study of adverse events following qHPV vaccination, utilizing routine data from inpatient and outpatient care. No increase in risks for autoimmune, neurologic or thromboembolic events for qHPV vaccinated women were observed. The observed decreases in risk for coeliac disease, Bell's palsy and epilepsy, may well be due to that women at risk for these diseases refrain from getting vaccinated against HPV.

**QUADRIVALENT HPV-VACCINE POPULATION EFFECTIVENESS ON GENITAL WARTS:
A POPULATION-BASED STUDY IN OVER 2.2 MILLION WOMEN**

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Objectives: To assess vaccine effectiveness and the population impact on genital wart (GW) incidence among women after opportunistic vaccination with the quadrivalent human papillomavirus (qHPV) vaccine.

Methods: We established a national population-based cohort of over 2.2 million women aged 10-44 living in Sweden between 2006 and 2010, which were identified through Swedish health care registers. The primary endpoint was GW either identified through the Prescription Drug Register (prescription of Podofyllotoxin or Imiquimod) or the Patient Registers (ICD10). Exposure status was time-varying qHPV vaccination dose, identified through the Prescription Drug Register and SVEVAC, a national HPV vaccination register. Time-to-event analysis using Poisson regression adjusted for attained age, age at first vaccination, and genital wart history prior to study follow-up, were performed to quantify vaccine effectiveness against genital warts in fully vaccinated women, and dose-effectiveness. Incidence rates among vaccinated and unvaccinated women were calculated with an average follow-up time of 4.4 (SD ± 1.3) years.

Findings: In Sweden, opportunistic vaccination began October 2006 and was partially subsidized for girls aged 13-17. Almost 124000 women were vaccinated from 2006-2010 and the vaccination coverage in the subsidized group was 25-33%. The amount of GW cases in the population avoided as a result of complete vaccination under the age of 20 was 418 per 100,000 person-years or 4300 cases per year in Sweden. Effectiveness was highest in girls who were vaccinated before age 14 (94%; 95% CI 75-98) and decreased as age-at-vaccination increased. Maximum protective effectiveness across all ages of vaccination was seen after three doses.

In conclusion: This study showed substantial reduction in GW cases as a result of the qHPV-vaccination. The qHPV-vaccine is highly effective in young women, and protection of the qHPV-vaccine against GW increased the younger the age at vaccination. Since this is an observational study, confounding from prevalent HPV infections cannot be ruled out as a source of bias explaining the decrease in effectiveness with increasing age at vaccination.

COULD A 9-VALENT HPV VACCINE MAKE A DIFFERENCE?

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The two licensed HPV vaccines (Cervarix[®], Gardasil[®]) are comprised of empty virus-like particles (VLP), self-assembled from the major capsid L1. Both incorporate VLP of high-risk types HPV16 and 18, which cause 70% of all cervical carcinomas. In addition the 4-valent Gardasil includes VLP of HPV6 and 11, which induce 90% of all genital warts.

Long-running clinical trials have reported the prophylactic vaccines' high efficacy against cervical, vulvar and vaginal dysplasia, and anal dysplasias and genital warts (quadrivalent vaccine), induced by the vaccine types.

Although limited cross-protection against types closely related to HPV16 and 18 (e.g. HPV31, 45) has been described, high-level antibody titers and long-term protection appears restricted to the types included in the vaccine. Consequently one third of all cervical cancers, caused by over 10 non-vaccine high-risk types, and a significantly smaller subset of carcinomas of the anus and of HPV-induced oropharynx cancers, are not targeted by current vaccines.

The manufacturer of Gardasil has designed a 9-valent HPV vaccine that includes VLP of 5 additional high-risk types to target HPV types 6, 11, 16, 18, 31, 33, 45, 52 and 58. This vaccine is currently in phase III clinical trials in young women and adolescents, and results are not yet available. Previously, combining 4 types into the 4-valent VLP vaccine has not resulted in immune-interference to HPV16, when compared to mono-valent HPV16 vaccination. Thus it is anticipated that a 9-valent VLP formulation will induce high-level and type-restricted antibodies against targeted 9 HPV types. Provided comparable level of safety, this vaccine may offer prophylactic efficacy against the vast majority of HPV induced mucosal cancers, in particular 90% cervical cancers worldwide.

As additional complexity compounds the already high costs of HPV vaccines, commitment of manufacturers and (non-) governmental organizations need to concentrate particularly on vaccination programs in developing countries to maximize impact on global cancer burden.

SS 13-2

IMMUNOGENICITY AND SAFETY OF A CANDIDATE TETRAVALENT HUMAN ONCOGENIC PAPILLOMAVIRUS VACCINE

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Objectives: A prophylactic human papillomavirus (HPV) vaccine that targets more oncogenic HPV types, in addition to HPV-16 and -18, could broaden protection against cervical cancer. A study was conducted to evaluate the immunogenicity and safety of a candidate tetravalent HPV L1 virus-like particle vaccine in healthy 18–25 year-old women, compared with the licensed HPV-16/18 AS04-adjuvanted vaccine.

Methods: In a Phase I/II, partially-blind, multicentre study (NCT00478621), subjects were randomised to receive bivalent control or 1 of 5 tetravalent HPV-16/18/33/58 vaccines formulated with different adjuvant systems (AS04, AS01_E or AS02_w), administered on 2 or 3-dose schedules (month [M]0,1,6; M0,3; or M0,6).

Results: At 1 month following last vaccination, all subjects (N=514) were seropositive for HPV-16 and -18 antibodies (ELISA) in all vaccine groups; anti-HPV-16 and -18 geometric mean antibody titres (GMTs) of 3-dose schedules are shown (Table). Anti-HPV-33 and -58 ELISA GMTs were higher for the 3-dose tetravalent AS01_E vaccine (21,505 and 10,897) compared with AS02_w (12,963 and 6,925) or AS04 (7,102 and 5,524). Memory B-cell and CD4+ T-cell responses to HPV-16 and -18 were generally lower or similar for the tetravalent vaccines compared with the bivalent control; the exception being the HPV-16 B-cell response, which was higher for tetravalent AS01_E and AS02_w vaccines. All formulations had an acceptable reactogenicity and safety profile, although solicited general symptoms were more pronounced for the tetravalent AS01_E vaccines.

Conclusions: All tetravalent formulations induced responses to HPV-33 and -58 but there was a trend for lower anti-HPV-16 and -18 antibody response when additional antigens were added to the HPV-16/18 AS04-adjuvanted vaccine; however, anti-HPV-16 GMTs were similar or higher for the 3-dose tetravalent AS01_E and AS02_w vaccines.

GMTs (EU/mL [97.5% CI]) 1 month post-vaccination (ATP immunogenicity cohort, initially DNA negative and seronegative subjects for corresponding antigen)

| | Anti-HPV-16 | Anti-HPV-18 |
|---|---------------------------|-------------------------|
| HPV-16/18 (20/20)* AS04 M0,1,6 (bivalent control) | 11246 [9133, 13847] N=58 | 4332[3521, 5330] N=70 |
| HPV-16/18/33/58 (20/20/20/20)* AS04 M0,1,6 | 6775# [5502, 8342] N=58 | 2987 [2428, 3675] N=70 |
| HPV-16/18/33/58 (20/20/10/10)* AS01 _E M0,1,6 | 27645 [22713, 33649]N=65# | 3008 [2441, 3706] N=69 |
| HPV-16/18/33/58 (20/20/10/10)* AS02 _w M0,1,6 | 17664 [14534, 21468]N=66# | 2155 [1766, 2629] N=76# |

*µg; #p-value <0.05 vs bivalent control (based on Tukey multiple comparison test) Only results from 3-dose schedules are shown

SS 13-3

RELATIONSHIP BETWEEN THE RISK OF NEW HPV-16 INFECTION AND NATURALLY-ACQUIRED TYPE-SPECIFIC ANTIBODY TITRES

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Objectives: To assess the quantitative relationship between the risk of new HPV-16 infection and naturally-acquired type-specific antibodies in sexually-experienced women enrolled in the control arm of the PATRICIA trial (NCT00122681) in the dataset from the end-of-study analysis.

Methods: Subjects enrolled in the control arm of the double-blind, randomised, controlled Phase III PATRICIA trial were followed during a 4-year period. Cervical liquid-based cytology samples were obtained every 6 months and cytological examination using the Bethesda system was performed every 12 months. The risk of new HPV-16 infection and development of atypical squamous cells of undetermined significance (ASC-US+) was modelled as a function of the baseline level of HPV-16 antibodies (measured using ELISA) in women aged 15–25 years from the control arm of the PATRICIA trial, using Poisson regression. The threshold level of HPV-16 antibodies for a 90% reduction of risk of new HPV-16 infection (ASC-US+) was derived from the model.

Results: The incidence of HPV-16 infection significantly decreased ($p < 0.0001$) with increasing HPV-16 antibody titre. An HPV-16 antibody titre of 371 EL.U/mL (95% CI: 242, 794) yielded a 90% reduction in HPV-16 incident infection and an HPV-16 antibody titre of 480 EL.U/mL (95% CI: 250, 5756) yielded a 90% reduction in HPV-16 associated ASC-US+. As a sensitivity analysis, a model including age of first sexual intercourse and smoking history as covariates showed very similar results: 315 EL.U/mL (95% CI: 215, 606) for 90% reduction in HPV-16 incident infection.

Conclusions: A significant quantitative relationship between naturally-acquired HPV-16 antibody titres and the incidence of new HPV-16 infection and HPV-16 associated ASC-US+ was observed. Although a correlate of protection cannot be inferred from these data, naturally-acquired antibody titres (measured by ELISA) confer a high degree of protection against HPV-16 infection and HPV-16 related ASC-US+. Further research is required on antibody functionality to identify a robust correlate of protection.

MOLECULAR BASIS OF PAPILLOMAVIRUS LATENCY**John Doorbar***National Institute for Medical Research, Mill Hill, London, United Kingdom*

HPV DNA can sometimes be detected in cervical smear samples in the absence of obvious disease, prompting the suggestion that the virus may exist in a latent state, or exist as an in-apparent infection. We have examined the molecular basis of such in-apparent infections using a mucosal animal model, and through the detailed analysis of clinical material.

The model system studies have been our primary focus to date, and have shown that in rabbits, papillomavirus DNA and papillomavirus transcripts can be detected at the sites of previous infections for at least a year after immune regression. DNA copy number declines slowly over time at such sites, consistent with long-term genome persistence in only a sub-set of the basal cells, such as the basal stem cells. Using laser capture methods, it appears that viral DNA is restricted to the basal layer, with perhaps sporadic bursts of genome amplification in the upper epithelial layers. Immunosuppression of such latently infected animals leads to a massive copy number increase at sites of previous infection, and may over time lead to the reappearance of lesions. We suspect that immune-mediated latency may reflect an inability of the immune system to clear viral genomes from the epithelial basal cells where viral gene expression is either very low or non-existent, and may differ from non-immunological latency that occurs following infection with low titers of virus.

Latency in humans is difficult to examine in such detail, but is likely to be similar in many respects to what is seen in the model systems. The analysis of clinical samples suggests that in some cases, 'apparent' latency may simply reflect the presence of subclinical micro-lesions. In these cases, HPV DNA can be detected in cervical smears. True immunological-latency is more likely to be associated with a failure to detect HPV DNA in cervical smears. Our results suggest that in such situations, viral copy number can rise following changes in the immune response such as may occur during aging.

HPV VACCINES UPDATE: STATE OF THE SCIENCE IMMUNOGENICITY OF HPV2 VS HPV4**Mark H. Einstein, MD, MS***Associate Professor of Obstetrics & Gynecology and Women's Health and Director of Clinical Research for Women's Health and Gynecologic Oncology Albert Einstein College of Medicine and Albert Einstein Cancer Center Montefiore Medical Center, USA*

The efficacy of prophylactic HPV vaccines for the prevention of HPV-associated diseases has been well-established. This session will describe some of the updates in our current understanding of the immune responses from vaccination with VLP-based vaccines. This includes the humoral responses and the role of adaptive immunity. Also, there will be discussion over how vaccine-induced immune responses are measured including seroassays and local cervicovaginal secretion measurements. It is still unclear if these responses will correlate with long term efficacy; which will need to be established from ongoing registries. There are recent published data regarding the correlation of all of these different assays measurements. A review of the recently published updated follow-up data regarding the head to head HPV2 vs. HPV4 immunogenicity trial will be discussed. Now that cross-protective efficacy has been seen with closely related HPV types, provocative data regarding cross-protective immunogenicity will be discussed. Efficacy against HPV-associated diseases in males has been shown for HPV4. Recently, correlative immunogenicity in males has also been published. Also, immunogenicity data in a special population of HIV-infected males will be presented.

SS 13-6

CHARACTERIZATION OF THE LOCAL IMMUNE RESPONSE IN CERVICAL AND VULVAR INTRAEPITHELIAL LESIONS**Sauer M¹, Hampl M², Schäfer K³, Schlotfeldt I³, Wentzensen N¹, Sinn P⁴, von Knebel Doeberitz M¹, Reuschenbach M¹***1) Department of Applied Tumor Biology, Institute of Pathology, University of Heidelberg, and Clinical Cooperation Unit, German Cancer Research Center, Heidelberg, Germany**2) Department of Obstetrics and Gynecology, University of Düsseldorf, Germany**3) Department of Obstetrics and Gynecology, University of Heidelberg, Germany**4) Institute of Pathology, University of Heidelberg, Germany*

Objectives: An insufficient immune response is suggested to contribute to the progression of HPV-associated lesions. To analyze stimulatory (activation of CD4+ Th-cells and CD8+ cytotoxic T lymphocytes (CTLs)) or inhibitory (activation of regulatory T cells (T_{reg})) immune pathways in women with lower genital tract lesions, the phenotype and density of lesion infiltrating T cells were investigated.

Methods: A total of 77 CIN/VIN patients were investigated. Biopsies were stained by immunohistochemistry. CD3 was used as Pan-T cell marker, Foxp3 as marker for regulatory T cells, CD8 for CTLs and Granzyme B for activated CTLs. Furthermore, the sections were analyzed for p16^{INK4a} and HPV.

Conclusions: The analyses of the local immune response in different stages of vulvar and cervical intraepithelial lesions revealed differences in the frequency and density of the T cell phenotypes. The density of lesion infiltrating and surrounding immune cells generally decreased with the severity of the disease in VIN and CIN patients. VIN2 lesions showed higher density of CD3+ and CD8+ cells ($p=0.043$, $p=0.024$) compared to VIN3. One VIN1 lesion available showed the highest epithelium/stroma ratios for all T cell populations. CxCa samples revealed a higher T_{reg} infiltration and a lower CTL infiltration compared to CIN3 ($p=0.017$ and $p=0.042$). These data imply that the local immune response in patients with high-grade lower-genital tract diseases is impaired, which might explain their higher progression risk towards invasive cancer whereas lower grade lesions show the trend towards having more protective mechanisms such as a higher CTL infiltration. The results are currently correlated with expression of the T cell receptor zeta chain of T lymphocytes infiltrating the lesions. CD3 zeta chain is an essential signaling element for the effective accomplishment of immune function of peripheral blood lymphocytes and its expression could be impaired in invasive cancer.

SS 14-1

ASSESSMENT OF COMPETENCE TO UNDERTAKE HPV TESTING IN A CERVICAL SCREENING PROGRAMME**Cubie HA¹, Moore C¹, Cuschieri K¹, Winder R², Patnick J²***1 UKHPV Reference Laboratory, Edinburgh, Scotland; 2NHS Cancer Screening Programmes, Sheffield, England*

Objective: To ensure competency and consistency across laboratories approved to provide HPV triage testing as part of the English cervical screening programme.

Methods: In March 2011, NHSCSP announced that laboratories conducting >35,000 routine cervical screening tests per annum could undertake HPV testing as part of the national screening programme provided several conditions were met, including competence testing of staff who would do the testing. HPV testing had to commence before the end of March 2012. The HPV Reference Laboratory in Edinburgh created validation panels from residual LBC material from routine screening samples. Samples were initially screened using Hybrid Capture 2 (HC2), pooled and diluted in PreservCyt[®] solution, then retested. Diluted material was then tested by several different HPV genotyping assays. Sufficient volume of each was prepared to supply a reasonable number of aliquots to requesting laboratories. Panels consisted of 88 unknown clinical samples (22 samples randomly distributed four times across the panel). At the time of writing, 50 panels have been distributed to 25 laboratories which had regional approval to undertake HPV testing. The first few results have been received and shown almost 100% accuracy. Full results from all approved laboratories will be presented.

Conclusions: Creation of validation panels from residual routinely collected LBC samples is achievable. Following a strict algorithm of pre-testing ensured consistent results when samples were tested by different staff in different laboratories. A high degree of accuracy appears likely when using an automated platform for HPV screening.

ROLE OF NATIONAL HPV REFERENCE LABORATORIES

Dillner J

WHO HPV LabNet Global Reference Laboratory (Sweden)

Background: The WHO HPV Laboratory Network was established in 2006 in order to “contribute to improving quality of laboratory services for effective surveillance and monitoring of HPV vaccination impact”. Global Reference Laboratories (GRLs) have focussed on development of international standards and standardisation of the quality assurance, notably using proficiency panels. Regional Reference Laboratories (RRLs) have e.g. been active in training, spreading of quality-assured practices and participation in global collaborative studies.

Objective: To describe role of National Reference Laboratories (NRLs)

Methods: Whereas GRLs and RRLs are appointed by WHO, NRLs are appointed by their respective countries and have different, national Terms of References. The WHO HPV LabNet is seeking to identify officially appointed NRLs in order to form a global network of NRLs. Apart from the laboratories that are also global or regional reference laboratories, NRLs have been identified to exist in at least England, Scotland, France, Germany, Norway, Canada and Slovenia. A networking with NRLs is intended to promote that the monitoring activities performed in the different countries worldwide are performed using internationally comparable standards. Collaboration with local and regional public health and research institutions, as well as with WHO and other international agencies, on monitoring HPV vaccination, the dissemination of knowledge on, and the use of, HPV international standard reagents to improve accuracy of genotyping, and serological measurements as well as participation in the exchange of information between national, regional and global reference laboratories should be the major means to achieve this goal.

Conclusions: In the countries that have appointed NRLs, they have an important role in the HPV monitoring programs. A global networking will be required to facilitate standardisation and further development of HPV monitoring programs.

WHICH HPV DNA TESTS CAN BE CONSIDERED AS CLINICALLY VALIDATED

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For screening it is important to have tests which detect hrHPV infections associated with ?CIN2 and differentiate them from hrHPV infections not associated with clinical disease to avoid unnecessary follow-up procedures. The FDA approved hc2 HPV DNA Test (Qiagen) showed in large prospective cohorts or randomized controlled trials clinical a sensitivity for the detection of \geq CIN2 of about 90-95%. This test and the GP5+/6+-PCR-EIA (Diassay) are considered clinically validated for screening purposes. For other tests a clinical sensitivity and specificity (criteria) comparable to the hc2 is demanded. The cobas[®] 4800 system (Roche) is a two component system consisting of the cobas x480 sample processing instrument and the cobas z480 amplification and detection instrument. The specificity and sensitivity of cobas[®] 4800 system in a population-based study of women aged 30+ is noninferior to that of Hybrid Capture 2 and is approved by the FDA since 2011. The Abbott RealTime HR HPV Assay uses the Abbott m2000sp machine and the Abbott m24sp sample preparation device. There is no cross-reaction with 15 other HPV types. A recent study compared RealTime HR HPV Assay with and showed that this test fulfils the criteria. The Cervista HR HPV test is a more recent liquid-phase signal amplification method from Hologic. In a recent population- based-clinical study comparing Cervista with Hybrid Capture 2 (Shenccast II) the sensitivity rates for \geq CIN3+ were comparable to Hybrid Capture 2, whereas the specificity of Cervista was higher than that of Hybrid Capture 2. This test fulfils the criteria on a provisional basis, since the population tested was slightly younger (25-60) instead of the required minimal age of \geq 30years. The PapilloCheck[®] HPV genotyping assay (Greiner Bio-One) is a low-density microarray based system for the detection of a fragment of the E1 gene of the HPV genome. The PapilloCheck[®] assay allows the simultaneous genotyping of 24 different HPV types. As the PapilloCheck detects many more types than the 13 high risk types detected by HC2 and GP5+/6+ PCR this test format would not fulfil the criteria to be considered as clinically validated. When the analysis was, however, restricted to the 14 hrHPV types targeted by the GP5/6-PCR-EIA, both the clinical sensitivity and the clinical specificity of the PapilloCheck assay were noninferior to those of the GP5/6-PCR-EIA. Using this restriction the Papillocheck fulfils the criteria partially on a provisional.

SS 15-1

NOVEL METHODS OF GENOTYPING, USING DEEP SEQUENCING OR MASS SPECTROMETRY

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Background: HPV monitoring puts new demand on HPV tests, as there is a need for a rapid, low cost large-scale testing for evaluation of the effects of different HPV vaccination strategies. Also, routine HPV testing methods that can identify HPV variant strains could be useful to monitor possible emergence of new variants.

Objectives: To develop i) a rapid, low cost large-scale testing method based on mass spectrometry and ii) a simple variant identification method based on deep sequencing of PCR amplicons.

Methods: We developed an HPV genotyping method using general primer PCR followed by mass spectrometry on the SEQUENOM platform. The method was calibrated to have a similar sensitivity for sixteen HPV types and was proficient in the WHO HPV LabNet 2010 proficiency panel. Evaluation using 44334 urine and swab samples submitted for *Chlamydia trachomatis* (CT) screening found an overall HPV prevalence of 31.4%. The most common HPV types in the sampled population were HPV 16 (8.0%), 51 (4.9%), 31 (4.3%), 18 (4.2%), 66 (4.2%), 52 (3.9%) and 6 (3.5%).

We also developed an HPV sequencing method based on deep sequencing of general primer PCR amplicons using a bench-top high throughput sequencing system (454 Junior). The method was compared to HPV genotyping using Luminex (reference method) and was found to have similar sensitivity (detection limit for the 16 HPV types included in the WHO HPV LabNet proficiency panel between 10-100 copies) and good concordance when testing clinical samples.

Conclusions: For HPV monitoring purposes we have established i) a high throughput HPV monitoring system that can readily assay many thousands of samples and ii) a simple method for detection of HPV variant sequences.

SS 15-2

CLINICAL VALIDATION OF HPV GENOTYPING TESTS

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Objectives: The VALGENT (validation of HPV genotyping tests) study aims: to define the potential role for HPV genotyping tests in clinical practice; to assess their accuracy in different clinical applications to detect cervical pre-cancer; and to develop a tool for evaluation and comparison of different HPV genotyping assays.

Methods: The VALGENT protocol consists in HPV genotyping with multiple assays of archived cervical cellular material remnant after routine cytological examination.

The study population is standardised: 1,000 continuous samples from women participating in screening enriched with 100 ASC-US, 100 LSIL and 100 HSIL samples.

The 300 pathological samples also come from the screening population and are also selected on a continuous basis to avoid selection bias and assure representativity.

Follow-up information is collected from the women whose samples are included in the study population and who are followed in agreement with current local guidelines. It is expected to find 90-130 CIN2+ cases and these samples are used to compute clinical sensitivity. Samples from women with a negative cytological result and having again a negative result at subsequent screening will be used to compute clinical specificity.

Results: A first panel of 1300 Belgian samples has been composed and genotyped with 4 assays. The generation of a second panel is being prepared in Scotland. Generation of more panels is considered. It is foreseen that at least one HPV assay is used that was already evaluated in a previous panel. HPV tests identifying cocktails of high-risk HPV types or tests identifying only a few types (HPV 16 and 18, for instance) can be included as well. This allows developing networks of test comparisons which can be pooled using MTM (multiple testing meta-analysis). Outputs of VALGENT will be: 1) comprehensive system for test validation and comparison; 2) listing of tests which fulfil minimal requirements; 3) development of a matrix of tests allowing translation of HPV prevalence studies from surveys using different tests.

CLINICAL VALIDATION OF HPV GENOTYPING TESTS USING THE SCOTTISH CERVICAL CYTOLOGY BIOBANK (VALGENT 2)

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2- Unit of Cancer Epidemiology, Scientific Institute of Public Health, Juliette Wytsmanstreet 14, B1050 Brussels

Objective: The VALGENT study aims to define the potential role for HPV genotyping tests in clinical practice, to assess their technical and clinical performance for different clinical indications and to develop a tool for evaluation and comparison of different HPV genotyping assays.

Methods: VALGENT relies on the collation of well characterised clinical samples which are subjected to HPV genotyping by a panel of different assays. The project is iterative; VALGENT 1 involved the assessment of 1,300 cervical liquid based cytology (LBC) samples (with associated clinical information) collated in Belgium and tested using in-house assays. VALGENT 2 will involve the collation of a further panel of 1,300 LBC samples in Scotland tested using an array of in-house and commercially available assays. Consistency of at least one genotyping assay across the separate iterations will facilitate assay comparison. Furthermore, VALGENT 2 will make optimal use of Scotland's bio-banking facilities and data linkages. Details of the project progress, methodology and planned outcomes will be presented

Conclusions: The VALGENT project will enable our understanding of how genotyping can contribute to effective and efficient cervical disease management.

ARE ALL HPV TARGET DESIGNS CREATED EQUAL? – A CRITICAL LOOK AT THE COMMONLY USED DETECTION STRATEGIES AND THEIR IMPLICATIONS FOR ASSAY PERFORMANCE

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Objectives: Carcinogenic (HR) HPV is a necessary co-factor for the development of cervical cancer. Four HR HPV tests are commercially available for IVD use in both the US and Europe. The BD™ HPV-GT Assay (BD Diagnostics, Sparks, MD, USA) is currently under development and will soon initiate US and European clinical trials. Among these five assays, the nucleic acid type and sequence region detected, the final reporting capabilities, and the detection technology are varied. The objective of this presentation is to provide an analysis of important design choices that impact an assay's ability to unambiguously report the presence of high risk HPV.

Methods: Peer-reviewed publications and package insert information were reviewed in order to critically compare the various assay design choices and their potential impact on clinical performance.

Conclusions: Assays vary in their genotype output capabilities, ranging from a pooled high risk HPV result to full reports of up to 6 independent types. Three of the assays reviewed provide HPV type 16 and 18 genotype information which is indicated in the most recent clinical guidelines. The length and placement of detection regions vary widely among assays. Careful selection of individual targets eliminates cross reactivity with low risk types while target gene selection eliminates the risk of false negatives due to target loss as a result of viral integration. Several assays detect multiple HR-HPV types using combined consensus primers or common probes in a reaction mixture. The use of carefully selected specific gene targets eliminates competitive PCR inhibition that can inhibit the ability to fully resolve mixed infections. The choice of RNA target over DNA has been reported to improve clinical specificity but this benefit appears to be small. Given the well documented difficulty of accurately defining CIN2+ endpoints, it is important to consider all aspects of a molecular assay's performance to provide the most accurate clinical information possible.

VALIDATION OF THE QIASYMPHONY® DSP AXPH DNA SYSTEM FOR PRESERVCYT® TESTING ON THE HC2 RAPID CAPTURE® SYSTEM

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Objective: Cervical specimens collected in PreservCyt (PC) liquid based cytology media (LBC) are validated for use with the digene® HC2 High-Risk HPV DNA Test® (HC2). HC2 sample preparation for PC requires manual manipulation and centrifugation. As previously reported, QIAGEN developed an automated PC Clinical Sample Concentrator and CE-IVD certified AXpH protocol and kit for the QIASymphony® SP platform. Here, we present results of formal system verification studies including PC specimens, the AXpH System (AXpH), and the Rapid Capture System (RCS) for HC2 (AXpH+RCS).

Methods: PC specimens were processed according to manufacturer recommendations for sample preparation and tested with HC2 on the RCS platform. These manual (MC) results were then compared to results of testing with the AXpH+RCS system. Testing was conducted using clinical PC samples (individual clinicals), pooled clinical PC samples (clinical pools) or tissue culture cells spiked into PC medium (cell models). Linearity was verified using cell models. Repeatability and Precision was tested with a multi-pool panel. Cross-contamination testing on the instrument was verified with cell models. Clinical performance testing was conducted with cytology-characterized individual clinicals.

Conclusions: We fully verified the new AXpH+RCS system in terms of linearity, reproducibility, and multivariable precision, cross-contamination and clinical performance and compared the performance to that of the currently approved MC+RCS system. A comparison of linearity yielded an R² of 0.995 and a y-intercept of 0.963. Repeatability and Precision comparisons showed no statistically significant differences. A pilot study of clinical performance resulted in an overall agreement of 98.8%, (95% Confidence Interval: 98.25-99.23) for a NILM population (N=1901, Kappa= 0.87, McNemar Chi-squared p-value=0.2088), and 95.5%, (95% CI: 92.97-97.12) for an ASC-US population (N=398, Kappa=0.91, McNemar Chi-squared p-value=0.6374). The newly verified AXpH+RCS system offers comparable performance on a fully automated platform.

COMPARISON OF THE HC2 –HR RESULTS USING QIASYMPHONY FULLY AUTOMATED SAMPLE PREPARATION AND MANUAL CONVERSION PROCEDURE FOR PRESERVCYT® SPECIMENS

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Objective: Cervical specimens collected in PreservCyt® (PC) are acceptable for use with the digene® HC2 High-Risk HPV DNA Test (HC2). The approved HC2 PC sample conversion protocol requires the manual processing of 4 ml of PC specimen. In this research study, we evaluated the feasibility of using the automated QIASymphony® AXpH protocol and kit for purification and concentration of DNA from cervical specimens collected in PC for testing with HC2 in order to compare manual and automated sample preparation methods. This work summarizes data generated in the verification process using the digene® HC2 High-Risk HPV DNA Test (HC2) for downstream analysis.

Methods: As reference, 4 ml aliquots of 273 clinical specimens, collected in PreservCyt® at ISPO (Cancer Prevention and Research Institute), were processed following the instructions for manual conversion of PreservCyt samples and tested using the digene HC2 HR-HPV test on the RCS system. For evaluation of the fully automated sample preparation, separate 4 ml aliquots of the PreservCyt specimens were processed on the QIASymphony using the AXpH DNA kit and tested using the digene HC2 HR-HPV test on the RCS system. The obtained RLU/CO values were compared and concordance analysis with the manual conversion method was performed. For samples with HC2 discordant results we repeated manual and QIASymphomy procedures.

Conclusion: The overall total agreement between QIASymphony AXpH and manual conversion was 91.5%, 83% for positive samples and 97% for negative. HC2-HR results performed in the same lab with manual conversion of PC samples and automated procedure with QIASymphony AXpH showed good reproducibility, with a k value of 0.81. The non-inferiority score test revealed that the clinical specificity of QIASymphony AXpH was not inferior (P 0.001) to that of manual conversion. The QIASymphony AXpH DNA protocol provides a fully automated sample preparation method for cervical samples in PreservCyt. A complete set of 96 samples can be processed in less than 4.5 hours and this significantly reduces the laboratory workload.

EFFECT OF MIXED INFECTIONS ON THE DETECTION OF HIGH-RISK HPV TYPES

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Objective: Informatics analysis suggests PCR competition may contribute to type-bias and the rate of false-negatives when PCR-based methods are used to determine the presence of "high-risk" (HR) types of HPV. Informatics predicted bias against the A5 and A6 species of high-risk HPV has previously been observed in a study of ASC-US subjects. This study examined the effect of mixtures of HR and non-oncogenic HPV types on the ability to detect the high-risk HPV using the Cervista® HPV HR test and consensus L1 PCR.

Methods: Plasmid DNAs containing portions of the genomes of HR and non-oncogenic HPV types were made in an aqueous solution of 10ng/μL tRNA containing 5000 copies of HR HPV types and 0, 5,000, 10⁴, 10⁵, or 10⁶ copies non-oncogenic HPV types and tested using the Cervista HPV HR test and consensus L1 PCR using the PGMV primer set. PCR amplicons were purified using Qiagen QIAquick® PCR purification kit, digested with *HaeIII*, separated electrophoretically and visualized.

Results and Conclusions: All samples containing HR HPV DNA produced positive results using the Cervista test, regardless of the amount of non-oncogenic HPV DNA in the mixtures. However, competitive inhibition of PGMV PCR amplification of HR HPV DNA was noted with increasing concentrations of non-oncogenic HPV DNA in the samples, with almost total quenching of the HR HPV DNA signal occurring when 10⁵ copies of non-oncogenic HPV DNA was present. These results confirm that non-oncogenic HPV DNA in a sample has no adverse effect on the ability of Cervista HPV HR to detect the presence of clinically relevant amounts of HR HPV. Significant competitive amplification interference of PGMV PCR amplification of HR HPV DNA can occur in the presence of non-oncogenic HPV DNA, similar to that of a mixed infection of oncogenic and non-oncogenic HPV types, potentially resulting in false-negative test results for HR HPV.

NOVEL CO-FACTORS FOR HETEROSEXUAL HPV TRANSMISSION

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Objectives: To assess the epidemiological evidence for a role of novel co-factors for heterosexual HPV transmission and progression to cervical lesions, namely IUD use and male circumcision.

Methods: We did a pooled analysis of individual data from two large studies by the International Agency for Research on Cancer and Institut Català d'Oncologia (ICO) research program on HPV and cervical cancer; one study included data from ten case-control studies of cervical cancer done in eight countries, and the other included data from 16 HPV prevalence surveys of women from the general population in 14 countries. A subset of seven case-control studies also enrolled current male partners to explore associations with male circumcision. A total of 2205 women with cervical cancer and 2214 matched control women without cervical cancer were included from the case-control studies, and 15 272 healthy women from the HPV surveys. Information on IUD use and male circumcision was obtained by personal interview. HPV DNA was tested by PCR-based assays. Odds ratios and 95% CIs were estimated using multivariate unconditional logistic regression models.

Results: After adjustment for potential confounders, circumcised men were less likely than uncircumcised men to have HPV infection (OR=0.37; 95% CI, 0.16 to 0.85). Monogamous women whose male partners had six or more sexual partners and were circumcised had a lower risk of cervical cancer than women whose partners were uncircumcised (OR=0.42; 95% CI, 0.23 to 0.79).

After adjusting for relevant covariates a strong inverse association was found between ever use of IUDs and cervical cancer (OR=0.55, 95% CI 0.42–0.70; $p < 0.0001$). A protective association was noted for both squamous-cell carcinoma and adenocarcinoma/adenosquamous carcinoma. No association was found between IUD use and detection of cervical HPV DNA among women without cervical cancer.

Discussion: Male circumcision is associated with a reduced risk of penile HPV infection and, in the case of men with a history of multiple sexual partners, a reduced risk of cervical cancer in their current female partners. In addition IUD use may also act as a protective cofactor in cervical carcinogenesis.

SS 16-2

SOURCES OF GENITAL HPV INFECTION

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Objectives: The sexual transmission of HPV is well established, although little is known about transmission dynamics and alternative routes of HPV transmission.

Methods: The natural history of genital HPV infection has been the focus of substantial research, but less studied are anal and oral HPV infection, and the dynamics of sexual transmission. Longitudinal studies have shown that anal HPV infection is a relatively common event among women. A high degree of genotypic concordance in the sequential acquisition of cervical and anal HPV infections has been reported. The anus of women appears to be a major source and target of heterosexual transmission, although few transmission studies have been conducted and the sample sizes have been modest. Transmission through non-penetrative sexual contact has been demonstrated between the female anus and the scrotum, as well as the female hand and male genitals. Male self-transmission has been noted, frequently involving the scrotum, and 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 and mouth may also serve as reservoirs of infection in both men and women. Autoinoculation involving the hands or oral cavity 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 16-5

OPPORTUNITIES FOR THE PREVENTION OF THE HETEROSEXUAL TRANSMISSION

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Objectives: Several hurdles exist in calculating transmission rates of HPV between couples including long testing intervals which dilute calculation and introduction of new sexual partners. In this study, we specifically sought to examine the rate of concordance and transmission in monogamous heterosexual couples over a relatively short observation period but with short interval testing.

Methods: Women in the SF HPV cohort study who had an incident HPV infection and reported at least 3 months of monogamy along with their partner were asked to participate. A baseline study visit (V1) was scheduled within 8 weeks. At all visits, samples for HPV DNA were obtained from the genital areas, intra-anal (women), perianal (men), palmar surface of hand and tongue/buccal mucosa. After V1, couples were asked to have sex on the upcoming Sunday, and then return within 24 hours for HPV testing (V2). Couples were then asked to abstain from sex and return at 72 hours (V3), 2 weeks (V4) and 6 weeks (V5) after V1 with no restrictions on sexual activity.

Results: 25 couples were enrolled. There was a high rate of concordance ranging from 71-90% of partners sharing at least 1 HPV type in their genitals at each visit. The highest rate (95%) was at V2 (24-hours post intercourse visit) compared to the other preceding and succeeding visits (rates ranged 64-74%). Rates of detecting the person's genital HPV type in the partner's hand ranged from 42-60% at any one visit and in the partner's perianal area 21-59%. Detection of the person's genital HPV was less common in the partner's oral cavity with a range of 0-9%. Of the persons who had HPV detected in the hand, 60-100% of their partners had at least 1 similar HPV type detected in their genital area, 29-60% in their partner's perianal area, 36-80% in their partner's hand, 0-7% in the partner's oral cavity.

When we examined exact match rates for all HPV types, the match rates were much lower. Out of 116 visits where HPV was detected somewhere in one partner, only 17 (15%) visits had the identical HPV types detected somewhere in the other partner. When examining discordant couples, transmission from F to M ranged from 428.6 per 100 person-months (p-m) between V 2 and V3 (shortest time-interval) to 24.8 per 100 p-m between V4 and V5 (longest interval). Similarly, highest transmission for M to F transmission was V2 and V3 (100 p-m) and the lowest between V4 and V5 (14 per 100 p-m). At each visit, the transmission rate from female-to-male was higher than male to female.

Conclusions: This study underscores the high rates of HPV transmission among heterosexual couples and demonstrates the predominance of female to male transmission. This study also underscores the complexity of HPV transmission dynamics in that even during periods of abstinence, transmission events appear to occur. The hand and perianal/intraanal areas remain likely reservoirs for transmission. However, transmission of HPV DNA likely does not result in infection in the majority of cases. This may suggest that simple good hygiene habits may decrease transmission rates.

VALIDATION OF CERVICAL CANCER SCREENING METHODS IN HIV POSITIVE WOMEN FROM JOHANNESBURG

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Objectives: Cervical cancer is a common in HIV-infected women, yet few data on available on best methods to screen these high-risk women. We aimed to evaluate the performance of three cervical cancer screening methods: cytology, visual inspection with acetic acid (VIA) and HPV testing in HIV-infected women from South Africa.

Methods: HIV-infected women aged 18-65 were recruited from a HIV clinic in a government hospital in Johannesburg. Women were screened using three different methods: i. HPV DNA testing (QIAGEN HC-2), ii. Pap smear and iii. VIA using 5% acetic acid. Each woman served as her own internal control. Women with a positive VIA or abnormal Pap smear were referred for a colposcopic-directed biopsy. A randomly-selected 20% of women with a negative Pap and VIA were also referred to histological confirmation to control for verification bias. A digital image of all VIA procedures was performed and reviewed by the study team for quality assurance. Sensitivity and specificity of VIA for histologic cervical intraepithelial neoplasia (CIN)-2 or greater were compared to those of Pap and HPV testing.

Conclusions: A total of 782 HIV-seropositive women participated, with a median age of 37 years and CD4 counts of 374 cells/mm³. Overall, 3.8% were diagnosed with atypical squamous cells of undetermined significance (ASCUS) by cytology, 35.8% with low grade squamous intraepithelial lesions (LSIL), and 30.2% high grade squamous intraepithelial lesion (HSIL). VIA positivity was 36.6%, whereas carcinogenic HPV prevalence was 61.9%. Using histology results of CIN-2 + as the gold-standard, the estimated sensitivity and specificity of Pap smears (HSIL+ cut-off) was 78.2% and 85.6%, respectively; that of HPV testing was 94.3% and 48.7%, respectively; and VIA was 69.4% and 74.2%, respectively. Of 496 women with negative VIA test results, 11.9% had CIN-2+. HPV testing had the highest sensitivity in women with CIN 2+. However, VIA picked up 69.4% of CIN-2 and may be an effective screening tool where other methods are not available.

HPV VACCINES IN HIV INFECTED INDIVIDUALS

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Objective: Since HIV infected individuals are particularly vulnerable to HPV-associated morbidities, vaccination is particularly important in affected children before sexual activity. Durability will be important to monitor since antibody response to several childhood vaccines appear to have less durability than seen in immunocompetent children. In addition, the mechanisms of protection of the HPV vaccines have not been completely identified. The objectives of this study was to characterize the immunogenicity and immune memory of a quadrivalent human papillomavirus vaccine (QHPV) in HIV-infected children.

Methods: HIV-perinatally infected children 7 to 12 years old received 3 doses of QHPV with or without a 4th dose after 72 weeks from baseline. Type-specific antibodies and cell-mediated immunity were measured up to 96 weeks from baseline.

Results: HPV6, 11 and 16 antibodies were detected in over 94% of children at 4 and 72 weeks, respectively, after the 3rd QHPV. In contrast, antibodies for HPV18 were 97% and 76%, respectively. Both HPV 6 and 18 antibodies were lower than those reported for immunocompetent children. Antibodies showed a rapid decay within the first 48 weeks after 3rd dose with slower decay thereafter. Assuming the slower decay reflects a steady state, 50% of children would become seronegative within 4-5 years. The strongest predictor for higher antibodies at week 96 was HPV antibody concentrations at week 28. Other variables significantly associated with week 96 antibody concentrations were CD4 cell counts.

Conclusions: Three QHPV doses generated robust and persistent antibodies to HPV6, 11 and 16, but comparatively weaker responses to HPV18 in the competitive Luminex Immunoassay. The decay seen for all vaccine genotypes is of concern. However, a 4th dose increased antibodies against all vaccine genotypes in an anamnestic fashion. Whether a 4th dose will be required is unknown. Similar decay for HPV 18 has been seen in immunocompetent women and yet high rates of efficacy remain. Efficacy studies in HIV infected persons are critical.

SS 17-3

ANAL HPV AND ANAL CANCER IN PEOPLE WITH HIV**Gulich, A.***Kirby Institute, University of New South Wales, Sydney, Australia.*

There are two separate reasons why people with HIV are at increased risk of anal cancer. First, in the absence of anti-retroviral therapy, all people with HIV will develop immune deficiency. This disrupts immunological control of anal HPV infection, and results in increased risk of anal cancer, irrespective of the cause of immune deficiency. Thus organ transplant recipients, and probably adult survivors of congenital immune deficiency, are at increased risk of anal cancer. Second, both HIV and HPV are sexually transmissible viruses, so people with HIV are at increased risk of HPV infection and its consequences. The overlap of these two risk factors is greatest in homosexual men with HIV, in whom anal HPV infection is close to universal, and control is disrupted in advanced HIV infection. Anal cancer incidence rates of 50-140 per 100,000 have been described in this population, and it is one of the most common cancers occurring in these men. Incidence of anal cancer is also increased, albeit more modestly, in other populations of people with HIV.

Since the availability of highly active anti-retroviral therapy in the mid 1990's, the incidence of AIDS-associated cancers has declined substantially, but stable or increasing rates have been described for anal cancer in people with HIV. The absence of a decline in rates is because anal cancer is much less closely related to current CD4 count than are AIDS-associated cancers. Nevertheless, emerging evidence suggests that a history of very low CD4 count, or a long duration with low CD4 count, does increase the risk of anal cancer. These data suggest that treating HIV early and maintaining a high CD4 count may reduce anal cancer risk.

Given the extremely high incidence of anal cancer in homosexual men with HIV, a cytology-based screening program has been proposed in a manner analogous to screening for cervical cancer. However, the extremely high prevalence of pre-invasive lesions, the lack of formal proof of effectiveness of therapies, and a high rate of subjective side effects has hindered the introduction of such a program. Research into the prevention of anal cancer, and the treatment of anal cancer precursors, is an urgent public health priority for people with HIV.

SS 17-4

HPV VACCINES IN HIV SEROPOSITIVE INDIVIDUALS**Garland SM1, 2***,*1 Dept. of Microbiology and Infectious Diseases, The Royal Women's Hospital, Melbourne, Australia**2 Murdoch Children's Research Institute, Melbourne, Australia*

Objectives and Methods: Individuals infected with Human Immunodeficiency Virus type 1 (HIV-1) are at an increased risk for HPV infection, mixed HPV infections, as well as at increased risk for HPV-related dysplasias and cancers.

Both bivalent and quadrivalent vaccines have been trialled in HIV positive individuals including as young as 7 years of age. In general, seroconversion has been reported to be high for both vaccines, although at lower titres than in HIV negative, age matched controls.

Conclusions: There are no increased higher adverse events relating to HPV vaccination in HIV(+) individuals.

HPV ONCOGENE MRNA TESTING FOR THE DETECTION OF ANAL DYSPLASIA IN HIV-INFECTED MEN

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Background and Objectives: Anal HPV-infection and anal dysplasia are very frequent in HIV-positive men who have sex with men (HIV+MSM), and progression of low-grade (LSIL) to high-grade lesions (HSIL) occurs faster than in HIV-negative persons. Detection of high-risk (HR)-HPV-E6/E7 oncogene-mRNAs has been reported to have higher specificity and positive predictive value (PPV) for the detection of high-grade cervical intraepithelial lesions compared to HR-HPV-DNA testing. We have evaluated the diagnostic accuracy of the APTIMA® HPV Assay (Gen-Probe incorporated) for the detection of anal dysplasia in HIV+MSM.

Methods: 414 intraanal swabs from HIV+MSM participating in a screening program including anal cytology, histology, and high-resolution anoscopy were analyzed. HR-HPV-DNA detection and typing were performed by PCR and hybridization with 20 HR type-specific probes using a bead-based multiplex genotyping assay. E6/7-mRNA detection of 14 HR-HPV-types was performed using the APTIMA HPV assay.

Results and Conclusions: 411 swabs had valid results in both test-formats (174 normal, 45 ASCUS, 136 LSIL, 56 HSIL by cytology/histology). For the detection of ASCUS+ (ASCUS plus LSIL plus HSIL) sensitivity, specificity, negative predictive value (NPV) and PPV were 89.0%, 28.7%, 65.8%, and 63.0% for HR-DNA-testing, compared to 80.2%, 44.8%, 62.4% and 66.4% for E6/E7-mRNA-testing. The respective values for the detection of LSIL+ (LSIL plus HSIL) were 90.1%, 26.0%, 75.0%, 51.6% for HR-HPV-DNA-testing and 83.3%, 42.5%, 74.4%, 55.9% for E6/E7-mRNA-testing. For the detection of HSIL, the values were 100%, 21.4%, 100%, 16.7% for HR-HPV-DNA-testing and 96.4%, 34.6%, 98.4%, 18.9% for E6/E7-mRNA-testing, respectively. A shift of the cut-off of the Aptima assay (from 0.5 to 5 S/CO) hardly affected sensitivity, but improved specificity (54.6%/50.7%/40.3%) and PPV (70.2%/59.2%/20.0%) for the detection of ASCUS+/LSIL+/HSIL, respectively. HPV oncogene mRNA detection has an (almost 2-fold) increased specificity, slightly decreased sensitivity and NPV, and a slightly increased PPV for the detection of anal dysplasia in HIV+MSM compared to HR-HPV-DNA-testing.

Conflict of interest: APTIMA HPV mRNA test kits were provided by Gen-Probe GmbH, Wiesbaden, Germany

HPV INFECTION AND INCREASED RISK OF HIV ACQUISITION. A SYSTEMATIC REVIEW AND META-ANALYSIS.

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Background: Human papillomavirus (HPV), one of the commonest sexually transmitted infections, may be a cofactor in HIV acquisition. We systematically reviewed the evidence for an association of HPV infection with HIV acquisition in women, heterosexual men and men who have sex with men (MSM).

Methods: Studies meeting inclusion criteria in Pubmed, Embase and conference abstracts up to 31/07/11 were identified. Random effects meta-analyses were performed to calculate summary hazard ratios (HR). Publication bias was formally tested using Begg's test and heterogeneity was assessed via the I² statistic. A components approach was used to evaluate bias within studies.

Results: Eight papers were included, with previously unpublished data received from five authors. Seven of the eight studies found an association between HPV infection and HIV acquisition. The risk of HIV acquisition in women doubled with prevalent HPV infection with any genotype (HR=2.06 (95%CI=1.44-2.94), I²=0%, n=3). Adjustment for confounders was often inadequate and the comparison group varied between studies. The effect seen was similar for high-risk (HR=1.99 (95%CI=1.54-2.56), I²=8.4%, n=5) and low-risk (HR=2.01 (95%CI=1.27-3.20), I²=0%, n=2) HPV genotypes with weak evidence of publication bias (p=0.06). In heterosexual men and MSM, penile and anal HPV infection, respectively, were associated with HIV acquisition. However, only two studies were identified and therefore meta-analysis was not performed.

Conclusion: Meta-analysis of studies in women showed an association between prevalent HPV infection and HIV acquisition, although included studies were at risk of bias and residual confounding. A similar association was seen in MSM and heterosexual men. If further studies validate this association, HPV vaccines may reduce HIV incidence in high-HPV prevalence populations, in addition to being highly effective in the primary prevention of cervical cancer. As HPV-vaccine programs are introduced, surveillance studies will be important to monitor the impact of HPV vaccination on HIV acquisition.

SS 18-1

AGE ISSUES OF HPV SCREENING

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Objectives: There has been considerable controversy over the appropriate ages for cervical screening and these issues also apply to cervical screening based on HPV testing. Recent guidelines agree that screening should not be offered to women under the age of 21 nor to those over the age of 65, but some are more restrictive (eg screening from age 30 to 60 only). Whereas the case for not screening women under age 21 is based on the assessment that the harms exceed the benefits, there is a lack of data regarding the incremental harms and benefits of continuing screening in older women.

Methods: As with cytology, there is a case for having longer screening intervals in older women and for HPV testing 10 yearly screening may be sufficient over the age of 40.

Two issues are of particular concern with regards to HPV testing. One is the low specificity for presence of CIN2 or worse in young women. For this reason most guidelines recommend against HPV testing in primary screening under the age of 30. The other is the use of HPV testing in women in their 60s. Although a negative HPV test in such a women may give additional reassurance that she need not be screened again, the difficulty is how to manage those women (around 5%) who are HPV positive with normal cytology. Repeat testing is likely to find that most of these are persistent infections, and adequate colposcopy and treatment are difficult in older women.

Conclusions: While HPV testing can be recommended for women aged 30-60, there are issues in both younger and older women. For older women detrimental issues seem harder to resolve, and may limit the upper age for HPV testing. The main concern with HPV testing under the age of 30 is that it could lead to over-treatment of CIN that would regress. Approaches to minimise such a risk will be discussed.

SS 18-2

CLINICAL OUTCOMES OF PRIMARY SCREENING FOR CERVICAL CANCER FOR DIFFERENT COMBINATIONS OF CYTOLOGY, POOLED HRHPV, AND HPV GENOTYPE 16/18 DETECTION: RESULTS FROM THE ATHENA HPV STUDY

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Objective: To analyze data from the cross-sectional phase of the Athena study on the performance of cytology and the cobas[®] HPV Test with HPV 16/18 genotyping, within established and investigational cervical cancer screening strategies.

Methods: In the ATHENA study, 41,955 eligible women ≥ 25 years of age (mean 41.9 year) were screened with cervical cytology and pooled high-risk HPV (hrHPV) testing with genotyping for HPV 16/18 (cobas[®] HPV Test, Roche, Pleasanton, CA). All women who were hrHPV+ and/or had abnormal cytology, as well as a subset who were hrHPV? with normal cytology, had colposcopy performed using a standardized protocol. Nine different strategies were investigated by evaluating the potential clinical utility of combinations of these 3 tests for recommending immediate colposcopic referral based on the sensitivity and false positive rate for the detection CIN2+ and on the estimated number of tests and colposcopies to detect each high-grade lesion.

Results: The risk for CIN2+ was highest for women positive for HSIL cytology (45.5%) followed by HPV16+ (24.2%) and lowest for women hrHPV neg (1.2%). Nearly 11% of hrHPV+ women had CIN2+. hrHPV testing with HPV 16/18 genotyping as the primary screen, followed by reflex cytology testing of all pooled 12-type hrHPV (non-HPV 16/18) positive, and sending to colposcopy all HPV 16/18 positive and \geq ASCUS offers the best compromise between sensitivity and false positive rate. Furthermore, it is in the bottom tier of number of initial screening tests required, in the number of colposcopies per CIN2+ detected (6), and in the middle tier of the number of colposcopies overall.

Conclusions: The challenge in any screening test is to find the best balance between sensitivity and specificity with acceptable rates of over and under-diagnosis. Primary screening with a pooled hrHPV test that includes HPV 16/18 genotyping appears to provide the best balance among the 9 cervical screening strategies.

**Clinical Outcomes of Different Models for Detection of CIN2+ Endpoint
(Sensitivity & Specificity of Each Strategy Relative to Cytology)
Crude Estimate**

| Strategy | Description of Model (All Women 25 years and older) | No. of Tests Performed | No. of Colposcopy Performed | Colposcopy to Detect One CIN2+ | CIN2+ Cases Identified | Sensitivity (%) | Relative Sensitivity | 100 - Specificity (%) | Relative Specificity |
|--|--|---------------------------|-----------------------------------|--------------------------------------|------------------------------|--------------------|-------------------------|-----------------------------|-------------------------|
| Standard Screening Options | | | | | | | | | |
| 1 | Cytology Alone | 40901 | 2186 | 10 | 222 | 51.5 | (1.00) | 26.6 | (1.00) |
| 2 | Cytology Alone with ASCUS Triage | 42532 | 1219 | 6 | 215 | 49.9 | (0.97) | 13.6 | (1.18) |
| 3 | Cotesting with 12 mo. f/u Pap-/HPV+ | 81,802 | 1219 | 6 | 215 | 49.9 | (0.97) | 13.6 | (1.18) |
| 4 | Cotesting with HPV 16/18 Triage | 81,802 | 1848 | 6 | 298 | 69.1 | (1.34) | 21.0 | (1.08) |
| Investigative Strategies | | | | | | | | | |
| Cytology followed by "reflex testing" | | | | | | | | | |
| 5 | ASCUS Triage & Pap-/HPV+ | 80816 | 3781 | 10 | 395 | 91.7 | (1.78) | 45.8 | (0.74) |
| 5.1 | ASCUS Triage | 42532 | 1219 | 6 | 215 | 49.9 | (0.97) | 13.6 | (1.18) |
| 5.2 | NILM triage to HPV | 38284 | 2562 | 14 | 180 | 41.8 | (0.81) | 32.2 | (0.92) |
| 6 | Cytology with ASCUS Triage & NILM 16+/18+ | 80816 | 1848 | 6 | 298 | 69.1 | (1.34) | 21.0 | (1.08) |
| 6.1 | ASCUS Triage | 42532 | 1219 | 6 | 215 | 49.9 | (0.97) | 13.6 | (1.18) |
| 6.2 | NILM triage to HPV 16/18 | 38284 | 629 | 8 | 83 | 19.3 | (0.37) | 7.4 | (1.26) |
| HPV testing alone or followed by "reflex testing" | | | | | | | | | |
| 7 | HPV alone | 40901 | 3502 | 9 | 380 | 88.2 | (1.71) | 42.2 | (0.79) |
| 8 | HPV reflex to cytology (HPV+/-ASCUS) | 45176 | 940 | 5 | 200 | 46.4 | (0.90) | 10.0 | (1.22) |
| 9 | HPV with 16/18 genotyping & reflex to cytology | 44009 | 1569 | 6 | 283 | 65.7 | (1.27) | 17.4 | (1.12) |
| 9.1 | Pap-/16/18 + | 40901 | 966 | 5 | 197 | 45.7 | (0.89) | 10.4 | (1.22) |
| 9.2 | 12 other hrHPV+ triage to Pap | 3108 | 603 | 7 | 86 | 20.0 | (0.39) | 7.0 | (1.27) |

SS 18-3

TRIAGE OF HPV-POSITIVE WOMEN: CYTOLOGY OR HPV GENOTYPING?

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Background: As HPV-positivity is common in the general population, primary HPV screening strategies need a second, triaging test before women are referred to colposcopy.

Objective: To compare strategies that triage HPV-positive women using either cytology, HPV genotyping and/or testing for HPV persistence using population-based screening databases from Sweden.

Methods: Comparisons of sensitivity, positive predictive values and number of colposcopies and tests required.

Conclusions: Distinction between different genotypes strongly affects PPVs, but HPV persistence is an even stronger risk determinant. Cytology has the advantage that the safety of screening intervals for cytology-negative women is very well established and can thus be used as a clinical routine.

SS 18-4

METHYLATION MARKERS FOR TRIAGE OF HPV-POSITIVE WOMEN**Steenbergen RDM, Hesselink B, Wilting SM, Heideman DAM, Meijer CJLM, Snijders PJF***Department of Pathology, VU University Medical Center, Amsterdam, the Netherlands*

Objectives: Progression of hrHPV-induced cervical intraepithelial neoplasia (CIN) to invasive cancer is a rare event driven by (epi)genetic alterations in the host cell genome. Current developments in population based cervical screening by primary hrHPV testing require objective biomarkers for risk stratification of hrHPV-positive women. Towards this goal DNA methylation events associated with HPV-induced malignant transformation were assessed.

Methods: Both a longitudinal in vitro model system and tissue specimens were analysed for DNA methylation alterations using quantitative methylation-specific PCR (qMSP). Following functional studies in HPV-transformed keratinocytes, the diagnostic potential of most promising methylation markers was evaluated on hrHPV-positive cervical scrapes.

An increasing number of genes was found to become methylated during hrHPV-induced carcinogenesis, including novel tumor suppressor genes and miRNAs. For CADM1, MAL and miRNA-124-2, DNA methylation-based gene silencing was demonstrated to functionally contribute to hrHPV-induced transformation. Based on their distinct onset during transformation and extensive evaluation on tissue specimens the combination of CADM1 and MAL methylation was tested on two independent series of hrHPV-positive cervical scrapes collected during population-based cervical screening studies using a training and validation approach. Testing for CADM1/MAL appeared as least as discriminatory for high-grade CIN+ as cytology either or not combined with HPV16/18 genotyping, the current golden standard.

Conclusions: Testing for DNA methylation alterations provides an objective triage tool for hrHPV-positive women. This opens up the possibility for complete cervical screening by objective, non-morphological molecular methods applicable to both conventional scrapes and self-sampled cervico-vaginal specimens.

SS 18-5

p16/Ki-67 DUAL-STAIN AS A TRIAGE MARKER FOR CYTOLOGY-NEGATIVE/HPV-POSITIVE WOMEN**Ikenberg H¹, Sideri M², Bergeron C³, von Knebel Doeberitz M⁴, Griesser H⁵, Bogers J⁶, Neumann H⁷, Schmidt D⁸, Ridder R⁹; for the PALMS Study Group***1 Cytomol, Frankfurt, Germany; 2 Europ. Inst. of Oncology, Unit of Prev. Gyn., Milan, Italy; 3 Lab. Cerba, Cergy Pontoise, France;**4 Applied Tumor Biology, Univ. of Heidelberg, Heidelberg, Germany; 5 Center for Pathology and Cytodiag., Cologne, Germany;**6 Labo Lokeren - Campus Riatol, Antwerp, Belgium; 7 Inst. for Pathology, Bad Münden, Germany;**8 Inst. of Pathology, Mannheim, Germany; 9 mtm laboratories, Heidelberg, Germany*

Objectives: Adding HPV testing to cytology based cervical cancer screening (co-testing) in women ≥ 30 years can increase sensitivity for CIN2+. However, there is a need for an efficient clinical management algorithm for women with Pap-negative/HPV-positive results. We analyzed the performance of a novel immuno-cytochemical dual biomarker that detects co-localization of p16 and Ki-67 expression within the same cervical cell as an indicator of cell-cycle deregulation, in triaging all Pap-negative/HPV-positive screening results from the PALMS study, a prospective screening trial in over 27,000 women.

Methods: Women enrolled to the PALMS trial were tested using Pap cytology (LBC or conventional Pap smear), HPV (hc2, Qiagen) testing, and p16/Ki-67 Dual-stained cytology (CINtec PLUS, mtm laboratories). Women aged ≥ 30 with any positive test result were referred to colposcopy. Adjudicated tissue diagnosis on cervical biopsies was used as reference standard.

Results: Out of a total of 19,205 women aged 30 and older enrolled to the PALMS trial, 1,023 women (5.3%) were Pap-negative/HPV-positive. p16/Ki-67 Dual-stained cytology provided positive test results in 225/1,023 (22.0%) women. Within this Dual-stain positive group, 29 out of a total of 36 (80.6%) biopsy-confirmed CIN2+ cases were found. Specificity of Dual-stained cytology at the CIN2+ threshold was 79.1%. The NPV of a negative Dual-stained cytology result was 98.7%.

Conclusions: These results from the prospective PALMS trial confirm the high sensitivity and specificity of Dual-stained cytology testing recently reported for triaging Pap-negative/HPV-positive screening results. The detection of co-localization of p16/Ki-67 expression in cervical cells identifies women that may benefit most from immediate colposcopy, whereas negative Dual-stain test results may exclude pre-cancerous cervical disease within this cohort with a high NPV.

OTHER MARKERS FOR HPV SCREENING TRIAGE**John Doorbar***National Institute for Medical Research, Mill Hill, London, United Kingdom*

High-risk HPV infections are graded on the basis of pathology criteria. Lesions classified as CIN2+ are routinely treated, while lower grade lesions are not. Despite its importance in patient management, pathology grading is highly subjective and has proved difficult to standardise. Accurate classification of CIN2, and the discrimination between viral and non-viral CIN1 are particularly difficult.

To improve accuracy and reduce subjectivity, molecular markers have been developed to distinguish low-grade disease from transforming infections. In our studies we have used a six-colour staining-approach to correlate five molecular markers of HPV infection with pathology as determined by H&E staining on the same tissue section. This overlay technique has allowed us to correlate molecular pathology based on viral gene expression with conventional pathology based on cell morphology. It appears that a combination of just two classes of marker, which discriminate between early and late life-cycle events, can allow establishment of disease severity and HPV causality.

The first marker-class includes MCM, p16 and Ki-67, which are already in widespread use. These markers help identify cells driven into cycle by E7, and the extent to which such cells persist above the basal layer correlates with CIN grade. The second marker-class includes E4, which becomes massively expressed as E7 levels decline during the switch to the late stages of the virus life cycle. Its presence in CIN1 and CIN2 can conclusively identify HPV causality and can be used to confirm HPV type.

The presence of E7-surrogates at the epithelial surface reveals a need for treatment irrespective of the infecting HPV type. By contrast, E4 at the epithelial surface indicates lower-grade disease, HPV causality and possibly also HPV type. The mapping of lesion-surface expression patterns is now allowing development of an in situ staining approach for the non-invasive identification of lesion grade and position prior to colposcopy.

HPV mRNA AS A TRIAGE TEST FOR HPV-POSITIVE CYTO-NEGATIVE WOMEN**Reuschenbach M¹, von Knebel Doeberitz M¹, Arbyn M²***1) Department of Applied Tumor Biology, Institute of Pathology, University of Heidelberg, Germany**2) Unit of Cancer Epidemiology, Scientific Institute of Public Health, Brussels, Belgium*

Objectives: The low specificity of HPV DNA detection in primary screening requires triage tests to decide who among the HPV-positives needs to be referred for colposcopy. Cytology is proposed as a triage option, but HPV DNA-positive, cytology negative women are at higher than average risk of cervical precancer and require further management. Detection of HPV oncogene mRNA might be more specific for transforming HPV infections than detection of HPV DNA and could serve as triage marker for HPV+/cytology- women. Performance characteristics of commercially available HPV mRNA tests vary, particularly depending on the number of detected HPV genotypes. The APTIMA assay detects 14 high risk types and the PreTect Proofer and NucliSENS detect the 5 HPV types associated with the highest progression risk.

Methods: Studies on different mRNA tests in the triage of different conditions will be summarized and discussed.

Conclusions: Numerous studies indicate a higher specificity of mRNA tests compared to HPV DNA tests. Detecting mRNA of 14 HPV types is less specific than detecting 5 types, but more sensitive, close to HPV DNA tests. Few studies show better specificity of mRNA testing compared to DNA testing in triage of low-grade and equivocal cytology results and better specificity of mRNA testing compared to cytology in HPV DNA-positive women. But the low sensitivity of tests detecting only 5 types probably requires a tight follow up of mRNA-negative women. Currently, there are no studies specifically evaluating mRNA tests in HPV-positive, cyto-negative women.

SS 19-1

THE VALUE OF RANDOM BIOPSIES**Cox JT***UC Santa Barbara, CA, US (Retired)*

The unparalleled success in cervical cancer prevention has come from the ability to detect and treat the precursor lesion to cervical cancer (CIN3) before it gains the capacity to invade, and is widely accepted to have occurred secondary to the partnership between cervical screening and treatment of colposcopically detected lesions. Despite this success, a number of evaluations have questioned the accuracy of colposcopy and have proposed taking both more biopsies and random biopsies in areas that look colposcopically normal. The primary variable in colposcopic sensitivity in the U.S. ALTS trial was the number of biopsies taken, with detection of CIN 3 increasing with 2 or more biopsies. Pretorius and colleagues demonstrated sensitivity of colposcopic biopsy for CIN 2+ of only 57%. Adding a random biopsy detected another 37.4% and 5.5% was found on ECC. The same group demonstrated that the yield of CIN 3+ per colposcopy was greater when colposcopically directed biopsy was augmented by up to 4 random biopsies in quadrants that appeared to be colposcopically normal. However, the mean average epithelial thickness of 33 biopsies of CIN 2,3 from cervical quadrants with a colposcopic impression of normal was only 57% of the thickness (184 vs 321 microm, $p < .001$) of the 111 biopsies of CIN 2,3 from quadrants with colposcopic impressions of CIN 1, CIN 2,3 or cancer. The authors concluded that the inability of expert colposcopists to visualize some CIN 2,3 is associated with thinner epithelium. It may be appropriate to ask whether detection of these colposcopically unapparent high-grade lesions is important, from the perspective of preventing cervical cancer. In the first few decades following the introduction of colposcopy, CIN 2,3 lesions that were detected were primarily large prevalent lesions. The median time between peak detection of CIN 3 (29 yrs) and microinvasive cervical cancer (42 yrs) gives reassurance that the oncogenic process typically spans many years and increasing size of CIN3 lesions has generally been tied to this passage of time. In the ALTS trial the median length of CIN 3 was only 6.5 mm and lesions in one third were so small that colpobiopsy did not leave any residual CIN 3 to be detected in the LEEP specimen. CIN3 associated invasion is, on average, seven times larger than CIN 3 without invasion, and certainly much thicker than the lesions found only on random biopsy. Will increased detection of these colposcopically invisible lesions provide more protection for women from getting cervical cancer; particularly those infrequently screened, or will the potential for over-treatment of small high-grade lesions that might regress create more harm?

SS 19-2

LEEP CONISATION INCREASES THE RISK FOR ADVERSE PREGNANCY OUTCOME-HOW MUCH?**Maija Jakobsson, MD.PhD.***Department of Obstetrics and Gynecology, Helsinki University Central Hospital, University of Helsinki, Finland*

Many investigators have reported increased preterm birth rates after LEEP conisation, but the predisposing mechanisms remain unknown. One explanation is that the procedures used might shorten the cervix and bring about mechanical weakness. Shortening of the cervix, however, has not been observed in all studies. According to another theory, there is decreased mucus production. In some studies the amount of lactobacilli, which belong to the normal ecological flora of the vagina, has been decreased after cervical conisation. Removal of the cervical glands, containing antimicrobial agents, leads to ascending bacterial colonization, elevation in the concentrations of prostaglandins, release of proteolytic enzymes and finally premature rupture of the membranes. The development of CIN is often associated with or preceded by cervical inflammation. The increased risk of infections may also be related to risk-taking behavior – more sexually transmitted infections and heavy smoking. According to another possible explanation, cervical scarring may play a role. General reorganization of the cervical stroma during the healing process could lead to preterm birth.

In Europe the preterm birth rate varies from 5% to 12%. LEEP conisation doubles the risk for preterm birth. Still the risk for individual patient is small and therefore these women should be counseled accordingly. In our own study we used internal controls i.e. women having a birth before and after LEEP conisation. The preterm birth rate was 6.5% before and 12.0% after the procedure (RR 1.94, CI 1.10-3.40; NNH 18). Adjustments for maternal age, parity, or both did not change the results. The risk for preterm birth was especially increased (RR 3.38, CI 2.31-4.94) among women without previous preterm birth. The risk for preterm delivery was increased almost three-fold and repeat treatments over five-fold after LEEP conisation compared to the background rate of preterm birth. If the size of the removed cone is large, the risk is two-fold when compared to small or medium-sized cones. Recent studies suggest that repeated treatments are the most dangerous.

CIN 2-3 DURING PREGNANCY – OBSERVATION OR TREATMENT?**Siegler E , Lavie O , Mackuli Lena , Amit A(1), Auslenrer R , Waisman A(1)***Colposcopic and Cervix Clinic, Gynecological and Obstetrics Department, Carmel Medical Center, Rambam Medical Center, (1), Rappoport Faculty of Medicine, Technion Institute of Technology, Haifa, Israel*

Background: Cervical Intraepithelial Neoplasia 2-3 (CIN 2-3) is considered a premalignant lesion. Loop Cone operation is the most recommended treatment for diagnosis and prevention of cervical cancer. During pregnancy the consensus guidelines are to repeat colposcopy and, perform cervical biopsy or Loop Cone only if invasive cancer is suspected. The rationale is that during pregnancy the evidence suggests that CIN 2-3 does not progress to invasive cancer, and spontaneous remission happened in many cases. Also during the 70-80's severe complications of treatments during the pregnancy, mainly knife cone were described. Excess haemorrhage, spontaneous abortions, premature deliveries high rate of incomplete excision were the major complications reported. There are few publications about Loop cone during pregnancy that shows that performing the operation during the first trimester is a safe procedure without significant intraoperative or postoperative complications

Objective: The aim of this study is to describe our experience with patients diagnosed with Cervical Intraepithelial Neoplasia 2-3 (CIN 2-3) during pregnancy. We examine the safety of performing Loop cone procedure during the first trimester of pregnancy, record the complications and report the histological diagnosis of the operation.

We compare the results to patients that were followed up and treated after delivery.

Methods: Between January 2008 and October 2011, 23 patients diagnosed with CIN 2-3 or invasive cancer during pregnancy were identified through our registries. The decision about treatment or observation was made in each case individually according to the risk factors, the colposcopic impression or pathological results and the patient's preference. All the Loop cone procedures were performed during the first 14 weeks of pregnancy under general anesthesia in most of the cases.

Results: Among the 13 patients treated conservatively, in postpartum histology in 3 women CIN 1 or normal histology were diagnosed, in 8 women Loop cone histology was CIN 2-3 and in two women cervical cancer was diagnosed.

In 10 patients Loop cone was performed during the first trimester. In 3 women the Loop was performed in consequence with the termination of pregnancy, and in one case together with a curettage due to a missed abortion. The histological report was normal in one case, CIN 2-3 was diagnosed in 7 patients and Invasive or microinvasive carcinoma were diagnosed in 2 women.

No major or minor complications were reported following the Loop procedure.

6 women continued the pregnancy and 5 patients had term deliveries and one pregnancy is ongoing at 34 weeks.

Conclusions: Loop cone procedure performed during the first trimester appeared to be a safe procedure with minimal complications and with the potential of diagnosing cervical cancer and permitting an optimal treatment early, preventing the devastating complications of an unobserved cancer during 20-30 weeks. We think it is time to reconsider the indications and contraindications of CIN 2-3 treatments during pregnancy and perform this operation with precaution more liberally during the first trimester.

**PAP SMEARS CLASSIFIED AS ATYPICAL GLANDULAR CELLS (AGC)
– OUTCOMES AND MANAGEMENT IN PREGNANCY.****Slama J, Freitag P, Fischerova D, Zikan M, Pinkavova I, Cibula D***Gynecologic Oncology Center, General Teaching Hospital and 1st Medical School of Charles University, Prague, Czech Republic*

Objectives: The incidence of cervical cancers increases with age. Due to the trend of increasing age of first pregnancy, abnormal Pap smears including those classified as atypical glandular cells (AGC) are being found more often in early pregnancy. Once invasive cancer is excluded, conservative management of squamous precancerous lesions in pregnancy is considered safe; however, optimal management of AGC is not well established.

Methods: The study included 22 patients referred to us in early pregnancy with AGC Pap smears and 48 pregnant patients with HSIL smears as the control group. All the women were initially examined by expert colposcopy and those with high-risk smears also by transrectal ultrasound to exclude invasive endocervical cancer. Follow-up controls proceeded every 8-12 weeks and if there were no signs of progression, reevaluation was scheduled 6-8 weeks after delivery. Progression to invasive cancer was not found in any of the patients in the study or control group. HPV test was used for triage in patients with persistent atypical glandular cells-not otherwise specified (AGC-NOS). Significant pathology was confirmed by conization or punch biopsy postpartum in 45.5% (10/22) patients in the study group and in 70.8% (34/48) patients in the control group.

Conclusions: Conservative management of women with AGC in pregnancy is safe where invasive cancer is excluded. As histopathology verification of glandular precancerous lesions by punch biopsy is not reliable and the postpartum regression rate cannot be determined precisely, conization should be performed in all cases with atypical glandular cells-favour neoplastic (AGC-FN) or adenocarcinoma in situ (AIS). Triage of persistent AGC-NOS with HPV testing is useful in distinguishing underlying lesion.

SS 19-5

HEXAMINOLEVULINATE (HAL) PHOTODYNAMIC THERAPY (PDT) – FEASIBILITY STUDY FOR A THERAPEUTIC TREATMENT OF CERVICAL NEOPLASIA (CIN)

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Objective: Treatment options for CIN are limited to excision or ablative procedures that are associated with undesirable side effects with a risk of preterm birth in future pregnancies. No treatment modalities are available for HPV. HAL PDT is being developed as a possible treatment modality for the treatment of CIN. The treatment causes apoptosis and necrosis of cervical lesions and it is hypothesized that this may stimulate the local immune system to eradicate the HPV infections. A novel easy-to-use drug and delivery combination device has been developed and tested to ensure safety in patients with CIN.

Method: Patients with biopsy proven CIN1 and 2 were recruited from 3 clinics in Norway and France. HAL 100mg (5%) or placebo ointment was administered by the gynaecologist using an intra-vaginal PDT device (Photocure, Oslo, Norway), after which the patient could resume normal daily activities. Following drug absorption to local cervical tissue, photoactivation was performed delivering a red (629nm) light dose of 100J/cm² over 5 hours. The patients removed the intravaginal device themselves after completion of treatment. Patients were followed closely for safety assessments for 6 months.

Results: 13 patients with a mean age of 38 (27-61) years were treated, 10 to active treatment – 3 to placebo. HAL was easily administered by the gynaecologists and easily removed by the patients. Only mild to moderate local discharge and discomfort/pain related to the treatment were reported in three patients. Seven out of ten patients on active treatment reported a complete response at 6 months, while one of three patients on placebo regressed spontaneously. HAL PDT for CIN was easy to perform and showed a favourable safety profile.

Conclusions: This treatment was safe and well-tolerated. In these preliminary results, there appears to be some efficacy that will be further evaluated in an expanded phase II ongoing trial in 262 patients with CIN1/2.

SS 19-6

LONG-TERM FOLLOW-UP OF POSTERIOR VESTIBULECTOMY IN THE TREATMENT OF LOCALIZED PROVOKED VESTIBULODYNIA

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Localized provoked vestibulodynia (LPV), (formerly vulvar vestibulitis syndrome, VVS) is a subset of vulvodynia. It causes vulvar pain and dyspareunia ruining the sexual life of many young women (1). LPV is characterized by severe pain in the vulvar vestibule provoked by touch or attempted vaginal entry. Prevalence rates up to 9% have been reported (2). The histopathology of LPV reveals typically chronic inflammation and increased density of superficial extension of nerve endings (3). Treatment of VVS is problematic since the etiology is unknown. Many patients benefit from conservative management (vulvar care measures, cognitive behavioral therapy, pelvic muscles bio-feedback therapy, and neuropathic pain medication). Surgery is the treatment of choice for the most severe cases with refractory LPV (4).

We studied a cohort of 70 severe LPV patients treated by posterior vestibulectomy during 1995 – 2007 in the Vulva Clinic, Helsinki University Central Hospital, to evaluate the safety and the effectiveness of posterior vestibulectomy. Data of the postoperative period of all patients were collected from hospital charts. Of the 70 women, 57 attended a long-term follow-up visit including face-to-face interview, gynecological examination with swab-touch test for vestibular tenderness, current VAS score for dyspareunia, and McCoy questionnaire for sexual problems.

Results: Ninety-one percent of the patients were satisfied with the outcome. VAS for dyspareunia decreased from a median of 9 to a median of 3 (66.7% decrease) ($p < 0.001$). Posterior vestibular tenderness was totally absent in 34 patients (64.2%). Six (8.6%) patients developed postoperative bleeding and 11 (15.7%) patients developed mild wound infection. Bartholin's cysts occurred in four (5.7 %) patients. No long-term sequelae occurred.

We then compared long-term wellbeing of the surgery group ($n = 39$) with a group of patients with equally severe disease at baseline who benefitted from conservative management and did not need surgery ($n = 27$). The results were comparable. The overall long-term patient satisfaction was 89% in both groups. VAS for dyspareunia decreased 66.7% in the surgery group and 78.1% in the conservative treatment group ($p=0.407$). McCoy questionnaire showed equal sexual well-being in both groups.

Conclusion: Posterior vestibulectomy is effective and safe in the treatment of severe LPV and provides long-term patient satisfaction. The response is as good as that achieved by conservative management among patients who do not need surgery.

AGREEMENT BETWEEN COLPOSCOPIC IMPRESSION AND HISTOLOGY AMONG HRHPV16 POSITIVE WOMEN

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Objectives: To study the agreement between colposcopic impressions of conventional and dynamic spectral imaging (DSI) colposcopy and histology, in hrHPV type 16 and non type 16 hrHPV positive women. Furthermore, we studied if lesion size influences the correlation between colposcopic impression and final histology.

Methods: The study was designed as a sub study of the DSI colposcope validation trial: a prospective, comparative, multicentre clinical trial. (1) At the colposcopy clinics of 3 Dutch hospitals women referred for colposcopy were included. First the colposcopist located and graded the lesion, using the DSI colposcope as a regular video-colposcope. Subsequently, the DSI impression was displayed and biopsies were taken from all suspected areas, plus one extra biopsy from an apparently normal site. A cervical sample was taken for HPV-typing. In total, 133 women were found eligible for inclusion in this analysis. Information on lesion size was determined by software counting the pixels on the DSI colour-coded map indicating high-grade disease.

Conclusions: The DSI colposcope identified more CIN2+ lesions correctly among hrHPV16 positive women than in non-16 hrHPV positive women (Table) and so achieved a significantly higher sensitivity among them (97% vs 74%). No such difference was detected for the conventional colposcopic impression; our analysis shows that the performance of conventional colposcopy is not affected by hrHPV16 status. Instead, it is mainly the lesion size that determines the accuracy of conventional colposcopy.

Colposcopic impression of DSI and colposcopist defined as normal/ low-grade (N/LG) vs high-grade (HG) CIN. Final histology CIN2+

| | Neg n (%) | hrHPV + n (%) | Non-16 hrHPV + n (%) | hrHPV 16 + n (%) | Total | p value* |
|--------------------------------|--------------|------------------|-------------------------|---------------------|-------|----------|
| DSI | | | | | | |
| N/LG | 5 (50) | 1 (20) | 10 (26.3) | 1 (3.1) | 17 | 0.009 |
| HG | 5(50) | 4 (80) | 28 (73.7) | 31 (96.9) | 68 | |
| Conventional colposcopy | | | | | | |
| N/LG | 4 (40) | 4 (80) | 15 (39.5) | 15 (46.9) | 38 | 0.630 |
| HG | 6 (60) | 1 (20) | 23 (60.5) | 17 (53.1) | 47 | |

* Fisher's exact test, 2-sided

POST-COLOSCOPY FOLLOW-UP OF WOMEN WITH NEGATIVE CERVICAL BIOPSY – A PROSPECTIVE STUDY

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Objectives: In Norway, cervical cancer screening is based on cytology, with recent introduction of HPV testing in triage of women with minor cytological changes. Cytology and HPV testing are also used in follow-up of women with negative cervical biopsy (normal or CIN1 histology). In 30-40 % of the cases the biopsy is not representative for the high grade lesion (CIN2+). The objective of this study was to evaluate cytology and HPV mRNA testing in post-colposcopy follow-up of women with negative cervical biopsy.

Methods: Our study comprises all women with negative biopsy after a repeated ASC-US / LSIL cytology and ASC-H / HSIL in the period 2005-2009 in Troms and Finnmark counties, Norway. The women were followed up through 2010.

Conclusions: Of women with negative cervical biopsy, 25 % had CIN2+ in follow-up biopsies. Of women with ASC-US / LSIL in follow-up, 30 % had CIN2+. Of women with ASC-H / HSIL cytology in follow-up, 80 % had CIN2+. Among women with positive HPV mRNA test at index cytology or after the first negative biopsy, >70 % had CIN2+ in subsequent biopsies. Not all women had additional HPV mRNA test. More detailed results will be available at the congress. In follow-up of women with negative cervical biopsy, our data suggest diagnostic conization for all women with HSIL cytology or a positive HPV mRNA result.

SS 19-9

**RISK OF CERVICAL CANCER AFTER TREATMENT OF CERVICAL INTRAEPITHELIAL NEOPLASIA:
ROLE OF LOSS TO FOLLOW-UP**

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Objectives: In most countries, women treated for cervical intraepithelial neoplasia (CIN) are recommended frequent follow-up for several years. They nevertheless have a higher risk of cervical cancer than other women. It has been considered, though not demonstrated, that this may be due to non-adherence to follow-up. We aimed to determine whether the excess risk of cervical cancer after CIN treatment is clustered among women lost to follow-up.

Methods: We identified women aged 15-84 years treated with conisation for the first time from 1996 onwards, and determined their follow-up histories until end of 2007 from the Danish National Patient and Health Service Registers, and the Pathology Data Bank. Diagnoses of cervical cancer were identified from the National Cancer Register. Conisations linked to cervical cancer in up to six months post-treatment, i.e. possible prevalent cases, were excluded. We calculated incidence rates of cervical cancer by adherence to follow-up.

Conclusions: In total, 46,020 treated women were included; 109 developed cervical cancer in 235,165 woman-years at risk. Compared to the general Danish population of the same age, treated women had a higher incidence of new CIN or worse only in the first six years after treatment, but the incidence of cancer remained significantly higher throughout the 12 years under study. These data are consistent with previous studies. Thereafter, we stratified the incidence by recency of follow-up prior to cervical cancer. Of the 109 women with cervical cancer, 72 (66%) women had last smear <three years prior to cancer (incidence 34/100,000), 10 (9%) had last smear only ?three years prior (incidence 89/100,000), and 27 (25%) had no follow-up after treatment (incidence 293/100,000). For comparison, the incidence in the general population was 18/100,000. As expected, women who were lost to follow-up indeed had a high risk of subsequent cervical cancer. However, two-thirds of cervical cancers in CIN-treated women were diagnosed among women who had recent smears. Despite their adherence to follow-up these women had twice the risk of cervical cancer as compared with the general population. In conclusion, cervical cancers diagnosed in women who, at least partially, adhered to follow-up contributed significantly to the observed excess risk of cervical cancer in CIN-treated women.

SS 19-10

**VAGINAL INTRAEPITHELIAL NEOPLASIA (VAIN 2/3):
COMPARING CLINICAL OUTCOMES OF TREATMENT WITH INTRAVAGINAL ESTROGEN**

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Objective: To determine the outcomes of women treated for VaIN 2/3 with intravaginal estrogen.

Methods: A retrospective chart review of 98 patients with VaIN 2/3 evaluated at a single institution between 2000 and 2008 was performed. Medical records were reviewed for demographic information, risk factors, HPV status, treatment type, pathologic diagnosis, and outcome information. Patients with VaIN 1 and invasive disease at the time of initial presentation were excluded.

Results: The mean age at diagnosis was 54.0 years (median 52.5, range 17.0-92.0 years). 83.7% of patients were post-menopausal and 88.8% had undergone prior hysterectomy. 59.2% of the patients reported prior treatment for preinvasive disease (cervical, vaginal or vulvar dysplasia). 46.9% reported prior and/or current tobacco use. 46.9% tested positive for high risk HPV subtypes. Treatment modalities included intravaginal estrogen, CO₂ laser ablation, 5-fluorouracil, wide local excision, loop electrosurgical excision procedure, and vaginectomy. Some patients underwent more than one treatment modality. Of those patients treated with intravaginal estrogen alone (n=38), 76.0% had regression or cure of high grade disease. Of those patients treated with intravaginal estrogen and one or more other treatment modalities (n=24), 80.0% experienced regression or cure. In contrast, 9.2% of patients undergoing treatment without intravaginal estrogen experienced regression or cure of high grade disease. The mean length of follow up for all patients was 40.2 months (median 34.0, range 2.0-115.0 months).

Conclusion: This cohort of women with VaIN 2/3 further delineates the demographic and clinical risk factors associated with VaIN 2/3. High rates of regression and cure were found in patients treated with intravaginal estrogen, whether alone or in combination with other treatment modalities.

A HAND-HELD ELECTRICAL IMPEDANCE SPECTROSCOPY DEVICE IMPROVES COLPOSCOPY PERFORMANCE**Tidy J**, Gynaecological Oncology, Royal Hallamshire Hospital, Sheffield, UK.**Brown B**, Medical Physics, University of Sheffield, Sheffield, UK.**Healey J**, Medical Physics, Royal Hallamshire Hospital, Sheffield, UK.**Daayana S**, Gynaecological Oncology, St Mary's Hospital, Manchester, UK.**Martin M**, Colposcopy Clinic, Tallaght Hospital, Dublin, Ireland.**Prendiville W**, Colposcopy Clinic, Tallaght Hospital, Dublin, Ireland.**Kitchener H**, University of Manchester, Manchester Academic Health Science Centre, Manchester, UK.

Objective: To determine if use of an Electrical Impedance Spectroscopy (EIS) device (APX100) as an adjunct to colposcopy, improves performance.

Methods: 474 women with abnormal cytology were enrolled at 3 colposcopy clinics. In phase one, EIS readings were taken from 12 points on the cervix before and after application of acetic acid. By comparing measured EIS spectra with 'finite element models' of cervical tissues, it was possible to derive a probability index for the presence of HG-CIN and a cut-off value for the detection of HG-CIN was derived. In phase two, 12 EIS readings were taken after application of acetic acid. EIS data collection and analyses were performed in real time but the clinician was blinded to the EIS reading. All examinations were video recorded for analysis of performance. 214 were eligible in phase one and 215 in phase two. Average age was 33.2 (median age 30.3; range 20–64) and 48.5% (208/429) had high grade cytology. In phase two, EIS increased the positive predictive value (PPV) to detect HG-CIN from 53.5% to 67% and specificity increased from 38.5% to 65.1%. Increasing the cut-off value of EIS measurements increases the specificity for detection of HG-CIN and can achieve a PPV >95%. Analysis of the receiver operator characteristic (ROC) to detect HG-CIN showed that the area under the curve (AUC) was 0.887 in phase two of the study, an increase on clinical performance alone.

Conclusions: The study demonstrated the safety of APX100. Performance of APX100 is independent of the application of acetic acid. Using APX100, as an adjunct, PPV to predict HSIL can exceed 95%. APX100 is a clinically useful device for the detection of or absence of HSIL. It could prevent over treatment when using a 'see and treat' management strategy and enhance patient care.

SEM 1-1

THE BURDEN OF HEAD AND NECK CANCER AND THE CHANGING RISK FACTORS.**LEFEBVRE JL**, Head and Neck department, Centre Oscar Lambret, Lille, France.

Head and neck cancers represent the 5th cancer in terms of incidence. More precisely, the worldwide incidence of head and neck cancers for both sexes is around 600,000 new cases a year. The death rate by head and neck cancer is approximately 54 %.

• For long three different groups of head and neck cancers were identified:

1. Cancers of the nasal and paranasal cavities

These cancers are quite rare (0.2 % of cancers) with a trend for a decreasing incidence. The annual incidence is around 1 per 100,000 in males and 0.5 per 100,000 in females. Glandular tumors are the most common pathology. These cancers are often due to professional or environmental exposures (wood dust, chemicals in particular). The treatment is surgical whenever possible often with postoperative irradiation. The most frequent failures are local recurrences.

2. Cancers of the nasopharynx

These cancers are very frequent in South-Eastern Asia (in particular the areas of Guangzhou, Hong Kong and Singapore) and in Chinese emigrating from these areas. The annual incidence may be as high as 30 per 100,000 inhabitants. The second area of high incidence is around the Mediterranean Sea and in particular in North Africa (and as for Chinese in emigrants from Maghreb) as well as in Eskimos from Greenland and Alaska. In these areas the incidence is around 15 per 100,000 inhabitants. These cancers are in the vast majority undifferentiated carcinomas. There is no doubt on the role of the Epstein Barr Virus (EBV), of particular genetic profiles and of some diet habits (salted fish in China). The treatment is based on chemotherapy and radiotherapy. Failures are loco-regional relapses and distant metastases.

3. Cancers of the oral cavity, pharynx and larynx

By far they are the most frequent head and neck cancers but with wide geographical variations ranging from less than 5 per 100,000 inhabitants to more than 30 per 100,000 inhabitants. Europe (in particular France) and India have the highest incidences. Latin Europe has a high incidence for larynx cancers. Males have a much higher incidence than females. However there is a notable trend for a decreasing incidence, in particular in males, in industrialized countries. These cancers are squamous cell carcinomas and are due to tobacco consumption and alcohol abuse. In Asia betel nut chewing is a frequent risk factor. Other factors may include some professional exposures (textile, metals, coal dust etc). The treatment is based on either surgery or irradiation for early diseases and on combined therapies (chemotherapy, biotherapy, surgery, and irradiation) for advanced diseases. The outcome remains disappointing due to three risks: disease relapses (local, regional, distant), second primary cancers (head and neck, lung, esophagus, bladder) and co-morbidities (cardiovascular, hepatic, pulmonary, neurologic etc) also induced by lifestyle.

• More recently a fourth entity has been individualized: Human Papilloma Virus (HPV)-associated oropharyngeal cancer

The potential role of HPV in head and neck cancer and in particular in oropharyngeal cancer was underscored in the 80s. A large review of the literature suggested that around 25 % of the oropharyngeal cancers were associated to HPV with some differences between Asia, Europe and the US. It appeared that HPV 16 (and to less extent, HPV18) was the virus type involved in this carcinologic process. In the US while the incidence of head and neck cancers was decreasing, oropharynx cancers was the only one primary site that continued increasing. Rapidly clinical research paid a particular attention to this situation. There are some data from the US suggesting that 2/3 of oropharyngeal cancers could be HPV-associated, or from Scandinavia (the Stockholm area registry) with impressive incidence up to 90 %. In Latin Europe there is no precise data on a large scale at the moment. The patients suffering from this particular disease are younger, with a better performance status, without (or with a minimal) tobacco consumption. Epidemiological studies support a sexual transmission (via bucco-genital contacts). The outcome is much more favorable than for tobacco-induced cancers. These tumors seem to be particularly chemo- and radiosensitive.

SEM 1-2

THE EPIDEMIOLOGY AND BURDEN OF HPV-OROPHARYNGAL CANCER**Professor Hisham Mehanna**

*PhD, BMedSc (hons), MBChB (hons), FRCS, FRCS (ORL-HNS)
 Director, Institute of Head and Neck Studies and Education, UK
 Consultant Head-Neck and Thyroid Surgeon & Honorary Professor*

The proportion of oropharyngeal cancer that is HPV -positive has increased significantly over the last decade. A recent meta-analysis shows that the global rate of HPV positivity has increased from 40.5% before 2000 to 72% after 2005. More recent studies report rates of over 90%. However there is considerable geographic and regional variation. We will discuss the details and impact of these changes in this session.

SEM 1-3

MOLECULAR CARCINOGENESIS OF HPV+ve AND HPV-ve HEAD AND NECK CANCER**Brakenhoff RH, Rietbergen MM, Bloemena E, Snijders PJF, Braakhuis BJM, Leemans CR**

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Squamous cell carcinomas in the head and neck (HNSCC) develop in the mucosal linings of the upper respiratory and digestive tracts. HNSCCs are caused by tobacco smoke exposure and alcohol consumption as well as by human papillomavirus infection. There are considerable differences in the molecular carcinogenesis of HPV-ve and HPV+ve HNSCC. HPV-ve HNSCC often develop within preneoplastic fields in the mucosal epithelium of genetically altered cells. These fields may have dimensions of multiple centimetres and might be visible macroscopically as leukoplakia lesions or microscopically as dysplasia. Initial genetic changes are mutations in TP53 and loss of CDKN2A (p16^{Ink4A}), targeting the p53 and pRb pathways. In previous research it was shown that these changes cause an immortal phenotype in primary oral keratinocytes. Persistence of these fields after treatment forms a major clinical challenge, since it may lead to local recurrences and second primary tumours that are responsible for a large proportion of relapses and deaths.

Molecular studies on these preneoplastic fields and HPV-ve tumors, revealed various genetic changes that accumulate more or less in parallel to the increasing histological changes. Signalling pathways in HNSCC that seem to be activated or inactivated are the epidermal growth factor receptor signalling pathway, the AKT/PI3K pathway, the NOTCH1 pathway, the NFkB pathway and the TGFb pathway, all in subgroups of tumors.

In HPV+ve tumors, there are no mutations in TP53 and no losses of CDKN2A (p16^{Ink4A}). Nonetheless, these same p53 and pRb pathways are targeted by the HPV E6 and E7 oncoproteins. In a conditional immortalized oral keratinocyte model we could recently show that any combination of p53 and pRb pathway inactivation led to an immortal phenotype. What is lacking at present is a genetic progression model of HPV+ve tumors, mainly as precursor lesions cannot be identified. Nonetheless, there are some preliminary indications that these do exist, but the lack of screening programs, visualization tools applicable to the head and neck region, and the location of lesions in the oropharyngeal and tonsillar epithelium, hampers their identification and molecular characterization. In this presentation the state of knowledge on carcinogenesis models in head and neck cancer will be discussed.

ORAL HPV INFECTION, ITS EPIDEMIOLOGY, TRANSMISSION AND SIGNIFICANCE AMONG SPOUSES**Stina Syrjänen, DDS, PhD, FDSRCSEd(hon),***Department of Oral Pathology, Institute of Dentistry, Faculty of Medicine, University of Turku, Finland*

The prevalence of asymptomatic oral HPV infection in adults (i.e., HPV DNA detection) varies between different studies from 0% to 81%, with the mean prevalence rate of 11%. HPV16 is the most prevalent HPV genotype. This wide variation in HPV DNA detection can be explained by the sampling site, sampling method, and HPV testing methods. Discrepancy exists as to the differences in HPV prevalence among the genders. Recent study from US reported that the prevalence of asymptomatic oral HPV infection is most prevalent in age group of 60-64 years and is higher among males (Gillison et al. JAMA, 2012).

The risk factors currently recognized for oral HPV infection include age, sex, number of sexual partners, current number of cigarettes smoked per day, persistent oral HPV infection of the spouse, and deep kissing. In the Finnish Family HPV Study, oral high-risk (HR) HPV detection rate varied from 16% to 27% in both spouses, during the 7-year follow-up. Of the baseline negative spouses, 10% had an incident oral HPV infection. Among those who tested HR-HPV+ at baseline, none of the men and only 5% of the women cleared their HPV within 2 years. Persistent oral HPV infection was detected in 9% of the adults. One can speculate that persistent oral HR-HPV could present a risk for precancer or oral cancer. According to a recent meta-analysis, it is nearly four-fold more likely to detect HPV DNA in oral potentially malignant disorders than in healthy oral mucosa (Syrjänen et al. Oral Diseases, 2011). However, the natural history studies on oral HPV infection are nearly lacking.

Sexual transmission is by far the most common route of spreading HPV. However, detection of HPV in virgins, infants and children suggests that vertical transmission and/or horizontal transmission from mouth to mouth or via fingers to mouth also exists, but the exact rates and routes have not been established. The importance of sexual transmission is confused by the fact that the HPV-type concordance between oral and genital infections seems to be poor among the sexual couples, and even so in the same person. Evidence also exists that oral HPV infection can be transmitted from mouth to mouth between the spouses.

PROGNOSTIC FACTORS OF HPV POSITIVE CASES AND SEROLOGICAL MARKERS IN FOLLOW UP**Jan Klozar¹, Ruth Tachezy², Eva Hamsíková², Eva Koslabová^{1,2}, Eliska Rotnáglová¹,***1 Department of Otolaryngology Head and Neck Surgery, 1st Faculty of Medicine, Charles University, Prague;**2 Department of Experimental Virology, Institute of Hematology and Blood Transfusion, Prague*

The overview presents recent knowledge and own results about two aspects of HPV relevance in head and neck (HN) oncology: 1. The influence of HPV on survival and different pattern of prognostic factors in HPV positive tumors, 2. The possible use of HPV specific antibodies in the follow up of patients with HPV positive tumors.

From clinical point of view the most important difference is the better prognosis of HPV positive patients. This is probably not treatment related. The traditional prognostic markers like extent of tumor and in particular nodal involvement seem to play a much less important prognostic role in HPV positive tumors.

The association of the presence of antibodies specific to HPV16 E6 / E7 antigens with the positivity of the tumor tissue for HR HPV DNA is statistically highly significant. In patients seropositive at enrolment the mean titers of HPV 16 E6 / E7 antibodies on the follow-up decrease, which is in contrast to the HPV VLP antibodies. The decrease of titers of HPV 16 E6 / E7 antibodies mostly corresponds with the absence of local – regional recurrence. These results suggest that serological methods could play a role in follow up of patients.

SEM 1-9

THE CHALLENGES OF PROSPECTIVE RESEARCH IN OROPHARYNGEAL CANCER**Professor Hisham Mehanna**

*PhD, BMedSc (hons), MBChB (hons), FRCS, FRCS (ORL-HNS)
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 Consultant Head-Neck and Thyroid Surgeon & Honorary Professor*

There is considerable interest in undertaking studies of interventions specifically tailored to patients' HPV status in HNC. However designing these studies can be very challenging, due to the fact that HOV + patients have very good outcomes. Therefore treatments are unlikely to result in additional benefit but are likely to be aimed at achieving the same survival benefit but with less toxicity.

This necessitates non inferiority designs which require large sample sizes, often not achievable in HNC populations. we discuss the obstacles and the potential solutions.

SEM 1-10

THERAPEUTIC VACCINATION IN HPV-ASSOCIATED HEAD & NECK CANCER – FACT OR FICTION?**T.-C. Wu, M.D., Ph.D,**

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It is now clear that approximately 20-30% of head and neck cancers are associated with human papillomavirus (HPV). The characterization of HPV typing in these cancers revealed that most of these cancers are associated with high-risk HPV-16. Furthermore, most of HPV-associated head and neck tumors are derived mainly from the oropharynx, including the tonsil and base of the tongue. HPV-16 has been shown to be a prognostic factor for enhanced overall and disease progression-free survival and might also be a predictive marker of response to treatment. Furthermore, the clear understanding of HPV as a necessary cause and the pathogenesis for the subset of head and neck cancer has created an opportunity to control cervical cancer through vaccination against HPV. Currently various forms of therapeutic vaccines targeting HPV-16 oncogenic proteins E6 and E7 antigens are currently being developed in preclinical models. Because HPV oncogenic proteins E6 and E7 are constantly expressed in the majority of HPV-associated malignancies and they are responsible for the malignant transformation and progression of HPV-associated cervical cancer, they represent ideal targets for the development of therapeutic HPV vaccines for the control of HPV-associated head and neck cancers.

The encouraging results from some of these preclinical studies have led to their translation to clinical trials.

SURGICAL TREATMENT OPTIONS FOR OROPHARYNGEAL CANCER

LEFEBVRE JL,

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Even if most of oropharyngeal tumors are treated by irradiation, there is still a large room for surgery either as the initial treatment or for salvage. In addition the tremendous improvements in reconstructive surgery have substantially reduced the post surgical functional and cosmetic sequels.

1) Transoral surgery

The oropharynx, in particular the soft palate and the tonsillar fossa are easily accessible transorally in case of limited tumors. For a long time electrocautery was used for transoral surgery but since the mid-70s the laser CO₂ knife has been more often used. If these techniques were in selected cases good alternatives to open surgery, their indications remained quite limited for the oropharynx due to the restricted access to the base of tongue and the glosso-tonsillar sulcus; More recently the transoral robotic surgery (TORS) has proven its reliability. This new tool provides an excellent visualization in all directions, with a magnified 3-dimensional view, allowing access to difficult areas and to vessels and therefore increasing the indication of transoral surgery.

2) Open surgery

There are two main approaches:

- The transmandibular approaches.

The lateral access to the oropharynx is compromised by the mandible. For long a hemi-mandibulectomy was requested to allow an oncological satisfactory resection of the tumor in continuity with the lymph nodes (the so-called commando procedure). More recently the mandibular swing approach provided the same access to the lateral oropharynx but preserving the mandibular arch via the osteosynthesis performed after tumor resection.

- The submandibular approaches.

Via the opening of the larynx by an anterior access between the hyoid bone and the base of tongue, a resection of the posterior wall of the oropharynx may be performed. Another technique is a supraglottic laryngectomy extended to the base of tongue for the removal of a tumor of the valleculae.

3) Reconstructive surgery

Limited resections most of the time do not require reconstruction. Local mucosal flaps (such as the FAMM, facial artery musculo-mucosal, flap) may be used in some cases. Pedicled flaps (dorsal flap, trapezius flap or the most commonly used: the major pectoralis myo-cutaneous flap) have significantly improves volume and surface reconstruction as well as prevention of postoperative complications in case of salvage surgery in irradiated fields. The introduction of microvascularized free flaps was an important milestone in oropharyngeal surgery. Some flaps are able to reconstruct a mucosal surface (radial forearm free flap) or tongue volume (dorsal free flap, anterolateral thigh free flap) or the mandible (iliac crest free flap, scapular free flap or preferably the fibula free flap). This means the surgery must be considered for decision making in oropharyngeal cancers. Based on a thorough clinical evaluation and a precise imaging mapping (MRI and CTscan) a multidisciplinary tumor board discussion may select the most appropriate therapeutic programme for each particular case.

SETTING UP DIAGNOSTIC SERVICE FOR HPV – HEAD & NECK CANCER

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Objective. For long an association between HPV and head neck cancer was suspected and eventually HPV was disclosed, and in 2007 also accepted as a risk factor for oropharyngeal squamous cell carcinoma (OSCC) by the IARC. In Western countries, due to an HPV epidemic the incidence of HPV+ OSCC has increased. In contrast, due to reduced smoking, the incidence of some head & neck cancer has declined. Moreover, patients with HPV+ OSCC have a better 5-year disease specific survival than those with HPV- OSCC and this is of clinical significance, since recently treatment for head & neck cancer has been intensified with induction chemotherapy and intensified radiotherapy. It is doubtful if intensified treatment, with also increased side effects, is beneficial for all patients with HPV+ OSCC. There, is thus a need to define both HPV status and other diagnostic markers that in combination can better predict response to treatment in HPV+ OSCC.

Methods. Presence of HPV can e.g. be analyzed by Southern blot, in situ hybridization, or immunohistochemistry for HPV E6, E7 or L1. However, these techniques are generally less sensitive than PCR based techniques, while the latter may due to their sensitivity detect HPV DNA that is nonfunctional in the tumors. For function, testing of HPV E6, E7 mRNA, or p16 expression may be useful. In fact, in the past p16 was used as a surrogate marker for HPV, but the correlation between HPV and p16 is not absolute, and accumulated clinical data on OSCC using p16 as a surrogate marker for HPV has not been acknowledged as being sufficiently accurate. So which tumors should we analyze, what should we test for, and which methodology should we set up?

Conclusion. Using a PCR based technique to detect HPV DNA in combination with expression of HPV E6 and E7 mRNA would be preferable. If this is not possible, the combination of PCR for detection of HPV DNA together with evaluation of p16 expression by immunohistochemistry could be useful. Nonetheless, even this is not sufficient to predict whether all patients with HPV+ OSCC will respond to treatment. The tonsil and base of tongue location is important, since OSCC outside these locations may less frequently be HPV+. Combining diagnosis of HPV functional status with additional markers, such as e.g. CD8, FoxP3, HLA class I expression, or CD44 may be helpful for even better predicting response to treatment and for individualizing therapy.

SEM 1-14

PITFALLS IN THE DIAGNOSIS OF HPV IN HEAD & NECK CANCER

**Peter J.F. Snijders¹, Michelle Rietbergen², Daniëlle A.M. Heideman¹, Bart Hesselink¹,
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It is currently widely accepted that detection of HPV DNA in head and neck squamous cell carcinoma (HNSCC) by PCR alone is insufficient to prove causality. This is supported by the fact that expression of HPV E6/E7 region mRNA, which is considered necessary for HPV-mediated carcinogenesis, is far less common in HNSCC than viral DNA detection by PCR. Moreover, HPV DNA positive tumors without E6/E7 mRNA are reminiscent of HPV DNA negative tumors in terms of chromosomal profiles, which are clearly different from HPV E6/E7 mRNA positive tumors. Previously, we evaluated various methods for their capability to detect biologically relevant HPV infections in formalin-fixed tissue specimens of HNSCC, using E6/E7 mRNA detectability on the corresponding frozen tissue counterpart as gold standard (Smeets et al., IJC 121:2465-72, 2007). The assays included type-specific short fragment E6/E7 reverse transcriptase (RT)-PCR, HPV DNA FISH, high-risk HPV consensus primer GP5+/6+-PCR and p16^{INK4A} immunostaining. Detection of E6/E7 region mRNA by RT-PCR appeared highly sensitive and specific. Other methods suffered either from suboptimal sensitivity or specificity for clinically meaningful HPV infections; p16^{INK4A} immunostaining and GP5+/6+-PCR were highly sensitive but not 100% specific, whereas DNA FISH, being highly specific, was less sensitive. Since short fragment RT-PCR is technically challenging we investigated combinations of the other, easier assays, in order to reach high accuracy for clinically relevant infections. It appeared that the algorithm that firstly uses p16^{INK4A} immunostaining, followed by consensus high-risk HPV GP5+/6+-PCR fulfilled this criterion. Recently, we have tested this algorithm on an independent test set consisting of a panel of 90 consecutive oropharyngeal SCCs from which both formalin-fixed and frozen tissue was collected. E6/E7 RT-PCR on the frozen samples served as gold standard. The algorithm showed an accuracy of 98% (sensitivity: 95%, specificity: 99%). In conclusion, the accuracy of this test algorithm could be confirmed in an independent series.

SEM 1-15

SCREENING FOR ORAL AND OROPHARYNGEAL CANCER

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Objective: To describe the role of screening in the early detection and control of oral and oropharyngeal cancer.

Methods: Findings and conclusions are described based on review of published studies.

Findings and conclusions: Oral and oropharyngeal cancer incidence and mortality are high in the Indian sub-continent, Papua New Guinea, Taiwan Eastern Europe, France and parts of South America. A causal association between human papillomavirus (HPV) infection and oropharyngeal cancer is increasingly recognised and an increasing trend in the incidence of oropharyngeal cancer has been reported from countries such as Canada (1.5% annual increase in men and 0.8% in women during 1992-2007), United States and Western Europe. Whereas HPV-related oropharyngeal squamous cell cancer is characterized by a distinct risk factor profile, a relatively consistent microscopic appearance, and a favorable prognosis, oral cancer is often preceded by clinically detectable precancerous lesions. Early oral and oropharyngeal cancers present as small indurated ulcer, surface thickenings, nodules, reddish velvety areas or ulceroproliferative growths. Early neoplasia can be readily detected by trained clinicians, nurses and auxiliary health workers, by a systematic visual oral inspection and by palpation. Oral visual inspection is a sensitive and specific test to detect oral neoplasia. In a randomized screening trial in India, three rounds of oral visual screening at 3-year intervals were followed by 33% reduction in oral cancer mortality among tobacco and/or alcohol users. Currently the routine use of adjunctive screening tests such as visualization aids, cytology and HPV testing cannot be recommended. No organized population-based oral cancer screening programmes exist in developed countries. Despite the highest risk of oral cancer, no screening programs exist in India, Pakistan and Sri Lanka. The impact of on-going national oral cancer screening programmes in Cuba and Taiwan on disease burden is not clear. It has been suggested that HPV-related non-cervical cancers (penile, anal, and oropharyngeal) may exceed cervical cancer within a decade in industrialised countries and thus preventing the non-cervical HPV cancers in men will require either "herd" immunity through high-vaccination rates in females or male vaccination in future.

EPIDEMIOLOGY AND BURDEN OF GENITAL WARTS

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Objectives and Methods: To describe the epidemiology and burden of genital warts, which are the commonest viral sexually transmitted disease seen in sexual health clinical settings. In some Western societies clinically apparent warts occur in around 1% of the population, with an estimated lifetime risk of developing genital warts being in the order of 10%. Genital warts have a short incubation period (median 2.8month, range 1-20 months), are readily transmissible (two thirds of those with overt lesions will transmit to a partner), are most commonly seen in <30 year-olds, with > 90% being caused by HPV 6/11. Treatment is relatively crude, being patient or clinician applied, and either ablative (eg freezing, surgery), chemotoxic (eg podophyllotoxin), or immunomodulatory (imiquimod). Relapses are common. Genital warts cause considerable psychosocial burden, as well as sizeable economic costs. The prophylactic HPV vaccine incorporating viral-like-particles for 6 and 11 have been shown protective for genital warts in those naive to 6,11. Where the quadrivalent vaccine coverage is high (in Australia ~70% of the vaccine eligible age received three doses), genital wart reduction is remarkable (73% reduction in genital warts in young women, and within a herd immunity effect in young men showing 38% reduction).

Conclusion: Whilst genital warts are very common and essentially incurable, prophylactic vaccination against HPV 6/11 has the potential to virtually eliminate this disease.

EPIDEMIOLOGY AND BURDEN OF VIN

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Vulvar intraepithelial neoplasia (VIN) and invasive vulvar cancer incidence increases annually by 2.5% mainly in young women. In the United States and Norway the incidence of the VIN increased by 411%.

The terminology of VIN has been changed lately (Table A). In the Netherlands, the incidence of usual VIN almost doubled from 1.2/100,000 patients in 1992 to 2.1/100,000 in 2005 and that of differentiated VIN increased nine-fold from 0.013/ 100,000 patients to 0.121/100,000. In contrast, the incidence of invasive vulvar cancer increased by only 20% from 1973 to 2000.

Young women suffer more: the incidence of the VIN increases until the age of 40-49 years and then decreases. Invasive vulvar cancer increase occurs only after 50 years of age. The association between the VIN and the human papillomavirus (HPV) is different from that in the cervix: only 30-40% of invasive cancers of the vulva are related to HPV, while the other carcinomas are related to other causes, mainly chronic irritation and pruritus, such as that associated with vulvar lichen sclerosus.

The incidence of vulvar cancer in white women older than 50 years of age is three times than that in black women and Hispanic women. For women under 50 years, the rates of invasive squamous cell cancer are similar between whites and blacks.

Vaccination against HPV has shown to effectively prevent development of VIN, and hence, VIN and vulvar cancer incidence may decrease in the next years.

Table A: VIN terminology

| PREVIOUS TERMINOLOGY | NEW TERMINOLOGY (ISSVD 2004) |
|--|--|
| VULVAR INTRAEPITHELIAL NEOPLASIA (VIN) | VULVAR INTRAEPITHELIAL NEOPLASIA (VIN) |
| 1. Squamous type (with or w/o HPV change) | VIN, usual type: |
| a. VIN I | VIN, warty type |
| b. VIN II | VIN, basaloid type |
| c. VIN III (Formerly: Squamous cell CIS, Bowen's disease, Erythroplasia of Queyrat, CIS simplex) | VIN, mixed (warty/basaloid) type |
| | VIN, differentiated type |
| 2. Nonsquamous type | |
| a. Paget's Disease | |
| b. Melanoma in Situ | |

SEM 2-3

MANAGEMENT OF GENITAL WARTS**Gerd Gross,***Department of Dermatology and Venereology, University of Rostock (Germany)*

HPV is one of the most common sexually transmitted infections among women and men. HPVs associated with external genitoanal lesions have been divided into low-risk and high-risk HPV types. Manifestations of genitoanal HPV infection comprise latent infections, subclinical infections and clinically benign genitoanal warts related to low-risk HPVs. Vulvar and penile cancer as well as the precursor lesions VIN and PIN have been associated in about 40 to 50 % of cases with high-risk HPVs. About half of vulvar and penile cancers are developing in association with chronic lichen sclerosus and are HPV negative. Genitoanal warts harbour in > 90% HPV 6 and HPV 11. About 20-44% of these benign lesions show coinfections of HPV6, HPV11 and high risk HPV types with HPV 16 the most frequently detected type. Early diagnosis and consequent treatment will contribute to reduce the burden of genitoanal warts and of intraepithelial neoplasias (vulvar intraepithelial neoplasia-VIN, penile and perianal intraepithelial neoplasia – PIN, PAIN). Diagnosis of genitoanal warts requires exclusion of other sexually transmitted conditions, 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 imiquimod and topical green tea derivatives, therapy of genitoanal warts remains a medical problem. Both physician- and self-administered therapies are not fully satisfactory. Several current therapies show high recurrence rates. The main progress achieved with imiquimod and green tea catechins, however, has been lowering the recurrence rate of genitoanal warts.

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

SEM 2-4

MANAGEMENT OF VIN**Hampel M***Department of Obstetrics and Gynecology, University Hospital of Düsseldorf; Germany*

The incidence of squamous cell intraepithelial neoplastic lesions of the lower female genital tract is increasing in the last years. A generalized infection of the female genital tract with human papillomavirus (HPV) is considered to be the cause of this disease and since HPV is transmitted by sexual intercourse in the majority of cases, multicentric intraepithelial dysplasias are most prevalent in young, sexually active women. Also cervical intraepithelial lesions are predominant, increasing rates of vulvar, anal and vaginal lesions are observed, often in combination.

It is well established that oncogenic subtypes of HPV are a necessary cause of cervical cancer and its immediate precursor lesions (CIN 2-3), whereas vulvar and anal cancer seem to have a dual cause: part of them are HPV-related, and some are not.

The most common precursor lesions on the Vulva are VIN lesions. In about 90% of cases, VIN lesions are HPV positive these lesions are called "classic" VIN, they are undifferentiated, non keratinized squamous intraepithelial lesions, either condylomatous, basaloid or mixed in histology and typically diagnosed in young women with multifocal disease on the vulva. HPV 16, 31, 33 and 6 were the most prevalent subtypes identified in VIN lesions.

In contrast, there is the rare, differentiated VIN in older women, HPV negative, developing in women with lichen sclerosus on the vulva. These lesions are unifokal and hard to diagnose.

Diagnosis of VIN is made by inspection, colposcopy and histology by punch biopsy.

Treatment consists of local excision in sano, laservaporisation after histology or immunotherapy with imiquimod, which is not officially licensed for this disease and therefore used in "off label use".

The treatment of VIN with imiquimod should only be indicated in experienced centers for this disease and under stringent follow up with colposcopy and re-biopsy in case of progress or persistence.

EXTERNAL GENITALIA AND HPV ASSOCIATED DISEASE: PREVENTION

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Background and Objective: Infection and diseases caused by human papillomavirus (HPV) are common in men. To date only one Phase III HPV vaccine efficacy trial has been conducted among males. The purpose of this trial was to test the safety of the quadrivalent HPV (types 6/11/16/18) vaccine (qHPV) and its efficacy against external genital lesions (EGL) and anogenital HPV infection in males.

Methods: A total of 4,065 healthy men (3,463 heterosexual men and 602 men who have sex with men) aged 16-26 years were enrolled from 18 countries into a randomized, placebo-controlled, double-blind trial. The primary efficacy objective was to demonstrate that qHPV vaccine reduced the incidence of HPV 6-, 11-, 16-, or 18-related EGL. Efficacy analyses were conducted in subjects receiving all three vaccinations naïve to the relevant HPV type at enrollment (PPE), and subjects receiving vaccine/placebo, regardless of baseline HPV status (ITT).

Results: Efficacy against HPV 6/11/16/18 EGL in the ITT population was 65.5% (95% CI: 45.8, 78.6) and against any EGL was 60.2% (95% CI: 40.8, 73.8). Efficacy against HPV 6/11/16/18-related EGL in the PPE population was 90.4% [95% CI: 69.2, 98.1]). Efficacy against HPV 6/11/16/18-related persistent infection and DNA detection at any time was 47.8% (95% CI: 36.0, 57.6) and 27.1% (95% CI: 16.6, 36.3), respectively in the ITT population and 85.6% (97.5% CI: 73.4, 92.9) and 44.7% (95% CI: 31.5, 55.6), respectively in the PPE population. Injection-site pain occurred significantly more frequently among those receiving qHPV vaccine vs. placebo (57% vs. 51%; $p < 0.001$).

Conclusions: Quadrivalent HPV vaccine effectively prevents HPV 6/11/16/18-related EGL and infection in men aged 16-26 years.

EPIDEMIOLOGY OF PAPILLOMAVIRUS INFECTION AND SKIN CANCER IN ORGAN TRANSPLANT RECIPIENTS

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Cutaneous squamous cell carcinoma (SCC) is the most common malignancy in organ transplant recipients (OTR), with an approximately 100-fold increased incidence compared to the general population. Functional data support a potential role for betaPV as co-carcinogens with ultraviolet light. Beta-papillomavirus (betaPV) abrogation of UV-induced apoptosis and interference with DNA repair and cell cycle arrest may contribute to skin carcinogenesis.

We examined the association between betaPV infection and SCC in Dutch, English, French and Italian OTR. A total of 210 OTR with previous SCC and 394 controls without skin cancer were included. The presence of 25 betaPV types in eyebrow hair follicles was determined using a human papillomavirus (HPV) DNA genotyping assay, and antibodies for the 15 most prevalent betaPV types were detected using multiplex serology. We used multivariate logistic regression models to estimate associations between various measures of betaPV infection and SCC.

BetaPV DNA was highly prevalent (>94%) with multiple types frequently detected in both groups. There were non-significant positive associations between betaPV DNA and SCC and betaPV antibodies and SCC. We found a significant association between SCC and the concordant detection of both antibodies and DNA for at least one betaPV type (adjusted OR 1.6; 95% CI 1.1;2.5). A borderline-significant association with SCC was found for HPV36 (adjusted OR 2.4; CI 1.0;5.4), with similar associations for HPV5, HPV9 and HPV24.

These data provide further evidence of an association between betaPV infection and SCC in OTR, but BetaPV is not an obligate risk factor for SCC and at most is likely to be a co-factor, of less importance than UV-damage. Assessing the actual level of risk conferred by betaPV in skin cancer is complicated by the multiplicity of different betaPV types carried in any one person, especially when that person is immunosuppressed.

*Members of the EPI-HPV-UV-CA group are presented in the American Journal of Transplantation 2011; 11: 1498–1508.

SEM 2-8

**MOLECULAR MECHANISMS OF BETA HUMAN PAPILLOMAVIRUSES
IN INDUCING KERATINOCYTE TRANSFORMATION****Massimo Tommasino,***Infections and Cancer Biology Group, IARC-WHO, Lyon, France*

More than 120 different human papillomavirus (HPV) types have been isolated so far, and they can be sub-grouped in cutaneous or mucosal according to their ability to infect the skin or the mucosa of the genital or upper-respiratory tracts. A sub-group of human mucosal HPVs, referred to as high-risk (HR) HPV types, is linked to cervical cancer and a sub-set of other genital cancers as well as oropharyngeal cancers.

In addition, emerging lines of evidence indicate that cutaneous human papillomaviruses (HPVs) of the genus beta are associated with the development of squamous cell carcinoma. Beta HPV types were first isolated in patients suffering from a rare autosomal recessive cancer-prone genetic disorder, epidermodysplasia verruciformis (EV), and are consistently detected in non-melanoma skin cancer (NMSC) from EV, immuno-compromised and normal individuals. However their precise and direct role in skin carcinogenesis in normal population is still under debate.

Studies on the mucosal HR HPV types have demonstrated that E6 and E7 are the major viral oncoproteins impacting on critical pathways in cell transformation and immune evasion. Similarly, the characterization of beta HPV E6 and E7 biological properties revealed their transforming activities in in vitro and in vivo experimental models. In particular, E6 and E7 from beta HPV types 38 and 49 induced immortalization of the primary human keratinocytes, the natural host of the virus. Both beta HPV types altered the cell cycle checkpoints targeting the product of the tumour suppressor gene retinoblastoma (pRb). In addition, they were able to inhibit the p53 functions, but with distinct mechanisms. HPV49, similarly to HR HPV16, promoted p53 degradation via the proteasome pathway. In contrast, HPV38 induced the accumulation of Np73 that in turn inhibited the capacity of p53 to induce the transcription of genes involved in growth suppression and apoptosis. Interestingly, HPV38 has developed two distinct mechanisms to increase the Np73 levels impacting on transcription regulation and protein stability.

Several of these novel mechanisms will be described in the presentation.

SEM 2-9

SKIN CANCER DEVELOPMENT IN BETA-HPV TRANSGENIC MICE**Pfister H.,***Uniklinik Köln, Institute of Virology, Cologne, Germany*

To evaluate the oncogenic potential of the betaPV HPV8, transgenic mice were generated expressing the complete early genome region (CER) or the E6 or E2 gene alone under control of the K14 promoter. All CER- and E6-mice spontaneously developed multifocal skin tumors and in about 6% of the cases squamous cell carcinomas. Infundibular hyperplasia and acanthosis spontaneously developed in a fraction of E2 mice and highly invasive squamous cell carcinomas with more or less pronounced spindle cell morphology later in life. Various observations point to the critical importance of the HPV8 oncogene transcription rate for oncogenesis. 1. The rate of tumor formation in three E2-transgenic mouse lines correlated with the different E2-mRNA levels (about A=6:B=3:C=1), e.g. all line A animals developed skin tumors within one year but only 60% of line B animals within their second year of life (1). 2. A single UV-irradiation of shaved skin increased HPV8 mRNA levels about 10-fold within 2 days and led to skin tumor growth within 3 weeks in all CER- and E6-mice and in 87% of E2B-mice (1-3). 3. A correlation between m-RNA and tumor induction was also noted after mechanical wounding (3). 4. A knock-down of E6 mRNA by tattooing HPV8-E6-specific siRNA led to a delay and a lower incidence of papilloma development (3). All these results suggest that starting from a low basal expression of viral genes in transgenic animals a certain threshold has to be transgressed to induce skin tumors.

(1 Pfefferle et al. *J Invest Dermatol* 128, 2310, 2008; 2 Marcuzzi et al *J Gen Virol* 90, 2855, 2009; 3 Hufbauer et al *Virology* 403, 128, 2010)

HPV – SKIN DEEP AND BEYOND

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In spite of the fact that Human papillomavirus (HPV)-genital infections represent a spectrum of significant dermatovenereological disorders acquiring attention of the both components of this specialty, the interdisciplinary team of different medical specialists should be involved in the comprehensive management of this complex issue. Anogenital warts (condylomata acuminata) are the most common HPV lesions, however, during the last decade the other HPV-associated exaggerated lesions such as condylomata plana, penile, scrotal, and anal intraepithelial neoplasias, as well as the penile, urine bladder and prostate cancer have been studied a little bit more extensively. Consistent studies are still sparse for male population. In general, the focus of scientific interest concerning the HPV infections has recently evolved from sexually transmitted diseases (STD) to different skin disorders such as seborrheic keratoses, skin cancers, psoriasis and beyond, not to speak about the laryngeal papillomatosis, and/or oral and tonsillar cancers.

More than 35 types of HPV infect the genital tract; types 16 and 18 inducing about 70% of high-grade intraepithelial genital neoplasias, such as penile, anal, scrotal, vulvar, vaginal etc. (thus not only cervical), and HPV 6 and 11 causing 90% of anogenital warts. However, the "banality" of anogenital warts should not be underestimated providing that the high risk HPV DNA 16 and 18 can be isolated (PCR) from "benign" HPV-associated genital lesions (anogenital warts) in 10-20% of patients, i.e. more than it is usually expected. On the other hand, the presence and the recalcitrant course of HPV DNA 6 and 11 associated diseases represent a significant physical and psychological problem for both men and women.

A prophylactic vaccine that targets these types should thus substantially reduce the burden of HPV-associated clinical diseases. It can be thus concluded that the HPV-infections represent a significant dermato-venereological issue, and the dermatovenereologists should definitely be the part of the HPV vaccine programme team.

THE EPIDEMIOLOGY OF ANAL HPV AND ASSOCIATED DISEASE IN HOMOSEXUAL MEN

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Objective: To summarise the literature on the prevalence and incidence of anal HPV-associated dysplastic and malignant disease in homosexual men.

Methods: We searched Pubmed, OVID Medline, and Embase for all studies published prior to Nov 1, 2011, that reported prevalence and incidence of anal HPV detection, anal intra-epithelial neoplasia (AIN), and anal cancer in MSM. We calculated summary estimates using random-effects meta-analysis.

Results: 53 studies met the inclusion criteria, including 31 estimates of HPV prevalence, 19 estimates of cytological abnormalities, eight estimates of histological abnormalities, and nine estimates of anal cancer incidence. Data for incident HPV and high-grade AIN were scarce. In HIV-positive men, the pooled prevalence of anal HPV-16 was 33.8% (95% CI 29.0–38.6). In the only published estimate, incidence and clearance of anal HPV-16 occurred in 13.0% (9.6–17.6), and 14.6% (10.2–21.2) of men per year, respectively. The pooled prevalence of histological high-grade AIN was 29.1% (22.8–35.4) with incidences of 8.5% (6.9–10.4) and 15.4% (11.8–19.8) per year in two estimates. The pooled anal cancer incidence was 45.9 per 100 000 men (31.2–60.3). In HIV-negative men, the pooled prevalence of anal HPV-16 was 12.5% (9.8–15.4). Incidence of HPV-16 was 11.8% (9.2–14.9) and 5.8% (1.9–13.5) of men per year in two estimates. The pooled prevalence of histological high-grade AIN was 21.5% (13.7–29.3), with incidence of 3.3% (2.2–4.7) and 6.0% (4.2–8.1) per year in two estimates. Anal cancer incidence was 5.1 per 100 000 men (0–11.5; based on two estimates). There were no published estimates of high-grade AIN regression.

Conclusion: Anal HPV and anal cancer precursors were very common in MSM. However, on the basis of restricted data, rates of progression to cancer seem to be substantially lower than they are for cervical pre-cancerous lesions. Large, good-quality prospective studies are needed to provide natural history data to inform the development of anal cancer screening guidelines for homosexual men.

SEM 3-2

THE NATURAL HISTORY OF ANAL HPV INFECTION AND DISEASE**Lacey, C.,***Centre for Immunology & Infection, Hull York Medical School, University of York, UK*

Anal HPV infections are not only frequent in men who sex with men (MSM) who give a history of receptive anal intercourse (RAI), but also in both men who have sex with women (MSW) and women without such a history of RAI. In unselected populations, prevalence rates of 47% in MSM, 12% in MSW and 27% in women of anal HPV infection have been recorded.

A small number of prospective studies of anal HPV infection have been published. Incidence rates of anal HPV infection of 0.31 in MSM, 0.10 in MSW, and 0.23 in women per 100 person years respectively have been recorded. In women a median duration of anal HPV infection of 0.41 years (95% CI 0.41-0.67) has been recorded. In a prospective study 32% of MSM and 4% of MSW had a persistent anal HPV over 6 months follow up. Current cigarette smoking and RAI are risk factors for HPV persistence among both MSM and women. Incidence, prevalence and persistence rates of anal HPV infection are even higher in MSM with HIV infection.

Much less data are available concerning the natural history of anal intra-epithelial neoplasia (AIN), and the data that are available are observational and confounded by selection bias. In two series of AIN3 cases 3/35 (9%) and 8/72 (11%) progressed to invasive anal cancer over 5.2 & 7 yrs respectively.

SEM 3-3

HPV AND AIN**Pepe Mullerat,***FRCS, Consultant Colorectal Surgeon, Director of Surgical Training, Basildon University Hospital, Basildon, UK*

The relationship between HPV and anal lesions mimics that of HPV and genital lesions. Genital HPV types are now being coined anogenital HPV types.

Anogenital HPV cause benign lesions (anal warts) - mainly HPV 6 and HPV 11, and carcinogenic strains (mainly HPV 16, HPV 18, HPV 31, HPV 33 and HPV 35 amongst others) cause anal dysplasia-anal SCC.

Since the early 1970's, HPV has been related to anal cancer. In 1987, the St Mark's group in London identified HPV serotype 16 as carcinogenic for anal cancer (Palmer, Lancet). Since then, with the development of increasingly better blotting techniques, and, in the late 1990's, with the introduction of more sophisticated type-specific PCR methods of detecting viral DNA – PCR, the relationship between carcinogenic strains of HPV with anal dysplasia and anal cancer has become more robust.

Ten years ago we carried out a study to characterise the distribution of the specific subtypes of HPV in the varying grades of anal dysplasia. We found that all (100%) patients with invasive anal SCC and 93% HG-AIN lesions contained carcinogenic HPV DNA types. HPV DNA types were also present in 82% of LG-AIN lesions and 27% of warts presented carcinogenic HPV types.

Identifying patients infected with carcinogenic HPV types could simplify the management of these lesions, and focus the efforts towards the diagnosis in high risk groups.

MANAGEMENT OF BOTH ANAL INTRAEPITHELIAL NEOPLASM (AIN) AND ANAL CANCER**Laurent Siproudhis,***Service des Maladies de l'Appareil Digestif , CHU PONTCHAILLOU, RENNES, FRANCE*

Anal squamous cell carcinoma and anal dysplasia are both related to human papillomavirus infection in western countries. Usually HPV infections are transient events where persistent infection induces dysplasia and invasive cancer in predisposed subjects (HIV populations, Smokers, MSM and those with a previous history or cervix carcinoma). In patients suffering from co-infection with HIV, the lesions are usually extensive with a faster progression of AIN.

Surgical excisions are preferred in carcinomas of the anal margin with limited extension. AIN with high grade component is usually treated with electro cautery or infrared coagulation when it is located in the anal canal. Alternatives are represented by imiquimod application in the lower part of the anal canal. Invasive carcinomas of the anal canal are treated with chemo radiotherapy.

Despite electro cautery and staged therapy, AIN recurrence occurs in 60% and the disease persists in 20%, especially in immunocompromised. The 5-year survival rate of invasive carcinoma was around 50 to 70% according to the TNM staging. However, loco regional failure rates vary between 10 and 30%: half has a true recurrence where others have persistent disease after the end of the radiotherapy. Most of recurrences are observed within the first 2 years of follow-up. During this period, endo anal ultrasound, MRI and Pet-scan are useful helps to clinical examination.

ANAL HPV INFECTION AND ITS CONSEQUENCES: PREVENTION AND PERSPECTIVES**Palefsky J,***University of California, San Francisco, CA, USA*

Like cervical cancer, anal cancer is associated with HPV infection, and proceeds through a series of precursor lesions such as intraepithelial neoplasia. High-grade anal intraepithelial neoplasia (HGAIN) is the likely precursor to anal cancer, and efforts to prevent it (primary prevention through HPV vaccination) or treatment of HGAIN (secondary treatment) may reduce the incidence of anal cancer. Recently a trial of the efficacy of the quadrivalent HPV vaccine to prevent AIN was published (Palefsky JM et al. NEJM 2011, 365: 1576-85). The results showed a 77% reduction in incident HGAIN among vaccinated HIV-uninfected MSM compared with the placebo group in the per-protocol analysis and more than 90% reduction in persistent anal HPV infection with vaccine HPV types. Based on this and other studies, vaccination of boys aged 9-21 years is recommended routinely, as it is in girls, with permissive vaccination of boys 22-26 years. Prevention of anal cancer has also been added to the list of indications for use of the quadrivalent vaccine in girls and women age 9-26 years.

Secondary prevention of anal cancer relies on screening for and identification of HGAIN, followed by treatment to remove the lesion. There are several different approaches to screening, depending on the clinical setting and prevalence of the disease in the screening population. Many experts examine at-risk individuals with high resolution anoscopy (HRA) and HRA-guided biopsy. Individuals at risk include those with a history of receptive anal intercourse, HIV infection, solid organ transplant, history of high-grade CIN or VIN, cervical or vulvar cancer and history of perianal condyloma. Given limitations in HRA resources, many clinicians triage their at-risk patients for HRA based on the results of a screening anal cytology. Future studies are needed to document the efficacy of primary and secondary prevention methods to reduce the incidence of anal cancer. All at-risk patients should also undergo a digital anorectal exam to palpate masses that may be anal cancer. Treatment of HGAIN relies on the physical removal of the lesion, and several different methods may be used, depending on the clinical setting, extent, number and location of lesions. Post-treatment follow-up is essential given the high risk of lesion recurrence, especially among HIV-infected individuals. Future studies are needed to document the efficacy of primary and secondary prevention methods to reduce the incidence of anal cancer.

WS 1-1

OVERVIEW OF CERVICAL CANCER PREVENTION IN EASTERN EUROPEAN COUNTRIES

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WHO Action Plan for Implementation of the European Strategy for the Prevention and Control of Noncommunicable Diseases 2012 - 2016 has become an important trigger in promoting a comprehensive approach to cervical cancer prevention and management. Mortality and morbidity rates vary widely among and within the 53 Member States in the WHO European Region.

The overview focuses on countries of eastern Europe and central Asia that are not members of the European Union (EU). According to the WHO Health for All database, cervical cancer incidence in these countries varies from 3.5 to close to 20 per 100 000 women; age-standardized death rate is 2 - 12 per 100 000 women. These outcomes result from differences in national policies and programmes, the availability of national guidelines, primary and secondary prevention services, and the availability of optimal treatment.

Human papillomavirus (HPV) vaccines are available in most of these countries, but no country in eastern Europe and central Asia outside EU has included HPV vaccine in its immunization calendar. During last 5 years several countries have revised their cervical cancer screening programmes and have worked to improve the quality of screening and increase its coverage, but none of the countries included in this overview has implemented organized population-based screening. In some countries, pilot projects are underway and may play an important role for implementation of nationwide screening. Many countries require international assistance to draw from available experience and accelerate progress in preventing cervical cancer.

WS 1-3

**CERVICAL CANCER IN SLOVAK REPUBLIC:
AN OVERVIEW ON ACTUAL SITUATION IN INCIDENCE, MORTALITY, SCREENING AND VACCINATION**

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Objective: To reduce both cervical cancer (CC) incidence and mortality is the primary objective of organized screening and vaccination. Thus, for any improvement the deep review of HVP related cervical cancer health care indicators are needed. Following this, we aimed to make an overview on cervical cancer incidence and mortality trends, current screening situation, actual problems in HPV infection and HPV vaccination programs in Slovak republic.

Methods: A retrospective analysis of regional and national registries containing data related to CC incidence, mortality and preventive strategies.

Results: The annual incidence in CC varies between 520-600 newly diagnosed cases (19/100 000), of which 190-220 are dying in Slovak republic (6-7/100 000). The trends in overall incidence and mortality from cervical cancer in the Slovak Republic are implacable. We are still observing the high incidence values of this condition that have stabilized long term without any signs of a significant decrease, what ranked us among the "developing countries" in Europe. For example there was recorded a stabilized trend in incidence with an estimated average annual change -0.008/100.000 and a moderately increasing trend in mortality with average annual increase 0.049/100,000 ($p < 0.0001$) over the period 1968-2006. The analyses of clinical stages during the period 1978-2003 showed that the number of cases in clinical stage I increased, the stage II rate declined, and cases in stages III and IV were still high with a rising tendency. The reason is the absence of organized screening. We offer only opportunistic one with low attendance of targeted population (20-25%). Although Slovak obstetrics and gynecology society worked out the guidelines for CC screening and management of patients with cervical cytopathology, the organized screening is not supported by the health care authorities. Thus, the only way to screen our women is through the legislation linked to mandatory gynecologic examination in every woman between ages of 23-64-years utilizing OC smears, in periodicity 1-1-3 years up to 64. There is no HPV related screening approach. The HPV test, mainly (HC2) is included as an adjunct in the management of women with ASCUS, ASC-H lesions. As for the vaccination activities there is possibility for vaccination by using tetravalent vaccine (HPV type 6, 11, 16, 18) in girls in age 9-15 years and in women aged 16-26 years or bivalent vaccine (HPV type 16, 18) in girls in age 10-14 years and in women aged 15-25 years since 2007. Moreover, there is an OBGY society recommendation to vaccinate women with CIN2,3 up to 45 years of age since 2012 (tetravalent type). The vaccination is provided by pediatricians or private gynecologists and lacks any organized form, not exceeding 10% rate of targeted population.

Conclusions: The trends in cervical cancer epidemiology in Slovakia are unfavorable and our results have confirmed the necessity of an immediate introduction of organized screening and vaccination programs.

**PRIMARY AND SECONDARY PREVENTION OF CERVICAL CANCER IN POLAND.
THE PROPOSAL OF NEW DIAGNOSTIC METHODS**

Prof. Wojciech Rokita

Nowadays, the cervical cancer is still a serious problem for the health care systems. This cancer is the second, after the circulatory system's diseases, reason of deaths in Europe. 1/4 of all the deaths in Europe are due to the cervical cancer. The mortality rate of cervical cancer in Poland is very high : 5,3/100.000 women, which gives the country one of the last places in European Union. To fight the cervical cancer effectively, the introduction and fusion of primary (vaccinations against HPV) and secondary (cytological screening) prophylaxis is needed. Polish Gynecological Society and Polish Society of HPV Infections Prophylaxis recommend the routine vaccinations against HPV of girls aged 11-12. Due to economical reasons, in Poland the vaccinations against HPV are done only locally and are financed by some local governments. In Poland the secondary cervical cancer prophylaxis is based on the National Screening Program, which was introduced in 2005. The Program states that each woman aged 25-59 should receive a personal invitation to a free of charge cytological examination that is to be performed every 3 years. The Program covered 2928798 women in the period from 01/01/2007 to 01/09/2010, that is the 22,45% of the number that was planned to be included. The abnormal results of the cytological examination were found in 2,6% of the cases. In the Program, the price of the diagnosis of one precancerous state cost about 150 EUR and in the case of cancer about 3500 EUR. From the moment of the introduction of the Program, the number of cases of cervical cancer decreased from 3263 in 2005 to 3102 in 2009. In spite of the fact that the financial expense connected with screening is still growing, the morbidity and mortality rate of cervical cancer remain on a certain level and do not decrease. This means that the current model of prophylaxis exhausted its capabilities and there is a need of finding new, more effective screening tools. The new diagnostic tools used in the screening of the cervical cancer are the tests HPV DNA, HPV mRNA and recently introduced immunocytochemical test based on the detection of the protein p16 and Ki67. Especially great hope is connected with the wide introduction of immunocytochemical tests as they have high sensitivity, specificity and are relatively cheap. It is possible that the immunocytochemical tests (p16, Ki67) will revolutionize the screening programs of the cervical cancer. However, before the tests could be introduced more widely, it is necessary to find if they would be useful in the screening of population.

THE EPIDEMIOLOGY OF THE CERVICAL CANCER AND HPV INFECTIONS IN POLAND

Prof. Ewa Nowak-Markwitz, MD, Professor

Poland is one of the countries with a medium incidence rate of the cervical cancer. In the year 2009 cervical cancer's incidence rate in Poland was 10,5/10⁵, which gave it the sixth place among all the cancers. The mortality rate of cervical cancer in Poland is very high: 5,3/10⁵ women, which gives the country one of the last places in European Union. The mortality rates rise constantly starting among women aged 25 and reaching the top between the age of 50-54. The diversity of the mortality rate is connected with the range of the incidence, the existence or non-existence screening programs and the access to the treatment. Standardised mortality rate of the cervical cancer was highest in kujawsko-pomorskie, zachodniopomorskie and lubuskie voievodship. The lowest mortality rate was observed in podkarpackie and opolskie voievodship. In the year 2009 3102 women were diagnosed with the cervical cancer and 1728 died caused by cervical cancer. The incidence rate of the cervical cancer in Poland was rising very quickly from the beginning of 1960s, reaching the top at the beginning of 1980s. The percentage of the 5 year survival rate among women with the invasive cervical cancer in Poland is 49%, while the European average rate is 63,5%. During the last decade the decrease in the incidence rate of the cervical cancer was observed, from 3597 diagnosed cervical cancers in the year 2000 to 3102 in the year 2009, which was the result of the introduction of the national cervical cancer's prophylactic program in 2005. It appears if these trends will continue and that in 2015 there will be approximately 2900 new cases and 1600 deaths caused by cervical cancer. Both incidence and mortality are showing a decreasing tendency since the 8th decade of the 20th century, although the decrease in mortality is smaller than that in incidence. If these trends continue, 2015 should see incidence drop to 9,3/10⁵ and mortality to 4,7/10⁵. In the last years the constant rise of the morbidity rate of the illnesses connected with HPV has been observed. It has been assessed that genital warts and other pathologies connected with the HPV infections can be found among more than 1% sexually active persons. Among 4-5% the subclinical infection can be found. The molecular studies confirm that in Poland among about 15-20% of sexually active people, there is an asymptomatic HPV infection. Only 25 % of polish women do not have virusological and immunological features of the infection with the genital types of HPV. Singular epidemiological studies showed the in the center and in the west of Poland the most common types of the HPV among the women with CIN2+ are 16,33,18,31,56. The types 16 and 18, against which the vaccinations are aimed, are in the group of three HPV genotypes mostly identified in CIN 2 and CIN 3 changes found among women in the middle-west Poland. It is necessary to carry a wider research that could show the epidemiology of HPV infections in Poland.

WS 1-7

HPV PREVENTION AND CERVICAL CANCER CONTROL IN BULGARIA – CURRENT RECOMMENDATIONS AND PERSPECTIVES

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Cervical cancer is a serious public health problem in Bulgaria because of the increased incidence and mortality rate registered since 1990. An organized cervical cancer screening was launched in 1970 and the data shows that the incidence and mortality were stable during the first fifteen years of screening activities followed by a constant increase, which became most obvious after the final interruption of organized screening. At present, there is an increase of crude incidence from 12.7 to 30.4/100,000 and mortality from 3.2 to 10.3/100,000 and only opportunistic screening exists, reaching approximately 20% of the women.

On condition that screening programme in Bulgaria is very deficient, the introduction of HPV vaccination as a primary prevention is expected to have significant impact on HPV-associated cytological abnormalities and on cervical cancer.

HPV vaccination was recommended by the Ministry of Health in July 2009, but without any reimbursement, and vaccine coverage remained very low. Recognizing the necessity to improve the cervical cancer control in Bulgaria, the Ministry of Health decided to adopt and finance a National programme for primary prevention of cervical cancer in Bulgaria for the period 2012 – 2017, aiming to decrease the disease incidence by reaching high HPV vaccine coverage in target age groups. Series of activities are planned to be performed to increase the public awareness and to ensure the acceptance of the HPV vaccine, including in the specific hard-to-reach population groups.

However, the lesson learned should be remembered: Twenty years had to pass from the introduction of universal hepatitis B immunization in Bulgaria in 1991 till the present success of the immunization programme. The visible results expected to be achieved by the HPV vaccination will not come any sooner. Moreover, significant impact on the cervical cancer burden in Bulgaria can be only achieved through implementing both available tools:

- Primary prevention by HPV vaccination
- Secondary prevention by restoring organised population-based cervical cancer screening.

WS 1-8

CERVICAL CANCER SCREENING PROGRAM IN LATVIA.

Ilze Viberga, *University of Latvia, Assoc. Professor, Ob/Gyn*

The organized cervical cancer screening program implemented in Latvia from January 2009 is based on the formation of target group of women-residents aged 25 – 69 years getting the personal invitation for testing that serves as the referral form every three years and on extensive opportunity for medical staff to take part in performing the screening manipulations. The general practitioners (GP), who have contracts with the HPC (Health Payment Center), can access the screening module in the HIS (Health Information System) that contains information about the relationship with the screening program of all females registered with the particular practitioner: the sending date of the invitation letter, reference No. of the letter and the screening examination date and findings. If in their practices cytological smears can be taken, GPs can perform this manipulation as a part of the program: they must contact the Screening Section of the HPC online and require the invitation letter and the screening examination form electronically if a woman has visited the GP due to any other reason and cannot produce the screening invitation letter. Gynecologists can act similarly — contact the Screening Section of the HPC and require the invitation letter and the screening examination form electronically if a woman has visited the doctor due to any other reason such as for advice on the selection of contraception. Private gynecologists and GPs may use the invitation letter and the examination account form to send the smear to the cytological examination as a part of the screening paid by the state and the data from the laboratory are delivered to the Screening module of the HPC. The 19 December 2006 Regulation No. 1046 of the Cabinet of Ministers „The Procedure for Organization and Financing of Health Care” provides that all manipulations performed as a part of the screening, including the taking of cytological smear, testing and the consequent examinations following the algorithm approved by the above regulations are fully compensated to the performer irrespective of any financial quota requirements. Moreover, the taking of cytological smear and testing as a part of the screening program may be also performed to inpatients and the payment for the manipulation is excluded from the payment for the treatment in the hospital.

Regardless of all the above mechanisms integrated in the implementation plan of the screening program, responsiveness to the invitation letters sent out in 2009 does not exceed 26% and it has reached 46% at the end 2011. The first round target population coverage is 44% that is less than it could be. There are unresolved issues remaining and that will need to be addressed. There is a lack of defined standards and inadequate quality control of some aspects of the program, including the taking of the cervical smear, the laboratory analysis, the follow-up of unclear or abnormal smears, and treatment. A needs assessment has also revealed a shortage of trained specialist staff, particularly for conducting further diagnostic tests like colposcopy in the case of suspicious smears and carrying out treatment, as well as a shortage of gynecological services, especially in rural areas. The use of the Leishman technique test in Latvia in place of the standard Pap test technique for analyzing cervical smear is also controversial. European guidelines recommend the Pap test because its accuracy in detecting warning signs of cervical cancer has been scientifically validated.

In September 2010, the national vaccination program against HPV 16, 18 for girls aged 12 years is started in Latvia. The first dose cumulative coverage of target group is 53.2% within period September 2010 till December 2011.

CERVICAL CANCER PREVENTION IN CZECH REPUBLIC – HOW TO START JUST IN TIME

Janetta Bogdanová

Director of ONKO UNIE, o.p.s.

Organisation that concentrates on activities concerning the prevention of breast cancer and gynecologic cancer. Running the organisation, doing fundraising, dealing with media and government. Responsible for preparation of projects, seminars and conferences. Cooperating with other patient's organisations.

Working in non-profitable environment for 4 years. Before ONKO Unie working for the organisation specialised on breast cancer, being the National delegate of Europa Donna.

ONKO Unie is preparing pilot project of prevention of cervical cancer and this project is targeted on young girls and their parents. This educational project should show the new possibilities of prevention of cervical cancer for young girls and also show the benefits of the vaccination. We want to start this pilot project this year and we want to go with this project to schools.

CONCENTRATION OF SELECTED BIOCHEMICAL PARAMETERS IN BLOOD OF PREGNANT WOMEN INFECTED BY HPV 16 AND 18

Prof. Agata Karowicz - Bilinska

Asymptomatic HPV infection in pregnant women may be connected with changes of pro-inflammatory cytokines and proteolytic enzymes which may condition the persistence of infection during pregnancy.

Aim: The main aim of the study was to evaluate the concentration of interleukin-1, TNF- α and α -1 antitrypsin in pregnant women infected by oncogenic types HPV.

Materials and method: The study was conducted in 2008-2009 at the Department of Obstetrics and Gynecology, Medical University Lodz, on pregnant women in the third trimester of pregnancy. The study group consisted on 19 pregnant women HPV-16, -18 infected. The control group consisted on 34 pregnant women at the same gestational age, HPV-negative. The concentration of interleukin-1, TNF- α and α -1 antitrypsin was estimated in blood.

Results: The results of concentration interleukin-1, TNF- α and α -1 antitrypsin were similar in both groups of pregnant women.

Conclusions: High risk HPV infection has no influence on the concentration of pro-inflammatory cytokines. In HPV infection during pregnancy the anti proteolytic defense did not decrease.

WS 2-2

CERVICAL CANCER PREVENTION IN RUSSIA**Academ. Sukhikh G.T., Prof. Vera Prilepskaya***Research Center for Obstetrics, Gynecology and Perinatology of the Ministry of Healthcare and Social Development of Russia*

Prevention of cancer, and in particular cervical cancer, has become an issue of most priority in Russia in light of the ongoing health care reforms. Statistic data indicate an increase in incidence and mortality from cervical cancer. In 2009, 6,187 women died in Russia from cervical cancer; the annual growth of the disease among young females is 4-5%. In Russia, regional screening programs exist that involve cytology studies for women at risk. In Moscow, the implementation of such programs has allowed to reduce annual morbidity and mortality from cervical cancer. However, in general, the currently existing regional screening programs are not effective and identify only 7% of cervical cancer and 20% of CIN. Epidemiological studies have identified an adequate awareness among Russian women and physicians about the importance of cytology studies; however, international studies show that until 2008 PAP-test and vaccination in Russia was used much less than in European countries. At the same time, many Russian women identify vaccination as one of the most effective ways to protect from cervical cancer. In 2007, following the Russian president approval of the concept of vaccination against HPV and cervical cancer, vaccination activities have been started in Moscow, and in Moscow Region was started a pilot project to vaccinate the girls, funded from the federal budget. Currently, vaccination is carried out in 273 hospitals. In 14 major regions of Russia - supported by the state funding. Possibility of adding HPV vaccination in the vaccination schedule is currently under discussion. There is a lot of work being done in the country for cervical cancer prevention, introducing new technologies. Russian scientists are participating in the international research on the effectiveness of vaccination and some of them are the members of the expert groups in the international organizations. Particular attention is paid to training of physicians and the public. Scientific Center for Obstetrics Gynecology and Perinatology organized two international congresses, trainings and master classes on cervical cancer prevention. A group of Russian experts has prepared a national guidelines on cervical cancer prevention, as well as a number of books and manuals. Russia has established centers of women's health and welfare centers for cancer prevention. We believe that only the combined efforts of government and public organizations, with a broad introduction of screening and vaccination can provide a woman lives without cervical cancer in the future.

WS 2-3

THE EDUCATION OF POPULATION AND PHYSICIANS IN HPV INFECTION PREVENTION IN RUSSIA**Podzolkova N., Rogovskaya S., Lipova E.***Russian Medical Academy of Postgraduate Education, Moscow*

The HPV prevalence in Russian Federation varies from 13.3% to 58.7% in general population according to regional studies. The burden of HPV-associated diseases for public health system includes management of genital warts, cervical intraepithelial lesions, cervical cancer and non-cervical pathology. HPV vaccination programs in many countries have shown that the implementation of anti-HPV vaccine is tightly connected with educational programs for doctors and patients. The outset of the region immunization programs in Russia demonstrated a lack of knowledge about papillomavirus infection and its consequences, both among adolescents and among their parents and teachers, as well as a negative attitude towards the immunization as a whole in society, and in particular to HPV immunization of young girls. The educational campaigns in schools produced generally their positive results, as the adolescent girls became significantly better aware of the prevention of sexually transmitted infections.

Since 2006 the educational program in HPV vaccination and cervical pathology management was introduced in Russia for physicians of various specialties (pediatricians, dermatovenerologists, gynecologists, oncologists, etc). The leading institutions and medical societies of Russia are involved into the program, providing regular master-classes within the frame of congresses and conferences around the country.

Russian Medical Academy of Postgraduate Education has been working for many years in area of cervical pathology. The regular courses for physicians from all parts of the country are provided in Moscow city and many cities around the Russian Federation and countries of former Soviet Union. Since 2011 year the Dpt of ob\gyn in a collaboration with other departments of the Academy started the new program for the faculty of medical universities of RF. The analysis of last achievements and failures in realization of the programs will be presented.

CYTOLOGICAL SCREENING – A LOOK OF CYTOLOGIST**Shabalova I.P.**

Department of clinical laboratory diagnostics of the Russian Medical Academy of postgraduate education, Moscow, Russia

The analysis of cytological screening at the present stage will be presented. Cytological screening of cervical cancer is an important link in the prevention and early detection of the disease. It is not limited to identifying intraepithelial neoplasia and carcinoma, but includes a wide range of pathological changes, developing in the vagina and uterus, which have an impact on the reproductive health of women. Being able to evaluate the state of the cervical epithelium from all of the surface, cytology helps to realize its ability to prevent the development of malignant tumors or to accept various risk factors of carcinogenesis. These capabilities can be extended through the integrated use of various diagnostic methods (molecular, biochemical, immunological, colposcopic, and others). The important points in the efficiency of cytological screening are: the correct organization of work at all stages from the active involvement of women to gynecological examination to the correct cytological report, the proper interpretation of it by gynecologist and professional treatment of any disease. Despite a relatively low sensitivity of cytological method, vaccination from HPV, the rapid development of molecular biology, that sometimes tends to replace cytological screening, cytological method, with its qualified and competent use remains the necessary diagnostic method with a great potential. The usage of liquid-based cytology, automatization, robotic search of cell with the signs of pathology can improve the quality of cytological screening. An important role in its optimization plays a high qualification of cytotechnologist and cytopathologist, normal workload, well organized workplace, the usage of information technologies, quality assurance and quality control in all steps of screening process.

HPV TESTING IN MANAGEMENT OF CERVICAL PATHOLOGY IN RUSSIA**Rogovskaya S., Podzolkova N.***Russian Medical academy of Postgraduate Education, Moscow*

There are few HPV tests available for the practical activity in Russia: PCR with domestic primers and Hybrid Capture II (HCII) have been used in majority of published studies in various regions. The test Cobas 4800 have been licensed recently.

The data in HPV prevalence in regions are mainly calculated for various populations without specified cytology and ranged between 13% and 58.7% among attendants of gynecological clinic, among attendants of dermatology-venereology clinics and among laboratory attendants data.

Most common type in general population was 16, and type 18 was ranged between 4 and 8 place in majority of studies.

Consistent with global estimates in Russia HPV type 16 is the most prevalent in samples of cervical neoplasia. Generally distribution of the most common high-risk HPV-types 16, 31, 33, 18, 45 seems to be similar in other geographical regions reported in the majority of studies which might be prevented by vaccination.

The few studies which have identified HPV genotyping in anogenital warts in regions have shown that generally types 6 and 11 were detected in most of publications. The prevalence rate of genital warts in population ranges between 20 and 30 per 100000 in RF.

The survey conducted in Russian Medical academy of postgraduate education revealed that gynecologists do not follow the clear guidelines recommending HPV test to use in specific clinical situations and often recommend it for young girls, pregnant women, etc. The practical issues of HPV testing in Russia for last years will be presented.

WS 2-6

DIAGNOSTIC AND PREDICTIVE VALUE OF ONCOPROTEIN E7 IN CERVICAL INTRAEPITHELIAL NEOPLASIA.**Ashrafyan L.A., Antonova I.B., Aleshikova O.I., Babaeva N.A Khunova L.Z.***The Russian Scientific Center for Roentgenoradiology of the Russian Ministry of Health and Social Development, Moscow (headed by Prof.V.A.Solodkiy)*

Introduction: The most important event in progression from pre-invasive to invasive stage of cervical cancer is accompanying with the integration of the HPV genome into the epithelial cell genome and induction of metabolite 16-a-OH. This results in negation of E1 and E2 gene regulatory functions leading to uncontrolled expression of viral oncogene E6 and E7. Thus, the most optimal and early marker of tumor progression is determination of E6 or E7 expression.

The aim of the study was to compare the frequency of HPV E7 over expression in pre-invasive and invasive cervical cancers

Methods and Materials: the research covers 707 patients who were examined during the period from 2003 to 2009. All of them were classified into 4 groups: group 1 that included 125 patients diagnosed with cervical cancer; group 2 that included 544 patients with I to III degree CIN; 3 group that included 38 patients with leukoplakia, ectopia, chronic cervicitis and other cervical conditions; and a control group 4 comprised of 68 healthy women.

A PCR method was used for detecting HPV. FISH was performed to detect E7 HPV serotypes 16 and 18, as well as a broad panel of monoclonal antibodies against various determinants of these proteins. All diagnoses are morphologically confirmed.

Study results. In each nosology results were ranged on 4 groups: The I group - HPV + E7 +; II group HPV-E7 group +; III group HPV + E7-; IV group HPV-E7-. At phone diseases: The I group - 4,6 %; the II group - 6,5 %; the III group - 71,4 %; the IV group - 18,5 %. At CIN1-CIN3: The I group - 27,4 %; the II group - 2,1 %; the III group - 39,7 %; the IV group - 30,8 %. At a cervical cancer: The I group - 67,5 %; the II group - 23,3 %; the III group - 4,1 %; the IV group - 5,1 %. At control: The I group - 2,3 %; the II group-; the III group - 36,7 %; the IV group - 61,0 %.

Conclusion: E7 expression is an important marker of poor prognosis in cervical neoplasia. Further research is required to measure the probability of tumor malignancy to prevent and reduce the incidence of malignant tumors.

WS 2-8

MANAGEMENT STRATEGY FOR PATIENTS WITH CERVICAL INTRAEPITHELIAL NEOPLASIA**Minkina G***Department of Obstetrics and Gynecology, Moscow State Medical Stomatological University, Moscow, Russia*

The management strategy for patients with CIN is based on a considerable number of evidence-based study conclusions. There are two options for patients with CIN 1: treatment of lesion using ablation or excision and monitoring of lesion with cytological examinations or HPV testing. CIN 2/3 are believed to be precancerous lesions and should therefore be treated. Follow-up is preferable for young patients with CIN 2, whereas CIN 3 necessitates treatment. In pregnant women CIN 2/3 should be monitored using cytological examination and colposcopy until after delivery. In the absence of invasive cancer, no treatment is acceptable for pregnant patients. The methods used for post-treatment follow-up are the same as for pre-treatment diagnosis: cytology, colposcopy, and HPV testing. The sensitivity of the HPV test in the identification of residual / recurrent CIN 2/3 and the predictive value of HPV negativity exceed the respective values of cytological examination. A negative HPV test at 6 months after treatment for CIN 2/3 allows switching to once-a-year cytological examination.

CERVICAL CANCER AND PREGNANCY**Urmancheeva A.F., Ulrikh E.A.***N.N.Petrov Reserch Institute of Oncology - I.I.Mechnikov State Medical University*

Cervical cancer is the 1st most common gynecological malignancy registered during pregnancy accounting 1-13 cases per 10.000 pregnancies and 1 per 1.000-2.500 deliveries. The median age of patients is about 30 years.

The incidence of CIN3 among pregnant women is 3:10.000.

Abnormal cervical cytology complicates approximately 5% of pregnancies. In our data CIN3 was detected in 22 (0,5%) cases among 4.230 pregnant women.

Conservative diagnostic-treatment tactics in cases of CIN2, 3 during pregnancy replaced aggressive ones.

1st: the risk of CIN2, 3 progression during such a short time interval is extremely minimal. 2nd: in several researches the high frequency of CIN2, 3 regress was observed after delivery. In our data, CIN3 was not detected after pregnancy terminating (delivery or abortion) in 6 patients (13%) among 46 women diagnosed CIN3 during pregnancy and received cone excision after pregnancy.

Thus, in case of CIN3 revealed during pregnancy and the desire to save the woman's pregnancy is possible to avoid traumatic procedures like cone biopsy, which can cause bleeding, spontaneous abortion, premature delivery, infection. Colposcopy, targeted biopsy is necessary in cases of suspected invasive process. All additional diagnostic and therapeutic measures (if invasive cancer is excluded) are postponed on the postpartum period, not earlier than 2 months.

Symptoms and clinical presentation of invasive cervical cancer during pregnancy does not differ substantially from the tumors in non-pregnant women. If it was possible to identify cervical cancer IA1, and the woman wants to keep the pregnancy, treatment may be postponed until after pregnancy. If the patient wants to continue to maintain reproductive function, then after abdominal delivery in 4-8 weeks extensive cervical conization performed. If the patient has no plans to continue to preserve fertility, along with a caesarean section is performed simple hysterectomy.

Treatment of pregnant women diagnosed cervical cancer IA2 is the same as the treatment tactics in cervical cancer stage IB.

Surgery is the first method in the treatment of cervical cancer stages IB, II in combination with pregnancy, even in stage IIB. Tactics depend on the gestation period.

Standard treatment for patients with cervical cancer stage III-IV is combined radiotherapy in combination with chemotherapy. Taking into account poor prognosis in advanced cervical cancer, treatment should begin immediately. If the fetus is viable, the Cesarean section is performed, followed by chemoradiotherapy (after 2-3 weeks). In I and II trimesters of pregnancy, external irradiation is started without the prior termination of pregnancy (after an average of 30-40 days, spontaneous abortion occurs).

Thus, the prognosis of early forms of cervical cancer (CIN3, IA, IB1) during pregnancy does not differ from nonpregnant women. The prediction of the biological behavior of advanced stages of the cervix tumors during pregnancy is extremely difficult. The choice of treatment tactics depends not only on the stage of disease and pregnancy period, but the decision of the woman regarding pregnancy and treatment.

GC 1-4

STEP BY STEP CLEARANCE OF THE PELVIC SIDE WALL.**László Pálfalvi**

Most of the recurrences in cervical cancer are local recurrences in the small pelvis. Most of the pelvic recurrences are situated on the pelvic side wall. So correct dissection of the lateral pelvic side wall - parametrectomy and pelvic lymphadenectomy - is mandatory, and influences the long term results of the procedure. The deficiencies of the surgery can not be compensated with adjuvant radiotherapy.

The aim of radical hysterectomy is not only the removal of the cervical tumour, but the removal of all pelvic ways of tumour spreading. Several studies have documented the location of parametrial positive lymph nodes, and found them randomly distributed, with an almost equal number of metastatic parametrial nodes in the medial and lateral parametria.

The subserous connective tissue of the pelvis has denser parts (ligaments) and spaces filled with loose connective tissue. Their anatomical interpretation is different. Their intraoperative dissection can be done using different natural and artificial planes.

The radicality of the surgery should be tailored according to the extent of the tumour. In the classical class III-IV radical hysterectomy the visceral branches of the hypogastric vascular system are divided, so the dissection plane is the medial surface of the hypogastric artery and vein.

The "Laterally Extended Parametrectomy" (LEP), is aimed at removing all the lymphatic tissue from the pelvic side wall. This procedure removes parametrial tissue not removed by a conventional class III-IV Wertheim hysterectomy by extending the lateral limits of the dissection to the true boundaries of the pelvic side wall rather than the medial surface of the internal iliac vessels.

GC 1-5

NEW HORIZONS IN PROGNOSTIC FACTORS OF CERVICAL CANCER

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A substantial amount of work aiming to elucidate the prognostic factors in cervical carcinoma (CC) has been done, including the clinical studies as well as histopathological assessment of the tumour specimens, either by conventional descriptive or semiquantitative (grading) approaches or adopting the techniques of quantitative pathology (morphometric measurements). Some years ago, a special volume of CME J. Gynecol. Oncol. was published, with several expert reviews on the divergent categories of prognostic factors in CC. In principle, the prognostic factors can be divided into two major categories: i) factors of the tumour origin, and ii) those of the host origin. These concepts are regularly referred to as tumour-host (inter)relationships, and these morphological manifestations of tumour-host reactions represent the first attempts to get insights in tumour immunology. After almost three decades of intense studies using immunohistochemical (IHC) techniques with a bewildering repertoire of biological tumour markers, many of the factors regulating the biological behaviour of cervical carcinoma still remain incompletely or purely understood. We recently completed a series of studies where 13 markers were analysed as predictors of intermediate endpoints in cervical carcinogenesis as well as in prognostication of CC: E-cadherin, ERK-1, 67-kd laminin receptor (LR67), topoisomerase-2, MMP-2, TIMP-2, NF-κB, nm23-H1, p16^{INK4A}, PCNA, Survivin, hTERT, and VEGF-C in a series of 150 (CC) and 152 CIN. In meticulous multivariate models, 3 marker panels were identified consisting of 5 independent predictors of CIN2 (E-cadherin, ERK-1, LR67, topoisomerase 2>, and VEGF-C), 3 predictors of HR-HPV (Survivin, p16^{INK4a}, and hTERT), and, importantly, 2 independent predictors of CC survival (nm23-H1 and TIMP-2). Prognostic factors for cervical adenocarcinoma (AC) are in some respects different from those of the squamous cell carcinoma, and unfortunately, also much less studied. Despite the rapid expansion of the biological marker studies, histopathology and related morphological methods still have an important role in routine assessment of the prognostic factors in cervical cancer. As evident from the published data, a substantial amount of research needs to be done before a panel of accurate predictors of CC will be at hand.

GC 1-6

HISTORICAL TRENDS IN CERVICAL CANCER IN GREENLAND: A 60-YEAR OVERVIEW.

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Objectives: At present, 24.8 per 100,000 women (World-standardised rate, ASW) in Greenland develop cervical cancer. The disease is caused by sexually transmitted Human Papillomaviruses (HPV). In Greenland sexual activity is initiated early and average number of sexual partners is high. However, data for Greenland have not been routinely included in historical volumes of major international cancer overview publications, making it difficult to assess the temporal trends. Our aim was to describe the incidence of cervical cancer from 1950 to 2009.

Methods: We systematically searched PubMed for articles reporting the incidence of cervical cancer in Greenland. We supplemented this search by obtaining novel population-based incidence data for 1980-2009, by searching own archives, and the electronic archives of the Danish Royal Library for scientific reports.

Conclusions: Incidence of cervical cancer was not always very high in Greenland. It was around 10 per 100,000 (ASW) in 1950-59. In 1960-69, the incidence increased to around 30 per 100,000 (ASW), and to around 60 per 100,000 (ASW) in the 1970's. From around 1985 onwards, the incidence of cervical cancer started decreasing until the current level of around 25 per 100,000 (ASW). It is a challenge to determine whether these changes, an initial increase and a later decrease, were true changes or due to data artefacts. We therefore sought further evidence that could substantiate either of the explanations. It appeared that under-reporting in the 1950's and the 1960's was small. Furthermore, the rate of out-of-wedlock births increased greatly from 1940 onwards. Sexual behaviour appeared to have changed in parallel with the economic development from the 1950's onwards, and an increase in other sexually transmitted infections like gonorrhoea and syphilis was observed. Use of tobacco also increased. In recent decades, data do not suggest that major changes in sexual behaviour have taken place. The decrease in the incidence since the 1980's would be consistent with introduction of screening, which started in the 1970's. A high burden of cervical cancer in Greenland appeared to have developed gradually. The data strongly suggest that observed trends in the burden in cervical cancer ran parallel to changes in sexual behaviour.

BURDEN OF HPV 6, 11, 16, 18, 31, 33, 45, 52, AND 58 IN INVASIVE CERVICAL CANCER**Serrano B¹, Alemany L^{1,2}, Tous S¹, Bruni L¹, F. Bosch X¹, de Sanjosé S^{1,2}; on behalf of RIS HPV TT study group***1 Unit of Infections and Cancer. Catalan Institute of Oncology. Barcelona, Spain**2 CIBER Epidemiología y Salud Pública, CIBERESP*

Objective: Our aim is to estimate the relative contribution (RC) of the 9 types (HPV 6, 11, 16, 18, 31, 33, 45, 52 and 58) included in a new broad spectrum vaccine, in ICC worldwide.

Methods: The estimations on RC were based on data from an international study providing information on 8,977 (85%) HPV DNA positive invasive cervical cancer (ICC) cases from 38 countries [1]. HPV DNA was detected by SPF₁₀-DEIA-LiPA₂₅ system. RC was expressed as the proportion of type-specific cases among all HPV positive samples. Contributions in multiple infections were added to single types in accordance with a proportional weighting attribution. Globocan 2008 and 2010 World Population Prospects as data sources were used to estimate current and future projections of new ICC cases. It was assumed that 100% of ICC cases are attributable to HPV infection.

Results: RC of the 9 HPV types was 89.4% (95%CI: 88.8-90.1), with some regional variations, from 84.6% in Central America to 95.4% in North America. RC of the 9 types varied by histology, ranging between 89.1% (88.4-89.7) in squamous cell carcinomas and 95.5% (93.3-97.2) in adenocarcinomas. HPV 16/18 or 45 was detected in 94.2% (91.7-96.2) of adenocarcinomas. HPV 16 and 18 were the most common types worldwide, accounting for 70.8% (69.8-71.7) of cases. HPV 31, 33, 45, 52 and 58 were detected in 18.5% (17.7-19.3). As expected, addition of HPV 6 and 11 did not modify the cancer burden estimates (RC: 0.1%). RC of the 9 types altogether decreased with age (trend test $p < 0.0001$); however, this was mainly explained by the decrease in older ages of HPV 16, 18 and 45. In contrast, HPV 31, 33, 52 and 58 were more frequent with increasing age (trend test $p < 0.0001$). Only due to population growth, projected global estimates of ICC cases attributable to the 9 types are expected to rise from 493,770 new cases in 2012 to 560,887 in 2025; with virtually all the increase in less developed regions.

Conclusion: Although the RC of each HPV type differ by region or by histology, the RC of the 9 HPV types counted altogether seems to be consistent across different variables. The addition of HPV 31, 33, 45, 52 and 58 to HPV types included in current vaccines (16/18) could prevent almost 90% of ICC cases worldwide.

HPV16 VIRAL LOAD AND RISK OF IN SITU AND INVASIVE SQUAMOUS CERVICAL CANCER**Sundström K*, Ploner A*, Arnheim Dahlström L, Palmgren J (1,2), Dillner J (3,4),****Adami HO (5), Ylitalo N (6), Sparén P.**

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Objectives: A strong association has been shown between high viral DNA load (VL) of human papillomavirus (HPV) type 16 and risk for cervical cancer in situ (CIS). However, little data is available for the significance of VL in invasive squamous cell carcinoma (SCC).

Methods: In two nested case-control studies among women participating to cervical screening, with a first cytologically normal smear, we collected 5665 smears from 621 women with CIS, 457 with SCC, and individually matched controls. All smears were tested for HPV, and the VL of HPV16 positive smears was quantified using realtime-PCR. The median follow-up until diagnosis of CIS or SCC was 6.1-7.7 years.

Conclusions: Low VL's of 5-10 copies/microliter were common among both CIS and SCC case women, until 1-2 years before diagnosis when a surge in VL occurred. The relative risk (RR) associated with low viral load of HPV16 was around 10 for CIS, and 10-20 for SCC throughout 10 years before diagnosis, compared to HPV-negative women. For women with medium to high VL, the risk for CIS was greatly increased from five years before diagnosis (RR=19, 95% confidence interval 7-48). In SCC, a high VL conferred an increased risk, but only from 3 years before diagnosis (RR=60, 95% CI 6-580). In this comprehensive study, we demonstrate differing risk functions associated with HPV16 viral load in CIS and SCC. We further show the potential significance of low viral loads early in the squamous carcinogenic disease process.

GC 1-9

THE EFFICACY OF CONSERVATIVE MANAGEMENT IN PATIENTS WITH CERVICAL CANCER STAGE FIGO IA1 TREATED WITH LASER CO₂ THERAPY

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Objectives: The goal of this study was to evaluate the efficacy of laser CO₂ conization therapy as gold standard for treating stage IA1 microinvasive cervical squamous carcinoma.

Methods: A retrospective study of the colposcopy database from 1998 to 2010 was conducted in sixty patients submitted to CO₂ laser conization therapy with histologic diagnosis of squamous carcinoma stage FIGO IA1 and then submitted to follow-up with PAP smear, colposcopy and biopsy at the Lower Genital Tract Physiopathology Centre Gynaecology, Perinatology, and Human Reproduction Department of Florence University. Prognostic risk factors for relapse, such as depth cervical stromal invasion, positive resection margins, positive Lymph-Vascular spaces infiltration, previous HPV related diseases, demographic risk factors, were evaluated with univariate analysis.

Conservative management with laser therapy was efficacy in more than 90% of the patients, without overtreatment. When disease persistence were detected (7%), patients underwent repeat laser CO₂ conization and followed-up without demolitive intervention. The risk of disease progression was 1.8%: only one case of invasive carcinoma.

Univariate analysis revealed that only depth of stromal invasion was a significant risk factor for relapse ($P < 0.04$), while positive resection margins, positive Lymph-Vascular spaces infiltration, previous HPV and demographical risk wasn't.

Depth of stromal invasion $< 3\text{mm}$ kept to be the only prognostic factor for disease relapse with specificity 78%, sensitivity 75% and likelihood positive ratio 3,47.

Five patients (8,3%) were excluded from the study because unable to be treated with conservative management so underwent hysterectomies: two (40%) due to Abnormal Uterine Bleeding and three (60%) due to cervical stenosis and consequently unsatisfactory follow-up.

Conclusions: Laser CO₂ conization alone appeared to be an effective and safe treatment for patients with stage IA1 microinvasive squamous cell carcinoma of the uterine cervix if careful post-treatment follow-up was guaranteed. The depth of stromal invasion has been confirmed as the most important risk factor and prognostic factor to be evaluated for disease relapse.

GC 1-10

EXPRESSION OF VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) IN THE EPITHELIUM AND STROMA OF CIN AND INVASIVE CERVICAL CARCINOMA

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Objectives: Cervical cancer develops from precancerous lesion, cervical intraepithelial neoplasia (CIN). Angiogenesis is crucial for local progression and development of distant metastasis and it is related with VEGF expressions. VEGF-A is involved in hemangiogenesis and VEGF-C takes part in the growth of lymphatic vessels. There are indications that in cervical neoplasia, a switch from a lymphangiogenic phenotype towards a hemangiogenic phenotype occurs with disease invasion and progression. Therefore, this study described and analyzed the expression of VEGF-A in epithelium and stroma cells of women with CIN 3 and invasive carcinoma.

Methods: This is a study design consisted of three groups. The first group corresponded to women with histological diagnosis of CIN 3, the second group consisted of women with invasive carcinoma associated with CIN 3 and the third group consisted of women with frankly invasive carcinoma.

Results: Expression of VEGF-A was greater in the stromal cells of frankly invasive carcinoma than CIN-3 alone or associated with cervical carcinoma, and these differences were statistically significant ($p < 0.05$). A borderline association was found between the expressions of VEGF-A in stromal cells in frankly invasive carcinoma and invasive carcinoma when associated with CIN 3. Considering the comparison of the VEGF-A positivity between stromal and tumoral cells in each group, the expression was significantly greater in epithelial tumor cells compared to stromal cells ($p = 0.0014$) in CIN 3 cases of group 1. **Conclusions:** These data suggest that VEGF-A expression could trigger angiogenesis to surrounding tissues of precursor lesions and early stage of cervical carcinoma, suggesting that the stroma cells play an important role in the hemangiogenesis.

BIOMARKERS AND ASSOCIATED FACTORS TO INVASIVE CERVICAL CANCER STAGE

Amaro-Filho S; Cunha CB; Pires A; Russomano F; Tristão A; Andrade CV; Oliveira MP; Salvatore G. De-Simone; Pereira LOR; Golub JE; Nuovo GJ; Nicol AF.

Cervical cancer is the second most common cancer among women worldwide. One of the most important prognostic factors is how early the cancer is detected and how far it has spread. Sociodemographic factors and co morbidity contribute to the probability of a delay in the diagnosis of cervical and developing to late-stage or end-stage disease. The aim of this study was to investigate the relationship between clinical, socio-demographic, behavioral factors, and biomarkers associated to the stage (at diagnosis) of cervical cancer.

Materials and Methods: This was a cross sectional study of 87 women diagnosed with invasive cervical cancer (ICC) attended at IFF/Fiocruz, RJ, Brazil. Tumors were classified using the FIGO classification. A TMA paraffin block was construct and were analyzed by means of immunohistochemistry to MCM2, p16 and ki-67. PCR was used for HPV DNA detection with primers GP5+/6+. All statistical analyses were performed using Stata software (10.0). Chi-squared, t-test and Fisher's test was used to compare mean values. The P value, odds ratio (OR) and 95% confidence interval (CI) were reported.

Results: The most prevalent HPV types found on the ICC specimens were HPV 16 (70.8%) and HPV 33 (11.2%) followed by HPV 35 (4.5%), 58 (2.3%), 18 (1.1%), 31 (1.1%) and others types (9%). There was an association among older age (55 years old; OD = 16,6; CI 95% = 3,5-79,2; p<0,001), pregnancies (> 4 ;OD = 7,2; CI95% = 2,4-21,5; p<0,001) and low education level (OD = 8,0; CI95% = 1,5-43,4; p=0,016) with advanced stage (III/IV) of Invasive cervical cancer. Regarding the biomarkers analyzed Ki-67, MCM2 and p16 showed 60%, 64% and 92% with ≥ 50-75% stained cells on the later ICC stages compared with the early stages (I/II) 40%, 72%, 95% respectively.

Conclusions: Advanced stages of ICC (III/IV) were associated with older age, > 4 pregnancies and low education level. Despite the prognostic value of the biomarkers analyzed, no significant association was found to differentiate the tumor stages. The most prevalent HPV types found in the 87 ICC specimens analyzed were HPV16 followed by HPV 33, 35 and 58 suggesting that new immunogenic targets of HPV vaccine should be implemented and evaluated in Rio de Janeiro, Brazil.

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CHEMORADIATION FOR CERVICAL CANCER

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Shortly after the discovery of Radium, cervical carcinoma became one of the first diagnosis that benefited from the discovery. With increasing knowledge of the physical properties of Radium, and with the development of a dosimetric system for its application, gradually there was an improvement in the treatment results of uterine cancers. The discovery of artificial radioactivity, new radionuclides for brachytherapy, as well as the immense technological development in the field of high-energy accelerators hand in hand with massive use of computerized technology have led radiotherapy from a mainly empirical treatment modality to a highly precise one. At the beginning of the 21st century it can be said that the treatment for cervical cancer from FIGO stage IIb and up, is mainly in the domain of radiotherapy, much as it has been in the past decades. Still, much has changed. The increased requirement for more precision in evaluating the extent of the disease – the extent of nodal involvement, as well as the exact evaluation of tumor size – has led to the use of imaging techniques like MRI, CT, PET on which the 3D planning of external radiotherapy, and more and more brachytherapy as well, depend. It is known that the volume of the primary tumor is a more important prognostic factor than the FIGO stage itself. Many studies have shown the benefit of using chemotherapy to augment the effects of radiation and thus improve the treatment results. To ensure the quality of the whole process of radiotherapy has become a paramount requirement today. The high demands on expensive technology and a knowledgeable personnel have led to modern teletherapy and brachytherapy being concentrated in fewer departments than in the past. The goal of modern radio-chemotherapy of cervical cancer is to cure the disease, but also minimize the side effects of radiotherapy to ensure a good quality of life.

Theoretically, we know today how to treat even advanced stages of cervical cancer with radio-chemotherapy, still the reality is not as satisfactory. Even across Europe, the availability of quality and timely radiotherapy is not given, and more so in developing countries.

The talk will highlight the indications for radiotherapy / radio-chemotherapy of patients with cervical carcinoma, the importance of cooperation, preparation and delivery of radiotherapy, and the current requirements for the provision of quality external beam radiotherapy as well as brachytherapy.

GC 2-2

CAN WE WRITE THE EPITAPH OF CERVICAL CANCER?**S. Dexeus¹, M. Cararach and D. Dexeus***1 Centro ginecológico Santiago Dexeus, Carrer Dr. Roux, 76, 08017 Barcelona, Spain*

It is quite evident that HPV vaccination is a very effective primary prevention for cervical cancer (cc). Cytology, as secondary prevention, has been an excellent way for reduction as well as the incidence as the mortality of cervical carcinoma. In Spain, with only an opportunistic screening, the incidence of cc is: 7/100.000 women and the number of cases is around 2000 per year with 50% of mortality.

These figures can support the idea that in our country cc is not a sanitary problem, but we must take into consideration that, in Europe The cc is the second most frequent cause of mortality in women between 15-44 years (Globocan 2008).

In Spain the rate of coverage for vaccination starting at 11-14 years old is 84,3% (Min 49,9 and maximum 91,1%). There are various concepts which make people reluctant to vaccination; the lack of proper information, some inadequate information not only in the mass media but also from the own G.P. and lets us say, also some gynecologists who are unable to answer properly the questions addressed by the patients. On top on this, we must consider that t more than 500.000 new cases appear every year in the world and among them almost the 80% in developing countries, with difficult access to global vaccination. Let us conclude that HPV vaccination is necessary but we can't still write the epitaph of uterine cervical cancer.

GC 2-3

MANAGEMENT OF VULVAR CANCER**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.

UPDATE ON TREATING WOMEN WITH ENDOMETRIAL CANCER**Peter Bösze***Saint Stephen Hospital, Budapest*

Adenocarcinoma of the endometrium is the most common genital malignancy, and has long been treated in a standardised fashion, including total abdominal hysterectomy with bilateral salpingo-oophorectomy and peritoneal washing followed by postoperative irradiation for decades. In the past, preoperative local radiation (first intrauterine radium or cobalt, later high dose afterloading) was the rule but it has been abandoned. Adjuvant external pelvic radiation with or without intracavitary treatment has been associated with acute and late toxicity in most cases, the latter being most severe and fatal occasionally. Extended radiotherapy was even more toxic. The complications of postoperative intracavitary afterloading alone are few and mostly mild.

With the introduction of lymph node dissection, postoperative pelvic external beam and intravaginal irradiation was limited to node positive women only, and thereby reducing the number of adjuvant radiation. Recently, even this practice has been challenged. There is evidence that postoperative pelvic external beam irradiation has no survival benefit provided adequate lymph node dissection took place, including total pelvic and para-aortic lymphadenectomy up to the renal vessels.

However, at least one third of early stage uterine cancer patients (30-40% of all women with endometrial cancer) are node negative and for them lymphadenectomy is an overtreatment. Lymph node sampling has been proven as inadequate procedure to determine lymph node status. The place of sentinel node sampling is under discussion.

During the last decade many authors have attempted to identify prognostic factors for stratifying women with uterine cancer into low- and high-risk group and treat them accordingly. And this will be discussed in details.

W 1-1

2011 UN DECLARATION ON THE IMPORTANCE OF NON-COMMUNICABLE DISEASES (BREAST, CERVIX)**Andreas Ullrich**

Over the last decade the global NCD burden which includes cancers such as breast and cervical cancer has increasingly affected health and economy of middle and even low income countries. Due to ageing populations and spread of risk exposure, the situation will worsen in the near future. During this decade, WHO has intensified its efforts to halt the epidemic by means of several global risk reduction strategies concerning tobacco, obesity and alcohol as well as infectious cause of cancer such as HBV and HPV. From 2009, the issue of NCDs was discussed also from a health economic perspective (ECOSOC) and – spearheaded by the CARICOM countries- put on the agenda of the UN general Assembly in 2011. The resulting political declaration expresses the political will of the signing UN Member States to halt the NCD “epidemic” by means of a series of interventions which are following WHO’s guidance in NCD control. Risk reduction strategies are complemented with health system strengthening measures in order to move towards access to care for people living with NCDs. Priority was given to cost effective interventions (“best buys” reference) which need to fulfill the criteria of being feasible and measurable. Among the series of interventions proposed in the political declaration, cancer screening (for women cancers) and immunization are the only specific recommendations listed. This will give the prevention and control of breast and cervical cancer a major momentum. As a first step in following up of the political declaration, WHO in its role secretariat has developed the methodological basis for the developing of targets and indicators by proposing a monitoring framework for NCDs which includes screening. Monitoring frame work and targets are currently under discussion with WHO Member States.

There is agreement, that target and indicators are an essential element for measuring progress and therefore a “conditio sine qua non” for passing from the political declaration to action. Once agreed, the targets and indicators will make decision makers accountable and will enable rational priority setting and evidence based public health planning. There is a unique opportunity that countries will set up national breast and cervical cancer programs in order to be able to fulfill the requirements of the political declaration and the related targets.

W 1-3

CENTERS OF EXCELLENCE AS A NEED FOR IMPROVING CERVICAL CANCER PREVENTION IN LOW-RESOURCES SETTINGS

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1 Instituto Nacional de Enfermedades Neoplásicas, 2 National Cancer Institute, Lima, Peru, 3 Jhpiego, Baltimore, MD, USA

Background: Even though cervical cancer is a preventable neoplasia, it still kills half a million women every year. VIA paired with cryotherapy has proven to be a feasible and effective strategy for detecting and treating pre-cancer lesions; but there is need for expanding the training of providers in areas with limited resources.

Methods: PATH is partnering with Jhpiego and the Instituto Nacional de Enfermedades Neoplásicas (INEN, Peruvian National Cancer Institute) to develop a validated package of training materials and cadres of master trainers in several Latin American countries. The master trainers are in charge of developing training courses on VIA and cryotherapy; then this newly trained group of providers are organized following the VIA/cryotherapy "service cluster" strategy to get most advantage of the scarce resources for providing treatment. The service cluster is formed by a group of health facilities with capacity for VIA connected with one facility equipped with cryotherapy. This cluster is also connected with a referral hospital where colposcopy and LEEP is available for those women considered not eligible for cryotherapy. Analysis was conducted to identify the strengths and weakness of the model.

Results: After two years of work using this model, a validated package of training materials for standard training activities in VIA and cryotherapy was developed. Our experience in Latin America shows that the training excellence center (TEC) has become a good resource that can be used by local ministries of health to expand the capacity of a country for providing VIA and cryotherapy. Currently the same model is being implemented in four Latin American countries. The main challenges identified during this project were the presence of other competing activities and obligations midwives and doctors have, as well as the limited number of human resources.

Conclusions: The TEC created in Peru has become a good source of expertise that was feasible to replicate in other countries in the region. Currently several countries are using the TEC capacity for training hundreds of providers on VIA and cryotherapy, and therefore expanding the capacity of the local governments for expanding cervical cancer screening.

W 1-4

POLISH COALITION AGAINST CERVICAL CANCER - A STRONG CALL FOR REAL CHANGE

Malgorzata Stelmach

MSD Women's Health Foundation, Coalition Member

Coalition is a unique social movement that joins together more than 80 most priority entities, including scientific societies, public institutions, non-governmental organizations and local authorities, determined to solve the problem of cervical cancer in Poland. In six months' time, with help of remarkable experts in many fields, basing on Polish and European guidelines, Coalition created a document - Recommendations of comprehensive change in 5 areas, crucial for cervical cancer prevention in Poland (e.g. education, primary and secondary prevention, registers, finance and system).

The document identifies the main barriers for an effective system of cervical cancer prevention and provides solutions of how to cope with them. The Recommendations were presented to the Ministry of Health in Poland in February 2012. Coalition members and experts believe, that implementation of the provided solutions will lead to a considerable decrease in cervical cancer morbidity and mortality - by half till 2020.

The Coalition also undertakes several activities - like CC Prevention Decalogue for pediatricians, GP's, gynecologists, nurses, society.

HEALTH CARE DATABASES – A TOOL TO STUDY POPULATION EFFECTS OF HPV VACCINATION: ASSESSMENT OF HPV VACCINATION IN GERMAN HEALTH INSURANCE DATA

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Objectives: In Germany, the Standing Committee on Vaccination (STIKO) recommends HPV vaccination for all girls in the age group 12 to 17 years since 2007. Following this recommendation, HPV vaccination is reimbursed by all health insurance companies. Initially, each federal state used different codes for HPV vaccination, but in July 2008 universal codes were introduced. The aim of this study was to assess vaccination status in a large health care database, as a precondition for future studies of vaccination effects on population level.

Methods & Results: We used 2008 data from one large health insurance company including nearly 7 million insured (around 8% of the German population). In addition to the recommended age range, this insurance company covers the costs of HPV vaccination for women up to the age of 26 years. We estimated the fraction of those vaccinated and the number of administered vaccine doses per 100 women by age and across federal states. Additionally, we estimated the fraction of completed vaccination series based on specific codes for the third vaccine dose (in federal states where specific codes were available). We also compared the extrapolated number of recorded vaccinations with the sales data for HPV vaccines. In 2008, most of the vaccinated women (76%) were 12-17-years old and nearly one quarter (22%) were 18-26-years old. Slightly more than 30% of those 12–17-years old in the study population received in 2008 at least one vaccine dose. Around one third of administered doses were coded as third doses, indicating completed vaccination series. Estimated vaccination doses matched sales data and their development over time. Unfortunately, the introduction of universal codes was not finalised in 2008 for all federal states.

Conclusions: We demonstrated that HPV vaccination codes can be identified in German health insurance data. At the same time, there were some limitations which have to be considered in future studies. Completed vaccination series seem to be common in Germany.

COVERAGE AND COMPLIANCE OF HUMAN PAPILLOMAVIRUS VACCINES IN FRANCHE-COMTE

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Objectives: Since 2007 and 2008 the French National Health Insurance began to reimburse two prophylactic vaccines (Gardasil[®] and Cervarix[®]) to prevent cervical cancer. The French guidelines recommend to vaccinate girls aged 14 with a catch-up program for 15 to 23 years old females. The aim of this study was to estimate vaccine coverage and compliance in the county of Franche-Comté.

Methods: Data were extracted from three major regional Health Insurance Information Systems recording all reimbursements of drugs including HPV vaccines. These systems represent 99% of the affiliated population living in Franche-Comté. The analysis was performed from the period of June 2009 until May 2011. Vaccination rates were estimated for the target and catch-up groups using the 2010 reference population. Furthermore this reference population from the three health insurance regimens was used as the denominator. The cumulative number of doses reached 18,857 during the 24-month period. About 9,928 girls have been reimbursed for at least one vaccine dose; of these 88.2% were 14-23 years old. A complete vaccination scheme was observed in 31% of the affiliates whereas only two doses were reimbursed for 27% and a single dose for 42% of females. The coverage rate in the target group with at least one dose of the vaccine remained low at 24.4% in 2009 decreasing to 11.2% in 2010. A complete vaccination scheme was achieved in 18.6% in 2009 and 12.4% in 2010 for 14 years old girls. In the catch-up group the highest rates for one or three doses were noticed for girls aged 15 years in 2010 (6.5% and 4.8% respectively). Despite a low average of compliance at 48% in 14-year-old girls this rate remained however the highest among all age groups.

Conclusions: Coverage and compliance rates appear to be low with the French program both in the target and catch-up groups. Other countries have shown better results with different recommendations and implementation strategies such as a target age below 14 and school-based programs.

W 1-7

HPV VACCINE ACCEPTABILITY IN ADULT JAPANESE WOMEN: DOES EXPERIENCE WITH HPV-RELATED CONDITIONS MATTER?

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Objectives: In Japan, the HPV vaccine is publically funded for girls aged 12-16yr. However, due to low screening rates (24%), studies have shown public funding to be cost-effective up to the age of 45yr. This study investigates correlates of HPV vaccine acceptance in adult Japanese women; in particular experience with HPV-related conditions.

Methods: A subgroup of 849 mothers aged 30-55yr recruited as part of a study on HPV vaccine acceptance for adolescent girls was included in this study. Median age was 42yr; 88.2% were married or cohabiting and 60.5% had more than a high school qualification. In all 55.3% of mothers had a pap smear in the past 2yr and 12.2% of participants had experienced an abnormal smear. A total of 1.2%, 1.4% and 1.5% reported receiving a diagnosis of cervical cancer (CC), HPV and genital warts, respectively. If free 85.5% said they would consider getting vaccinated, but this decreased to 1.5% at the current retail price (>\$400). While 51.1% of mothers had heard of HPV, only 6.3% knew it caused CC and only 2.6% thought they themselves had been infected. However, 35.9% thought they had a medium-high chance of being infected in the future. Factors significantly associated with free HPV vaccine acceptance were: being aged <40yr (OR=1.94, 95% CI:1.16-3.25); recent pap smear (OR=1.49, 95% CI:1.02-2.19); believing vaccines are necessary to prevent disease (OR=7.73, 95% CI:3.26-18.32); believing susceptible to HPV infection (OR=2.33, 95% CI:1.47-3.68); believing medium-high future chance of developing CC (OR=1.81, 95% CI:1.23-2.67) and recommendation from a doctor (OR=4.87, 95% CI 3.12-7.51). Experience with HPV-related conditions, including abnormal pap smears, was not associated with HPV vaccine acceptance.

Conclusions: Health beliefs about HPV and preventative healthcare are more important factors for HPV vaccine acceptance than past experience with HPV-related conditions

W 1-8

PHARMACIST VACCINATION AUTHORITY OF THE HUMAN PAPILLOMAVIRUS VACCINE: A STATE-BY-STATE ANALYSIS OF THE UNITED STATES OF AMERICA

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Objectives: Current initiation and continuation rates of human papillomavirus (HPV) vaccination in the United States remain relatively low. CDC's 2010 cross-sectional national teen vaccination data showed that less than half of girls in the US had received the first dose of the HPV vaccine in 2010, and just under a third had received all three recommended doses. Pharmacies represent an alternate delivery site to potentially increase HPV vaccination coverage. Pharmacists' authority to administer vaccines to patients varies depending on their state of practice. We sought to identify and separate individual states in the United States based on the level of authority their pharmacists have to administer HPV vaccines.

Methods: A standardized telephone questionnaire was administered to members of state pharmacy associations, state boards of pharmacy or faculty at schools of pharmacy. One response from each state was collected. Survey participants were identified by online rosters of pharmacy associations and boards of pharmacy, or by searching school of pharmacy websites for faculty involved with immunization implementation.

Conclusions: Of a total of 50 potential states, including the District of Columbia, representatives from 36 states have been interviewed. Of these 36 states, 77% can provide vaccination against HPV. Thus far, we have recognized that some states' pharmacists can immunize children as young as age nine without physician oversight, while other states' pharmacists cannot administer HPV vaccine at all. Complete survey results from all 50 states will be presented. Pharmacists are currently underutilized as HPV immunizers and some states' laws prevent pharmacists from offering HPV immunization services.

1 [Centers for Disease Control and Prevention (CDC). National and State Vaccination Coverage among Adolescents Aged 13 through 17 Years --- United States, 2010. MMWR. 2011d; 60(33):1117-23.]

COLPOSCOPY AND CERVICAL PATHOLOGY EDUCATION IN RUSSIA**S. Rogovskaya, N. Podzolkova, G Minkina, E. Lipova***Russian Medical Academy of Postgraduate Education, Moscow*

The opportunistic cytology screening in Russian Federation exists. However it is not highly effective because of low coverage, low sensitivity and low availability in many regions of the country. Colposcopy service is more accessible and often being used as a primary screening tool. Lot of women's clinics are equipped by colposcops. As far as colposcopy requires intensive training and retraining due to new classifications, terminology and new management approaches modern educational program is needed. HPV vaccination programs in many countries have shown that the implementation of anti-HPV vaccine is tightly connected with parallel education program for doctors and patients.

Since 2006 the intensive educational program in HPV vaccination and cervical pathology management was introduced and intensified in 2011. The main leading institutions and medical societies of Russia are involved into the program. Recently founded Russian Association of genital infections and neoplasia (RAGIN) provides regular master-classes on cervical pathology and colposcopy around the country.

Russian Medical Academy of Postgraduate Education is working for many years in area of cervical pathology. The regular courses for physicians from all parts of country are provided in Moscow city and many cities around the Russian Federation and countries of former Soviet Union. This year the Dept of ob\gyn starts the new program for the lecturers of medical universities of RF. The analysis of last achievements and failures in realization of this program will be presented. Last survey of ob\gyns has shown the increased awareness of screening necessity and HPV-vaccination.

AWARENESS OF HPV, CERVICAL CANCER AND HPV VACCINE IN TURKEY, ATTITUDES FROM A MUSLIM COUNTRY**Güdücü N, Gönenç G, Iflçi H, Yi iter AB, Dünder I***Istanbul Bilim University, Medical Faculty, Gyn / Obs Istanbul Turkey*

We assessed the knowledge of healthcare workers and students of İstanbul Bilim University and affiliated hospitals about HPV and cervical cancer. Sixhundredthree subjects responded (81,15%). Nurses and students of nursing had better knowledge about smear test when compared to students of medicine (first 3 years) and other healthcare personnel. Only 52% accepted to get a daughter vaccinated. The main reasons for vaccine rejection were the concerns about vaccine safety (41%), cost of the vaccine (10%) and sexual promiscuity (5%) after vaccination. Religion was not considered as an obstacle. We have to increase awareness of HPV, cervical cancer and HPV vaccination.

W 1-11

KNOWLEDGE AND HEALTH BELIEFS AMONG SYRIAN HEALTH WORKERS ABOUT CERVICAL CANCER PREVENTION AND EARLY DETECTION

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Objectives: Variations between developed and developing countries in implementation and effectiveness of health preventive programmes; reflect either differences in cervical cancer management or disparities in behaviours and preventive practices. Healthcare workers are the main providers of health education and their knowledge, health beliefs and health practice may have a profound effect on the success of any health education program. In order to make recommendations for preventive programmes in Syria, a study was conducted regarding knowledge, beliefs and practice regarding HPV and cervical cancer among healthcare workers from general hospitals and health centres in Damascus City.

Methods: A cross-sectional study using a self-administered questionnaire assessed the impact of knowledge and beliefs on screening and vaccine acceptability. Two conceptual models, the health beliefs model and the knowledge model, were tested on predictability of healthcare workers' behaviour using a regression model.

Conclusions: The findings show that there is a major discrepancy between practicing preventive behaviour and intentions. Even though there was only moderate knowledge regarding cervical cancer and HPV, healthcare workers did not report many barriers for their own preventive behaviour. Respondents appreciated the benefits of Pap-test highly, though they perceived the benefits of HPV vaccine moderately. Participant perceived low susceptibility to cervical cancer and HPV, even though they could recognize the seriousness of both. Knowledge about cervical cancer did not affect participants' practice and intentions directly.

A combined health beliefs-knowledge model was shown to have a higher ability to predict preventive health behaviours for this study population. In addition, the study showed the confounding role of each of the health beliefs concepts on each other as well as the role of knowledge as an effect-modifier. These findings suggest the importance of including these aspects as central parts in the process of designing a cervical cancer control programme.

W 1-12

RISK AND PREVENTIVE BEHAVIOURS AMONGST HPV VACCINATED AND UNVACCINATED YOUNG WOMEN

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Objectives: To compare indicators of risk and preventive behaviours in HPV vaccinated and unvaccinated young women attending sexual health clinics.

Methods: 364 participants eligible for HPV vaccination (Cervarix) in the UK vaccination programme (born after 1/9/1990) were recruited from genitourinary medicine clinics in the towns of Warrington and Halton in North West England. Collection of routine clinical data was standardised and additional information about HPV vaccination status and alcohol use was requested. The majority of participants were White British (98.1%, n=357) and the mean age at clinic attendance was 18 years. 63.7% (n=232) of participants had received at least one dose of HPV vaccination and 31.3% (n=114) were unvaccinated. The vaccination status of 4.9% (n=18) is unknown. Markers of sexual risk and other risk behaviour were compared between vaccinated and unvaccinated women. Data was analysed using logistic regression and adjusted for vaccination cohort.

Conclusions: Vaccinated women were taking fewer sexual risks than the unvaccinated women. Of those tested, Chlamydia positivity was higher amongst unvaccinated (19.8%, n=16/81) compared to vaccinated (9.5%, n=18/189) women (Odds Ratio= 2.3, 95%CI, 1.1-5.0, p=0.034). 78% (n=64/82) of unvaccinated women were current smokers compared to 63.2% (n=98/155) of vaccinated women (OR=2.0, 95%CI 1.0-3.8, p=0.036). Alcohol use twice a week or more was higher amongst unvaccinated (31.9%, n=36/113) than vaccinated (24.2%, n=56/231) women (OR=0.84, 95%CI, 0.49-1.4, p=0.52). Unvaccinated women were more likely to have symptoms when they attended (63.4%, n=71/112 versus 51.1%, n=118) (OR=1.81, 95% CI, 1.1-2.9, p=0.018). We conclude that in the UK, where vaccine coverage is high, failure to initiate HPV vaccination is a marker of high risk behaviour amongst genitourinary medicine attendees.

BENEFIT VERSUS RISK OF HPV VACCINES**Lopalco PL,***Head of Vaccine Preventable Disease Programme, European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden*

Communicating the risk is not easy and it is crucial when promoting vaccination in the field. Vaccine acceptance is the result of a complex decision making process that involves rational, emotional and ethical aspects of the personality. In the case of HPV vaccines the issue is even more complicated being the communication mostly addressed to adolescent women that should take such decision during a very delicate phase of their physical and psychological development. Communication based on evidence is more effective as regards the rational part of the decision. The benefit of HPV vaccination are clear and easy to communicate, even though some major effort needs to be put in place for better communicating the residual risk of HPV infection (and consequently cancer) from non-vaccine types. The subsequent need for undertaking screening tests should be stressed as well. Risk communication should be focused on the very low risk of getting vaccinated using a vaccine product that demonstrated a very good safety profile. Transparent and authoritative information is paramount. Much harder is the communication when involving emotional/irrational arguments or ethical/religious aspects. For such purpose specific communication should be developed, adapting both content and channels to the characteristics of the target populations.

COST- EFFECTIVENESS ISSUES: ARE THEY QUESTIONABLE?**Brisson M ,***Laval University, Faculty of Medicine, Canada*

With promising efficacy results from randomized control trials of human papillomavirus (HPV) vaccines and the availability of new screening paradigms, policymakers are being asked to make recommendations and decisions regarding the optimal strategies in reducing HPV infection and disease. Such decisions are increasingly being made with significant input from mathematical and economic models. The demand for modeling has resulted in an exponential rise in the publication of mathematical models looking at the cost-effectiveness of HPV vaccination. However, the appropriateness of using cost-effectiveness as criteria for decision making in the field of immunization has often been questioned, either on equity or philosophical grounds.

The objectives of this presentation are to describe:

- 1) the general concepts related to cost-effectiveness,
- 2) its role in decision making and
- 3) the advantages and limitations of economic evaluation of HPV vaccines.

W 2-4

**ETHICAL ISSUES OF HPV VACCINE INFORMATION:
IS THERE ANY DEVIATION?**

Cain, JM

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HPV Vaccine offers the promise of significantly reducing the burden of cervical cancer globally through primary prevention with virtually no risk to the population vaccinated. The ethical issues that should be raised with any vaccine effort are those of benefit and harm. Benefit—the obligation to assure that a goal of medicine is being met is defined for this intervention by its ability to control and eliminate a serious and life threatening disease. A concurrent goal of medicine—that of educating a patient or a population about the disease—should also be an obligation of health professionals and those serving the public interest. To do so, education must be based on evidence and the pathogenesis of the disease. The harms of vaccination so far documented have been limited to rare allergic events and local irritation at the site of injection.

Why then has there been misinformation put forward about this particular medical advance? Concerns about spontaneous abortions, autoimmune diseases, and even fainting have been laid aside by review of the research. Concerns about changes in sexual behavior likewise have been proven to be unwarranted and unsupported by evidence in multiple vaccine programmes throughout the globe. It is also no more logical that someone receiving hepatitis immunization would, on the basis of immunity, start injecting illicit drugs than a child would seek out sexual exposure on the basis of immunity to HPV. The parental assurance that they can protect their daughters from sexual exposure and therefore they do not need vaccination has been proven to be false in every resource setting. Even following every restrictive cultural and sexual convention in a region or country and entering marriage as a virgin does not protect a woman from HPV exposure if her spouse has a high risk HPV. The harm that is done by fostering false information, and propelling parental fears about sexual promiscuity is unethical for any community leader—but it breaches the most important ethical boundary for those in the health professions who engage in this behavior. It causes harm, and doing no harm (nonmaleficence) is the most important ethical tenet for all health professionals.

W 2-5

**WHY DO WE NEED A BROAD HPV VACCINE PROGRAM TO PREVENT CERVICAL CANCER
IN DEVELOPED AND DEVELOPING COUNTRIES?**

Eduardo Franco (*McGill University, Montreal, Canada*)

Few if any delegates at an EUROGIN Conference doubt the validity or question the weight of evidence from the phase II and phase III randomized controlled trials by pharmaceutical companies that have provided the essential answers concerning efficacy and safety of the available HPV vaccines. The two available vaccines have been licensed in most countries. School-based vaccination programs of pre-adolescent or adolescent girls in Australia, the UK, and Canada have attained very high coverage rates and are accepted as models of cost-effective and equitable delivery of HPV vaccines to the population. Centralized procurement and purchasing mechanisms via GAVI, the WHO, PAHO, and other organizations have also attempted to provide HPV vaccines to low resource countries, which bear the greatest burden of cervical cancer. The obvious primary goal of such programs is to prevent cervical cancer in the long term as a socially just benefit to the population. Cervical cancer is a disease that serves as a sentinel of inequality. Implementing HPV vaccination simply as an opportunistic program risks augmenting the existing inequality in cervical cancer control. Simple reimbursement for out-of-pocket vaccination costs may end up benefitting only the daughters of women who are already captive to cervical cancer screening programs and are thus of low risk of cervical cancer. Women targeted by vaccine promotion messages already afford health care and the benefits of having frequent Pap smears. These women are not the ones who eventually develop cervical cancer because they are assiduous clients of screening offered them on an opportunistic basis by their physicians. Nor are they candidates for vaccination because of their age, but will learn that their daughters can benefit from receiving it. Uptake is thus higher among girls from these affluent families. Like their mothers, however, these young women, even without vaccination, would not be at high risk for cervical cancer later in life because they are highly likely to become clients of the intensive, elitist screening that benefited their mothers. On the other hand, women without access to health promotion who cannot afford private health care and thus have to depend on the public system (which has low quality, spotty, or nonexistent screening) are not being screened adequately or at all. They do not know about HPV vaccines nor will their daughters be offered vaccination. Their daughters will be like them, unvaccinated and unprotected by screening, and 20 years later they may sadly end up contributing to the cruel statistics of cervical cancer.

STC 2-1

VULVAR DISEASE COURSE INTRODUCTION**Bornstein J**

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Bar-Ilan University, Faculty of Medicine - Nahariya, Israel*

The vulvar diseases course is sponsored by ISSVD and EUROGIN. The ISSVD is a multidisciplinary organization, whose aims are to promote international communication among gynecologists, pathologists, dermatologists and related disciplines, and to establish international agreement on terminology and definitions of vulvovaginal diseases; to promote clinical investigation, basic research, and dissemination of knowledge in this field; to promote international communication among gynecologists, pathologists, dermatologists, and related disciplines, and to establish international agreement on terminology and definitions of vulvovaginal diseases; to promote clinical investigation, basic research, and dissemination of knowledge in this field. Registration to the society is through the website – www.issvd.org.

This course is an educational program for clinicians who treat patients with persistent or recurrent vulvar symptoms. The learning objectives of the course are:

- To gain awareness of the pathophysiology and treatment of certain benign vulvar conditions
- To understand the diagnosis and treatment of vulvar pain
- To expand their knowledge on HPV associated disease
- To learn the diagnosis, terminology, prevention and treatment of vulvar intraepithelial neoplasia (VIN) and vulvar carcinoma
- To gain awareness of the sexual aspects of vulvovaginal diseases

STC 2-2

BENIGN HPV LESIONS, DIAGNOSIS AND TREATMENT**Hampel M**

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Benign HPV induced lesions are in the majority of cases condyloma or VIN 1 lesions.

The incidence of condyloma of the lower female genital tract is increasing in the last years. An infection of the female genital tract with human papillomavirus (HPV) Typ 6/11 (in over 90% of cases) is considered to be the cause of this disease and since HPV is transmitted by sexual intercourse in the majority of cases, condylomas are most prevalent in young, sexually active women. The peak of disease prevalence is in the age group of the 20-24 years old women, but also in elder women or children the infection with HPV may causes warts.

Diagnosis of condylomas is made by inspection, colposcopy and histology by punch biopsy in cases VIN is suspected to exclude high grade disease.

Treatment consists of local treatment with substances like podophyllotoxin, imiquimod, trichlor acitic acid or catechines. In addition surgical excision, laservaporisation or electrocauterisation are widely used in addition to alternative medicine. Treatment options are discussed with advantages and disadvantages within the course.

STC 2-3

VULVAR PAIN: ETIOLOGY AND DIAGNOSIS**Bornstein J***President of the International Society for the Study of Vulvovaginal Disease (ISSVD)**Department of Obstetrics & Gynecology, Western Galilee Hospital and**Bar-Ilan University, Faculty of Medicine - Nahariya, Israel*

Localized Provoked Vulvodynia (LPV) or Vestibulodynia, previously named Vestibulitis, is a common cause of dyspareunia. Until 1981, dyspareunia was considered a result of vaginismus. Since then it was discovered that dyspareunia can be caused by a physical condition characterized by hyperesthesia of the vestibule. In a population based study, 16% of women aged 18-64 reported histories of chronic vestibular burning, knifelike pain, or pain on contact that lasted three months or longer. This highly prevalent condition causes many women to abstain from intercourse. Using immunostaining by C-kit, Mast cell Tryptase and heparanase, S-100 and PGP-5,9 nerve staining, we detected increased local heparanase expression, as well as subepithelial and intraepithelial neuroproliferation, and a significant increase in sub-epithelial inflammatory infiltrate, in cases of Vestibulodynia, compared to normal controls. A search for the local presence of H. Pylori proved negative.

Diagnosis of LPV is stepwise: chronic dyspareunia should prompt vulvar examination. Transient local conditions such as candida vulvovaginitis as well as chronic skin disorders as Lichen Sclerosus should be ruled out. Typically, vestibular sensitivity in seven foci is documented. The tampon test and various algesiometer test are also available. Tissue diagnosis is unnecessary, but if a biopsy is taken, increase in mast cells, neuroproliferation are usually seen.

STC 2-5

VIN- PREVENTION AND TREATMENT**Joura E,***Medical University Vienna, Vienna, Austria*

Objective: To discuss epidemiology, diagnosis, treatment and prevention 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. Differentiated VIN is associated with Lichen sclerosus, and not with HPV. It can be found in older women and has a high risk of progression to invasive cancer. 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.

STC 2-6

EARLY STAGE VULVAR CANCER: MANAGEMENT**Michel Roy***MD, FRCS, Québec, Canada*

he first step in the management of vulvar cancer is a good investigation. Of course, a biopsy is necessary to confirm an invasive lesion. If the deepness of invasion is not available with a biopsy and/or a lesion is reported as a VIN but is clinically suspicious of invasion, a wide excision is indicated before a definitive treatment.

Because in younger patients, HPV plays a major role in the development of VIN and cancer, an evaluation of the cervix, vagina and anus is mandatory.

In very early stage (Ia), with invasion less than 1mm, wide excision alone without inguinal lymph node excision is the treatment of choice. When invasion is more than 1mm, radical surgery is indicated. Two major changes have been proposed in the last years in the surgical approach of vulvar cancer: The search and excision of sentinel lymph nodes (SLN) and more conservative excisions: Wide radical excisions, unilateral vulvectomy and plastic surgery when necessary.

Complications after inguinal total lymphadenectomy happen in more than 50% of the cases. SLN mapping has shown that in 97% of the cases, excision of the sentinel node alone is satisfactory and avoids 90% of the complications related to complete lymphadenectomy. Furthermore, the ultrastaging of the SLN helps detect very small metastasis that would not be identified in routine histopathological evaluation. We think that, nowadays, excision of the inguinal SLN mapping is mandatory. Further studies should show that the excision of the SLNs alone does not lower the chances of cure for the patient.

STC 2-7

VULVAR PAIN: SURGICAL AND NON-SURGICAL TREATMENTS**Bornstein J***President of the International Society for the Study of Vulvovaginal Disease (ISSVD)**Department of Obstetrics & Gynecology, Western Galilee Hospital and**Bar-Ilan University, Faculty of Medicine - Nahariya, Israel*

While our understanding of Vulvodynia continues to grow, there is a growing conflict between care givers considering the ideal treatment. Obviously, management should be carried out according to the exact etiology of Vulvodynia. However, the treatment of Localized Provoked Vulvodynia (LPV), the main cause of Vulvodynia, is still a matter of major disagreement. In 1981, surgical removal of the vulvar vestibule has been proposed and was accepted, becoming a popular treatment of LPV. This popularity was reflected in publications, including a randomized trial by our group that has confirmed the curative role of that procedure. A preferred surgical technique is excising the whole vestibule. Review of the studies of surgical treatment published so far depict a success rate of 85%. The reasons for surgical failure include: inadequate tissue removal, inappropriate choice of patients, performing surgery on patients with unprovoked or mixed Vulvodynia.

However, with time, a plethora of non-surgical treatments have been recommended. The main drive behind advocating medical treatments is to avoid surgery in a young woman. The popular treatments are rehabilitation of pelvic musculature using biofeedback techniques, topical oils or anesthetic creams, behavioral therapy, low dose tri-cyclic antidepressants, certain anti-convulsants, such as Gabapentin and Pregabalin, low oxalate diet, capsaicin. Botox and local interferon injections.

Taking all issues into account, surgery remains the most successful approach to the treatment of Vulvodynia.

STC 2-8

PSYCHOSOMATIC ISSUES IN VULVAR DISEASE**Marc Steben MD,***Unité infections transmissibles sexuellement, Institut national de santé publique du Québec, and medical director of Clinique A, Montréal, Québec, Canada*

Recurrent and persistent vulvo-vaginal problems and sexual and psychological distress are frequently associated. The association can be in the way of the biological problems causing the mental problems or the reverse. Whether the association is the former or the latter, the sexual and psychological distress is frequently not diagnosed or not attended. Patient with history of chronic pain, especially associated with fibromyalgia, migraine, irritable bowel syndrome, fatigue, insomnia, pelvic pain and endometriosis have a greater risk of depression, anxiety or both. Presence of violence or abuse as been irregularly associated with chronic genital problem and should be evaluated. Conjugal violence or problem predispose to such pain. The use of standardize evaluation forms such as the Beck depression inventory or Hamilton-D helps recognition of underlying anxious or depressive disease and should be used in first evaluation and in phases of patient deterioration.

Depressive symptoms can be associated with adaptation problem, dysthymia or frank depression. Anxious symptoms can be associated with adaptation problem, post-traumatic stress disorder, generalized anxiety, obsessive compulsive disorder and social phobia. Ignoring the depressive or anxious state will lead to failure of proposed treatment in many cases. The use of antidepressant and anxiolytic is associated with some side effects of which some may even aggravate pre existing condition such as low sexual drive, orgasmic difficulty or asthenia. Knowledge of these drugs is important before prescribing to avoid the side effect that may make the patient shy away from adequate treatment. Multidisciplinary approach of those patients increases the success of treatment of those patients with chronic genital pain.

STC 3-1

BEST PRACTICES IN PUBLISHING CLINICAL AND PUBLIC HEALTH RESEARCH**Eduardo Franco***(McGill University, Montreal, Canada)*

The objectives of this short course are: 1) to provide an overview of principles of good scientific practice as applied to research on human subjects; 2) to discuss elements of scientific reasoning that are used in clinical and public health research and mentorship; 3) to discuss the process of research communication and publishing; 4) to discuss the key features that enhance the quality of a paper and its value to the scientific community; and 5) to discuss rules of authorship, strategies to avoid conflict, criteria to select journals, and issues to be avoided. Instruction will be via interactive lecture to take advantage of students' own experiences as authors; emphasis on issues pertaining to study design, ethics, data analysis, scientific integrity, and strategies of communicating and interpreting results of clinical and public health research. This course is intended for public health practitioners, epidemiologists, and other clinical researchers in the early stages of their careers or those who are switching from clinical or public health practice to research. Public health practitioners and policymakers may also find the contents valuable in understanding the process of scientific research and publishing. Researchers whose mother tongue is not English may find the course particularly helpful in assisting them to navigate the process of preparing, submitting, and revising manuscripts for publication in international biomedical journals.

The instructor has extensive experience as an author, editor, and in overseeing the publication process in epidemiology and clinical research in oncology. He has published over 340 scientific articles, 50 chapters, and two books on cancer epidemiology and prevention. He has served as Guest Editor for nine journal supplement issues on the topic of his research (cancer epidemiology and prevention). He has served in the editorial board of the following journals: American Journal of Epidemiology (1993-98); Cancer Detection and Prevention (2001-08), Cancer Epidemiology, Biomarkers & Prevention (1995); Epidemiology (1993-2009), International Journal of Cancer (2009-), Medical and Pediatric Oncology (2000-04), Oral Diseases (2005-), PLoS-Medicine (2004-), Preventive Medicine (2008-) among other journals. He has also served as ad hoc manuscript reviewer for dozens of other biomedical journals.

HPV GENOTYPING AND COFACTORS IN THE DETECTION OF HIGH-RISK PATIENTS AFTER CONISATION

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2 RIATOL, Laboratory for Clinical and Molecular Pathology, Sonic Healthcare Benelux, Antwerp, Belgium.

Background: High-risk types of the Human Papillomavirus (HR-HPV) are the essential causative agents for cervical cancer and improvement in effectiveness of the cervical screening program can potentially be achieved by combined analysis of cytology and HR-HPV detection to predict high grade disease with high sensitivity and specificity for clinical management.

Objectives: The aim of this study was to investigate the role of the HR-HPV types before and after treatment in triaging women for follow-up post conisation and the effect of different factors (age, parity, contraception, smoking and immunosuppression) in predicting persistence after conisation for high-grade cervical intraepithelial neoplasia or cancer (CIN2+).

Methods: This study analysed data from 248 consecutive women (mean age 39.7 year, range 19 – 81), referred for colposcopy and conisation between January 2008 and December 2011. Women were prospectively submitted to cytology and HPV genotyping by PCR prior to surgery and at 6 months follow-up. Type specific HPV viral load, age, parity, smoking, anti-conception and immunosuppression were evaluated as possible covariates of residual disease.

Results: Before treatment, 90.32 % of the women were HR-HPV positive. HPV 16 and HPV 33 were significantly the most prevalent types in the CIN2+ biopsies [OR = 5.64, 95% CI= 2.90-10.98 and OR= 9.96, 95% CI= 1.26-78.49 respectively]. HPV genotyping before and after treatment identified persistent viral infection in 37.5 %. At the 6-month visit, HPV 52 [OR=2.21, 95% CI= 1.01- 4.85], HPV 56 [OR= 6.36, 95% CI= 1.99-20.40] and HPV 58 [OR= 3.19, 95% CI= 1.37-7.45] were significantly associated with persistence. Multiple regression analysis revealed that there were no other significant covariates in predicting HPV persistence.

Conclusion: HR-HPV testing is the most sensitive means of identifying persistence and is capable of optimizing follow-up after treatment. The presence of infection with HPV 52, 56 or 58 before conisation was significantly associated with higher rates of residual disease after conisation. Independent of the HR-HPV type, no other risk factor seemed to significantly increase the rate of persistence after conisation.

HIGH-RISK HUMAN PAPILLOMAVIRUS E6/E7 mRNA AND L1 DNA AS MARKERS OF RESIDUAL/RECURRENT CERVICAL INTRAEPITHELIAL NEOPLASIA

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3 Department of Medical Epidemiology and Biostatistics Karolinska Institutet, 171 77 Stockholm, Sweden

Objective: To assess the use of human papillomavirus (HPV) E6/E7 mRNA testing in the follow-up of women treated for cervical intraepithelial neoplasia (CIN) by conization. To compare the prognostic value of HPV E6/E7 mRNA to HPV L1 DNA and cytology.

Methods: 143 women underwent cytological/histological testing, HPV DNA genotyping by Linear Array, and HPV E6/E7 mRNA testing by APTIMA HPV Assay during follow-up after surgical treatment for histologically verified CIN.

Results: High-grade residual/recurrent disease (CIN2+/HSIL+) was identified in 7 (4.9%) women, and low-grade disease (CIN1/LSIL) in 25 (17.5%). At the inclusion visit 33 (23%) women were HPV DNA-positive; 13 (9.0%) were HPV E6/E7 mRNA-positive. HPV E6/E7 mRNA did not identify two women with high-grade disease. Presence of high-risk HPV DNA at the inclusion visit predicted 100% (95% CI 64.6-100) of high-grade residual/recurrent disease, with a specificity of 80.9% (95% CI 73.5-86.6); cytology had a sensitivity of 85.7 %, and a specificity of 87.5%. HPV E6/E7 mRNA testing was a poor predictor of treatment failure, with a sensitivity of 57.1% (95% CI 25.0-84.2), but high specificity (93.4%; 95% CI 87.9-96.5).

Conclusions: Detection of high-risk HPV DNA after treatment by conization identified 100% of women with residual/recurrent high-grade disease, whereas HPV E6/E7 mRNA testing was a poor predictor of treatment failure. This study suggests that a negative HPV mRNA result cannot exclude the risk of malignant progression, and that HPV E6/E7 mRNA testing by APTIMA HPV Assay is not useful in the follow-up of women treated for CIN.

FC 1-3

E6 TESTING FOR PRIMARY SCREENING OR TRIAGE EVALUATION: EXPERIENCE FROM CHINA**Jerónimo J¹, Zhao FH², Schweizer J³, Chen W², Bai P², Li J¹, Peck R¹, Castle PE⁴, Qiao YL²**1. *PATH, Seattle, USA;*2. *Cancer Institute/Hospital, Chinese Academy of Medical Sciences/Peking Union Medical College, Beijing, China;*3. *Arbor Vita Corporation, Fremont, USA*4. *American Society for Clinical Pathology Institute, Washington DC, USA*

Background: HPV-DNA testing is highly sensitive for detection of cervical precancer or cancer of the uterine cervix. However its specificity is sub-optimal because the detection of HPV cannot distinguish between benign HPV infections and those associated with cervical precancer or cancer. The detection of E6 protein may be useful biomarker for making that distinction. The goal of our study is to evaluate the performance of a prototype test that detects the expression of E6 protein of HPV 16, 18 and 45.

Methods: approximately 7500 women aged 25 to 65 years from Shanxi Province (China) were enrolled between 2010 and 2011. After informed consent all women were trained to self-collect a vaginal sample for careHPV and Hybrid Capture 2 (HC2) testing. Then they had a pelvic evaluation and cervical samples were collected for HPV E6 Testing, careHPV and HC2, and finally the provider performed visual inspection with acetic acid (VIA). Women positive at any test and a 10% random sample of women negative on all tests had colposcopy and biopsy.

Results: The analysis of results of the first 5,621 women enrolled shows that the positivity rate for the E6 test was 1.6% compared to 13.3% for careHPV test and 13.9% for HC2. The sensitivity for cervical intraepithelial neoplasia grade 3 or more severe (CIN3+) of HPV E6 was 67.4% compared to 98.0% for careHPV test and HC2, and 42.9% for VIA.

Conclusions: The HPV E6 test for genotypes 16, 18 and 45 detected a significant percentage of CIN3+ cases with a very low referral rate. The test could be beneficial as a primary screening test in settings with limited capacity for evaluation and treatment of women with CIN3+. The HPV E6 test could also be used for triage of women with positive HPV-DNA results.

FC 1-4

PERFORMANCE OF TWO HR HPV-mRNA DETECTION TESTS ON THIN-PREP SAMPLES FROM HPV-DNA POSITIVE WOMEN SELECTED FOR COLPOSCOPIC EXAMINATION**Matinato C., Lunghi G., Orlandi A., Zoccoli A., Bono P., Torresani E.***UOS Virology – Laboratory analysis, Fondazione IRCCS Cà Granda Ospedale Maggiore Policlinico di Milano*

Objectives: Aim of this study was to evaluate performance of the two tests for High Risk (HR) HPV-mRNA detection APTIMA HPV Assay, (Gen-Probe) and NucliSENS EasyQ HPV v1.0, (Bio-Merieux) on 203 Thin-Prep samples of women positive to HPV-DNA and selected for colposcopic examination.

Methods: A Pap test for cytological examination was performed. All samples were submitted to HPV-DNA and mRNA detection. The extraction procedures were performed by automated system NucliSENS Easy-Mag (Biomerieux), according to manual instructions.

HPV-DNA genotyping was performed using INNO-LIPA HPV Genotyping Extra (Innogenetics) reverse hybridization which provides for the identification of 28 different HPV genotypes: 18 high-risk (16,18,26,31,33,35,39,45,51,52,53,56,58,59,66,68,73,82), 7 low-risk (6,11,40,43,44,54,70), and 3 (69,71,74) defined as intermediate risk.

Two tests for HR-HPV mRNA were performed:

APTIMA HPV Assay, a multiplex nucleic acid test that detects HPV E6/E7 mRNA from 14 high-risk HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) and that provides a qualitative result (positive/negative) for the presence/absence of these HR-HPV types, but does not determine the specific HPV type present in the specimen.

NucliSENS EasyQ HPV v1, a real-time nucleic acid sequence-based amplification (NASBA) assay for the qualitative determination of E6/E7 mRNAs of 5 HPV types (16,18,31,33 and 45).

To establish concordance between the two mRNA tests, Cohen's Kappa was determined.

Results and Conclusions: 72 ASCUS (35%), 101 LSIL (48%) and 30 HSIL (15%) were detected with cytological examination. 68% of samples were infected with only one genotype of HPV-DNA, 32% were mixed infection with 2 or more HPV genotypes involved. Genotype 16 was the most frequently detected (20%), 31 was detected in 18% of samples, 53 in 16%, 52 in 8% and 18 only in 4%. HPV-mRNA was detected in 42% of samples tested with NucliSENS EasyQ HPV v1 and in 59% of samples tested with APTIMA HPV Assay. Concordance between the two HPV-mRNA tests was moderate (K= 0,44).

HPV-DNA TITRES IN SQUAMOUS CERVICAL INTRAEPITHELIAL LESIONS (SIL): CORRELATION WITH LESION SEVERITY AND VIRAL FINDINGS

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Objectives: The role of HPV load in the development and progression of SIL is unclear and its relationship with other virological features is controversial. We aimed to define the correlation of HPV titres with relevant clinical and virological findings in women undergoing colposcopy for abnormal cervical cytology.

Methods: We evaluated cervical scrapings collected in ThinPrep-Preserv-Cyt solution from 501 women (mean age 37 yrs) undergoing colposcopy; 79% had targeted biopsy (42.5% CIN1, 32% CIN2+). Full HPV genotyping by INNOLiPA SPF-10 was available in all samples (43% multiple infections, 13.5% untypable). DNA extraction was performed using an automatic instrument (Magstration system 12GC, VODEN) and HPV DNA quantification was obtained by real time PCR (RotorGene, Explera) using SPF10 primers. beta-globin gene was quantified to normalize HPV loads.

HPV DNA titre and HPV/beta-globin ratio significantly correlated with severity of cytological (p 0.001) and histological lesions (p 0.0011), multiple infection (p 0.000), number of HPV types (p 0.000), and class of oncogenic risk (multiple HR types and HR-LR types had higher titres than single HR and LR infections, p 0.0001). Untypable samples had lower titres than other groups. Viral titres were positively correlated with infections by HPV type 6 (p 0.006), 31 (p 0.025), 53 (p 0.003), 56 (p 0.016), 66 (p 0.010) and 82 (p 0.018); no correlation was found for HPV 16. Age and menopausal status didn't affect viral load.

After correction for number of viral types and lesion severity, normalized HPV titres were directly correlated with infections by HPV 31, 44, 66 and 74 and inversely correlated with HPV 18 and 35.

At multivariate analysis, viral titres significantly correlated with the number of infecting types (p 0.000) and the presence of low grade SIL (p 0.043).

Conclusions: In our series, HPV loads correlate with SIL grade, multiple infection, number of viral types and class of oncogenic risk. The presence of HPV 16 does not significantly influence viral titres. Untypable infections have lower viral titres. Larger series are necessary to clarify the role of viral load in the clinical management of SIL.

CERVISTA® HPV HR PERFORMANCE EVALUATION USING SAMPLES COLLECTED IN SUREPATH® PRESERVATIVE FLUID

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Objectives: The analytical and clinical performance of the CE Mark Cervista HPV HR test has been previously determined using ThinPrep® cervical specimens collected in PreservCyt® solution. The objective of the current investigation was to evaluate CE Mark Cervista HPV HR performance using samples collected in SurePath® preservative fluid.

Methods: 418 women were enrolled in a co-collection study to obtain paired cervical specimens collected into SurePath preservative fluid and PreservCyt solution. The SurePath specimens went through a conversion process and then DNA was extracted from both specimen types and tested with the CE Mark Cervista HPV HR test. Reproducibility and repeatability were determined by testing a sample panel consisting of residual SurePath samples with varying levels of HPV or negative samples and performing the test across multiple sites, operators, runs and days. In addition, sample stability and manual versus automated test performance were evaluated.

Results and Conclusions: Results from the co-collection study demonstrated 92% agreement for specimens collected in SurePath Preservative fluid compared to specimens collected in PreservCyt solution. In a separate study, 192 pooled residual SurePath samples were processed using either a Cervista manual method or medium throughput automation (MTA) system. The positive and negative percent agreement for the manual versus MTA method was 95.7% and 96.0% respectively. The results from the reproducibility study confirmed that the SurePath sample process, when used with the CE Mark Cervista HPV HR test, generates reproducible and repeatable results*. Cervical specimens collected in SurePath preservative fluid demonstrated stability at room temperature (20-30°C) for up to 6 weeks. The results of these studies support the use of SurePath cervical specimens with the CE Mark Cervista HPV HR test.

* Not for sale in the United States. CE Mark Cervista HPV HR test, and use with cervical specimens collected in SurePath preservative fluid, is limited to EU and CE countries.

FC 1-7

HISTOLOGIC COMPARISON OF CERVICAL BIOPSIES IN ASCUS/HPV-HR DIAGNOSED PATIENTS**Dierick A***, **Sturtewagen Y***, **De Mey A***, **Vastenavond H***, **Ali-Risasi C***, **Lambein K***, **Padalko L****, **Praet M****Departments of Pathology* and Clinical Biology, Virology**, University Hospital, Gent, Belgium*

Objectives: ASCUS/HPV HR reflects a broad spectrum of cytologic changes characterized by slight dysplastic changes insufficient to call them CIN. More often cervical biopsies/hysterectomies are performed to rule out neoplasia.

Methods: 126 formalin fixed paraffin embedded cervical specimens containing cervical biopsies (n=95), pipelle biopsies (n=8), LLETZ (n=19) and hysterectomies (n=4) from 89 patients, presenting with abnormal smears, classified as ASCUS on liquid based cytology and positive for HR-HPV on HPV-testing (GenoID, Amplicor or Abbott test). All biopsy specimens were reviewed by two pathologists on 4 μ HE sections and after immunostaining for p16^{INK4a} and Ki-67 with deeper cuts when necessary.

Conclusions: 8 % (n=10) contained HSIL lesions. In 29 % (n=37) LSIL lesions were found. 35 % (n=44) were characterized by inflammation at the transformationzone with metaplastic changes, including atypical immature metaplasia. 19% (n=24) were normal or slightly inflammatory, 9 % (n=11) were not representative.

FC 1-8

COMPARISON OF HPV ONCOTECT AND NUCLISENS EASY Q TEST IN PRENEOPLASTIC LESIONS OF THE CERVIX**Rongioletti M¹**, **Papa F¹**, **Vaccarella C¹**, **Majolini MB¹**, **Neri F¹**, **French D²**, **Liumbruno GM¹***1 Laboratory of Clinical Pathology "San Giovanni Calibita" Hospital Rome Italy.**2 Department of Clinical and Molecular Pathology, "Sapienza" University of Rome, Italy.*

Objectives: As well known, in High risk HPVs the expression of oncoprotein E6 causes the degradation of p53, while E7 inactivates pRb and causes the progression to S phase of cell cycle, both sustaining the conversion to and the maintenance of malignancy.

The aim of this study is to compare the evaluation of the E6 and E7 mRNA expressions of HR HPV using the NucliSENS Easy q test (bioMerieux) with HPV Onco Tect (IncellDx) a test based on Flow cytometry-FISH method for detection E6/7 HPV mRNA in cervical cells.

Methods: We enrolled 50 patients previously tested positive for HRHPV DNA and referred for evaluation of the NucliSENS Easy q test (bioMerieux). All enrolled patients were tested for the HPV types 16, 18, 31, 33, 45 E6/E7 mRNA expression and underwent a pap smears and histological evaluation. All the 31 patients resulted positive for RNA expression with Nuclisens were selected for HPV Onco tect assay. The patients were divided into two cohorts: patients with a low grade lesions (CIN1), and patients with high grade lesions (CIN2+) histological diagnosed.

Conclusions: Patients with CIN1 tested positive were 26 with NucliSENS Easy q test and 13 with HPV Onco Tect. Patients with CIN2+ tested were 5 with NucliSENS Easy q test and 5 with HPV the NucliSENS Easy q Real time rtPCR method is more sensitive than flow cytometry, but flow cytometry is able to distinguish the cylindrical cells from squamous cells and this could be an added value in the diagnosis of endocervical lesions. This preliminary results preliminary results seem to indicate that HPV Onco tect have a better specificity than NucliSENS Easy q test, while more samples needs to assess the difference in sensitivity. In conclusion HPV Onco Tect seems to be a very interesting method to evaluate the preneoplastic lesions of the cervix thats requires further study to determine the effectiveness for clinical decisions.

TYPE-DEPENDENT E6/E7 MRNA EXPRESSION OF SINGLE AND MULTIPLE HR-HPV INFECTIONS IN CERVICAL NEOPLASIA

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Objectives: To characterize the tendency of 12 HR-HPV to express E6/E7 mRNA, correlated to the severity of the cervical lesion. Expression of oncogenic E6/E7 mRNA has previously been shown to correlate with risk of malignancy, but commercial assays for genotype-specific E6/E7 mRNA detection of all HR-HPV are lacking. Furthermore, we wanted to analyse mRNA expression in multiple infections, in order to establish which genotype may be responsible for cellular transformation.

Methods: 245 samples from women with normal histology, various grades of dysplasia (cervical intraepithelial neoplasia grade 1-3) and cancer, were analysed for presence and genotyping of HPV DNA and mRNA using an in house real-time PCR test.

Conclusions: The genotype most prone to express mRNA in high-grade lesions was HPV45, followed by HPV16 and HPV31. Less prone was HVP59. The frequent expression of E6/E7 by HPV45 may promote oncogenicity and could be of clinical importance. In multiple HPV infections, more than one genotype expressed E6/E7 mRNA in a majority of cases (52%), a phenomenon increasing with severity of lesion. Further and larger studies using complementary techniques are required to identify the genotype that causes transformation. However, presence of mRNA could more often be detected in samples with multiple infections than in samples with single infections, indicating that multiple infection with HR-HPV could be a clinically important finding.

STABILITY OF CERVICAL SPECIMENS COLLECTED IN SUREPATH® PRESERVATIVE FLUID FOR USE WITH COBAS® 4800 HPV TEST

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Objectives: The cobas® 4800 HPV Test (Roche Molecular Systems Inc.) is a new fully automated real-time PCR system for the detection of 14 high-risk HPV types with differentiation of genotypes 16 and 18. This system is intended for cervical specimens collected in either PreservCyt (Hologic Inc.) or SurePath® (Beckton, Dickinson and Company) preservative fluid. While the stability of PreservCyt is acceptable at ambient temperature for up to 6 months, the use of SurePath® is restricted to specimens refrigerated soon after collection and tested within 4 weeks. As continuous refrigeration poses practical difficulties in our setting, the stability of SurePath® preserved specimens maintained at ambient temperature for use in the cobas® 4800 HPV test was investigated.

Methods: Cervical specimens (n = 308) were collected in SurePath® preservative fluid, pooled and aliquoted. One aliquot was tested employing the cobas® 4800 HPV Test at baseline (Control 1) as well as the Hybrid Capture 2 (HC; Qiagen) assay as per manufacturer's instructions. Aliquots were stored at refrigeration and ambient temperatures for a minimum of 10 weeks and retested employing the cobas® 4800 HPV Test. The refrigerated aliquots served as Control 2. The aliquots stored at ambient temperature were also tested employing the pre-analytic cobas Sample Preparation Buffer (CSPB) protocol as per manufacturer's instructions (Test 1) and without CSPB pre-treatment (Test 2).

Results and Conclusions: Agreement between cobas baseline (control 1) and HC results was 85.1% and cobas control 1 vs. 10 weeks post-refrigeration (Control 2) was 94.2%. Cobas control 1 vs. 10 weeks ambient storage with CSPB pre-treatment (Test 1) was 96.1%. Cobas control 1 vs. 10 weeks ambient storage without CSPB pre-treatment (Test 2) was 93.2%. The CSPB pre-treatment appeared to improve the overall agreement between the control and test results. There was no significant difference (P < 0.05) between the proportion of positive results obtained with specimens at baseline and those held at ambient temperature for up to 10 weeks. In conclusion, cervical specimens stored in SurePath® preservative fluid are stable for up to 10 weeks at ambient temperature for HPV testing with the cobas® 4800 HPV Test.

FC 2-2

IMPACT OF THE URINE FRACTION (FIRST-VOID VERSUS MID-STREAM) ON HPV DNA DETECTION.**Vorsters A¹, Micalessi I^{1,2}, Van den Bergh J¹, Ieven M^{1,3}, Bogers J², Van Damme P¹.***1 Vaccine & Infectious Disease Institute, University of Antwerp, Antwerp, Belgium**2 Laboratory of Cell Biology & Histology, University of Antwerp, Antwerp, Belgium**3 Laboratory for Medical Microbiology, Antwerp University Hospital, Antwerp, Belgium*

Introduction: Detection of human papillomavirus (HPV) DNA in urine, a specimen easily obtained by a non-invasive self-sampling method, has been the subject of a considerable number of studies. As reported previously the methodology between these different studies varies considerably. From a total of 41 studies, 17 reported to have analysed first-void urine, 3 used mid-stream urine and 21 did not disclose which fraction was examined. [1] These findings show clearly that there is no consensus on which urine fraction to collect and that many investigators are not aware of the potential impact the collected urine fraction may have on the outcome of their study.

Objective: To determine the optimal urine fraction for HPV DNA detection.

Methods: Women with a history of a cytological normal but HPV DNA positive cervical sample were asked to provide approx. 50 ml first-void urine and the subsequent fraction in two separate collection containers. DNA was obtained according to an optimised in-house urine collection and extraction method. HPV DNA and human DNA (hDNA) were quantified using real-time PCR. Sample pairs of 10 women were analysed.

Results: The first-void fraction was in most cases more turbid than the subsequent fraction. For HPV as well as for hDNA a significant higher copy number was found in the first-void fraction compared to the mid-stream fraction. For HPV 5 to 160 times more HPV DNA copies were found in the first void fraction. For hDNA the median ratio of first-void hDNA copies over mid-stream hDNA copies was 5.6.

Conclusion: These data confirm that there is a substantial impact of the urine fraction used for analysis and that the first-void fraction is the most appropriate fraction for HPV DNA detection.

[1 A Vorsters, I. Micalessi, J Bilcke, M Ieven, J Bogers, P Van Damme. Detection of human papillomavirus DNA in urine. A review of the literature. *Eur J Clin Microbiol Infect Dis.* 2011 DOI 10.1007/s10096-011-1358-z]

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FC 2-3

HPV mRNA IN CERVICAL AND VAGINAL SAMPLES FROM AN APTIMA COLLECTION AND TRANSPORTATION KIT**¹Chernesky, M., ¹Jang, D., ¹Gilchrist J., ²Elit, L., ²Lytwyn, A., and ¹Smieja, M.***1 St. Joseph's Healthcare/McMaster University, Hamilton, ON, Canada**2 Juravinski Hospital Colposcopy Clinic, Hamilton, ON, Canada*

Objectives: When traditional Pap testing is performed without a liquid-based Pap (L-Pap) system, a cervical sample collected for HPV testing would require an appropriate collection device, transportation to the laboratory and a validated fluid to detect high risk (HR) HPV in an assay. Vaginal self sampling may provide an alternative sample from women who are not routinely receiving cervical examinations. A specimen collection and transportation (SCT) kit (Gen-Probe) was used to evaluate cervical and vaginal collections and was compared to PreservCyt (PC) and SurePath (SP) L-Pap samples for the presence of HRHPV mRNA with the APTIMA HPV assay (Gen-Probe Inc.).

Methods: A total of 577 women attending a colposcopy clinic signed consent for a physician to collect one vaginal SCT, and three cervical samples (cervical SCT, PC and SP). Each women self-collected a vaginal SCT. All samples were tested for HPV mRNA and the PC sample was also tested for DNA by HC2 (Qiagen). Test and sample agreement with Kappa statistic were calculated. Sensitivity and specificity for detection of pathology results were calculated. Patients expressed strength of agreement and disagreement with ease and comfort involved with each step of the self-collection process.

Conclusions: Raw agreement between cervical and vaginal sampling using the SCT kit ranged between 80.7 and 81.3 (k 0.60-0.62). Agreement of self and physician collected vaginal SCT was 84.4% (k0.68). Agreement between HC2 and AHPV on PC specimens was 90.8% (k0.81), on cervical SCT was 89.0% (0.77) and on SP specimens 85.6% (0.69). Agreement of AHPV testing of cervical SCT was 91.6% (k0.82) with PC and 85.4% (0.69) with SP. AHPV testing of cervical SCT was 100% sensitive and 59.7% specific in detecting 30 CIN 2+ cases compared to 96.6% and 66.2% for PC (AHPV); 96.7% and 62.9% for PC (HC2) and 90.9% and 69.7% for SP (AHPV). The vaginal SCT samples ranged from 79.3 to 86.2% sensitivity and 60.0 to 68.5% specificity. The SCT samples tested by the APTIMA HPV assay compared favourably to L-Pap sampling. Vaginal collection with the SCT, which was easy and comfortable, identified most cases of CIN 2+ when tested by the AHPV assay.

COMPARISON OF HPV TESTING METHODS: HPV DIRECT FLOW CHIP, LINEAR ARRAY, GENOMICA CLART HPV2 AND HYBRID CAPTURE 2

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Objectives. HPV testing is being introduced in routine gynecological examinations due to its high sensitivity and high NPV in cervical cancer screening. This study evaluated the clinical performance of the HPV DIRECT FLOW CHIP (DF) genotyping test -based on PCR and reverse dot blot hybridization, with no DNA extraction required- by comparison with Linear Array (LA), Clart HPV2 and Hybrid Capture 2 (HC2) assays.

Methods. Randomly selected anonymized samples from women attending routine HPV cervical inspection were tested. DNA was purified for LA and Clart HPV2 tests. Digene Transport Medium was used directly for HC2, while total cell extracts were utilized as PCR template for DF. Comparison of the different methods was done by analysis of each sample by two diagnosis systems: DF vs LA (n=108), DF vs Clart (n=104) and DF vs HC2 (n=100). Clinical sensitivity and specificity, multiple infection rates and distribution among diagnosis groups were studied. Comparison with HC2 is currently ongoing and results will be presented in the conference.

Conclusions. Rates for HPV detection were: LA 64.8% vs DF 71.3% (kappa 0.82, CI 95% 0.7-0.9) and Clart 62.5% vs DF 72.1% (kappa 0.74, CI 95% 0.6-0.8). Multiple infection rates were LA 31.5% vs DF 42.6% and Clart 24% vs DF 43.9%. Clinical sensitivity for HSIL+ was highly concordant between systems (LA and DF, 1; Clart 0.8), whereas specificity was higher for LA (0.4 vs DF 0.3) and Clart (0.4 vs DF 0.3). Preliminary results for DF vs HC2 comparison showed excellent agreement for HPV+ and clinical sensitivity. The accuracy of LA, Clart and DF for detecting HSIL+ was similar, although DF detected significantly higher rates of both multiple infections and HR-HPV positivity in \leq LSIL patients. Presence of HPV16 and 18 was respectively: 24.3%-1.3% LA vs 28.2%-5.4% DF and 26.1%-6.1% Clart vs 24%-8% DF. In conclusion, systems evaluated here yielded comparable results. High sensitivity of DF makes it a useful tool in cervical cancer screening and follow-up of lesions, with the added value of not requiring the DNA extraction step.

THE INTERNAL CONTROL IN THE CERVISTA® HPV HR ASSAY IDENTIFIES CLINICAL SPECIMENS WITH INSUFFICIENT CELLULARITY

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Introduction Detection of high-risk HPV in cervical cytology specimens is an important part of cervical cancer screening. HPV detection assays must have a very high sensitivity; false-negative results fail to identify women who may be at increased risk of developing cervical cancer. The internal control in the Cervista® HPV HR assay is intended to distinguish negative results from false negative results caused by inadequate cellularity.

Methods We reviewed our database for specimens that were unsatisfactory for cytologic evaluation and underwent HPV testing. We identified 181 specimens tested with the Cervista HPV HR assay and 172 specimens tested with the Hybrid Capture® 2 assay (hc2). For each cohort, we reviewed the cause of the unsatisfactory cytology and HPV test results to determine whether the internal control identifies specimens with insufficient cellularity.

Results In the Cervista cohort 94 (51.9%) specimens were unsatisfactory for cytologic evaluation due to low cellularity. Of these, 51 (54.3%) had an insufficient signal from the internal control, and 43 (45.7%) reported a result (40 negative, 3 positive). The remaining 87 specimens (48.1%) were unsatisfactory for cytologic evaluation for reasons other than cellularity including interfering substances. Of these, 14 (16.1%) had an insufficient signal from the internal control, and 73 (83.9%) reported a result (66 negative, 7 positive). All specimens with an insufficient internal control signal would have been reported as negative in the absence of the internal control. In specimens satisfactory for cytologic evaluation, our laboratory's rate of low internal control signal in the Cervista HPV HR assay is ~ 1.1%. In our hc2 cohort, 82 (47.7%) specimens were unsatisfactory for cytologic evaluation due to low cellularity; 78 (95.1%) tested negative, 4 (4.9%) positive. Ninety specimens (52.3%) were unsatisfactory for cytologic evaluation for reasons other than cellularity; 77 (85.6%) tested negative, 13 (14.4%) positive.

Conclusions In specimens satisfactory for cytologic evaluation, our rate of low internal control signal for the Cervista HPV HR assay is ~1.1%; for specimens with inadequate cellularity the rate was 54.3%. The internal control in the Cervista HPV HR test is designed to identify samples with insufficient cellularity and may reduce the potential for a false negative result.

FC 2-6

COMPARISON OF SELF COLLECTED CERVICAL SPECIMEN AND GYNECOLOGIST-COLLECTED CERVICAL SPECIMEN FOR DETECTION OF HUMAN PAPILOMAVIRUS DNA

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Objective: We evaluated if there was a difference between self-administered cervical specimen (Kato device) compared to gynecologist collected sample for detection of HPV DNA.

Method: Design: Cross sectional comparative study. A total 211 women age between 20 to 70 who came to Primary Health Clinic, Jempol Negeri Sembilan, Malaysia were volunteered into study. The women collected the specimen using self-administered cervical Kato device (SACD) and after one hour underwent a second smear by appointed gynecologist. The DNA were extracted and its concentration were measured for DNA quantity and quality. The human papillomavirus DNA detection were conducted on the specimen using nested polymerase chain reaction system (primer MY 9/11 and GP5+/6+). Positive HPV DNA were sent for automated sequencing services for HPV genotype. The difference of DNA concentration and concordance on HPV DNA detection between the two pair specimens were analyzed (IBM SPSS 19 statistical software)

Results: There was no significant difference of human DNA concentration collected by each technique. A high level of concordance between self-collection and gynecologist-sampling with 87.7% (CI 95% 83.3% to 92.1%) was obtained for detection of HPV DNA with good kappa agreement 0.627 (95% CI, 0.498 to 0.668) $P < 0.05$. 19.4% (41/211) were positive for HPV in self-collection while 22.3% (47/211) were positive in gynecologist collected samples. For positive HPVs, variation of HPV type was noted between the two collection techniques, especially type HPV type 16 and 18 which were 32% and 32% for self collection and 28% and 49% respectively for gynecologist sample.

Conclusion: A good agreement between self-collection sample and gynecologist sampling were shown in this study. We conclude that, self-collection using Kato device for HPV DNA can be used as an alternative to the conventional physician cervical scrapping especially in settings where healthcare resources are limited and therefore would improve women participation for cervical screening.

FC 2-7

RANDOMIZED COMPARISON OF VAGINAL SELF SAMPLING FOR HPV TESTING BY STANDARD VS. DRY VAGINAL SWABS

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Objective: To compare wet and dry transport for samples for HPV DNA testing.

Method: The study enrolled women who visited the colposcopy clinic of University Hospitals of Geneva. Women were asked to perform self-sampling HPV testing with kits including a vaginal specimen transport medium (STM) and a vaginal dry swab (DRY). Randomization determined the order of the tests.

Conclusions: A total of 112 women were included in the study. Median age was 31 years (range 21-63 years). The percentage of HPV infection was 68.7% in STM group and 54.4% in DRY group. The overall agreement between the STM and DRY specimens was good (kappa = 0.70, 85.7%). Sensitivity was lower with the DRY swab. Self-sampling using a vaginal dry swab for HPV testing may be an acceptable alternative to STM. Its easy and cheap use may be an alternative for low resource setting.

CAN SUREPATH™ FIXATIVE, USED FOR FIXATION OF CERVICAL BIOSPIES BE A SUBSTITUTE FOR PARAFFIN SECTIONS IN HPV-GENOTYPING BY PAPILOCHECK®?

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Objectives: The purpose of this study is to compare HR-HPV genotyping on SurePath™ fixative used for fixation of cervical biopsies and paraffin sections from the same biopsies by using the PapilloCheck® system.

Methods: The study included 24 cervical biopsies received in SurePath™ vials. The biopsies were fixed over night in the SurePath™ fixative, and processed according to the laboratory's guidelines for rapid processing without formalin fixation.

DNA extraction from SurePath™ vials (group 1) was performed manually using the PapilloCheck® DNA extraction kit from Greiner Bio-One. Sections were cut from the paraffin embedded cervical biopsies (group 2) for manual DNA extraction using the QIAamp DNA FFPE Tissue Kit from Qiagen. HPV genotyping was performed on all extracted DNA using the PapilloCheck® microarray test kit and the CheckScanner from Greiner Bio-One.

Conclusion: Major discrepancy is defined as HR-HPV found in only one group, and minor discrepancy as different types of HR-HPV (other than 16 and 18) found in both groups.

DNA for HPV-genotyping was extracted successfully from all vials, but the biopsy was cut through in 5 cases and hence only 19 cases were enrolled in the final study. It is worthwhile to note that HPV 16 was found SurePath™ vials in 2 of the excluded 5 cases.

No HPV was found in any group in 6 cases. All of these were histologically benign.

HPV 16 was found in 7 cases in group 1 but missed in one case in group 2. The missed HPV 16 was part of a multiple infection, where other HR-HPV types were detected.

HPV 18 was missed in one of the specimens in group 1, but found in group 2 as a solo infection.

Full agreement between HR-HPV types including type 45 were found in 13 cases including the benign cases. Minor discrepancies were found in 4 cases most of them in multiple infections.

These preliminary results shows that HPV-genotyping can be done on the SurePath™ fixative used as fixation for cervical biopsies with good correlation to HPV-genotyping on DNA extracted from tissue. Discrepancies might be caused by the fact that the tissue in the block was cut through, which maybe an advantage in favor of HPV testing on SurePath™ fixative used for fixation of the tissue. The results also shows that DNA extracted from paraffin embedded tissue fixed in SurePath™ fixative can be analyzed on the PapilloCheck® system.

CERVISTA HPV HR ASSAY. BIRMINGHAM CITY HOSPITAL EXPERIENCE USING HOLOGIC HPV PLATFORM IN A ROUTINE CYTOLOGY LABORATORY

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Context : Pilot studies of human papillomavirus (HPV) testing concluded that HPV triage and Test of Cure was feasible and cost-effective as part of a National Screening programme. Funding for the roll out of HPV Testing in the National Health Service Cervical Screening Programme (NHSCSP) in England for women with low grade cervical cytology abnormality has been approved with implementation beginning in early 2012.

Objectives : The aim was to assess the Hologic Cervista HPR HR equipment, the Genfind DNA Extraction Kit and the Cervista HPV HR assay kits in order to ascertain its ease of use and reliability of results, obtain a better understanding of the chemistry and processes involved and assess how HPV testing would fit into the laboratory work flow when implemented into a routine cytology environment.

Method: Liquid based cytology (LBC) samples reported as showing low-grade abnormalities were selected and tested for HPR HR using the Hologic Cervista assay in order to triage those women who required further treatment. In addition, any test result showing low grade abnormality following treatment for Cervical Intraepithelial neoplasia (CIN) were also included as Test of Cure. The trial was conducted on 478 anonymised samples.

Results: 478 HPV test were carried out during this evaluation.

| HPV Result | Triage Repeat cytology sample | Triage Referral cytology sample | Test of cure sample |
|------------|-------------------------------|---------------------------------|---------------------|
| Inadequate | 2 | 4 | 4 |
| Positive | 92 | 140 | 18 |
| Negative | 76 | 52 | 90 |

Conclusions: The results demonstrated the reliability of the Cervista HPV HR assay with only a 2.09% inadequate rate. The Hologic platform fitted in well with the work flow within the department. Carrying out these manual tests has given a good understanding of the chemistry involved and the potential problems that can arise. Manual DNA extraction requires accurate laboratory techniques in order to obtain results. Our experiences within the laboratory have indicated that implementing HPV HR testing on a large scale will require automated platforms.

FC 2-11

COMPARISON OF THE F-HPV TYPING™ AND HYBRID CAPTURE II® ASSAYS FOR DETECTION OF HIGH-RISK HPV GENOTYPES IN CERVICAL SAMPLES

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Objectives: The detection of High-Risk HPV in cervical samples has been proposed as a valid alternative to improve the efficacy of cervical carcinoma screening programs and HPV genotyping methods have been considered to identify HPV positive women with persistent HR-HPV infections. Furthermore, HPV genotyping tests might be effective tools for monitoring the effectiveness of HPV vaccination, these approaches requires access to fast, easy and high-throughput technology. The aim of this study was to compare a new commercialized method (f-HPV typing™) with Hybrid Capture II® (HC2) to detected HPV infection.

Material and Methods: This new test is a multiplex PCR based on 15 fluorescently labelled primers recognising 13 high-risk (HPV-16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68) and two low-risk (HPV-6 and 11) HPV type within E6 and E7 regions of the HPV genome, the sequences most likely to be retained after viral integration. The F-HPV typing™ also includes a polymorphic Short Tandem Repeat (STR) as internal PCR control. A subset of 157 cervical samples was performed with both assays targeting the same set of HR HPVs. HPV DNA was positive by HC2 in 23.8% (37/155) and by f-HPV typing in 32.6% (49/155). Concordant results were found in 133/155 (overall agreement, 85.8%; Cohen's kappa = 0.65). Twenty-two cases discordant were retested with Linear Array® (LA). Seventeen samples were f-HPV positive/ HC2negative, 9 samples were LA+ and in 8 samples LA-. Five were HC2 positive/f-HPVnegative, 3 were LA-, and in two samples were detected HPV-66 and in another HPV-16 by LA. The analytical sensitivity and specificity of f-HPV were 97.6 and 93 respectively.

Conclusions: This study shows that the f-HPV assay provides a good alternative to HC2 to detect HPV infection, allowing simple and rapid HPV genotyping and detecting multiple infections.

FC 2-12

COMPARISON OF INNOLIPA HPV GENOTYPING EXTRA ASSAY AND ABBOTT REALTIME HIGH RISK HPV TEST ON THREE DIFFERENT CERVICAL COLLECTION MEDIA FOR HR HPV DETECTION

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Objectives: High risk (HR) HPV DNA testing is a highly sensitive method to screen women at risk of CIN2+ lesions. But many assays and various media for cervical sample collection are available. So, we aimed to compare the Abbott RealTime High Risk HPV test and the Innolipa HPV Genotyping Extra (Innogenetics) on cervical samples collected on Abbott, PreservCyt (Hologic) and Easyfix (Labonord) media.

Methods: 166 samples were collected: 44 in duplicate with brushes (Digene Cervical sampler for routine diagnosis and Abbott Cervi-Collect Specimen Collection Kit), and 122 in liquid based cytology media (38 PreservCyt and 84 Easyfix). Distribution of cytodiagnosis results was: 18,7% normal, 56% ASCUS, 20,5% LSIL and 4,8% HSIL). Samples were routinely tested with the Innolipa assay after extraction with Easymag (Biomérieux). An aliquot was also tested with the Abbott assay. Discordant results were analyzed with the universal GP5+/6+ PCR.

Conclusions: Among the 166 samples, 7 were ruled out because amplification of internal control failed with Abbott assay (Ct > 35). As it occurred only with Abbott media collected after the samples used for routine analysis, we suppose that quality of the second sample was not sufficient for analysis. HR HPV prevalence was more elevated by Innolipa (67.9%) than Abbott assay (57.8%) and overall agreement between both assays was good (90%; kappa 0.78). Regarding the samples collected with Digene and Abbott brushes, agreement between both assays was 83.8% (kappa 0.68). For liquid-based cytology media, agreement between both assays was very similar: 89.5% (kappa 0.78) for PreservCyt and 92% (kappa 0.82) for Easyfix. Analysis of discordant results confirms the high sensitivity of Innolipa PCR. The Abbott assay 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. This test is therefore well adapted for HR-HPV based cervical screening.

ANALYTICAL PERFORMANCE OF HPV DIRECT FLOW CHIP COMPARED WITH SEQUENCING ANALYSIS**Herraez-Hernandez E¹, Guill R², Olmo A¹, Espigares B¹, Lara E¹, Garcia-Garcia JA², Martorell M².***1 R&D Department, Máster Diagnóstica. Granada, Spain.**2 Pathology Department, Hospital General Universitario de Valencia, Valencia, Spain.*

Objectives. HPV DIRECT FLOW CHIP (DF) is a new system for HPV detection and identification of 36 different genotypes. The procedure can be completed in less than 3 hours and is based on PCR followed by automatic reverse dot blot hybridization and analysis. The DF assay efficiently genotypes samples using as PCR template either cell extracts or purified DNA. Here, performance of DF was evaluated by comparison with a sequencing method.

Materials. A collection of 106 samples were tested. DNA was purified by standard phenol-chloroform and proteinase-K methods. Nested PCR (MY11/09 and GP5+/GP6+ primers) amplicons were analyzed by direct sequencing. DF included GP5+/GP6+-based PCR and reverse dot blot hybridization with genotype-specific probes.

Conclusions. From the 106 samples, 86 were concordant (81.1%). The 20 discordant results corresponded to: 8 cases with genotypes not included in the DF panel (HPV 13, 32, 83, 102 and 107); 4 samples positive by sequencing for HPV 52, 54, 81 and 84 respectively, but negative by DF; and 8 cases positive for HPV infection by both methods, but with differences in genotype-specific positivity. Specific genotypes were more efficiently detected by DF, i.e.: HPV 6 (13 DF vs 8 seq), 31 (6 DF vs 3 seq), 35 (6 DF vs 2 seq), 39 (7 DF vs 2 seq), 42 (11 DF vs 2 seq), 44/55 (8 DF vs 2 seq), 45 (7 DF vs 2 seq), 51 (13 DF vs 3 seq), 52 (8 DF vs 3 seq), 59 (7 DF vs 3 seq), 62/81 (17 DF vs 4 seq) and 68 (13 DF vs 3 seq); while other HR genotypes were highly concordant: HPV 16 (11 DF vs 11 seq), 18 (7 DF vs 8 seq), 33 (5 DF vs 3 seq) and 45 (7 DF vs 2 seq). Sequencing could only identify one or two genotypes per sample, whereas DF detected samples that harboured from 1 to 7 genotypes. Multiple infection rate was 4.7% for sequencing and 62.3% for DF. In summary, high concordance was found between both methods, although HPV DIRECT FLOW CHIP showed a higher efficiency for genotype identification than direct sequencing, with the added advantages of quick sample processing, reduced hands-on time, high analytical sensitivity in multiple infection detection and the possibility of using cell crude extracts as PCR template.

PREDICTION OF CERVICAL INTRAEPITHELIAL NEOPLASIA OF GRADE 2+ (CIN2+) USING HPV DNA TESTING IN THE REGULAR CERVICAL SCREENING ACTIVITIES OF THE NATIONAL HEALTH SERVICE IN CATALONIA, SPAIN.**de Sanjosé S, Ibáñez R^{1,2}, Rodríguez V¹, Roura E¹, Bruni L¹, Bosch FX¹ on behalf of CATCERVIX³***1 Unit of Infections and Cancer. Cancer Epidemiology Research Programme. IDIBELL. Catalan Institute of Oncology. Barcelona. Spain
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Background: Catalonia has a population of 2,802,504 million women aged 25 and older, (census 2008) and has an opportunistic screening program. Since June 2006 a protocol for cervical cancer screening among sexually active women in the age range of 25 to 65 years was introduced to increase coverage and to recommend a three-year interval between screening cytology in Catalonia (Spain). Further, Human papillomavirus (HPV) was offered as a triage test for women with a diagnosis of atypical squamous cells of undetermined significance (ASC-US), as a rescue test together with cytology for women aged ≥ 40 with more than a 5-year interval since their last pap and post treatment surveillance after CIN surgery.

Methods: In Catalonia there is a public health system in which the population has a free of charge access to medical care and cancer screening interventions. Primary health doctors and primary health midwives were regularly required to evaluate the adequate uptake of women to the general screening recommendations. Within screening activities, during 2007-08 a total of 611 women from five reference laboratories in Catalonia with a novel diagnosis of ASC-US were referred for high risk HPV(hrHPV) triage using high risk Hybrid Capture (hrHC2). Using routine record linkage data, women were followed-up for three years to evaluate hrHPV testing efficacy for predicting CIN2+ cases. A total of 37,711 hrHPV tests were performed during the years 2007-2008 in the context of Catalonian screening protocol. Of these, 5,861 hrHPV tests were associated to a diagnosis of ASC-US and 16,956 with rescue of women with inadequate screening.

Conclusions: Triage of ASC-US with hrHPV testing showed a high sensitivity for the detection of CIN2+ and a high negative predictive value (NPV) after three years of follow-up. The full evaluation of the rescue test is ongoing.

FC 3-2

EXPRESSED PROSTATE SECRETIONS (EPS) FOR THE STUDY OF THE EPIDEMIOLOGY OF HPV INFECTIONS IN THE MALE**Smelov V^{1,2,3,4}, Eklund C¹, Bzhalava D¹, Novikov A², Dillner J¹***1 Karolinska Institutet, Dept of Laboratory Medicine, Stockholm, Sweden,**2 Northwestern State Medical University, Dept of Urology and Andrology, St. Petersburg, Russia,**3 St. Petersburg State University Outpatient Clinic, St. Petersburg, Russia,**4 D.O. Ott Research Institute of Obstetrics and Gynaecology Outpatient Clinic, St. Petersburg, Russia*

Objectives. More knowledge about the HPV ecology in males is important for epidemiological and transmission studies as well as for the studies on a possible link between genital HPV infections and development of uro- and anogenital cancers. Expressed prostate secretion (EPS) is a readily obtainable sampling material for the study of HPV epidemiology in the male urogenital tract.

Methods. Multiplex high-throughput HPV genotyping was performed, comparing urethral and EPS samples obtained from 752 men, aged 18-60, and attending urology outpatient units in St. Petersburg. HPV prevalence in the study population for all, oncogenic and multiple HPV types was detected in 25.9% and 32.6%, 24.5% and 27.7%, 5.2% and 8.1% in urethral and EPS samples. Among oncogenic types, HPV-16 was the type most often detected in both the distal urethra (8.9%) and EPS (10.8%). The concordance between the two sampling sites was low: 6.4% and 10.1% for all and oncogenic HPV infection, respectively. Investigation of EPS samples may thus greatly increase the number of HPV-positive men as compared to the urethral samples: in the present study it resulted in detection 26.2% HPV-positive men additionally ($p < 0.0001$).

Conclusions. Prostate samples are valuable for a full understanding of the HPV infections in the male.

FC 3-3

POPULATION-BASED HPV PREVALENCE, GENOTYPE DISTRIBUTION AND RISK-FACTORS FOR HPV INFECTION IN 20-25-YEAR-OLD WOMEN IN GERMANY**Deleré Y¹, Remschmidt C¹, Haußig J², Schuster M¹, Schneider A², Wichmann O¹, Kaufmann AM²***1 Robert Koch Institute, Department for Infectious Disease Epidemiology, Berlin, Germany**2 Charité-Universitätsmedizin, CBF/CCM, Clinic for Gynaecology, Berlin, Germany*

Background Persistent Human Papillomavirus (HPV) infections can progress over years to cervical precancer and cancer. Cervical cancer is one of the most common cancers among women worldwide. Previous studies have shown that self-sampling by cervicovaginal lavage is an attractive and reliable method to detect HPV in women. We conducted a cross-sectional study using self-sampling devices to determine HPV prevalence, genotype distribution and risk-factors among women aged 20 to 25 years.

Methods We identified women through the German registration offices and sent self-sampling kits and a questionnaire to those who had agreed to participate in this study. The self-collected cervicovaginal samples were analysed by a general primer GP5+/GP6-mediated PCR-Luminex-based genotyping method for detection of 18 high-risk and 6 low-risk HPV genotypes (Multimetrix, Heidelberg, Germany).

Results Samples of 675 women could be tested. Of those, 441 had not been HPV vaccinated. 56% of unvaccinated participants did never take part in cervical cancer screening (95% confidence interval (CI), 50 to 60). HPV prevalence of any type was 36% (95% CI, 31 to 40) and 28% (95% CI, 24 to 32) for high-risk HPV-strains. Multiple infections with two or more HPV types were observed in 46% (95% CI, 38 to 54). In univariate analysis a higher number of sexual partners in the last 12 month, a higher life-time number of sexual partners, earlier age of first intercourse and current smoking were significantly associated with HPV infection. In multivariate analysis only variables coding for sexual behaviour were independently associated with HPV infection.

Conclusion Our results are in line with recent population-based studies on HPV prevalence in the US and in Denmark (1; 2), in which 45% of women aged 20-24 years were HPV positive and HPV detection was mainly influenced by sexual behaviour. In a study conducted in Germany (3) in which women attending routine screening were recruited, HPV prevalence was 28% in 20 to 22-year-olds. To evaluate the benefit of HPV vaccination, knowledge about population-based HPV prevalence and type distribution prior to or shortly after HPV vaccination introduction is necessary. Our study provides valuable baseline data in unvaccinated 20 to 25-year-old women to monitor the impact of vaccination in future

HPV IN YOUNG WOMEN IN GERMANY – A POPULATION BASED EPIDEMIOLOGICAL STUDY

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Objectives: In Germany little data are available on the epidemiology of HPV infections and associated diseases in women aged 20 to 30 years. WOLVES (Wolfsburg HPV epidemiology study) is the first population based surveillance study in Germany to measure the burden of HPV related diseases in a two different birth cohorts where vaccination coverage is low.

Methods: All women born 1983/84 and 1988/89 who were registered in the city of Wolfsburg are invited by letter. Participants filled in a standardized questionnaire and underwent pelvic examination with Pap smear and HPV testing (HC2-LR and HR). HPV genotyping (LiPA) was done in all HC2 positive samples. Women with genital warts (GW) or atypical smears were transferred to colposcopy.

Results: 43.8% (n=659) of the 1983/84 and 44.5% (n=599) of the 1988/89 target populations were enrolled from October 2009 to December 2010. The overall HPV prevalence (HC2 HR & LR) was 26.5%. Between the two age groups no significant differences were observed (25.5% vs 27.5%). 0.63% of participants were diagnosed with GW and another 1.7% had a history of GW, while 1.06% were diagnosed with biopsy proven CIN2+ and 3.2% had atypical Pap smear results associated with HPV. In both birth cohorts HPV 16 had the highest prevalence (7.4%) of all high-risk HPV types, followed by HPV types 51 (5.9%), 31, 53 and 66 while HPV6 was the most important low risk HPV type (1.9%). Complete vaccination series was received by 6.1% (1983/84) versus 21% (1988/89).

Discussion: In young women in Germany HPV infections are very common and the burden of HPV associated diseases is relevant. These data could be useful for further evaluations of the impact of HPV vaccination and screening.

AGE-SPECIFIC PREVALENCE OF CERVICAL HPV INFECTION AND CYTOLOGICAL ABNORMALITIES IN WOMEN IN GAUTENG, SOUTH AFRICA

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Objectives: This cross-sectional study describes the age-specific prevalence of HPV infection and cytological abnormalities among a largely unscreened urban population with a high background prevalence of sub-optimally treated HIV-1 infection.

Methods and results: Women (n=1524) attending public sector primary health care clinics were invited to participate in a cervical cancer screening study from 2009 to 2011. All participants were screened with conventional cytology and HPV genotyping was done using the Linear Array (Roche Diagnostics®). Of 1472 women with valid cytology results, abnormalities were detected in 17.3% (95% CI 12.6-23.3), of which ASCUS 4.7% (95% CI 3.9-5.7), LSIL 3.0% (95% CI 2.0-4.4), HSIL 9.1% (95% CI 5.6-14.5), and squamous carcinoma 0.5% (95% CI 0.2-1.7). Of the 1445 women with complete data, the overall and high risk HPV (hrHPV) prevalence were 74.6% (95% CI 69.5-79.1) and 54.3% (95% CI 53.0-56.5), respectively. The "top 8" cancer causing hrHPV types were detected in 43.5% (95% CI 41.3-45.8) and HPV type 16 and/or 18 in 19.5% (95% CI 12.0-15.0) of women. Infection with multiple hrHPV types was more common than single hrHPV type infections in patients younger than 40 years of age. The age-specific hrHPV prevalence peaked at 66.0% (95% CI 57.6-74.4) among women younger than 25 years of age and only slightly decreased with age, presenting a "flat" or "plateau-like" curve.

Conclusions: The prevalence of HPV infection and abnormal cytology were significantly higher than previously reported in general populations in South Africa and elsewhere. Higher age-specific prevalence and similar age-specific epidemiological curves have previously only been described in studies among HIV-positive women. These findings underscore the urgent need for improved cervical cancer screening and prevention programs in sub-Saharan Africa. The higher HPV prevalence, especially in older age groups, may affect the efficiency of low-cost HPV screening programs in developing countries with largely unscreened populations with a high background prevalence of HIV-1 infection.

FC 3-6

HUMAN PAPILLOMAVIRUS GENOTYPE DISTRIBUTION IN CERVICAL INTRA-EPITHELIAL NEOPLASIA: A PROSPECTIVE STUDY FROM 2008-2011 IN BELGIUM

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Objectives: Identification of the HPV genotype distribution in different grades of cervical Intra-epithelial neoplasia in Belgium.

Methods: 254 patients were referred for conisation in our hospital, from January 2008- March 2011. An audit study of this consecutive series of patients was designed. Both HPV genotype analysis and histopathology of the cones specimen was performed centrally. Index cytology was performed in the cytopathology laboratory of the referring doctor.

HPV typing in a period of one month before conisation was performed. Age, parity, smoking habits, cytology and HPV presence and type were parameters for uni- and multivariate analysis. Variables related to CIN 2+ lesions in our population were identified and odds ratios were calculated.

The main outcome measure was the presence of biopsy proven CIN 2+.

Conclusion: HPV types 16, 31, 51, 52, 53 and 58 were most prevalent in our population. Presence of any HPV was statistically significant related to CIN 2+ lesions at an odds ratio of 10.3. The odds ratio for a single infection with HPV 16 was 7.5; in infections with multiple types the odds ratio was 3.5 for HPV 16 and 8.9 for HPV 33. For cytology \geq ASC-H and HSIL the odds ratio was 5.8. Other variables were not statistically significant in this series.

HPV infection, especially HPV type 16 and 33, is independently correlated with CIN 2+ as well as high grade cytology (ASC-H or H-SIL). HPV 16 or HPV 33 are even stronger predictors than high-grade cytology for the presence of CIN 2+.

FC 3-7

EPIDEMIOLOGICAL STUDY OF HIGH-RISK HPV IN MIDI-PYRENEES (FRANCE) WITH ABBOTT HIGH-RISK HPV REALTIME PCR IN 2011

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Objectives: This work aims to obtain the first epidemiological data on the distribution of high-risk HPV in Midi-Pyrenees (France).

Over a period of 8 months in 2011, 351 patients were studied for high-risk HPV and positive samples were genotyped in three different groups (HPV 16, HPV 18 and others HPV HR).

The analyzes were performed on an automated platform Abbott M2000, validated according to the SH GTA 04 COFRAC guideline for accreditation according to ISO 15189.

Results: Ninety-six samples were detected positive for HPV HR (27.4%) and 255 samples were negative (72.6%). The distribution of genotypes in positive samples is as follows: HPV 16 in 29.2% (n = 28) and HPV 18 in 9.4% (n = 9) and others HPV HR (between 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68) in 82.3% of positive samples (n = 79).

Twelve samples were positive for HPV 16 alone, three samples for HPV 18 alone and 61 samples for others HPV HR alone. The remaining 20 samples are positive in at least two different HPV HR (two samples with HPV 16 and HPV 18, 4 samples with HPV 18 and others HPV HR and 14 samples with HPV 16 and others HPV HR).

The distribution of the prevalence of HPV depends of the matter of the prescription. In the 230 samples of ASC-US women, 31.3% of the samples were positive and 62% were negative, In the 83 women included for "screening" or "systematic", only 8.4% of the samples were positive and 91.6% were negative. In the 38 remaining women tested the distribution was higher than the "screening" group as follow, 13 women "CIN" control or CIN (30.8% positive and 69.2% negative), 4 patients with an history of HPV "(50% positive and 50% negative), unspecified 21 patients (47.6% positive and 52.4% negative).

Conclusions: HPV 16 and HPV 18 are detected in less than 40 % of the positive samples. The others HPV HR group are mainly detected on more than 80% of positive samples. When HPV 16 or HPV 18 are detected, they are frequently associated with an other HPV HR in more than 50% of cases.

The ASC-US patients have a prevalence of HPV HR significantly higher with over 30% of samples detected positive while patients performing the test for "screening" have a prevalence of less than 10% of HPV HR.

This study is the base point for following the evolution of HPV genotypes in Midi-Pyrenees, especially with the advent of vaccination, which target genotypes 16 and 18.

AGE-SPECIFIC DISTRIBUTION OF HPV INFECTION AMONG YOUNG FEMALES

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Objectives: HPV genital infection is highly prevalent in young female population from all countries. Data about its real distribution in young women are scarce in literature.

The aim of this study was to describe the age-specific distribution of HPV types and related lesions among girls under 25 years and to provide a first knowledge on Italian adolescents' HPV infection occurrence.

Methods: In 2009-2010 a prospective cohort study was carried out at the Colposcopy Office of Careggi University Hospital in Florence, including 85 girls aged 16-25 referred to our unit after a repeated abnormal smear result. Patients underwent an HPV test, a colposcopy and eventually a cervical biopsy. Treatment was performed by destructive or excisional laser CO2 therapy.

Conclusions: We observed a particular age-specific stratification of HPV types and related disease, which appeared to be characterized by a cut-off at the age of 20.

We observed a predominance of low risk infections among girls aged 16-19 years: the overall prevalence of LR HPV amounted to 80% in this age group. On the contrary, high risk infections were predominant among girls aged 20-25, with an overall prevalence of 85.5% in this group.

The univariate analysis of chosen characteristics of HPV disease (presence of HR HPV, coinfection, presence of CIN 2/3, disease relapse and multi site lesions) demonstrated the statistically significant difference of this infection between the two age groups ($P < 0.005$).

On the basis of our data we are able to observe that the initial age (25 years) of Italian cervical screening programs seems to be older than the starting of HPV pathology in young population. Among girls aged 16-19 we observed that 70% of CIN 1 were associated with florid genital warts. This association makes the HPV pathology of the youngest a clinically manifested disease in most cases, not requiring institutionalized screening. Otherwise girls aged 20-25 were affected by cervical isolated HPV lesions in 96.4% of cases, without external manifestations of the infection. Moreover these girls are the ones prevalently infected by HR HPV, suffering by CIN 2/3. This observation may suggest the inclusion in the Italian cervical cancer screening programs of girls aged 20-24, as other countries did.

HPV INFECTION AND GENOTYPES' DISTRIBUTION IN CERVICAL CANCER IN SAUDI ARABIA: IMPLICATION FOR SCREENING AND PREVENTION

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Objectives: Uterine cervical carcinoma is one of the most common neoplasia among women worldwide; however, it ranks number 15 in Saudi Arabian women forming only 2.2% of newly diagnosed cancer cases. The causes of this low incidence are unknown but environmental and cultural factors were suggested to lower the rate of infection with human papillomaviruses (HPV). National data on HPV and cancer are still scanty. Therefore, the aim of this study was to assess HPV infection and genotypes' distribution in invasive cervical cancer to provide information for screening and prevention.

Methods: HPV detection and genotyping were performed in 174 paraffin-embedded cervical tumors using the Linear Array kit (Roche Diagnostic) that enables the detection of 37 most common mucosal HPV's including 13 high-risk subtypes.

Conclusions: Patients age ranged between 28 and 106 (median=46) years old. By histology 79% had squamous cell carcinoma while 21% had adenocarcinoma. HPV was detected (positive) in 145 patients (83%), which is at the lower range of what has been estimated worldwide (85-99%). Fifteen different HPV genotypes were detected, 12 high risks (16, 18, 31, 33, 39, 45, 51, 52, 56, 59, 73 and 82) and 3 low risks (6, 64 and 70) with 15 patients having double infections involving mainly HPV-16 and 18. The most common single genotypes were 16 (66%), 31 (7%), 45 (6%), 18 (5%) and 73 (2%). With double infections, HPV-16 and 18 were the two most common genotypes present in 67% of cervical cancers, and forms 80% of all HPV-positive patients. Although the incidence of cervical cancer in Saudi Arabia is low, national screening and HPV-vaccination programs are expected to protect predisposed women from invasive cervical cancers. Supported by KACST grants LGP-12-4, ARP-27-12, and KFSHRC grants (RAC#2060-027, 2060-029, 2100-010).

FC 3-10

HIGH-RISK HUMAN PAPILLOMAVIRUS GENOTYPE FREQUENCY AMONG BRAZILIAN SAMPLES

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Objective: Human papillomavirus virus (HPV) infection is the most common sexually transmitted infection and the major cause of cervical cancer worldwide. The high risk HPV types are associated to cervical dysplasia. A vaccine against the types 16 and 18 is now available. The aim of this study was to describe the HPV high risk genotype distribution among Brazilian samples.

Methods: 305 samples (244 females and 61 males) were submitted to Papillocheck – HPV Screening (Greiner bio-one) for the identification of 24 human genital papillomavirus genotypes.

Results and conclusion: A total 129 women (129/244 - 52.9%) and 24 men (24/61 - 39.3%) presented high risk HPV genotypes. Among the female population with single genotype HPV infection (55/129 - 42.6%), the most prevalent genotypes were 33 (6/55 - 10,9%), 51 (7/55 - 12,7%) and 53 (9/55 - 16,4%). Multiple genotype infection was observed in 57.4% (74/129) of this group and the most common genotypes among these women were 16 (14/74 - 18,9%), 53 (16/74 - 21,6%) and 56 (14/74 - 18,9%).

Among men, single genotype infection was found in 58.3% (14/24) of the individuals and the most prevalent genotypes were 16 (3/14 - 21,4%), 39 (2/14 - 14,3%), 51 (2/14 - 14,3%) and 56 (2/14 - 14,3%). Multiple genotype infection was observed in 41.7% (10/24) and the most common genotypes in this specific situation were 16 (4/10 - 40%) and 68 (5/10 - 50%).

Our results show a high prevalence of multiple infections among Brazilian individuals. In addition, the most common subtypes are not covered by current vaccines profiles.

FC 3-11

SEROPREVALENCE OF HPV-16 AND -18 AMONG HETEROSEXUAL MEN, WOMEN AND MEN WHO HAVE SEX WITH MEN, ATTENDING AN STI CLINIC IN THE NETHERLANDS

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Objective: To examine whether seroprevalence of HPV-16 and HPV-18 is related to gender, type of sexual intercourse, number of sexual partners, or HIV infection. We investigated this in 4 risk groups; heterosexual men, women, HIV-negative Men who have Sex with Men (MSM), and HIV-positive MSM.

Methods: An anonymous cross-sectional survey was conducted among attendees of the STI clinic in Amsterdam. A subset of the participants enrolled in 2008-9 was randomly selected for this study. Participants answered detailed questions on sexual behavior, and serum samples were tested for antibodies to HPV and HIV. HPV was tested with a Luminex-based multiplex assay; a sample was considered seropositive for HPV-16 or -18 if the Median Fluorescence Intensity was >400.

Conclusion: The study population consisted of 784 heterosexual men, 784 women, 649 HIV-negative MSM and 249 HIV-positive MSM. The median age of the total study population was 29 years (IQR 24-40) and 1428 (58%) were Dutch. The median lifetime number of sexual partners (LSP) was 15 (IQR 9-30), 8 (IQR 5-14), 80 (IQR 30-200) and 200 (IQR 100-1000) for heterosexual men, women, HIV-negative MSM and HIV-positive MSM, respectively. The HPV seroprevalences differed significantly between these groups: 8%, 21%, 28% and 63% ($p < 0.0001$), respectively for HPV-16, and 5%, 14%, 24% and 50% ($p < 0.0001$), respectively for HPV-18.

For further analyses the group of HIV-negative MSM was divided into two subgroups: reporting to have had (1) insertive anal sex only (insMSM) and (2) insertive and receptive anal sex (recMSM). In pair-wise multivariable analyses adjusting for age, LSP and age of sexual debut HPV-16 was significantly more common (i) in women compared to heterosexual men (OR=4.3, 95%CI 3.0-6.2), (ii) in recMSM compared to heterosexual men (OR=4.1, 95% 2.7-6.1), (iii) in recMSM compared to insMSM (OR=1.8, 95% 1.2-2.9), and finally (iv) in HIV-positive MSM compared to HIV-negative MSM (OR=4.2, 95%CI 3.0-5.8). HPV-18 results were comparable. We conclude that: (1) HIV infection is strongly associated with HPV seroprevalence in MSM; and (2) HPV-16 and -18 seroprevalence appears to be associated with type of sexual intercourse.

SEROPREVALENCE AND DETERMINANTS OF HIGH RISK HPV TYPES IN HIV-INFECTED AND HIV-NEGATIVE MEN WHO HAVE SEX WITH MEN

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Objectives: Men who have sex with men (MSM), in particular HIV-infected MSM, are at increased risk for HPV infection and HPV-related diseases. Our goal was to assess prevalence and determinants of type-specific antibodies against high risk HPV types in HPV-unvaccinated HIV-negative and HIV-infected MSM; here we present our preliminary findings.

Methods: MSM aged ≥ 18 years were recruited from: the Amsterdam Cohort Studies, an STI clinic and an HIV treatment centre in Amsterdam, the Netherlands. Participants completed a questionnaire regarding socio-demographic characteristics, health-related issues and sexual behaviour. Serum samples were analysed at the RIVM-Clb using a fluorescent bead-based multiplex assay, detecting VLP antibodies against 7 high risk HPV types. Cut-off values were previously determined using a one-sided 99% prediction interval method, based on children aged 1-10 years.

Conclusions: 797 MSM were enrolled in the study; 760 MSM with data available were included in this analysis. The median age was 40.2 years (IQR 34.9-47.5) and 309 (40.7%) were HIV-infected. Seroprevalence of HPV-16 was 47.7% in HIV-negative and 73.8% in HIV-infected MSM ($P < 0.001$); seroprevalence of HPV-18 was 40.6% in HIV-negative and 58.9% in HIV-infected MSM ($P < 0.001$). Similar patterns were observed for HPV types 31, 33, 45, 52 and 58, ranging between 18.9-47.9% in HIV-negative and 43.4-78.6% in HIV-infected MSM ($P < 0.001$ for each HPV type). In multivariable analyses (adjusting for age, sexual behaviour, smoking and recreational drug use), HIV infection was a strong independent risk factor for HPV-16 seroprevalence. Increasing number of lifetime male sex partners was significantly associated with HPV-16 seroprevalence in HIV-negative MSM, but not in HIV-infected MSM. We conclude that seroprevalence of 7 oncogenic HPV types is high among MSM. HIV infection is a strong independent risk factor for HPV-16 seroprevalence, which we hypothesize is due to increased persistence of HPV-16 infection in HIV-infected MSM.

ROLE OF CONTRACEPTION IN THE EVOLUTION OF HIGH-RISK HUMAN PAPILLOMAVIRUS INFECTIONS AND CERVICAL INTRAEPITHELIAL NEOPLASIA

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Objective: To evaluate the role of combined oral contraceptives and intra uterine contraceptive device (IUCD) in cervical high-risk human papillomavirus (HR-HPV) infections, in high-grade cervical intraepithelial neoplasia and cancer (CIN2+) and in the evolution of HPV infections and cervical cytology 6 months after a large loop excision of the transformation zone (LLETZ).

Methods: A consecutive series of 248 women who underwent LLETZ between January 2008 and June 2011 was studied using logistic regression to analyse the covariates of contraceptive use, followed by multivariate modelling for predictors of HR-HPV and CIN2+. Our series was stratified by parity, smoking, age and immunosuppression. We analysed cervical cytology and HR-HPV genotypes prior to surgery and at 6 months follow-up.

Results: In our study the age at LLETZ varied from 19 to 81 years with a mean age of 40 years. There was no age difference between IUCD users and non-users. HR-HPV infection was a consistent risk factor of CIN2+. More than 90% of the patients were HPV-positive at the time of LLETZ. HPV testing 6 months after treatment showed 62.5% clearance of HPV infection whereas 37.5% of the initially HPV-positive cases revealed a residual or recurrent HR-HPV infection.

At the moment of LLETZ 12.9% of the study population was using an IUCD, 49.2% used combined oral contraceptives, 20.6% used other contraceptive methods (sterilisation, barrier method, combined injectable contraceptives, vaginal ring, contraceptive implants), 14.1% consisted of postmenopausal women while 3.2% lacked information on their contraceptive method.

Conclusions: No evidence was found for IUCD to have a protective role in cervical HPV infection or in cervical carcinogenesis. Even when corrected for age, there was no significant difference in HPV infection and CIN2+ between IUCD and combined oral contraceptive use, although there was a trend for combined oral contraceptive toward positive HPV testing at pre-LLETZ ($P = 0.0646$) and higher risk of CIN2+ ($P = 0.0754$), more specifically in women younger than 36 and older than 45 years. In our study there was no correlation with parity, smoking or immunosuppression.

FC 4-1

THE IMPACT OF HPV GENOTYPE ON THE PERFORMANCE OF COLPOSCOPY IN YOUNG WOMEN**Cruickshank ME¹, Gillespie C¹, Cotton S¹, Busby-Earle C², Moore C³, Cubie HA³ and Cuschieri K³***1 University of Aberdeen, Aberdeen, UK, 2 New Edinburgh Royal Infirmary, Edinburgh, UK, 3 Scottish HPV Reference Laboratory, Edinburgh, UK*

Background: In 2008, vaccination against the two most common HPV types, 16 and 18, was introduced across the UK. Cervical screening will continue but CIN could be missed in HPV immunised women if HPV 16 & 18 infection create the colposcopic features widely considered by colposcopists to represent high grade CIN.

Aims: To determine if there is an association between HPV genotype and HPV mRNA expression and colposcopic features in young women with abnormal cervical cytology.

Methods: 200 women aged 20-25 years in the East and North-East of Scotland referred to colposcopy routinely, as a consequence of preceding cytology abnormalities were recruited. Colposcopic findings and a Reid's Colposcopic Index were recorded and HPV DNA typing - using Linear Array - (Roche) and mRNA testing using the PreTect Proofer (Norchip) was performed on residual cytology material..

Results: From the first 105 women, of whom only one had received HPV vaccination, 20% had an infection with one HPV type, 31% had infection with two types, 17% with three types, 19% with four types, and the remaining 10% had five or more types. Just over half of the women (53%) were infected with either HPV16 or HPV18 or both types.

One third of women had CIN2/3 and the proportion was higher in those with HPV16 infection (50%) compared to those with no HPV16 infection (21%). A similar pattern was observed in women with HPV16 and/or HPV18 infection. The PPV of a high-grade colposcopy impression for CIN2/3 was higher for women infected with HPV16 and/or HPV18 (85%) compared to those not infected with HPV16 or HPV18 (50%); and this difference in positive predictive value was statistically significant ($p=0.04$).

Of the first 68 women to have mRNA testing result, half had a low-grade colposcopic impression and half had a high-grade colposcopic impression. Histology subsequently showed that 95% of women with CIN2/3 tested mRNA positive for at least 1 HPV type. As expected, the most frequently detected type using the mRNA assay was HPV 16. Further type-specific mRNA data and association with clinical outcomes will be presented.

Conclusion: The PPV of colposcopy appears to be lower in the absence of infection with HPV16 and HPV16/18. In vaccinated women, these HPV types are likely to be eliminated. This suggests that the performance of colposcopy may be compromised in the HPV immunised women.

FC 4-2

MANAGEMENT OF ADENOCARCINOMA IN SITU OF THE UTERINE CERVIX USING AN OUTPATIENT LOOP EXCISIONAL PROCEDURE.**Machado, G; Moreira, M; Machado, M; Castro, M**

Objectives: To evaluate the results of a colposcopically guided outpatient loop excisional procedure in the management of adenocarcinoma in situ of the uterine cervix.

Methods: Seventy one patients with adenocarcinoma of the uterine cervix were diagnosed and followed in our colposcopy unit between 04/16/1991 and 02/25/2012. Meanwhile, we also followed all the 1227 outpatients treated for cervical squamous intraepithelial lesions with the same technique.

Results: We reviewed the seventy one (71) cases of adenocarcinomas of the uterine cervix among which fifty (50) were adenocarcinomas in situ (AIS), five (05) microinvasive adenocarcinomas (MA) and sixteen (16) invasive adenocarcinomas (IA). In the same period we performed 1227 outpatient excisional procedures for cervical squamous intraepithelial lesions (24.54 procedures for squamous lesions for each similar procedure in AIS patients). Among the 50 cases of AIS, 37 outpatients were treated only with excisional loop surgery. We were not able to follow three patients after diagnostic biopsy. One patient had an endocervical polyp with only its distal end positive for AIS, and polypectomy was performed. Nine patients with AIS had undergone a hysterectomy after loop excision, considering that they had set their offspring. The average age of the 38 patients who had not undergone a hysterectomy was 28.84 years (21-40), 23 of these patients were nulliparous. The edges of the surgical specimen were negative in 22 cases, positive in 8 and, in 8 the evaluation was not possible. After a mean follow-up of 35.67 months (1-154.8) no recurrent disease occurred. The mean age of the AIS patients that underwent to subsequent hysterectomy was 37.77 years (28-52) and, all of them had at least one successful pregnancy before diagnosis. The mean period of follow-up in this group was of 18.66 months (1-60). Furthermore, in this particular group, only one patient had negative edges on the surgical specimen and, there were also no recurrence. We conclude that conservative management seems to be a safe approach for AIS; however, careful surveillance is required in addition to other prophylaxis resources that have been tested recently, such as HPV vaccines.

HPV E6/E7 mRNA AND HPV DNA TESTING IN POST COLPOSCOPIC TREATMENT SURVEILLANCE OF CERVICAL INTRAEPITHELIAL NEOPLASIA

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Objective: Disease recurrence following 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 infection. This study evaluates the utility of HPV mRNA testing in determining the risk of residual CIN2+ disease post-treatment.

Method: We are prospectively following a cohort of 307 women treated by LLETZ for suspected high-grade disease. Cervical specimens are taken at first visit prior to colposcopic procedure at 6, 12 and 18-24 months. HPV DNA is detected using HC II (Qiagen) and HPV mRNA detected using HPV PreTect Proofer (Norchip).

Results: The prevalence of high-risk HPV DNA and mRNA prior to treatment was 92.4% and 70.1%, respectively. HPV16 was the most predominant HPV type representing 66.7%. Histological examination revealed 80.7% had CIN2+, 15.9% CIN1, and 3.1% Normal. Post colposcopic follow up at 6-12 months post-treatment, showed 20.72% with abnormal cytology and indicated HPV DNA persistence in up to 20.01% of cases and HPV mRNA persistence in 9.61%. HPV data was correlated with margin positivity and residual/recurrent CIN2 disease within 18-24month follow up.

Conclusions: HPV DNA testing is useful for predicting recurrence of CIN in women post-treated and can be used as test of cure in the colposcopy setting. HPV mRNA testing is more specific but less sensitive than HPV DNA testing, however sensitivity may be more central in post treatment disease surveillance.

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HPV16 GENOME METHYLATION AS BIOMARKER IN CERVICAL ADENOCARCINOMA AND OROPHARYNX CARCINOMA

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Background: Infection with human papillomavirus (HPV) is the cause of cervical carcinomas, both squamous carcinoma (SCC) and adenocarcinoma (AdCa), and is also associated with about 35% of head and neck carcinoma.

HPV16 DNA methylation has been recently suggested as a marker for squamous cervical intraepithelial carcinoma. In previous studies examining HPV16 genome methylation, the authors concluded that the methylation status of the L1 and LCR gene might be biomarkers for neoplastic progression (Ding et al. 2009, Sun et al. 2011).

Objective: Determine the role of HPV16 DNA methylation (L1 and LCR regions) in less studied HPV-associated tumours, as cervical AdCa and oropharyngeal cancer (OPSCCs), and correlate the methylation with HPV E6/E7 mRNA detection and viral integration. The meaning of methylation marker could be the correct characterization of AdCa histotypes, with different therapy and prognosis, and the definition of causal role of HPV16 in OPSCCs.

Methods: DNA was extracted from bioptic tissues of AdCa and OPSCCs. Methylation was quantified, after bisulfite treatment, using PCR followed by pyrosequencing (Pyromark Q24 system - QIAGEN) at 19 CpGs throughout the LCR and 3'L1 regions. The methylation state was correlated with E6/E7 mRNA detection (EasyQ, bioMérieux) and viral integration (home-made E2/E6 real time-PCR quantitative method).

Results: All analyzed CpG sites had a significantly high mean methylation percentage in AdCa respect to SCC, with a methylation pattern similar to CaSki cells. In particular, the endometrioid histotype showed a higher mean methylation percentage for the LCR-E6 promoter than the mucinous histotype. For both the histotypes we observed E6/E7 expression confirming the causal role of HPV in these cancers.

The same sites in OPSCCs had a lower mean methylation percentage, with a pattern more similar to SiHa cells and SCC. HPV transcription activity was proved by E6/E7 mRNA detection and the viral genome was mainly or totally episomal in about 50% of the samples.

Conclusions: Methylation of specific CpG sites of HPV16 DNA should be a biomarker associated with specific AdCa histotypes and indicating the HPV16 as causal role of oropharyngeal cancer, allowing a correct management of the patients and improved therapy.

FC 4-5

CONSERVATIVE TREATMENT OF MICROINVASIVE ADENOCARCINOMA (MIAC) OF THE CERVIX: THE PROGNOSTIC SIGNIFICANCE OF CLEARANCE APEX IN THE CONE BIOPSY.

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Objectives: (MIAC) is a rare pathology that may affect women in their reproductive age. Usually it is treated with hysterectomy. Very few cases managed with conservative treatment are listed in medical literature. Because MIAC rise mostly close to the transformation zone, and skip lesions account for about 12-20% of cases, we study what may be a safety clearance from the apex of the cone to the invasive lesion to avoid persistence of skip lesions.

Methods: from January, 1st, 1995 and December, 31st, 2011 we perform 1025 laser cone biopsy and 61 cold knife conization because of SIL/CIN, AGC or suspected AIS, finding out 15 MIAC, endocervical type, without lymph-vascular spaces invasion, 13 out of 15 clear margins (13 stage IA1, 2 stage IA2). Seven women (stage IA1) underwent simple hysterectomy, 8 (6 stage IA1 and 2 stage IA2) asked for conservative treatment: 6 underwent follow-up, 2 underwent a new cone biopsy and then follow-up. After informed consent they had follow-up with pap smear, colposcopy, endocervical curettage, abdominal/pelvic ultrasound, CTscan at 12 months if stage IA2. We have linked the clearance from the invasive lesion to the apex of the cone and with persistent/recurrent disease.

Conclusions: 2 stage IA1 patients were positive in the apex of the cone; one underwent hysterectomy, and show residual disease (5mm longitudinal extension, 3 mm in depth, total lesion with 6 mm longitudinal extension, 3 mm in depth, final stage IA1), the second because of age underwent to new cone biopsy, without residual disease. The clearance from the apex was available in 12/13 cases. In 5/12 cases it was >10mm: 4/5 had negative follow-up after conservative treatment, 1/5 had negative histology in the hysterectomy specimen. In 7/12 patients clearance was <10mm; 3 had negative follow-up after conservative treatment, 4 underwent hysterectomy and 2/4 show microinvasive adenocarcinoma in one case and atypical glandular hyperplasia in their specimens. We don't know what could be the security clearance to suggest the conservative treatment of MIAC, endocervical type. Our data suggest that clearance ≥10 mm may be a safety index. Further investigation on larger series are needed to give indication on conservative treatment of MIAC out of clinical studies.

FC 4-6

ACCURACY OF PAP AND BETHESDA ONCOCYTOLOGY FOR PREDICTION OF CIN3+ IN WOMEN WITH SQUAMOUS INTRAEPITHELIAL LESIONS INDICATED FOR CONISATION: CORRELATION WITH HISTOLOGIC FINDINGS

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Objective: The human papillomaviruses play specific role in ethiology of cervical cancer and cause cytopathic effect on squamous epithelium. Such changes are the base for cytologic screening of pre/invasive lesions by applying Papanicolaou (PAP) or Bethesda system. However, many patients are surgically overtreated without detection of severe histologic findings. Thus, our aim was to examine accuracy of PAP and Bethesda system in prediction of CIN3+ lesions based on correlation between cytologic (OC) and histologic findings in patients with intraepithelial lesions.

Methods: A retrospective analysis of 658 patients operated for cervical lesions undergoing LEEP or cold-knife conisation based on PAP and/or Bethesda. OC results from cervical smears were correlated with histologic findings in assessment of cytology test ability to predict CIN3+ lesions. Specific analysis of negative histologic results (test false positivity) was retrieved for each classification.

Results: There was a significant correlation between OC results categorized accordingly PAP or Bethesda system and histology ($p < 0.0001$). However, trend for accurate diagnosis was better if OC smears were evaluated by Bethesda ($r = 0.302$ vs. $r = 0.223$ for PAP). There was insignificant trend to severity of OC with decreased age at menarche ($r = 0.035$) when used Bethesda contrary to opposite situation in PAP smears (increase trend of OC with increased age at menarche ($r = 0.078$)). The sensitivity of Bethesda for detection of positive histology (CIN3+) was 79.2% and specificity 53.7% with 1.71 positive likelihood ratio and 0.39 negative likelihood ratio (AUC=0.660, $p < 0.0001$). The sensitivity of PAP system for detection CIN3+ lesions was 49.5% and specificity 60.0% with 1.60 LR+ and 0.73 LR- (AUC=0.601, $p < 0.0001$). There was revealed insignificant rising trend of CIN3+ with increased parity, smoking, and significant increased trend with advanced age ($r = 0.1519$, $p < 0.0001$). The positive correlation between CIN3+ findings and indications to conisation based on cytopathology was in 41.7%. Unfortunately, 58.3% patients indicated to conisation due to impaired OC showed negative histology (\leq CIN2). After adjustment to L/H-SIL we have revealed 52.3% and up to 83.9% rate of negative histology after surgery in H-SIL and L-SIL classified lesions, respectively. Moreover, only 11.7% of L-SIL lesions in women \leq 40-years of age showed CIN3+ histology after surgery.

Conclusions: The sensitivity of Bethesda system in detection of CIN3+ lesions is higher than PAP. However, both system cannot detect true biologic behavior of cervical lesions, what increases rate of negative histologic findings. New specific markers of cervical carcinogenesis are needed as adjunct in indication protocols for achieving better therapeutic outcome and reduction of overtreatment.

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COMBINED CONDYLOMATOSIS AND BUSCHKE-LOEWENSTEIN TUMOR: CASE REPORT & SYSTEMATIC REVIEW**Mynbaev O¹, Balmasova I³, Griffin, H⁵, Doorbar J⁵, Medvedev M⁴ and Eliseeva M²***1The Experimental Researches & Modeling Division, 2 of Obstetrics and Gynecology, 3The Laboratory of Pathogenesis and Treatment Methods of Infectious Diseases, Moscow State University of Medicine & Dentistry, Russia, Moscow;**4 The Department of Obstetrics and Gynecology, Dnepropetrovsk State Medical Academy, Dnepropetrovsk, Ukraine;**5 National Institute for Medical Research, London, UK*

Objectives: An undesired manifestation of HPV is the transformation of infected cells into neoplastic cells, with the subsequent development of precancerous intraepithelial lesions and in some instances cancer. The generally benign manifestations of HPV include asymptomatic and subclinical disease as well as anogenital condyloma acuminatum, warts and verrucosis. Certain HPV manifestations are however associated with poor outcome for the patient, and these include giant and multiple condylomas such as Buschke-Löwenstein tumor (BLT), and respiratory papillomatosis and their relapses. These conditions decrease the patient's life quality, and have the potential to progress to malignancy, and are a particular problem for practitioners. Management of these patients is complicated due to the absence of therapies that can eliminate HPV and prevent wart recurrence, or medication that can cure genital warts and condylomas. Indeed, although there are many clinical reports describing the aggressive manifestation of genital warts in immune compromised individuals, the triggering mechanisms for such atypical lesions is not yet understood. Our objective here is to present a case report of combined condylomatosis and BLT in a patient with Henoch-Schönlein purpura and simultaneously infected with six HPV genotypes (6, 11, 18, 31, 43 & 56), and to systematically review the literature in order to provide a better insight into these conditions.

Methods: Clinical and laboratory parameters as well as a video of surgery of female patient (30 ys) with combined condylomatosis and BLT (the informed consent was obtained). Clinical and research literature over the last 15 years focusing on the immunological aspects of papillomavirus interaction with the innate and adaptive immune systems, and interference with the cellular and humoral host responses. Particular attention is given to the nature of papillomavirus clearance and persistence.

Conclusions: Atypical manifestation of HPV was in our case related to systemic disease involving an autoimmune host response and long-term glucocorticoid treatment. Our review confirms that such atypical manifestations and the long-term persistence of HPV are often linked to disturbances in the hosts immune system. More specifically these comprise changes in the content of lymphocyte subsets (helper, cytotoxic and regulatory T-cells), NK cells and macrophages at HPV infected sites and in the blood, as well as changes in lymphocyte function related to the expression of INF- α & β , IL-2; IL-4, IL-10, TGF β , and other cytokines.

OBSTETRIC OUCOME AFTER LASER CO2 CONIZATION IN TREATMENT OF CERVICAL INTRAEPITHELIAL NEOPLASIA AND MICROINVASIVE CERVICAL CANCER**Pieralli A., Longinotti M., Tarani S., Lozza V., Corioni S.**

Objectives: Conservative techniques in particular excisional ones are currently considered the gold standard for treatment of high-grade CIN and microinvasive cervical cancer, even if these methods have often been associated in literature with adverse obstetric outcomes in subsequent pregnancies.

A review of the literature shows pregnancy-related morbidity after cervical conization, this procedure is associated with obstetrical problems in subsequent pregnancies, as incompetent cervix, premature rupture of the membranes and above all preterm delivery.

The goal of this study was to estimate the preterm delivery incidence in young women who made a laser CO2 conization to treat high-grade CIN.

Methods: A retrospective matched cohort study was designed, starting from a group of patients selected by colposcopy electronic database, who underwent laser CO2 cervical conization in the University Hospital of Careggi in Florence within 1 January 2000 / 1 June 2009, aged at the time of the surgery 39 years or less. The final study population consisted of a selected group of these women who have had one or more pregnancies after treatment.

Carried out a univariate analysis of preterm delivery risk factors by Fisher's exact test for discontinuous variables. Then statistical significance has been validated by a multivariate analysis choosing logistic regression as statistical test.

Conclusions: In the cohort study, were 64 pregnancies with favorable results one of which was a monochorionic twin pregnancy, therefore total births were 65. The preterm delivery incidence in the study group was 7.6%.

This study aims to provide laser technique cannot expose the selected patient at high risk of premature delivery because according to data reported the preterm delivery incidence in Italian population is within 5-10%.

The superiority of laser technique is linked to a better organic and functional restitutio ad integrum of cervical tissue after laser conization compared to other excisional methods.

FC 4-9

APPLICATION OF TELECYTOLOGY FOR PAP SMEARS DIAGNOSTIC

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Objective: The study aimed evaluation of the effectiveness of telecytology for routine diagnostic of pap smears under the conditions of Georgia.

Methods: Gynecological cytology cases (n=420) were taken from the clinical laboratory. Cases were diagnosed routinely by one of four certified cytologists who provided cytology diagnoses. Digital images were obtained on all cases and were evaluated as computer images by a panel of cytologists.

Conclusions: There was 94% concordance in average between routine versus digital images diagnostic. Intracytologists concordance averaged 95.5%. Image sharpness and quality were rated "good" and "excellent" in 97% cases. With respect to image color, 96% of the images were rated as "excellent" or "good". Digital images for cytology diagnostic of pap smears produces images of adequate quality and diagnostic concordance rates.

FC 4-10

CONDYLOMA ACUMINATUM VS CONDYLOMA LATUM: SIMILAR NAME, DIFFERENT DISEASE

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Condyloma acuminatum refers to an epidermal manifestation attributed to the epidermotropic human papillomavirus (HPV). More than 100 types of double-stranded HPV papovavirus have been isolated. Many of these have been related directly to an increased neoplastic risk in men and women. Approximately 90% of condyloma acuminata are related to HPV types 6 and 11. These 2 types are the least likely to have a neoplastic potential. Risk for neoplastic conversion has been determined to be moderate (types 33, 35, 39, 40, 43, 45, 51-56, 58) or high (types 16, 18), with many other isolated types. The picture is complicated by proven coexistence of many of these types in the same patient (10-15% of patients), the lack of adequate information on the oncogenic potential of many other types, and ongoing identification of additional HPV-related clinical pathology.

The incidence of human papillomavirus infection in men and in women is still rapidly increasing. The goal in treating human papillomavirus infection is the elimination of lesions, eradication of the virus is not yet possible.

Syphilis is a sexually transmitted disease known to have varied presentations and hence it is known as the 'Great Imitator', caused by *Treponema pallidum*. Syphilis is still a serious disease with diagnostic difficulties. The common manifestations of secondary syphilis are rash (75-100%), lymphadenopathy (50-80%) and mucocutaneous lesions like mucous patches and condyloma lata (40-50%). Other symptoms common at this stage include fever, sore throat, malaise, weight loss, headache, alopecia and enlarged lymph nodes.

The lesions of secondary syphilis that appear in the mucocutaneous areas are called as condyloma lata. Generally, they are reddish-brown or purple, flat-topped and moist, flesh colored or hypopigmented, macerated papules or plaques, and are seen in the anogenital region. Their surface may be smooth, papillated or covered with cauliflower-like vegetations.

Condyloma lata (syphilis caused) and condyloma acuminata (HPV caused) can result in vaginitis or vaginosis which require clinical treatment. Both lesions are smooth and flat, with many clinical similarities.

Differential diagnosis of the condyloma acuminatum includes condyloma latum, seborrheic keratoses, nevi, pearly penile papules and neoplasms.

The lecture will show how both diagnosis are clinically similar and will recommend for further diagnostic procedures to distinguish both diseases.

AUTOMATED DETECTION AND CLASSIFICATION OF CERVICAL DYSPLASIA THROUGH HOLOGRAPHIC MICROSCOPY AN OBJECTIVE CLASSIFICATION ON 3-DIMENSIONAL CELL INFORMATION

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OBJECTIVES: Is 3 dimensional Optical Height Delta combined with Nucleus/Cell Ratio measurement a new objective and automated scoring parameter in diagnosis of cervical cells? This method was analysed as to sensibility as to specificity.

METHODS: 16 selected patients previously diagnosed by the Thinprep® liquid based cytology confirmed by HPV Abott® assay or histology diagnosis for CIN II and III, were analysed on the new Holocyt® diagnostic intelligence software by use of the Holographic Digital Microscope (DHM) using partially coherent laser light. Residual liquid from the Thinprep® sample was analyzed in a non-destructive way. DHM enables a quantitative multifocal phase contrast imaging that has been found suitable for quantitative and qualitative inspection, and for three-dimensional cell imaging. Cells were identified and measured in an automated way on nucleus/cell ratio (NCR) and Optical Height Delta (OHD) was extracted in the 3D holographic image. The Optical Height Delta is the difference between Nucleus top height minus Cytoplasm average height. NCR and OHD were separately determined in CIN1, CIN 2 + CIN 3 patients, compared with normal cells either patients with normal cytology diagnosis. Data were imported in the global data sheet and statistical ROC analysis was performed.

| | Negative | Positive | p value ANOVA | AUC (ROC) |
|-------|---------------|---------------|---------------|-----------|
| CIN1+ | | | | |
| n | 66 | 122 | | |
| NCR | 0,30 +/- 0,23 | 0,39 +/- 0,17 | 0,002 | 0,71 |
| OHD | 0,22 +/- 0,09 | 0,34 +/- 0,14 | <0,0001 | 0,75 |
| CIN2+ | | | | |
| N | 83 | 105 | | |
| NCR | 0,29 +/- 0,21 | 0,41 +/- 0,17 | <0,0001 | 0,76 |
| OHD | 0,23 +/- 0,10 | 0,35 +/- 0,14 | <0,0001 | 0,75 |

CONCLUSIONS: Cell population analysis of the objective measurement reveals an increased NCR and OHD in the dysplastic cells. By use of DHM, combined with an improved scoring algorithm, the field of cancer cell diagnosis has entered a new era, providing a non-destructive and objective method for analyzing cell samples. A correlation can be observed between the nucleus' optical height and the diagnosis. This correlation can be used for improving the automatic scoring factor algorithm, while all cells in the sample are preserved for further complementary tests such as for HPV. The first study confirms the feasibility of this new technology as a powerful tool applying non destructive methods. Further tests will be performed to validate the cut-off values for the algorithms.

AMPLIFICATION OF THE CHROMOSOME 3Q26 REGION IN LIQUID CYTOLOGY SPECIMENS AS A MARKER FOR CLEARANCE OF LESION POST-TREATMENT IN CERVICAL CANCER

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Objective: There are a number of potential clinical uses of testing for 3q gain in liquid cytology specimens (LBC). One is to identify subsets of women with ASC-US or LSIL cytology who are at greatest risk of CIN 2+ and would thus benefit most from immediate colposcopy. To evaluate the potential utility of testing for 3q gain in clinical practice, we performed 3q testing using an automated fluorescence in-situ hybridization (FISH) assay in a series of LBC specimens obtained from patients who had been prospectively followed during a 2-year period.

Methods: This is a longitudinal study of 28 patients with ASC-US or LSIL who underwent colposcopy and were diagnosed with biopsy-confirmed CIN 2+. All patients were treated using LEEP or conization and all had negative cytology results post-treatment. Residual LBC specimens from the referral cytology were analyzed for high-risk HPV (hrHPV) as well 3q gain by FISH, using an automated system developed specifically for rare-cell detection and analysis. Specimens were classified as positive for 3q gain if ≥2 cells with more than four copies of the 3q26 probe were detected. All patients had up to three post-treatment follow-up visits negative LBC results and residual LBC from these visits were also analyzed for 3q gain by FISH.

Results: 3q gain was detected in 71% of the initial LBC specimens from hrHPV (+) women with biopsy-confirmed CIN 2+, and this increased to 76% for women with CIN 3+. Length of follow-up ranged from 4-33 months, with a median length of 11.3 months. Seven (25%) of the 28 women who were cytologically negative post-treatment continued to be 3q gain positive by FISH, whereas 21 (75%) became 3q gain negative.

Conclusion: This study highlights the sensitivity for CIN 3+ of testing for 3q gain by FISH in women with a cytological result of ASC-US or LSIL. It also suggests that a significant proportion (25%) of women who are cytologically negative post-treatment for CIN 2+ may continue to harbor residual foci of high-grade neoplasia.

A SYSTEMATIC REVIEW OF CHROMOSOMAL GAINS AND LOSSES IN LOWER GENITAL TRACT LESIONS

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Objectives: Chromosomal instability is a hallmark of many cancers and develops during HPV-induced carcinogenesis. We wanted to identify the most common chromosomal aberrations which are characteristic for lesions of different grades and localizations of the lower genital tract in order to elucidate alterations which are potentially relevant for carcinogenesis or might serve as diagnostic markers.

Methods: A systematic literature search was performed in PubMed for studies that analyzed chromosomal alterations by comparative genomic hybridization (CGH) in HPV-positive and negative cancers or premalignant lesions of the anogenital tract (cervix, anus, vagina, penis, vulvar). Data were extracted and analyzed from 31 studies.

Conclusions: The frequency of chromosomal gains and losses at distinct chromosome arms increased from premalignant lesions to invasive cancer. The most common alterations in cervical cancer were gains at 3q with a weighted average frequency of 55% (240/436, 95%CI 44.8-65.3%) and losses at 11q (147/436, 33.7%; 25.2-42.2%), followed by 2q losses (141/436, 32.3%; 23.7-41.0%). Most of these alterations could be mapped to the terminal chromosome regions (3q25-3q29, 11q14-25 and 2q33-37). Gains at 3q and losses at 2q were observed already at premalignant stages with gains at 3q in 33.6% of CIN2/3 (47/140; 21.5-45.7%), and losses at 2q in 13.6% in CIN1 (8/59; 2.5-24.6%), and 20.0% in CIN2/3 (28/140; 8.6-31.4%). HPV16-positive cervical squamous cell cancers displayed a higher frequency of gains and losses at most chromosome arms and significantly more losses at 3p than HPV18-positive cervical squamous cell cancers. Gains at 20q appeared less frequent in HPV16-positive cancers than in cancers with other HPV-types. When comparing HPV-positive with HPV-negative vulvar cancers the most obvious difference were significantly more 8q gains in HPV-negatives but generally lower frequencies of alterations compared to HPV-positive vulvar squamous cell cancers. In summary, there are common alterations characteristic for HPV-induced and non-HPV induced cancers of the lower genital tract of which some occur already at premalignant stages. Distinct alterations differ between cervical squamous cell and adenocarcinoma and between lesions induced by different HPV genotypes.

VALUE OF A NOVEL PANEL OF CELLULAR BIOMARKERS IN CERVICAL CANCER SCREENING AND TRIAGE

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Objectives: New methods, protocols are proposed to increase positive predictive value of cervical cancer screening and maintain effective cervical cancer prevention measures. In this evolution of methods and protocols, HPV testing already gained a significant role, but cytology is still the mainstay for most of the screening programs. Recently a new panel of cellular biomarkers is proposed to potentially replace tedious and subjective cytology. The test has the potential to distinguish normal healthy from diseased with cervical intraepithelial neoplasia grade 2 or worse (CIN2+) women based on the gene expression status of exfoliated cervical cells.

Method: To access its value a case-control study using Hungarian and Irish cervical screening populations was set up. Cases comprised 750 women who developed CIN2+ or worse confirmed by histology, and were stratified by age group (under 29 and over) and disease grade (CIN2, CIN3, and carcinoma). Controls (n=250) were women who identified disease free by cytology and were the same age on average as cases, the controls were also stratified by age. Biomarker reverse transcriptase PCR measurements using routine exfoliated cytological samples, high-risk HPV testing, cytology and/or histology were performed and used to evaluate different protocols of testing including combinations of the tests with different test cutoffs. The Bayesian estimates of critical parameters of test performance were calculated and compared for the different stratified groups.

Conclusions: For the detection of CIN2+ cases the biomarker panel specificity [0.93 95%CI 0.90-0.96] and sensitivity [0.89 95%CI 0.86-0.91] were better than the values of HR-HPV test [specificity 0.85 95%CI 0.81-0.88, sensitivity 0.88 95%CI 0.85-0.90] or cytology alone with ASCUS+ cutoff [specificity 0.83 95%CI 0.77-0.88, sensitivity 0.70 95%CI 0.66-0.73] or with LSIL+ cutoff [specificity 0.97 95%CI 0.93-0.99, sensitivity 0.54 95%CI 0.51-0.55]. Bayesian point estimates of positive (PPV) and negative (NPV) predictive values for a hypothetical, natural history based, screening population with 1% of CIN2+ prevalence are [PPV: 0.12, 0.05, 0.04, 0.17; NPV: 0.999, 0.998, 0.996, 0.995], respectively. Favoring high screening specificities to keep screening costs low for special settings an optimal cutoff value for the biomarker panel test was selected. This was achieved at a compromise of sensitivity for CIN2+ [0.50 95%CI 0.48-0.50], however the point estimate of PPV improved significantly to 0.55 and the corresponding NPV is still high (0.994). Nevertheless this cutoff still provides high sensitivity for the detection of cervical carcinoma [0.83 95%CI 0.70-0.86].

HPV16 EARLY TRANSCRIPT EXPRESSION IS NOT NECESSARILY REGULATED BY INTEGRATION IN CERVICAL SMEARS, THE ROLE OF DNA METHYLATION

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Objectives. HPV16 is the most carcinogenic high-risk HPV genotype. Persistent infection is necessary for the development and progression of HPV-induced lesions to invasive cervical cancer. We previously described that HPV16 load and viral DNA integration allow the identification of women with high-grade lesions. Along neoplastic progression, viral DNA integrates into the host genome. This often results in the disruption and loss of E2 and E5 gene expression and increased levels of E6/E7 transcripts. Methylation of the viral promoter can also regulate E6/E7 expression. The aim of our study is to correlate HPV16 early transcript expression with viral markers and viral DNA methylation in cervical smears with different cytological diagnosis.

Methods. (1) Real-time quantitative PCRs (qPCR) targeting HPV16 E6, E2 and albumin gene were carried out to quantify the viral load and to determine the physical status of the viral genome (E2/E6 ratio). (2) Reverse Transcription (RT) qPCRs have also been developed to quantify E6, E2, E5 and beta 2 microglobulin mRNA in HPV16+ cell lines and cervical samples infected by HPV16. To improve the method sensitivity, a cDNA pre-amplification approach has been validated. (3) High resolution melting (HRM) PCR has been set up to evaluate the methylation of HPV16 DNA (sequences under patent pending) after bisulfite conversion.

Results - conclusions. Until today, 149 samples have been analyzed (39 WNL, 34 ASC-US, 33 LSIL, 33 HSIL and 10 carcinomas). (1) As expected HPV16 DNA load is higher in cancer samples (median of 26,810 copies/103 cells) than in normal samples (median of 1,529 copies/103 cells). (2) The proportion of samples harboring integrated HPV16 genomes increases with the lesion grade. (3) At least one HPV16 early transcript is detected in 77 to 94% of samples according to cytology. (4) The levels of HPV16 E6 and E2 transcripts increase with the lesion severity and are surprisingly not inversely correlated. Taken together, our data suggest that another mechanism than integration can be involved in the regulation of E6 expression. The methylation of HPV16 DNA is under investigation and will be correlated to integration and transcript expression

DISTINCT HPV 16 URR METHYLATION IN PERMISSIVE AND TRANSFORMING INFECTIONS

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Objectives. Persistent infection with high risk human papillomaviruses (HR-HPVs) can cause cervical cancer. The transition from the permissive infection mode to the transforming mode is driven by deregulated expression of the viral oncoproteins E6 and E7. Expression of E6 and E7 is regulated by upstream regulatory region (URR). The E2 protein is the master regulator of the viral gene transcription and replication, binds to four conserved E2 binding sites (E2BS) in the URR. Methylation of CpG dinucleotides within E2BSs is known to inhibit E2 binding thereby abrogating its regulatory activity on viral onco-gene expression, might contribute to E6/E7 deregulation. Thus, we aimed to analyze the methylation pattern of the HPV 16 URR in cervical lesions at different stages of progression in order to identify potential changes in methylation pattern which might be associated with oncogenic transformation.

Methods. HPV 16 positive formalin-fixed paraffin-embedded tissue sections were immunohistochemically stained for p16^{INK4a} to identify epithelial regions with permissive (p16^{INK4a} negative) and transforming (p16^{INK4a} positive) HPV infections. Basal and parabasal cell layers were microdissected and DNA was isolated and bisulfite treated. Methylation levels at 15 CpGs within the HPV 16 URR were determined by pyrosequencing. In total, HPV16 URR methylation of 31 regions with permissive, non-transforming infections was compared with 40 regions with transforming infections (23 CIN2+ and 17 SCC samples).

Conclusions. We observed that transforming HPV infections marked by p16^{INK4a} overexpression had significantly higher methylation levels in the promoter, more precisely at the proximal E2BSs 3 and 4, than permissive, non-transforming infections indicating that changes in promoter methylation are associated with oncogenic transformation.

These data suggest that distinct shifts of the HPV 16 methylome, especially at E2BSs may trigger neoplastic transformation by preventing E2 binding and thereby contributing to E6 and E7 deregulation.

FC 5-6

AMPLIFICATION OF TERT AND TERC GENES IN CYTOLOGY SAMPLES FROM CERVICAL CANCER AND CERVICAL INTRAEPITHELIAL NEOPLASIAS

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Objective: Telomerase is the enzyme consisting of two parts: RNA component (3q26 - gene TERC) and catalytic component (5p15 - gene TERT). It is well known that telomerase has a crucial role in the process of carcinogenesis. Current screening methods for cervical cancer do not tell as much about the prognosis and malignant potential of HPV DNA infection. Thus, the detection of amplification of telomerase subunits TERT and TERC by fluorescent in situ hybridization could provide the missing information about future behavior of cervical lesions.

Methods: Slides for FISH analysis were prepared from 13 liquid-based cytology samples taken during the gynecologic examination after signing informed consent by the patient. Patients according to cytological diagnosis were divided into following groups: NILM (n=3), ASC-US (n=2), L-SIL (n=2), H-SIL (n=4) and SCC (n=2). Samples were analyzed for the amplification of genes TERC (3q26) and TERT (5p15) using a four-color FISH probe (FHACTION™) on 100 cells per slide. The results of in situ hybridization were compared with cytologic (Bethesda) analysis of the cervical smears.

Results: The average ratio of analyzed gene copies (3q26:5p15:cen7) was 2,15:2,04:2,01 in case of NILM, 2,26:2,01:2,00 in cases of ASC-US, 2,60:2,05:2,00 in cases of L-SIL, 2,61:2,10:2,045 in cases of H-SIL, and 2,82:2,19:2,00 in cases of SCC. Average number of TERC and TERT copies correlates with Bethesda cytological classification ($p < 0.0001$ for both genes). The number of cells with 4 and more copies of TERC gene per 100 cells was 4 (NILM), 9 (ASC-US), 14 (L-SIL), 20 (H-SIL) and 26 (SCC). Average number of cells with 4 and more copies of TERC gene correlated with cytological results. Furthermore, correlation between amplification of TERC and TERT gene was also statistically significant ($p < 0.0001$).

Conclusions: FISH diagnostics of TERT and TERC genes could identify cervical lesions with malignant potential in advance. Moreover, it can help to lower the overtreatment of diagnosed cervical lesions as an adjunct to cytology and HPV DNA testing.

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FC 5-7

THE P16^{INK4A} AND E6 MRNA QUANTITATIVE EXPRESSION IN CERVICAL SQUAMOUS LESIONS FROM PATIENTS WITH HPV 16/18 INFECTION

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Objective: The HPV type 16 and 18 are the most prevalent subtypes responsible for 70% of cervical carcinomas. The progression of cervical lesion into carcinoma is related to over-expression in E6 and E7 viral oncoproteins. The p16 is a cellular correlate of increased E6/E7 mRNA expression. In line with this, we aimed to determine the level of p16^{INK4A} and E6 mRNA transcripts in cervical smears from HPV 16/18 positive women with squamous cervical lesions.

Methods: A total of 98 women were included and underwent colposcopy, oncocytology (OC) and biopsy from suspicious lesions. The OC smears were classified according to Bethesda and analyzed for the presence of HPV DNA and mRNA level of p16^{INK4A} and E6 in HPV16 and 18 positive cases. HPV genotypes were confirmed by sequencing analysis and p16^{INK4A} expression by relative quantification (RQ). The level of p16^{INK4A} was normalized to housekeeping gene ACTB and compared to controls (OC from healthy women, HPV free). For E6 mRNA expression we used qPCR.

Results: There were 34 samples classified as L-SIL, 52 as H-SIL and 12 as squamous cell carcinoma (SCC). The hrHPV infection was found in 85.7% of specimens. HPV16 and/or 18 were present in 52.9% of L-SIL, 61.5% of H-SIL and 71.4% of SCC. The mean level of p16^{INK4A} and E6 mRNA transcripts gradually increased with severity of lesions, and were expressed on p16^{INK4A} RQ as 1.42-fold in L-SIL, 8.03-fold in H-SIL, and 25.32-fold in SCC lesions higher compared to controls and significantly differ to each other ($p < 0.001$). Similarly, an increased trend of E6 mRNA copies on qPCR was revealed toward severe lesions giving significant difference between L-SIL and H-SIL ($p = 0.002$), L-SIL vs. SCC ($p < 0.001$), and H-SIL vs. SCC ($p < 0.001$).

Conclusion: There is significant increased trend in p16^{INK4A} and E6 mRNA expression level with increased severity of squamous cell lesions among HPV 16/18 positive women in cervical smears. Thus, obtained results may have a great potential for detection of women at risk of CIN2+ progression due to squamous lesions, and for determination of lesion behavior.

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HPV PREVALENCE & ABNORMAL CYTOLOGY IN HIV-SEROPOSITIVE WOMEN FROM HIV-DISCORDANT COUPLES**Cooper J¹, Rositch A², Guthrie B³, Bosire R⁴, Choi R³, Kiarie J⁴, Farquhar C^{3,5}, and Smith J⁶***1 Howard University College of Medicine, Washington, DC, USA**2 Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA**3 Department of Global Health, University of Washington, Seattle, WA, USA**4 University of Nairobi Institute of Tropical and Infectious Diseases, Nairobi, Kenya**5 Department of Medicine, University of Washington, Seattle, WA, USA**6 Department of Epidemiology, University of North Carolina, Chapel Hill, NC, USA*

Objective: HIV is associated with an increased risk of high-grade cervical neoplasia and invasive cervical carcinoma. We evaluated the prevalence of high-risk (HR) HPV infection and cytology in HIV-1-infected women from HIV-1-discordant couples.

Methods: A cross-sectional study of HIV-1-seropositive women, aged 18 to 50 years, from a larger prospective cohort was conducted in Nairobi, Kenya. Between September 2007 and December 2009, cervical cells were collected from women for HPV DNA identification and cervical cytology. High and low-risk HPV types were detected and genotyped with Linear Array (Roche).

Conclusions: Among 283 women with a valid HPV test, the mean age was 29.1 years (SD ± 6.5), the median CD4+ count was 460.5 (IQR: 304-677), and most women were married (95%). The HPV prevalence was 62.2%. Of these HPV-positive women, 73.8% (134/176) were infected with high-risk (HR) HPV genotypes. The most common genotypes were HPV53 (10.6%), HPV51 (9.2%), and HPV35 (8.5%). Nearly a quarter of women in this study had abnormal cytology (24.4%). Among those with high-grade lesions, 93.8% (15/16) were also HPV-positive. The most common HPV subtypes in women with high-grade lesions were HPV31 (25%), HPV 35 (12.5%), HPV51 (18.8%) and HPV58 (18.8%). HPV16 was detected in only 1 (6.3%) woman with a high-grade lesion. Women with a CD4+ count <350 cells/μl had a higher prevalence of HPV than those with ≥350 cells/μl (71% (56/79) vs. 59% (120/204)), although, this difference was not statistically significant. The majority of women in the study population were in stable, married partnerships with an HIV-negative partner, yet the prevalence of HR-HPV infection and cervical abnormalities was high. Cervical cancer screening is needed in Kenya, and perhaps with particular attention to special groups like HIV-Positive women from HIV-discordant couples.

PROSPECTIVE EVALUATION OF CERVICAL SCREENING METHODS IN HIV POSITIVE WOMEN IN AFRICA (HARP STUDY): BASELINE RESULTS**KELLY H¹, MUZAH B², SAWADOGO B³, DIDELOT M⁴, MICHELOW P², LOMPO O³, DOUTRE S⁴, GILHAM C¹, VON KNORRING N², ZAN S³, DELANY S², OMAR T², MEDA N³, DRABO J³, WEISS H¹, LEGOOD R¹, NAGOT N⁴, SEGONDY M⁴, COSTES V⁴, MAYAUD P¹***1 London School of Hygiene & Tropical Medicine, UK**2 Wits Reproductive Health and HIV Institute, Wits University, South Africa**3 Centre de Recherche Internationale sur la Sante, Ouagadougou University, Burkina Faso**4 University of Montpellier 1 & INSERM U1058, France*

Objectives: To compare strategies for screening HIV-positive women in Africa for CIN, through a prospective design. This abstract presents epidemiological data at enrolment.

Methods: The HARP (HPV in Africa Research Partnership) study aims to enrol 1200 HIV-positive women aged 25-50, equally distributed between those on antiretroviral therapy (ART) or not eligible for ART (CD4 > 350 cells/mm³). At enrolment, participants undergo specimen collection for 1) conventional Pap smear, 2) HPV testing by CareHPV and genotyping by InnoLipa, 3) VIA/VILI, and 4) colposcopy. A decision for taking a 4 quadrant cervical biopsy is based on abnormalities present by any of the strategies. HIV plasma viral load, CD4 count, WHO clinical staging are also collected. Enrolment is due to finish in May 2012.

Conclusions: As of February 2012, 162 women have been enrolled in Burkina Faso (BF) and 176 in South Africa (SA), with overall 276 on ART and 62 not on ART. The prevalence of high-risk HPV is 66% in BF and 59% in SA, with the 4 most common types in BF being HPV52 (17%), HPV35 (16%), HPV16 (13%), HPV51 (9%) and in SA being HPV52 (20%), HPV51 (14%), HPV35 (10%) and HPV16 (10%). The prevalence of abnormal cytology (≥LSIL) is 9% in BF and 48.5% in SA, with HSIL being 3% in BF and 9.1% in SA. Data on VIA/VILI, colposcopy, biopsy rate and histology results will be presented. As expected the prevalence of high-grade cervical abnormalities and HR-HPV are high in HIV-positive African women.

FC 6-3

CERVICAL AND ANAL HPV DETECTION AMONG WOMEN DRUG USERS LIVING WITH HIV IN MIAMI, FLORIDA.**Rosa-Cunha I, Hooton TM, Cardenas GA, Metsch LR.***University of Miami Miller School of Medicine, Miami, FL, USA.*

Objectives: Human papillomavirus (HPV) can cause cervical and anal dysplasia and cancer in women. 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 HPV detection and related anogenital diseases among women drug users living with HIV. The objective of our study was to assess the rate of HPV detection and correlation with cervical and anal Pap smears among drug users living with HIV in Miami, FL.

Methods & Results: Project HOPE (Hospital is an Opportunity for Prevention and Engagement) is a two-site study carried out 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. In the Miami site 29 women had cervical and anal Pap smear screening and the samples were tested for cytology and for 37 high- and low-risk HPV genotypes using Roche LIN-EAR ARRAY HPV genotype testing[®]. The study population was 88% Black, the mean age was 45. Half of the participants were on antiretroviral therapy. Overall abnormal anal Pap smears (21/29) were almost as common as abnormal cervical Pap smears (23/29). Concomitant abnormal cervical and anal Pap smears were found in 70% (16/23) of participants and among the few ones with a negative cervical Pap smear, the majority (5/6) had an abnormal anal Pap smear. HPV was detected in 86% of the cervical samples (in 21/23 abnormal and in 4/6 negative Pap smears) and in 52% of the twenty-two anal samples available for HPV testing (in 10/14 abnormal and in 3/8 negative anal Pap smears). The majority of participants were infected with two or more HPV types (23/25 cervical and 13/14 anal) and mostly (23/25 cervical and 12/13 anal) non-16, non-18 types.

Conclusions: Preliminary data suggest that HPV detection and abnormal cervical and anal Pap smears are common in women drug users living with HIV in Miami and the data highlights the need for further studies on prevention and screening of cervical and anal dysplasia and cancer in this group population.

FC 6-4

CLINICAL RELEVANCE OF SEXUAL TRANSMITTED DISEASE PROFILE AND HUMAN PAPILLOMAVIRUS (HPV) INFECTION IN THAI FEMALES**Nuttavut K¹, Thaniya S², Chutima C², Narongrit S³, Chantane T⁴, Naree C⁴, Siriporn S⁴, Gaidkanok S, Waraphorn K***1 Gynecologic Oncologist; 2 Pathologist 3 Master of Science 4 RN Cervical Cancer Care Team, Chulabhorn Hospital, Bangkok, Thailand*

Objectives: HPV is a causal risk factor for cervical cancer development, and also the most commonly found sexual transmitted disease (STD). In many cases, one profile of STD can be related to another. This study aimed to identify the correlation between STD and HPV infection.

Methods: A number of 3,046 HPV unvaccinated Thai women, aged 20-70 years (mean age, 44.7 years; range 20-70 years), were recruited in the cervical cancer screening at Chulabhorn hospital, Bangkok, Thailand, during July 19, 2011 – February 22, 2012. HPV genotyping (Linear array, Roche, USA) and liquid-based cytology (Surepath, Becton and Dickinson, USA) were used as the screening tools. All females were tested for HIV, HBsAg, and VDRL. Residual liquid based cytology solution was implemented for the detection of Chlamydia Trachomatis (CT) and Neisseria Gonorrhoea (NG) by Cobas 4800 CT/NG test (Roche, USA). Candida infection was defined by cytological diagnosis.

Results: 31 females were excluded. The overall HPV prevalence in 3,015 females was 15.2%, with 6.9 % high risk (HR) HPV, 9.8 % low risk (LR) HPV, and 1.5% probable HR. The positive results for HIV, VDRL, HBsAg, CT, NG, candida infection were 0.2%, 1.4%, 6.9%, 1.2%, 0%, and 7.3 %, respectively. Using univariate logistic regression analysis, odd ratio (95%CI, p value) for the prediction of HPV infection in HIV positivity, VDRL, HBsAg, CT, candida infection was 8.42 (1.40-50.55, 0.02), 1.32 (0.61-2.87, 0.48), 0.68 (0.43-1.06, 0.09), 5.77 (2.98-11.18, <0.001), and 1.10 (0.76-1.60, 0.62), subsequently. NG could not be analyzed and no event was found. Multivariate analysis showed that HIV and CT infections were still predicting factors for HPV infection. According to the correlation with HR-HPV, only CT positive result was significant with odd ratio of 5.40 (95%CI=2.29-11.77, p=0.001).

Conclusions: HPV is a very common STD in Thai women with the prevalence of 15.2 %, and 6.9 % HR-HPV. Interestingly, coexisting hepatitis B infection was frequent in this cohort, but no association with HPV infection was found. The association of HIV infection with HPV was observed and Chlamydia Trachomatis was the only STD profile to predict both HPV and HR-HPV infections.

HPV FLARE IN AN HIV POSITIVE PATIENT

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Cutaneous HPV infection is very common both in immunocompetent and immunocompromised individuals as well. HIV positive patients with low number of CD4+ T lymphocytes are generally in high risk of HPV infection. Worsening of preexisting HPV infection can be also paradoxically associated with successful initiation of combination antiretroviral therapy (cART). The increased number of the CD4+ T lymphocytes leads to a strong inflammatory reaction to preexisting or latent opportunistic infections. This type of immune response resulting in a flare of opportunistic infections such as viral, bacterial and fungal is called immune reconstitution inflammatory syndrome (IRIS).

A clinical case of the flare of cutaneous HPV infection in HIV positive patient due to IRIS will be presented.

JOINT BIOMARKERS IN HPV16 INVASIVE ANAL CARCINOMA

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Objectives. Most studies have reported that the majority of invasive anal carcinomas contains high-risk human papillomaviruses (HPV), HPV16 being the most prevalent type. This study aimed to investigate HPV status and cellular biomarkers of HPV-oncoproteins in invasive anal cancers.

Methods and results. HPV genotype distribution was determined in 83 anal cancers by the INNO-LiPA assay. Then, HPV16 positive samples were tested for viral DNA load and physical state with type-specific real-time quantitative PCR targeting E6, E2 and albumin genes. Samples were also subjected to immunohistochemical analysis of p16, Ki-67, p53 and p21. Of the tumors, 98.7% were positive for alpha-HPV DNA. HPV16 was the most prevalent genotype (89.7%), followed by HPV39 (3.8%) and HPV33 (2.6%). HPV16 viral load was high, ranging from 2x10³ to 1.5x10⁷ copies/10³ cells. The integration of HPV16 DNA estimated by the E2/E6 ration was detected in 77.8% of tested cases, with 70.4% of mixed integrated and episomal DNA cases and 7.4% of fully integrated DNA. The later cases were associated with a low HPV16 DNA load compared with cases containing either episomes or mixed integrated and episomal DNA. As expected, the majority of HPV16 positive tumors expressed p16 (89.4%) with a high proliferative index, while a minority of them overexpressed p53 (15.1%). p21 expression did not appear to correlate with p53 expression. At last, p16, p21 and p53 will be evaluated as possible prognostic markers in terms of disease free survival and overall survival.

In conclusion, HPV16 was most exclusively detected in our series of invasive anal cancers, at high viral load, and can exist as episomal, fully integrated or mixed forms. The expression patterns of cell-cycle proteins differed in the series. The combination of HPV features with p16, p53 and p21, constituting downstream pathways of viral oncoprotein activity, as joint biomarkers, may provide a unique opportunity to predict clinical outcomes.

FC 7-2

**PREDICTIVE FACTORS FOR ANAL HPV PREVALENCE IN HIV NEGATIVE WOMEN:
COLPOSCOPY CLINIC PATIENTS VS. SEX WORKERS**

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Objective: To investigate predictive factors [smoking, oral contraceptives (OC), a history of anal sex, of sexually transmitted infections (STI), or of genital warts] of anal HPV infection in 2 HIV negative populations: colposcopy clinic patients (P) and sex workers (SW).

Method: Of 250 consecutive women [101 SW, 149 P] one anal and one cervical swab were taken (Cervex-Brush®). Presence of 18 HPV genotypes was determined using TaqMan-based RT PCR's. Samples were considered adequate when B-Globin and/or HPV DNA were found.

Statistical analysis: t test, X², and Mantel-Haenszel Common Odds Ratio Estimate.

Results: Patients were significantly older (36.4 vs 30.3 years), and had significantly more abnormal pap smears and genital warts. Sex workers smoked more and had had more STI's (both significant). The use of OC was identical.

Cervical samples of both groups were adequate. The anal samples of P and of SW were adequate in 64.4% (96/149) and 29.7% (30/101), respectively.

In P, HPV DNA was found in 56.3% (54/96) of anal and in 53.7% (80/149) of cervical smears. In SW, HPV DNA was found in 46.7% (14/30) of anal and in 38.6% (39/101) of cervical smears.

In the P group, full and partial concordance of genotypes was observed in 37.0% (20/54) of samples. In the SW group, full and partial concordance was found in 35.7% (5/14) and in 42.9% (6/14), respectively.

We found no difference in prevalence of HPV 16 / any hrHPV in the anal and cervical samples of both groups.

Assessment of the predictive factors, related to the presence of anal HPV 16 / any hrHPV, showed no difference.

Conclusions: Predictive factors of both groups differ. In addition, sex workers have a higher exposure to HPV. The prevalence of anal HPV 16 / any hrHPV is identical.

An explanation may be that other factors may be of importance (e.g. immune system). It is difficult to determine which women need anal screening.

FC 7-3

**EVALUATION OF HR-HPV MRNA AND P16/KI67 TESTING IN SCREENING
FOR ANAL INTRAEPITHELIAL NEOPLASIA IN HIV INFECTED PATIENTS**

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Objectives: Anal intraepithelial neoplasia (AIN), anal cancer and anal high-risk human papillomavirus (HR-HPV) infections occur at increasing rates in HIV infected patients. Cytology and HR-HPV DNA analyses are sensitive but most of the time positive in this high-risk population. The aim of this study was to evaluate the performance of HR-HPV E6/E7 mRNA and p16/Ki67 dual-stain cytology to identify low-grade (LSIL) and high-grade lesions (HSIL) in HIV infected patients.

Methods: Anal swabs were collected from HIV infected patients, during clinical examination, in a liquid based medium for cytological analyses. HPV-DNA detection of 19 HR-HPV- and 16 LR-HPV types was performed by Clart HPV 2® clinical arrays (Genomica), while HR-HPV E6/E7 mRNA of 14 HR-HPV-types was detected by the TMA-based APTIMA® HPV Assay (Gen-Probe Incorporated). Most patients with abnormal cytology were tested for p16^{INK4a} /Ki67 dual staining by CIntec® PLUS (Roche mtm laboratories).

Conclusions: Of these 118 patients (12 women and 106 men), 50 (42,4%) were considered to have normal cytology, 26 (22,0%) ASC-US, 35 (29,7%) LSIL and 7 (5,9%) HSIL. 77 (65%) and 67 (56,8%) of anal swabs were HR-HPV DNA and HR-HPV E6/E7 mRNA positive, respectively. 21 (35,6%) of the 59 swabs tested for p16^{INK4a} /Ki67 were positive. For the detection of both LSIL and HSIL, sensitivity, specificity, negative predictive value (NPV) and PPV were 92,9%, 50,0%, 92,7%, 50,7% for HR-HPV DNA testing, 92,7%, 39,6%, 86,4%, 56,7% for HR-HPV E6/E7 mRNA testing, and 61,3%, 89,7%, 68,4%, 86,4% for p16/Ki67 testing. An elevated cut-off of the HR-HPV E6/E7 mRNA assay (S/CO of 10 instead of 1) decreased sensitivity (82,9%) but really improved specificity (62,5%) and slightly improved PPV (65,4%). Whereas HR-HPV DNA has a very good sensitivity, HR-HPV E6/E7 mRNA assay and p16^{INK4a} /Ki67 testing have significantly higher specificity and PPV. It may indicate their use for anal cancer screening.

PAIN AND BLEEDING ASSOCIATED WITH ANAL CANCER SCREENING STRATEGIES IN HOMOSEXUAL MEN

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Objectives: To characterise adverse effects (AE) and participant experience of anal Pap swab, digital anal examination (DAE) and high resolution anoscopy (HRA).

Methods: The Study for the Prevention of Anal Cancer (SPANC) is a 3-year community-recruited prospective study of homosexual men in Sydney, Australia. Participants undergo anal swab for cytology, DAE and HRA at each visit and complete an AE questionnaire within one month.

Conclusions: By end December 2011, 179 participants (median age 49 years; 34.6% HIV positive) had enrolled, of whom 40% had also attended a 6-month visit. DAE, anal swab and HRA were reported to be fairly or very uncomfortable by 9, 35 & 47% of men, respectively. Reported discomfort was non-significantly greater at 6 months compared with baseline for the anal swab ($p=0.055$) and HRA ($p=0.081$) but not DAE ($p=0.56$). Similarly, moderate or severe pain associated with DAE, swab and HRA was reported by 6, 25 & 34%, respectively. Pain lasted a few days or longer in 36% and 20% of all men required analgesia. Having a biopsy and increasing number of biopsies were both associated with increasing pain severity ($p=0.043$ & $p=0.020$, respectively) but not increasing pain duration. After HRA 68% of men reported some bleeding. Bleeding lasted a few days for most men but 6% reported bleeding that lasted longer. Having a biopsy and increasing number of biopsies were strongly associated with increasing severity of bleeding ($p=0.001$ & <0.001 , respectively). Longer duration of bleeding was associated with having a biopsy ($p=0.012$) but not with increasing number of biopsies taken ($p=0.137$). A physician review was sought by 1 man for pain and 4 men for bleeding. After controlling for number of biopsies taken, more severe pain and bleeding was experienced by men who predominantly practise the insertive position in anal sex ($p=0.004$ & $p=0.050$, respectively). Thus far in SPANC no serious adverse events have occurred, but 7 participants have withdrawn, of whom 3 cited excessive pain and/or bleeding post-procedure. Overall, fewer than half the men screened reported that they found an anal Pap swab and HRA to be "acceptable" procedures. Such common procedure-related adverse effects may affect the utility of these tests as screening tools.

RISK FACTORS FOR ANAL HPV INFECTION IN PATIENTS WITH SEVERE CERVICAL DYSPLASIA

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Objectives: Human papillomavirus (HPV) infection is strongly associated with the development of anal cancer. Epidemiology of anal HPV infection among women with cervical dysplasia is not well described. Some studies, mainly in HIV-positive subjects, showed that high degree of genotype-specific concordance could suggest that both - the cervix and anus may serve as reservoirs for HPV infection.

Methods: We performed a cross-sectional study of women attending 2 university-based colposcopy clinics, who underwent conization for high-grade cervical intraepithelial dysplasia. Participants received anal HPV genotyping and cervical HPV genotyping using Cobas HPV test (Roche Molecular Systems, CA), completed a questionnaire detailing medical history and sexual risk factors. Altogether 99 women were enrolled in period between September 2011 and January 2012. All of them completed a questionnaire, 97 samples from cervix and 96 samples from anus were adequate for final analysis of HPV DNA. Median age of patients was 36 years and 7 months (19 – 72 years), 58,5 % (58/99) women used oral contraception, only 26,3 % (26/99) women did not smoke, 21,2 % (21/99) women had early koitarche and 17,2 % (17/99) women had more than 10 sexual partners. More than half of subjects (56/99) did not reported any anal intercourse. Cervical HPV infection was confirmed in 81 women (81,8 %) - low-risk (LR) genotypes only in 6 patients, high-risk (HR) genotypes only in 59 patients, LR and HR types together in 16 patients. Anal HPV infection was detected in 46 women (46,5 %). Concurrent anal and cervical HPV infection was found in 41 patients (41,4 %), in all of them only HR genotypes were detected with the dominance of HPV 16. Genotyp 16 was found in 38,3 % of infected women with cervical infection (31/81) and even in 54,3 % of infected women with anal infection (25/46).

Conclusions: Anal high-risk HPV infection is common in women with high-grade cervical intraepithelial dysplasia irrespective of other risk factors including sexual behavior. Other unidentified reasons may play a role in the development of anal HPV infection.

FC 7-6

HPV GENOTYPE PREVALENCE IN ANAL SPECIMENS AMONG HIV-POSITIVE MEN WHO HAVE SEX WITH MEN (MSM): CAN HPV VACCINATION HELP TO PREVENT ANAL HPV-RELATED LESIONS?

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Background: MSM, especially those with HIV infection, have one of the highest risks of anal cancer and other HPV-related lesions. HPV vaccination with bivalent (serotypes 16 and 18) or quadrivalent (serotypes 6, 11, 16 and 18) vaccine is effective in women for prevention of cervical and anogenital diseases attributable to vaccine-type HPV. Data supporting routine male vaccination are few.

Methods: A prospective cohort study was performed in a tertiary-care university hospital. A total of 305 consecutive out-patients HIV-positive MSM underwent a systematic examination for macroscopic human papillomavirus (HPV)-related lesions through high resolution anoscopy, anal cytology, HPV testing and histological confirmation when necessary. Sexual behaviours and medical history were assessed using a direct questionnaire

Results: Median age was 42 (IQR 22-79) years; median elapsed time since HIV diagnosis was 8.4 (0.5-24) years; median CD4 cell count was 605 (IQR 400-770) cells/mm³; 60.6% of them had HIV-RNA viral load < 50 copies/mL. Results of anal cytology were: 148 (48.5%) normal, 88 (28.8%) LSIL, 6 (0.2%) HSIL and 63 (20.6%) ASCUS. Ninety-six (31.4%) patients presented with intranal and/or perianal condylomas. Fifty-five (14.7%) patients were diagnosed of anal intraepithelial neoplasia (AIN) of any degree. Among the whole cohort, the most frequent HPV-serotypes were: 16 (10.9%), 59 (7.6%), 11 (5.9%) and 31 (5.6%). In those patients with ASCUS in the anal cytology the frequencies were: 16 (8.8%), 59 (8.3%), 52 (7.8%) and 31 (7.3%). In patients with condylomas: 16 (10.3%), 11 (10%), 6 (8.7%) and 59 (6.7%). Among those with diagnosis of AIN: 59 (10.7%), 16 (8.3%), 18 (8.3%) and 33 (8.3%).

Conclusions: On the basis of the results, we can conclude that HPV vaccination with HPV-quadrivalent vaccine would be useful in MSM population, not only as a protection against AIN and so against anal cancer but also contributing to control anogenital condylomas that also mean an important morbidity in this set of population.

FC 8-1

EFFECT OF THE MEDIA ON CERVICAL CANCER INCIDENCE: TIME-TREND ANALYSIS OF POPULATION BASED CANCER REGISTRY DATA AND INDIVIDUAL SCREENING HISTORIES

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Objective: To investigate why recently published statistics for England recorded 18% more cases of cervical cancer in 2009 than in 2008 with greater numbers aged 25-39 than in any year since 1990.

Methods and Findings: Trends in published incidence of cervical cancer and of cervical intraepithelial neoplasia (CIN3) were analysed for England, Wales and Scotland. Data on cancers diagnosed between April 2007 and March 2010 from the National Audit of Invasive Cervical Cancers were analysed by month of diagnosis. In England there was a 75% (95% CI 47%-108%) increase in cervical cancer incidence rates in women aged 25-29 in 2009 relative to 2007 and a 35% (21%-50%) increase in women aged 30-39. There has been a gradual increase in rates of CIN3 (particularly for ages 25-29) since 2003, with a more dramatic increase in 2009. A cytology test triggering referral to colposcopy was recorded within 4 months of diagnosis in 75% of cancer cases aged 25-39. On average there were 52 such "diagnoses" per month, but there was a substantial increase in the five months with media coverage of Jade Goody's cervical cancer: with 139 such cases in the month of her death. Overall an excess of 238 cancer cases were observed during the months surrounding her diagnosis and death. The biggest increase was in lapsed former screening attendees, but there was an increase in all groups other than those that were purely symptomatic.

Conclusions: The dramatic increase in cervical cancer in England in 2009 cannot be attributed to the lack of screening of women aged 20-24, nor to a general decrease in the coverage or quality of cervical screening. The media attention surrounding a young celebrity dying of cervical cancer led to the earlier diagnosis of over 200 cancers in young women and affected national cancer statistics.

**DURATION OF PROTECTION OFFERED BY CERVICAL SCREENING TO WOMEN OVER AGE 65:
EVIDENCE FROM A POPULATION-BASED CASE-CONTROL STUDY OF PROSPECTIVELY RECORDED DATA**

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Objective: To establish the duration of protection offered by screening at ages 50-64 to women over age 65.

Methods and findings: Cases of invasive cervical cancer diagnosed in England between April 2007 and March 2011 in women (N=1,040) aged 65+ and two controls individually matched on age and area of residency were identified. We estimated the OR (odds ratios) for strength of association between time since last adequate cytology test (under age 65), age at last adequate test, number of negative tests and the risk of subsequent cervical cancer. The risk of developing cervical cancer within 8 years of an adequate test taken between age 55-64 is reduced by 81% (OR 0.19, 95% CI 0.15-0.25). The risk of developing cervical cancer age 67-84 was reduced by about 60% if your last screen was between the ages of 58-65. At least three negative tests between ages 50-64 are need to reduce the risk of cervical cancer at ages 65-84 (OR 0.22, 95% CI 0.16-0.31).

Conclusions: Offering screening up to age 64 more than halves the risk of developing cervical cancer between ages 65-74. Women can be safely ceased from the screening programme if their last test was after age 57, they have three or more negative tests between ages 50-64 and all abnormal tests have been resolved.

THE IMPORTANCE OF BEING REGULAR: ANALYSIS OF CERVICAL SCREENING INTERVALS IN NEW ZEALAND.

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Objectives: To monitor trends in short and long interval rescreening over the past decade by age and ethnicity in New Zealand.

Cervical screening reduces the risk of cervical cancer. Research has shown that even a single screen provides some benefit. However, the more regular the screening, the greater the benefit. The optimal interval for most New Zealand women is 3 years: the longer the interval between screens the greater the risk of interval cancers. On the other hand, the shorter the interval the greater the risk that adverse effects of screening may exceed the benefit.

While the recommended screening interval for eligible women (ie sexually active, non hysterectomised women 20-69 years) has been 3 years since the inception of the National Cervical Screening Programme (NCSP) in 1990, no previous analysis has examined the actual distribution of screening intervals achieved (the regularity of screening).

Methods: Data was extracted from the NCSP Register. Records of women who were outside the 20-69 age group and who had had a hysterectomy were excluded. The length of time between each screen over the study period (2000-2010) was calculated. This was compared with the recommended screening interval, allowing enrolled women to be classified as 'short' (<2 years), 'appropriate' (2-4 years) and 'long' (>4 years) interval screens.

Conclusions: Over the study period, the proportion of 'long interval' women being screened increased (from about 5% to about 15%). At the same time, the proportion of 'short interval' women being screened decreased from (from about 40% to about 20%). The proportion of 'appropriate interval' women being screened increased slightly (from about 55% to 60%). Variation in these trends by age and ethnicity were also examined.

A substantial proportion of enrolled women are not being screened at intervals recommended by the NCSP. To maximise benefits and simultaneously minimise harms from screening, the NCSP needs to improve regularity of screening, not just coverage.

FC 8-4

EFFECTS OF OFFERING MORE FLEXIBLE CERVICAL SCREENING OPTIONS FOR UNDER AND UNSCREENED WOMEN IN DUMFRIESSHIRE, SCOTLAND.**Wedisinghe L^{1,4}, Currie H¹, Baxter G¹, Khalifa L¹, McCullough A¹, Breen D¹, Wilson A², Canham M³, Cubie H³, Sasini P⁴.***1 NHS Dumfries & Galloway, Dumfries, DG1 4AP, UK. 2 Monklands Hospital, Monklands. 3 Scottish HPV Reference Centre, Edinburgh. 4 Wolfson Institute of Preventive Medicine, Queen Mary University of London.*

Objectives: To examine effects of offering more flexible cervical screening options for under and unscreened women in Dumfriesshire. Primary outcomes are uptake rates of the standard cervical smear (liquid based cytology) and self collection of a vaginal sample for Human Papillomavirus (HPV) testing. Methods: All under and unscreened women over 30 years of age in Dumfriesshire is the study population. They will be sent an invitation letter either to have a standard cervical smear or to collect a vaginal sample for HPV testing. Their choice will be facilitated. Cervical smear testing will be offered at a different venue apart from their standard options. Those with an abnormal cytology will be managed as per current routine practice. Women will also be given the option to self collect (at home or a clinic) a vaginal sample with Rovers Evalyn device for HPV testing with Hologic Cervista. Respondents who test positive for HPV will be called for a smear test and thereafter managed as per routine practice. Those with a negative HPV test will be advised to attend their next screening appointment. Women who decline any form of screening will be asked to complete a detailed, validated questionnaire and to comment regarding this decision. Original data of the 1000 women pilot will be presented.

Conclusions: We hope that this study will not only contribute to improving the cervical screening coverage itself, but also in providing evidence on how we might increase it, thereby reducing cervical cancer incidence, morbidity & mortality.

FC 8-5

INCREASE OF CIN DETECTION RATES IN THE NETHERLANDS; IS LBC TESTING THE UNDERLYING CAUSE?**Rozemeijer K., de Kok I.M.C.M., Matthijsse S.M., van Rosmalen J., van Ballegooijen M.***Department of Public Health, Erasmus MC, Rotterdam, The Netherlands*

Objectives: The detection rates of cervical intraepithelial neoplasia (CIN) in the national screening programme of the Netherlands have rapidly increased since approximately 2004. This could be due to an increased sensitivity of the screening programme and/or an increased background risk in screened women. A potential explanation for an increased sensitivity is the adoption of a new screening test, e.g. Liquid Based Cytology (LBC) or primary human papillomavirus (HPV) testing. Primary HPV testing has not yet been implemented in the Netherlands, but LBC testing was implemented on a regional basis, independent of age. Increased background risks can be explained by changes in behaviour (i.e. sexual behaviour), and these changes can differ between age groups.

Methods: Information on all cervix uteri examinations in the Netherlands was retrieved from the nationwide registry of histo- and cytopathology (PALGA). CIN detection rates and estimated annual percent changes (EAPC) of the detection rates were calculated for the period 2004-2009 by age, region and CIN grade. No data were available on which test was used. In addition, we assessed the positive predictive values (PPV) of the result of the primary smear (borderline/mildly dyskaryotic (BMD) vs. >BMD smears) for CIN I, CIN II and CIN III.

Conclusions: Higher EAPCs were found for CIN I (+13.5%, 95% CI 11.5-15.5) than for CIN II (+8.5%, 95% CI 6.6-10.4) and CIN III (+6.1%, 95% CI 4.4-7.9). We found significantly increased, but equal, EAPCs for all age groups. However, EAPCs between regions did differ significantly (range for CIN I: 4.3% (95% CI -0.4, 8.3) vs. 24.4% (95% CI 8.3, 43.0)). Also, we found that the PPV of a BMD smear has increased in the last 5 year from 7.13% to 8.24% for CIN I, from 6.10% to 6.34% for CIN II and decreased from 6.80% to 5.64% for CIN III detection. The PPV of a >BMD smear has increased from 6.35% to 8.15% for CIN I, from 16.65% to 17.48% for CIN II and from 53.76% to 54.41% for CIN III detection. Our results indicate that an increased sensitivity of the screening programme and likely also the implementation of the LBC test are the underlying causes of the increase in CIN detection rates. Future analyses will include linkage of the observed trend in CIN detection to LBC implementation, by studying the period of LBC implementation and CIN detection rates by region.

REVIEW OF CYTOLOGY AND HISTOPATHOLOGY AS PART OF THE NHS CERVICAL SCREENING PROGRAMME AUDIT OF INVASIVE CERVICAL CANCERS

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Objective: To audit cytology slide reporting in the Cervical Screening Programme in England by reviewing cytology slides from women subsequently diagnosed with invasive cervical cancer.

Methods and Findings: Between April 2007 and March 2011, 8566 women diagnosed with cervical cancer were identified. Cervical cytology slides taken within 10 years of diagnosis were identified and where possible reviewed after a nationally agreed protocol. Reviewers were not blinded to the original reading of each sample. Of 6,284 women with a test within 10 years of diagnosis 65% have a review result recorded. Of 11,242 cytology slides with a review result, 44.4% were conventional cytology, 42.5% liquid based cytology and for 13% the cytology type was unknown. The review result was identical for 61% of slides. Of 5480 originally normal slides, only 51% were normal on review: 9% were inadequate, 21% low grade (borderline or mild dyskaryosis) and 19% high grade (moderate dyskaryosis or worse). Overall 55% of LBC slides originally recorded as negative in women who subsequently developed cervical cancer were upgraded upon review. However this increased from 35% of slides taken more than 5 years prior to diagnosis to 52% in those taken 3-5 years prior to diagnosis and 61% in those within 3 years of diagnosis.

Conclusions: In spite of the excellent quality of cytology in England, a large number of cancers diagnosed within 5 years of a negative screening test are, with the benefit of hindsight, diagnosed following a missed cytological abnormality as opposed to being due to rapidly progressing new disease or poor sampling. Continuing these reviews, with a strong focus on education, will ensure a clear understanding of these slides and further reduce the risk of developing cervical cancer.

MCM BIOMARKER OVER-EXPRESSION ENHANCES STANDARD LIQUID-BASED CYTOMORPHOLOGY IN DETECTING CIN2+ CERVICAL DISEASE IN WOMEN ≤ 35 YOA

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Objective: Cervical disease screening in women ≤ 35 years of age (yoa) is challenging given the prevalence of HPV infection within this age group. Current modalities of HPV testing and cytology screening in younger women each has their limitations with unacceptable false positive and false negative rates, respectively. This study investigated the ability to detect biopsy confirmed CIN2+ disease within a ≤ 35 yoa cohort using a novel test that combines MCM (mini-chromosome maintenance) protein biomarker expression and standard cytology.

Methods: Preliminary data was prospectively collected from a cohort of 3107 women. Cervical samples were collected in SurePath vials to produce liquid based cytology (LBC) slides. Two slides were made from each sample, 1) a standard SurePath Pap and 2) a novel stain that combines the immunocytochemical detection of MCM proteins within the nuclei of cells that are Papanicolaou counterstained. Cytology and histology results were adjudicated via a standard protocol. The utility of the novel MCM biomarker/LBC test was compared to standard LBC performance.

Conclusions: The combination of MCM biomarker expression and LBC abnormal morphology resulted in the identification of 73 additional HSIL cases compared to LBC alone (89 vs. 16). Upon the biopsy of these HSIL patients, there were an additional 24 CIN2+ cases found compared to LBC alone (37 vs. 13). In total, 87 CIN2+ cases were identified with abnormal morphology (ASC-US+) with the novel combined test. Of these 87 cases, 77 were positive for MCM biomarker expression. Within the entire cohort, 345 cases with abnormal cytology and MCM biomarker expression were found to be ≤ CIN1 upon initial biopsy. These patients are currently being followed longitudinally for 3 years to correlate biomarker expression with disease progression. Within our ≤ 35 yoa test cohort, more high grade cytologic abnormalities and biopsy confirmed CIN2+ cases were identified with the novel MCM biomarker/LBC test as compared to standard LBC.

This biomarker expression shows promise in directing management of young women who are at increased risk for developing invasive cervical cancer.

FC 8-8

SCREENING HISTORIES OF CERVICAL CANCER PATIENTS: THE EFFECT OF USING INCOMPLETE DATA

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Objectives: Screening histories of cervical cancer patients are often evaluated using incomplete historical data. We aimed to determine the impact of completeness of cytology registration on the proportion of cervical cancer patients without a recent screening history.

Methods: For Danish women diagnosed with cervical cancer in 2003-2007, we retrieved 7.5-year screening histories from the nationwide Danish Pathology Data Bank (PDB) and the Danish National Health Service Register (NHSR). The data were linked using the unique personal identification numbers. In five steps with increasing level of comprehensiveness of the data sources, we computed the proportions of women who were screened prior to their cancer diagnosis. At first step we included only cytology from the cancer diagnosing laboratories (PDB). Subsequently, we added the laboratories from the same county and later on from the whole country. We thereafter added cytology from the NHSR. At the final step, our analysis was nationwide and based on complete data.

Conclusions: The proportion of women with prior screening was 40% in the first step covering only the local laboratory. This proportion increased continuously when we added other laboratories and new data sources. At the final step, the corresponding percentage was 55%. This represents a 25% reduction in the proportion of women who appeared not to have been screened prior to developing cervical cancer. This effect was due to screening tests undertaken by other laboratories, and to the women's changing of residence. This study shows that it is very important to use comprehensive data sources when evaluating screening histories. Studies that do not use comprehensive data will underestimate the proportion of screened women and inadvertently shift the priorities for cervical cancer control.

FC 9-1

CLINICAL VALIDATION AND REPRODUCIBILITY OF ABBOTT REALTIME HIGH RISK HPV TEST IN A SCREENING SETTING OF WOMEN ≥30 YEARS OLD

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Objectives: Randomized trials produced sound evidence about the efficacy of screening with HPV DNA test in reducing cervical cancer incidence and mortality.

We evaluated the clinical performance and the reproducibility of Abbott RealTime High Risk HPV test in comparison with the Digene HC2 assay.

Methods: A random sample of 949 cervical specimens of women ≥30 years old (881 <CIN 2 and 68 CIN 2+) collected in the Florence and Catania Cervical Cancer Screening Programs was retrospectively evaluated with the Abbott RealTime High Risk HPV and compared to historical HC2 results.

Absolute specificity was 92.4% (95% CI: 0,912-0,947) for both, the Abbott and the HC2 test.

Absolute sensitivity was 95,6% (95% CI: 0,868-0,988) and 97% (95% CI: 0,888-0,994) for Abbott and HC2, respectively.

The non-inferiority score test revealed that the clinical sensitivity and specificity of the Abbott test were non-inferior (P=0,010 and P=0,003, respectively) to those of HC2. Overall agreement between the two assays was 96.5% with a k value of 0.85 (CI 95%: 0,798-0,899).

We evaluated the intra-laboratory reproducibility retesting 521 samples at least 4 weeks later the first test; the crude agreement between first and second test was 98.5% with an overall k value of 0.97 (CI 95% 0.95-0.99).

Conclusions: Abbott RealTime High Risk HPV test fully satisfied the validation requirements for clinical specificity and sensibility for a HPV testing in primary cervical cancer screening.

Moreover, this assay allows to simultaneously differentiate between HPV16, HPV18 and 12 high risk non-HPV16/18 types in every specimen; this information could be useful to evaluate new strategies to triage hr-HPV positive women in a screening setting.

HPV E6 ONCOPROTEIN-BASED PLATFORM FOR DETECTION OF CERVICAL PRE-CANCER AND CANCER

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A key step in reducing cervical cancer-related mortality is the implementation of appropriate screening methods for early detection and treatment of cervical pre-cancer and cancer in developing countries. Arbor Vita Corporation (AVC), in collaboration with PATH and CICAMS, has developed HPV E6 oncoprotein-based screening technology. Elevated expression of E6 and E7 have been shown to correlate with cervical neoplasia, and hence an HPV E6-based test promises to identify specifically those women who need clinical follow-up and intervention amidst the many more women who have an HPV infection without clinical relevance. HPV E6 oncoprotein-based screening has the potential to reduce costs and burden associated with unnecessary colposcopies, and it may enhance feasibility of a screen-and-treat approach.

The "HPV E6 Strip Test" is a lateral flow test for detection for E6 of HPV types 16, 18, and 45, the HPV genotypes that cause 75-80% of all cervical cancer worldwide. This test does not require complex machinery or a cold chain and therefore is well suited for use in lower-resource settings. A large clinical study (START-UP) consisting of screening of 7,500 women including a one-year follow-up in three rural sites in China is ongoing. Study results obtained up to date have demonstrated a high clinical specificity and excellent positive predictive value (>30%) of the HPV E6 Strip Test for cervical pre-cancer and cancer. In addition to the HPV E6 Strip Test, AVC is developing a cytometric bead-based platform for HPV E6 oncoprotein detection. This platform will be suited for high-throughput screening in a centralized clinical laboratory, and will include the detection of additional high-risk HPV types. Lastly, AVC is developing a HPV E6 oncoprotein-based immunocytochemical assay for clinical settings that rely on Pap testing and wish to improve specificity of cytology.

These three different E6 based screening test formats, and an update on results of the START-UP study in China will be presented.

**HPV TESTING IN PRIMARY CERVICAL CANCER SCREENING IN ROUTINE PRACTICE
– A COMMUNITY-BASED DEMONSTRATION STUDY**

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Objectives: The Viral Testing Alone for Screening Cervical Cancer in Routine Practice (VASCAR) study was designed to provide demonstration of the applicability of HPV DNA testing alone with cytology triage in a routine primary screening context. To our knowledge, it is the first real-world implementation of high risk HPV testing for primary screening in a community-based, public health care context.

Methods: All women aged 30 years and older presenting for cervical cancer screening are tested for the presence of HPV DNA with the HC2 assay (Qiagen). The recommended management for women with negative results is to repeat testing at 3-year intervals, whereas women with positive results undergo triage using conventional cytology. Among the latter, colposcopy is indicated in those with a cytology result of ASCUS or worse, whereas those with negative cytology smears repeat both tests in one year. VASCAR was implemented on March 1st 2011 to replace a policy of conventional cytology screening only, with colposcopy referral based on the cytology results. Detection rates for high grade lesions on biopsy, number of cytology tests averted, and colposcopy referral rates before and after the implementation of VASCAR were compared to assess the predictive value and the impact of the new policy. In addition, the frequency of women who were not managed according to VASCAR recommendations (i.e. protocol violations) was evaluated in this community-based context.

Conclusions: Since March 2011, 19,330 women aged 30 or above have been screened with HPV DNA testing, of whom 6.8% had positive results. Of the HPV positive women for whom cytology results were available, 32% had positive cytology triage, and a total of 21.5% of the latter had high grade lesions on biopsy. Data on the yield of lesion detection, visits averted, and colposcopy referrals will be presented by comparing the historical Pap cytology era with the implementation phase of VASCAR.

FC 9-4

HPV E6, E7 MRNA QUANTIFICATION (HPV ONCOTECT) SIGNIFICANTLY INCREASES SPECIFICITY WITHOUT LOSS OF SENSITIVITY FOR CIN 2+ AND CIN 3+ COMPARED TO HPV DNA: A PROSPECTIVE STUDY**Results from the Inter-institutional Study to Increase Specificity (ISIS) in Cervical Cancer Screening****G. Lallas¹, T. Zafiroopoulos¹, C. Kottaridi², A. Spathis², P. Karakitsos²***1 Flowcytogen Laboratories Ltd., 2 Cytology Laboratory of Attikon University Hospital of Athens, 3 IncellDx, Inc*

Objectives: The ideal assay for primary screening of cervical cancer is one that maintains high sensitivity with greatly improved specificity. To demonstrate whether increased specificity could be achieved without loss of sensitivity, we performed a prospective study comparing the performance of cellular quantification of HPV E6, E7 mRNA and cellular size (HPV OncoTect) to HPV DNA arrays (CLART[®] HPV 2) and HPV E6, E7 mRNA genotyping (Nuclisense Easy Q HPV).

Methods: To test the performance of our novel analysis strategy, we performed all three assays using manufacturer's recommended protocols on greater than 4000 liquid-based cervical cytology samples with 205 subsequent biopsy results.

Results: HPV DNA genotyping exhibited a sensitivity of 91% (95% CI, 82.0, 97.0) and HPV E6, E7 mRNA quantification yielded 90% (95% CI, 80.0, 96.0) for CIN 2+ and 91% (95% CI, 76.0, 98.0) and 94% (95% CI, 80.0, 99.0), respectively, for CIN 3+. HPV E6, E7 mRNA genotyping sensitivity of 87% (95% CI, 77.0, 94.0) for CIN 2+ and 84% (95% CI, 67.0, 95.0) for CIN 3+ was lower. HPV E6, E7 mRNA quantification 74% (95% CI, 65.0, 81.0) and HPV E6, E7 mRNA genotyping 76% (95% CI, 68.0, 83.0) both had statistically significant increases (McNemar's $p < 0.0001$) in specificity for CIN 2+ relative to HPV DNA 38% (95% CI, 30.0, 47.0) and in specificity for CIN 3+ 62% (54.0, 70.0) and 62% (54.0, 69.0) compared to 30% (23.0, 38.0), respectively

Conclusions: HPV OncoTect significantly increases specificity for high grade cervical disease without concomitant decreases in sensitivity compared to HPV DNA.

FC 9-5

START-HPV: FRENCH HPV-BASED PRIMARY SCREENING PILOT PROGRAM FOR CERVICAL CANCER**Dalstein V¹, Rousselot-Marche E², Charlier B², Botokeky J², Mereb E³, Graesslin O⁴, Jovenin N⁵, Birembaut P¹, Clavel C¹ and the START-HPV Study Group***1. CHU Reims, Laboratoire d'Histologie – Cytologie – Biologie Cellulaire, Reims, France**2. SAC08, Charleville, France 3. CHR Charleville, Gynecology Department, Charleville, France**4. CHU Reims, Gynecology Department, France 5. ONCOCHA, Regional Cancerology Network, Reims, France*

Background: New cervical screening algorithms need to be tested "in the real life" before implementation as national screening programs. The INCa (French National Institute of Cancer) is supporting the first French primary screening pilot study using solely HPV testing for 31-65 year-old women and cytology for 25-30 year-old women, referred to as START-HPV (STudy of primary screening in the ARdennes department by Testing for HPV infection).

Objectives: To determine in a general population: (i) the optimal management of women tested HPV positive; (ii) the compliance for an extended screening interval (3 - 5 yrs) between 2 negative HPV tests; (iii) the feasibility of a distinct screening strategy according to age.

Methods : 50,000 women aged 25-65 years living in the Ardennes Department, who did not get a Pap smear since more than 3 years, will be invited to participate in the screening program by mail. Non-responders will receive a second and third invitation. At the third invitation, an auto-sampling device will be proposed for HPV testing (DelphiScreener[®]). Women aged 25-30 yrs will be screened using cytological testing. Women aged 31-65 years will be screened using solely HPV testing (Hybrid Capture 2[®]) using STM[®] or DelphiScreener[®] devices.

Cytology positive women will be managed according to the French national guidelines. HPV+ women will be triaged by cytology and HPV genotyping will also be performed. Women with normal or negative tests will be recalled at 3 or 5 years, according to the primary screening test, cytology or HPV respectively. After one year of organization, this study begins in March 2012. First results will be presented.

Conclusion: The detailed methodology of the study will be presented. The START-HPV pilot study will provide a considerable amount of data concerning the organization, the management of HPV+ women and the education and feedback of physicians and population. These data will serve for the guidance of further implementation of large-scale HPV-based screening programs with cost/efficacy analysis.

TWO YEARS OF ROUTINE PRIMARY HIGH-RISK HPV SCREENING FOR CERVICAL CANCER IN ABRUZZO (ITALY): RESULTS AND COST-EFFECTIVENESS ANALYSIS

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Objectives: To determine whether HR-HPV DNA testing is superior to liquid based and conventional cytology to identify high-grade cervical intraepithelial neoplasia (CIN2+) in women ages 25 to 64 years in Abruzzo, Italy, and to perform cost-effectiveness of primary HPV screening method.

Methods: In 2010-2011, 50,728 women 25-64 yrs have performed HPV testing, following by PapTest for positive one. We compared primary HPV screening test(a) and ARINT study (2006-2009) results: 111,415 Pap tests were performed (47.3% Liquid-Based Cytology & ThinPrep Imaging System(b) and 52.7% Conventional Cytology & FocalPoint System(c)).

Women with abnormal Pap test results (ASC+) underwent colposcopy and biopsy if clinically indicated. Costs were calculated by Standard Cost method considering a same number of women examined per year and per screening test.

Conclusions: HPV Test+ were 5,189 (10.2%) and following Pap Test+ were 2,290 (4.5%). This positive rate is comparable with Pap Test only (4.7% ARINT). The effectiveness of cytological diagnosis of HPV testing in 2 years of 1st round is marked by: 1) lower uncertain diagnosis (0.17% vs 2.65% ASC-US & AGC); 2) increase HSIL+ (0.59% vs 0.43%); 3) increase CIN 2+ (0.53% vs 0.40%); 4) 5.6% recall rate (1 year) HPV+ Pap- cases (partial data) with 0.8% HPV positivity rate, 0.3% Pap Test positivity rate and 0.01% CIN2+ detected.

With a three-year round the HPV Test determines a cost of nearly Euro 5.54 million against 5.11 million of Pap tests. Considering a broader round for HPV testing (4-7 years) this screening is more convenient than a 3-years Pap test (from Euro 7.39 million to 12.93).

Indicators are more cost-effective already with a 3-years round of HPV test: Euro 6,155 vs 7,815 for HSIL+ and Euro 6,839 vs 8,393 for CIN 2+ lesion detected.

Obviously the results must be revalued in the following rounds of screening.

THE STUDY OF 503 CASES OF CERVICAL CYTOLOGY INTERPRETED AS ATYPICAL SQUAMOUS CELLS WHICH CANNOT EXCLUDE HIGH GRADE SQUAMOUS INTRAEPITHELIAL LESION (ASC-H)

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Objective: To explore the risk of CIN II or greater in patients with cytology interpreted as ASC-H

Method: Patients with ASC-H accepted HPV test, colposcopy-directed biopsy, and endocervical curettage.

Surepath was used for cytologic diagnosis.

HC II was applied to detect HPV DNA.

Results: 503 patients accepted HPV testing, colposcopy-directed biopsy, and endocervical curettage.

1. The average age was 42.2 years old.

2. 37.3%, 188/503, patients were diagnosed CIN II or greater, including 78 cases of CIN II, 103 cases of CIN III, 6 cases of cervical squamous carcinoma, 1 case of cervical adenocarcinoma.

There is no cancer be found in women younger than 40years old.

3. 75.5%, 379/503, patients were HPV positive

4. 48.5%, 184/379, were diagnosed CIN II or greater in HPV positive group and 3.2% (4/124) in HPV negative.

5. We divided the patients in 5 groups by age. In patients younger than 30 years old, 30-39 years old, 40-49 years old, 50-59 years old and ≥60 years, the HPV positive rate was 83.6%, 83.4%, 70.3%, 71.4%, 60.5%, respectively. Women who were 60 or older had low HPV positive rate than other groups and the difference was statistic significant.

The risk of CIN2 or greater in the five group were 37.3%, 56.6%, 44.4%, 31.7%, 38.5%, respectively.

Conclusions: 1.37.3% patients with cervical cytology interpreted as ASC-H were diagnosed CIN II or greater and 1.4% were invasive cervical cancer.

2. HPV positive rate was decreased when the patient was older.

3. The negative prospective value of HPV DNA in this study were 96.8% (120/124).

4. HPV DNA test was a helpful tool for cervical cancer screen especially for older than 60.

FC 9-8

ASSESSING THE PERFORMANCE OF CERVISTA HPV HR TEST IN A ROUTINE CERVICAL SCREENING POPULATION.**Keegan H**^{1,2}, **Pilkington L**^{1,2}, **O' Brien R**², **Jamison J**³, **Wilson RT**³, **Carson J**³, **Martin CM**^{1,2}, **O'Leary JJ**^{1,2}¹ Department of Histopathology, School of Medicine, University of Dublin, Trinity College, Dublin 2, Ireland.² Department of Pathology, Coombe Women and Infants University Hospital, Dublin 8, Ireland.³ Department of Cytopathology and Molecular Pathology, Antrim Area Hospital, Antrim, Northern Ireland.

Objectives: Cervical screening programmes worldwide are moving towards HPV DNA testing as part of the population screening process. In this study, we evaluated the CERVISTA HR HPV test (Hologic Ltd.) on a subset of a routine cervical screening population which had the following cytological breakdown: Normal (426/540; 78.8%), BNA (12.4%; 67/540), Mild (6.3%; 34/540), Moderate (1.5%; 8/540), Severe (0.9%; 5/540).

Methods: Cervical cytology specimens from 540 women aged between 16 and 74 (average age 37.7 years) were recruited and HPV tested. HPV was tested by Hybrid Capture (hc2, Qiagen Ltd.) and by Cervista for the same 13 high-risk types as the hc2 test with the addition of HPV66. A subset of HPV-discordant specimens was chosen for HPV genotyping by Linear Array HPV genotyping test (Roche Ltd).

Results: Overall, the prevalence of HPV in this cohort was 26.3% by hc2 and 31.5% by Cervista. The concordance rate of the two tests was 85.9% (Cohens Kappa = 0.68). A discordant result is a positive result by one assay and negative by the other test. In the mild or greater abnormalities category the percentage of HPV positive cases was 85.1% by both tests.

Conclusions: There was a good rate of concordance between the hc2 assay and Cervista. In the mild or greater abnormal cytology categories, the hc2 and Cervista performed similarly, however Cervista detected more positive specimens in the normal cohort.

FC 9-9

COMPARISON OF HPV DNA TESTING USING COBAS® 4800 AND PAP TESTING AS A METHOD OF PRIMARY SCREENING FOR CERVICAL CANCER. A MULTICENTER STUDY IN GREECE.**Agorastos T**¹, **Katsamagkas T**¹, **Chatzistamatiou K**¹, **Theodoridis T**¹, **Boni E**², **Skenteri A**², **Papanicolaou A**³, **Tarlatzis B**³, **Sevastiadou P**⁴, **Athanasίου E**⁴, **Destouni H**⁵, **Tsarouchas D**⁵, **Kaplanis K**⁶, **Daponte A**⁷, **Nepka H**⁸, **Constantinides T**⁹, **Constantinides TC**⁹- Aristotle University of Thessaloniki, "Ippokrateio" General University Hospital, Thessaloniki, Greece, ¹ Department of Obstetrics and Gynecology, ² Department of Cytology, - Aristotle University of Thessaloniki, "Papageorgiou" General University Hospital, Thessaloniki, Greece, ³ Department of Obstetrics and Gynecology, ⁴ Department of Cytology, - "Theageio" Cancer Hospital, Thessaloniki, Greece, ⁵ Department of Cytology, ⁶ Department of Gynecologic Oncology,- University of Thessaly, General University Hospital, Larisa, Greece, ⁷ Department of Obstetrics and Gynecology ⁸ Department of Cytology, - ⁹ "Dimokritio" University of Thrace, Alexandroupoli, Greece, Department of Hygiene and environmental protection.

Objectives: The comparison of Pap testing and HPV DNA testing as a method of primary screening for cervical cancer, as well as the assessment of the effectiveness and accuracy of the COBAS® 4800 System, Roche, as a primary screening method for cervical cancer prevention.

Methods: A multicenter Greek study on non-pregnant women, 25 to 55 years old, who visit the outpatient clinics of the Obstetric and Gynecologic Departments and the Centers of Preventive Medicine in Greece's largest cities in order to have a Pap test for screening purposes. The Pap test is performed using the ThinPrep® liquid cytology method. After the cytologic evaluation the material collected is used to detect the DNA of 14 oncogenic types of the Human Papilloma Virus [HPV 16, HPV 18, and 12 other high risk HPV types: 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68]. The HPV DNA testing is performed using the COBAS® 4800 system, ROCHE.

The objective of the screening is to detect lesions \geq CIN2 (CIN2+). For that reason, women found positive, either at Pap test (\geq ASCUS) or at HPV DNA test are subjected to colposcopic evaluation. In case of abnormal colposcopic findings, multiple biopsies are performed from the abnormal cervical epithelium and women are managed properly according to the histopathological findings. If the colposcopic evaluation proves normal, women are subjected again to HPV DNA test and Pap test in one year. At that time, if the HPV DNA test and the cytologic evaluation are negative, women are reevaluated in three years. On the other hand if one of these tests is positive, colposcopy is performed as well as biopsy, if needed, as mentioned before.

Conclusions: Up to now we have collected 1003 samples. A percentage of 4.7% of the examined samples was found to have abnormal cytologic findings (\geq ASCUS) and 9.7% was hrHPV(+). There was, till now, no high grade lesion (CIN2+) found after the colposcopic and histopathological evaluation. Different algorithms will be formed which will use HPV DNA testing and/or HPV 16/18 detection and/or Pap test according to the analyses of the study's results. Each approach, for cervical cancer screening, formed that way will be assessed in terms of cost and effectiveness.

HOW MANY CASES OF CERVICAL CANCER AMONG YOUNG WOMEN COULD HAVE BEEN PREVENTED WITH A MORE SENSITIVE SCREENING TEST?

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Objective: Despite the well performing cervical cancer screening programme, still approximately 100 women under the age of 35 are diagnosed with cervical cancer in the Netherlands annually. Some of these cases were missed by the cytology based screening programme and may be identified if we use the more sensitive primary HPV DNA test. On the other hand, since (transient) HPV infections are more prevalent in young women, the lack of specificity of the HPV test is potentially problematic at younger ages. The use of HPV DNA testing below the age of 35 is therefore not obvious. We estimated the potential gain in prevented cervical cancer incidence, by the use of HPV DNA testing at age 30 instead of cytology testing.

Methods: We analysed the screening history of women aged 29-34 years diagnosed with cervical cancer in the period 2006-2010 from the nationwide registry of histo- and cytopathology (PALGA), which contains all information on cervix uteri examinations in the Netherlands.

Conclusions: Of the 346 women aged 29-34 years diagnosed with cervical cancer in the period 2006-2010, 134 (39%) had a previous primary smear. In 99 (29%) cases, this previous smear was performed less than 5 years before cervical cancer was diagnosed. Of these 99 smears performed at most 5 years before the cancer diagnoses, 94 were classified as 'normal'. This means that 27% of the cervical cancer cases diagnosed between ages 29 and 34 were newly developed cancers within 5 years or missed by previously performed cytology. These cases could possibly have been prevented using the more sensitive HPV test. However, it is likely that not all of these missed cases would have been found using the HPV test, due to the small percentage lack of sensitivity of the HPV test. Also, the number of false positive primary HPV tests needs to be included when considering to implement primary HPV screening under age 35.

BENEFIT OF HPV-BASED PRIMARY CERVICAL CANCER SCREENING

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Objectives: The objective of this systematic benefit assessment produced by the German Institute for Quality and Efficiency in Health Care (IQWiG) was to evaluate the benefit and harms of HPV testing alone or in combination with cytology compared with cytology alone in primary cervical cancer screening.

Methods: A systematic literature search was conducted in MEDLINE, EMBASE and the Cochrane Central Register of Controlled Trials until 01/07/2011 for randomized controlled trials (RCTs) investigating HPV-based primary cervical cancer screening strategies compared with cytology alone. The following patient-relevant outcomes were analysed: overall survival, cause-specific mortality, incidence of invasive cervical cancer, CIN3/CIS, CIN3+, screening-related harm, and quality of life. All methods for study selection, risk-of-bias assessment, data extraction, and aggregation were based on standardized methods published online in the IQWiG methods paper. Results of meta-analyses are presented as relative risks (RR) with 95% confidence intervals (CI).

Conclusions: Six RCTs involving a total of 235,613 randomized women were included in the systematic review. The studies varied in several aspects, such as screening and work-up strategies, age of included women, and follow-up time. Four RCTs presented data for a second screening round. Overall, the data showed a reduction in invasive cervical cancer incidence in those women who underwent an HPV-based screening strategy in the first screening-round compared with those women screened with cytology alone (RR: 0.22; CI: 0.08 to 0.67; $p = 0.007$). The number needed to screen to prevent one additional case of cervical cancer was 4800 (2700; 20,800) given a screening interval of 3 to 5 years. Valid data on cancer mortality, quality of life and screening-related harm were lacking. In conclusion, these results provide supporting arguments for a benefit of HPV-based primary cervical cancer screening. However, potential harms could not be evaluated due to a lack of data. No recommendation for a specific screening and work-up strategy can be made because of the high variability of the algorithms applied in the studies. (1 <https://www.iqwig.de/methods-procedures.926.en.html>)

FC 9-12

EVALUATION OF A NEW CERVICAL CANCER SCREENING PROTOCOL WITH PAP AND HPV

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Objective: : To compare cervical cancer screening efficiency between using conventional PAP smears (PAP) and high-risk HPV-DNA testing (HPV) with the use of HPV only in abnormal PAP.

Method: From a population of 50065 women in a selected area (Barbastro), 26936 from 25 to 65 years old were enrolled between 1st January 2010 (72, 8%) and 31th December 2011(80.9%). The screening is carried out mainly in Primary Care and in 2010 was applied the Spanish Gynecologist Guidelines published in 2006 (PAP negative release 3 years); only the 5 years interval was set in a pilot centre with PAP and HPV testing. During 2011, new 2010 Spanish Gynaecologists guidelines were followed (women 21 to 29y PAP every 3 years and ≥30 years PAP and HPV every 5 years).

Hybrid-Capture HC2 and cobas HPV Test Testing technology were used. Our Pathology Laboratory is PAP accredited according to ISO 15189 and PAP and HPV results are recorded independently. After the PAP diagnosis the concordance has been reviewed with the histological results as the ≥CIN2. Previous PAP was reviewed indeed.

In 2010, 4770 PAP were performed and 5235 PAP in 2011, representing a coverage of 36.80% to 50.99% respectively.

| | 2010 | | 2011 | | Pilot centre | |
|---------------------------------|-----------|--------------|-----------|--------------|--------------|-----|
| | Barbastro | Pilot centre | Barbastro | Pilot centre | | |
| Abnormal PAPs: | N | % | N | % | N | |
| •ASC-US | 68 | 1.43 | 13 | 104 | 1.98 | 8 |
| •ASC-H | 6 | 0.13 | 1 | 9 | 0.17 | 0 |
| •LSIL | 54 | 1.13 | 6 | 99 | 1.89 | 9 |
| •HSIL | 21 | 0.44 | 0 | 35 | 0.67 | 6 |
| •Carcinoma | 0 | | | 1 | 0.02 | |
| •ASC/SIL | 0.99 | | | 0.86 | | |
| Positive Predictive Value (VPP) | | 95 | | | 90 | |
| Sensitivity | | 70.83 | | | 78.94 | |
| HPV | 784 | | 337 | 3560 | | 452 |
| •HPV+ | 83 | 10.58 | 19 | 267 | 7.5 | 37 |
| •HPV+ with normal PAP | 40 | 5.1 | | 147 | 4.12 | |
| New CIN 2-3 cases | 18 | | | 36 | | |
| Invasive Carcinoma | 3 | | | 2 | | |

| New CIN 2-3 | 2010 | 2011 |
|------------------------------------|------|------|
| •Follow-up of ASC-US and HPV + | 2 | 2 |
| •Follow-up of HPV+ with normal PAP | 1 | 1 |
| •Revision of HPV + and normal PAP | 0 | 1 |

The introduction of screening using PAP and HPV in 2011 has allowed the improvement of the detection in a 100% of CIN2/3 without a significance increase of PAP (9.7%) and without appreciable variation in invasive cancers. 6/36 cervical cancer cases were detected by the 2nd year in the pilot centre.

Conclusions: New CIN 2/3 cases were found in women with ASC-US and normal PAP when HPV test was positive, this is directly related with the use of HPV testing during 2nd year working with both methods and keeps expecting an increase over the next years.

The increase in coverage is another important factor that contributes to detect pre-cervical disease.

FC 10-1

MOLECULAR BIOLOGY OF HPV-ASSOCIATED HNSCC

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HPV-induced carcinogenesis has been unveiled due to extensive research in the most widely accepted HPV-related malignancy, cervical cancer. HPV-associated cancers continuously express the HPV viral oncogenes even in advanced stages, and repression of viral oncogene expression can prevent the growth or survival of HPV+ cancer cells. This finding raises the possibility that even late-stage HPV-associated cancers can be cured by HPV-targeted approaches, such as medicines that interfere with the expression or function of viral oncoproteins and therapeutic vaccines that elicit a cytolytic immune response to cells expressing these oncoproteins. This lecture summarizes the main events of HPV-induced carcinogenesis with an emphasis on the implications of these carcinogenic mechanisms on research, treatment and prevention of HPV-associated OPC.

INCIDENCE OF HPV INDUCED OROPHARYNGEAL CANCER IN SPAIN AND ITS PROGNOSTIC SIGNIFICANCE

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Objectives: To analyze the incidence of oropharyngeal squamous-cell carcinomas induced by human papillomavirus (HPV), and to determine the prognostic significance of HPV status among patients with oropharyngeal cancer treated with concurrent chemoradiotherapy.

Methods: We performed a retrospective analysis on 102 patients with stage III-IV oropharyngeal cancer, treated with curative chemoradiotherapy in four university hospitals between 2000 and 2008. Immunohistochemical (IHC) expression of p16 was analyzed in matched pretreatment paraffin-embedded tumour blocks from these patients. Tumours were classified as p16 positive in case of a confluent nuclear and cytoplasmatic staining pattern, and p16 negative in case of absence of staining. Quantitative real-time polymerase chain reaction (qRT-PCR) assay for HPV16 mRNA was also performed. The influence of p16 status on disease-specific survival, and overall survival after treatment was evaluated.

Conclusions: p16 positivity by IHC was found in 27 tumours (26.7%). HPV16 E6 mRNA was positive in 10 tumours (10.5%). RNA integrity was very low, due to high RNA degradation in the deparaffinization process. All positive E6 mRNA patients were also positive by p16 IHC. No statistical significant differences were observed between the p16 positive and p16 negative groups regarding tumour stage, gender, age or tobacco use. Overall survival was improved for p16 positive tumours compared to p16 negative, with 5-year actuarial values of 74% versus 52% ($p=0.053$). Disease-free survival was also improved for HPV-positive patients, although not significantly (62.9% vs 48.3%, $p=0.2$, at 5 years).

The incidence of HPV-related oropharyngeal carcinomas in Spain is similar to that reported in other European countries, and apparently lower than the incidence in North America. HPV-positivity by p16 IHC was associated with improved overall survival in patients treated with chemoradiation.

HLA CLASS I EXPRESSION AND TUMOR INFILTRATING CD8+ AND FOXP3+ T-CELLS IN HPV +/- TONSILLAR CANCER IN RELATION TO DISEASE FREE SURVIVAL

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Objectives: To investigate if HLA class I expression or number of tumour infiltrating CD8+ T-cells and FoxP3+ regulatory T-cells in tonsillar squamous cell carcinomas (TSCC) can be correlated to the presence of high risk HPV and/or patients disease free survival.

HPV is a major causative factor for TSCC and patients with HPV positive (HPV+) TSCC have an improved 5-year survival after treatment as compared to patients with HPV negative (HPV-) TSCC. There is thus a need to find biomarkers that can be used in combination with HPV status to predict patients response to therapy.

Methods: Formalin fixed paraffin embedded TSCC from 83 patients, formerly analyzed for the presence of HPV DNA, were divided into four groups depending on HPV status and the clinical outcome of the patients. Tumours were stained by immunohistochemistry and evaluated for expression of HLA class I antigens, the number of tumour infiltrating cytotoxic (CD8+) and regulatory (FoxP3+) T-cells. The result obtained with regard to HLA class I antigens and tumor infiltrating lymphocytes (TIL) was correlated to disease free survival.

Conclusions: For HPV+ TSCC a low expression of HLA-A,B,C while for HPV- TSCC, a normal expression of HLA-A,B,C was significantly correlated to a disease free survival. These correlations were more pronounced for membrane staining of HLA-A,B,C as compared to cytoplasmatic staining. For HPV+ TSCC, a high CD8+ T-cell infiltration was significantly positively correlated to disease free survival. Similarly, a high CD8+/Foxp3+ TIL ratio was correlated to a disease free survival for HPV- TSCC. Furthermore, HPV+ TSCC had in comparison to HPV- TSCC, higher numbers of infiltrating CD8+ and Foxp3+ T-cells.

This results indicates that both HLA-A,B,C expression and tumor infiltrating CD8+ cells may potentially serve as a biomarker for response to treatment for HPV+ TSCC patients. Likewise, for HPV- TSCC both HLA-A,B,C expression and the CD8+/Foxp3+ cell ratio can potentially be used for the same purpose.

FC 10-4

IS HPV16 DETECTION IN CERVICAL LYMPH NODES OF OROPHARYNGEAL CANCER A MARKER OF METASTASES?

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Objectives: Oropharyngeal squamous cell carcinomas (OSCC) are associated with high grade Human papillomavirus (HPV) infection in 20 to 30% of cases. HPV16 DNA has been detected in cervical lymph node metastases of HPV16-positive OSCC. However the meaning of HPV16 DNA detection in lymph nodes remains controversial. Does the presence of HPV16 in lymph nodes correlate with their metastatic involvement or is it just a consequence of lymph nodes filter function?

Methods: Viral load quantification using RT-PCR was retrospectively performed in primary tumours and in cervical lymph nodes, originating from level IIa, IIb and III, in 11 patients affected with HPV16-positive OSCC and in 3 control patients affected with HPV16-negative OSCC.

Conclusion: A total of 45 lymph node levels were analysed. HPV16 DNA was not detected in HPV16-negative OSCC lymph nodes. No statistically significant difference was found between primary tumors and metastatic lymph nodes viral load ($p > 0.01$). Viral load was significantly higher in metastatic lymph nodes than in tumour free lymph nodes ($p < 0.01$). Among 27 tumour free lymph node levels, the viral load was undetectable in 16, low or medium ($< 10^5$ copies per million cells) in 8 and high ($> 10^5$ copies per million cells) in 3. HPV16 DNA detection in lymph nodes of patients affected with HPV16-positive oropharyngeal cancer is in favor of metastatic involvement. Tumor free lymph nodes with a high viral load value would suggest the presence of occult lymph nodes metastasis and the opportunity to use HPV16 DNA as a metastatic marker. Further investigations are needed.

FC 10-5

DEMOGRAPHIC DIFFERENCES IN THE PREVALENCE OF ORAL HPV IN U.S. ADULTS; CROSS SECTIONAL ANALYSIS OF NHANES 2009-2010

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Objectives: Oral Human Papillomavirus (HPV) 16 is the most common HPV type found in HPV positive oropharyngeal cancer¹. We assessed the prevalence of HPV high (HR) and low risk (LR) types and their associations with ethnicity, country of birth and selected factors among US adults.

Methods: We analyzed data on 4847 men and women aged 18 to 69 years from the US National Health and Nutrition Examination Survey 2009-2010. Oral rinse specimens were analyzed for 37 HPV DNA types using a HPV probe array (Roche) and classified as HR and LR types². HPV prevalence was analyzed by selected demographic and sex behavior variables; multivariable logistic and multinomial regression were used to obtain odds ratios with 95% confidence intervals using sample weights to account for the complex sampling method.

Conclusions: The prevalence of oral HPV was 7.3% (95% CI 5.9, 8.7); 3.64% (95% CI 2.9, 4.4) for LR and 3.68% (95% CI 2.7, 4.6) for HR types. HPV16 was the most prevalent type (1.06%, 95% CI 0.73, 1.38), accounted for 28.7% of HR types, and its prevalence was higher in men (30.2%) than in women (22.6%) ($p > 0.05$). Overall HPV prevalence was higher in men than in women (11% vs. 3.7%) ($p < 0.05$) and the HR:LR HPV ratio was 0.6 in women and 1.2 in men. HPV prevalence was higher among Blacks than among Whites and Mexican Americans (MA) (11.3%, 6.9%, and 6.5%, respectively); HR HPV prevalence was similar in Blacks and Whites (4.4% vs. 4.0%), but lower in MA (2.3%). However, Whites had higher prevalence of HPV16 than Blacks and MA (1.3% vs. 0.8% and 0.3%, respectively). MA in the US had similar HR HPV prevalence as those born in Mexico (7% vs. 5.9%). Consistent with previous findings³, HPV55 was higher among Mexicans born in Mexico than born in the US (3.1% vs. 0.1%). Multivariable logistic regression showed associations between any type of HPV with Black race (vs. MA), increasing age, male sex, and smoking. Sexual orientation (heterosexual or not), oral sex, and income were not significantly associated. A multinomial logistic regression model showed associations of HR HPV with Black ethnicity and being male. Ethnic differences exist in the prevalence of HR and LR HPV as well as specific HPV types among US adults.

HPV STATUS ALTERS THE MIRNA EXPRESSION IN TONSILLAR CANCER

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Objectives: All over the western world oropharyngeal cancer (OC) is rising epidemically presumably in part due to human papillomavirus (HPV). HPV-positive (HPV+) OC appears to be distinct from HPV-negative (HPV-) OC with regard to a number of different clinical, molecular and pathologic characteristics. The role of HPV in OC is still not clear though. microRNAs (miRNAs) are post-transcriptional regulators of genes involved in cancer development why miRNA profiles are promising new diagnostic tools and disease specific miRNAs are potential new biomarkers and future therapeutic targets. We explore the miRNA expression in HPV+ and HPV- tonsillar cancer (TC) and in normal control tonsils to contribute to the molecular understanding of HPV+ and HPV- TCs.

Methods: Thirtyone formalin fixed, paraffin embedded TCs and 13 control tonsils collected during the period 2008-2010 were included after confirmation of diagnosis by pathologist. We extracted DNA and subjected it to HPV PCR with general - and HPV16 specific primers and tissue sections were stained for p16. HPV+ TCs were defined as specimens with positive p16 staining and positive HPV PCR. Ten HPV+ TCs, 10 HPV- TCs and 10 control tonsils were laser microdissected, RNA was subjected to miRNA expression profiling and array results were validated by qRT-PCR. The data were analysed by Student's t-test.

Conclusion: miRNA profiles can clearly distinguish between and characterize HPV+ TCs, HPV- TCs and normal tonsils. We found 116 differentially expressed miRNAs in HPV+ TCs compared to controls and 111 differentially expressed miRNAs in HPV- TCs compared to controls - thirtysix miRNAs were differentially expressed in HPV+ - compared to HPV- TCs. The most up regulated, specific miRNA in HPV+ TCs was miR-363 with a foldchange of 10.

COMPARATIVE MIRNA PROFILING IN HUMAN PAPILLOMA VIRUS RELATED HEAD & NECK AND CERVICAL CANCER

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Objectives: While the role of HPV in cervical cancer (CSCC) is well established, the role in head and neck cancer (HNSCC) is less clear. MiRNAs play a role in cancer development and HPV status may affect the miRNA expression pattern in HNSCC. The aim of this study is to compare miRNA profiles in HPV+HNSCC and HPV-HNSCC with those of HPV+CSCC to explore the distinct oncogenic role of HPV. By making a link to CSCC, we aim to explore the common virus induced molecular changes in the cell that leads to the malignant state.

Methods: Total RNA was extracted from fresh frozen and laser micro-dissected paraffin embedded tissue samples obtained from patients with HPV+/HPV- HNSCC, CSCC and controls. Microarray analysis were performed on all samples and differentially expressed miRNAs in the HPV+ and HPV- HNSCC samples compared to controls and matched up to the differentially expressed miRNAs in the CSCC samples.

Conclusion: The miRNA profile showed significantly more similarity between HPV+ HNSCC and CSCC than HPV- and CSCC. A set of HPV core miRNAs were identified. Of these especially the miR-15a/miR-16/miR195/miR-497 family, miR-143/miR-145 and the miR-106-363 cluster appear to be important within the known HPV pathogenesis. The study adds new knowledge to the known pathogenic pathways of HPV and substantiates the oncogenic role of HPV in subsets of HNSCCs.

FC 10-8

VALIDATION OF A NOVEL DIAGNOSTIC STANDARD IN HPV POSITIVE OROPHARYNGEAL SQUAMOUS CELL CARCINOMA.**Schache A^{1,2}, Liloglou T¹, Risk J¹, Jones T^{1,2}, Ma X-J³, Wang H³, Bui S³, Luo Y³, Sloan P⁴, Shaw R^{1,2}, Robinson M⁴.**

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Objectives: HPV testing is now widely advocated in the work up of oropharyngeal squamous cell carcinoma (OPSCC). The 'gold standard' for oncogenic HPV detection is the demonstration of transcriptionally active High Risk HPV in fresh tumour tissue. For clinical utility, HPV testing strategies have necessarily focused on formalin-fixed paraffin-embedded (FFPE) tissue, but this has been at the expense of reduced sensitivity and specificity for oncogenic HPV infection.

This study compares the performance of a novel High Risk HPV RNA in situ hybridisation test on FFPE tissues, against the reference test, quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) for High Risk HPV mRNA, performed on matched fresh frozen tissue samples.

Methods: A tissue-microarray comprising FFPE cores from 79 OPSCC was tested using High Risk HPV RNAscope (Advanced Cell Diagnostics, USA), which detects HPV-16, -18, -31, -33, -35, -52, and -58. Analytical accuracy and capacity for prognostic discrimination was determined by comparison with qRT-PCR for HPV-16, -18 and -33. Demographic and tumour parameters were compared by Chi-square and Kruskal-Wallis tests. Test sensitivity and specificity were calculated against the reference test. Kaplan-Meier survival estimates were constructed to assess prognostication.

Conclusions: We demonstrate that High Risk HPV RNAscope can be used to detect oncogenic HPV in FFPE OPSCC samples and that it has both excellent analytical performance (sensitivity and specificity of 97% and 93% respectively) and prognostic performance against the 'gold standard' test for oncogenic HPV (Kaplan-Meier estimates of disease specific survival, $p=0.002$ and overall survival, $p<0.001$).

The diagnostic capacity of High Risk RNAscope was noted to be superior to the frequently advocated HPV diagnostic tests, p16 immunohistochemistry (IHC) and the combination of p16 IHC and High Risk HPV DNA qPCR.

These results raise the possibility that High Risk HPV RNAscope could be adopted as the standard test for OPSCC in clinical practice. As the oncology community approaches therapeutic de-escalation based on HPV status, such a reliable and efficacious test may have immediate application.

FC 10-9

HPV DETECTION BY PCR IN SUSPECTED TONSILLAR LESIONS: SMEAR-BIOPSY AGREEMENT**Vairel B¹, Alshehri S¹, Delord JP², Courtade-Saidi M³, Vergez S¹, Woisard-Bassol V¹.**

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Objectives: Our main objective is to evaluate the correlation between biopsy and smear in the detection of HPV by PCR in the suspicious lesions for tonsillar malignancy.

Our second objective is to compare the quality of the smear of suspected tonsillar lesions to the quality of the smear for the healthy contralateral tonsil depending on cellularity, inflammation and necrosis.

Methods: Bicentric prospective study conducted from June 2009 to August 2011.

Smears and biopsies were taken from the same area for patients with suspected tonsillar lesion. HPV detection by PCR was performed on both the smear and the biopsy.

The primary target was to find a matching between sampling methods (smear and biopsy) estimated by Cohen's Kappa with its confidence interval at 95%.

A second smear was performed on the healthy contralateral tonsil.

A McNemar's test was used to compare the smears' quality of the sampled area (healthy or pathological) according to the cellularity, necrosis and inflammation.

24 patients were enrolled in our study of which 20 had squamous cell carcinoma (SCC) of the tonsil. High risk HPV was detected in 25% of cases with SCC of the tonsil.

The Cohen's Kappa coefficient was equal to 0.881 (0.654 – 1,000) which an almost perfect matching between the two sampling methods (biopsy and smear).

There was no significant difference between the smear of the suspected tonsil comparing to the healthy contralateral tonsil in terms of cellularity ($p=1.000$) and inflammation ($p=0,250$) In contrast, necrosis was significantly greater ($p=0.001$) in pathological tonsils.

Conclusion: Having a non-invasive, valid method for the detection of oropharyngeal HPV is essential. We believe that detection of HPV in a smear by PCR is a promising tool especially in monitoring the oropharyngeal cancers and a particular interest in screening of HPV in general population.

ORAL MUCOSA EXFOLIATED CELLS FOR HPV TYPING BY SPF10 IN HEAD AND NECK CANCER

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Objective: The presence of HPV in the superficial cells of the oral mucosa could reflect the HPV status of head and neck cancer. Discordant literature data exist on the agreement between HPV status in non-neoplastic oral mucosa and in tumor tissue from the same patient, mostly due to the use of heterogeneous analytical methods.

Methods: We compared HPV status and viral typing in paired cytological and biopsy samples from 56 patients with head and neck neoplastic and preneoplastic lesions using the highly sensitive SPF10 LiPA assay, which has recently been validated for formalin fixed paraffin embedded tissue using paired cervical cytology and biopsy samples. Kappa statistics were used to measure interrater agreement.

Conclusions: The overall agreement with respect to HPV status was 94,64%. For 80,34% of subjects ($\kappa = 0,7435$), the same numbers of HPV types were detected in cytological and biopsy specimens. The overall positive typing agreement was 91,1% (95% CI, 85,4 to 94,7), for 134 out of 147 individual HPV type analyses. Agreement was moderate for HPV -44 ($\kappa = 0.4682$), good for HPV-18, -45, -54 and -66 ($\kappa = 0.6585$ to 0.7910), excellent for HPV-6, -40, and -52 ($\kappa = 0.8250$ to 0.8993), and absolute for HPV-11, -16, -31, -33, -35, -39, -51, -53, -59, -74, and -69-71 ($\kappa = 1.0000$). The high sensitivity of SPF10 LiPA and its excellent performance both in recognizing HPV infection and in identifying the viral types present in tumor tissue and in oral exfoliated cells makes it a useful tool in the assessment of HPV status of head and neck cancer patients. The excellent agreement for HPV status and genotyping in paired samples suggests that oral exfoliated cells can be used for HPV detection in head and neck region in clinical settings.

HUMAN PAPILLOMAVIRUS INFECTION AND P16INK4A/KI-67 CO-EXPRESSION IN THE HEAD AND NECK SQUAMOUS EPITHELIUM

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Objectives: A proportion of head and neck squamous cell carcinomas (HNSCC) is caused by the human papillomavirus (HPV) and the tumor suppressor p16^{INK4a} is regularly overexpressed in these lesions. At the same time, little is known about the role of HPV in the stages that precede the invasive state in the head and neck and on the general p16^{INK4a} expression pattern in this tissue. The aim of our study was to gain insight into the relevance of HPV infection and the meaning of p16^{INK4a} in non-invasive stages by determining the presence of HPV DNA and p16^{INK4a} in normal, premalignant and malignant head and neck tissue. We further analyzed the proliferative potential in p16^{INK4a} overexpressing areas by simultaneously studying the expression pattern of the proliferation marker Ki-67.

Methods: The co-expression profile of p16^{INK4a}/Ki-67 in normal (n=50), premalignant (n=34) and malignant (HNSCC; n=147) head and neck tissue was studied using p16^{INK4a}/Ki-67 immunohistochemistry. 14 HR-HPV genotypes were detected by Luminex technology.

Conclusions: 20/147 (13.6%) HNSCC were HPV-positive. A strong correlation between HPV DNA presence and diffuse p16^{INK4a} expression (with simultaneous Ki-67 co-expression) was observed ($p < 0.001$), confirming the involvement of HPV in a proportion of HNSCC. The diffusely p16^{INK4a} expressing cells lost cell cycle control as they co-express Ki-67. 4/50 (8.0%) normal tonsils were HPV-positive, but negative for diffuse p16^{INK4a} or p16^{INK4a}/Ki-67 co-expression, thus indicating no HPV oncogene expression. 14/34 (41.2%) premalignant lesions were HPV-positive, but HPV DNA presence did not correlate with diffuse p16^{INK4a} expression. Irrespective of HPV DNA presence, all diffusely p16^{INK4a}-expressing premalignant lesions displayed p16^{INK4a}/Ki-67 co-expression. This finding does currently not allow a conclusion regarding HPV oncogene expression in the premalignant lesions.

FC 10-12

HPV/P16^{INK4A} STATUS IN SQUAMOUS CELL CARCINOMAS OF THE ORAL CAVITY**Reuschenbach M¹, Thiele O², Kansy K², Hoffmann J², von Knebel Doeberitz M¹, Freier K²**1) *Department of Applied Tumor Biology, Institute of Pathology, University of Heidelberg, Germany*2) *Department of Oral and Maxillofacial Surgery, University of Heidelberg, Germany*

Objectives: While the prevalence and prognostic relevance of HPV for oropharyngeal cancers becomes more and more clear, data for squamous cell cancers (SCC) located in the oral cavity are inconclusive. We here retrospectively investigated the prevalence of HPV and p16^{INK4a} overexpression and the prognostic impact of the HPV/p16^{INK4a} status in oral SCC.

Methods: A total of 299 patients with oral SCC treated at the University of Heidelberg and Tübingen, Germany were included. Tumor tissue was analyzed for HPV-DNA by GP5+6+ PCR and p16^{INK4a}/Ki-67 expression by immunohistochemistry.

Conclusions: HPV-DNA could be detected in 24.7% (74/299) of the tumors. Diffuse p16^{INK4a} overexpression was detected in 5.7% (17/299) and focal p16^{INK4a} expression in 16.4% (49/299). Some p16^{INK4a}-expressing cells in diffusely positive tumors co-expressed proliferation marker Ki-67, while this was not found in focally p16^{INK4a}-expressing tumors. Only 3.7% (11/299) tumors were positive for HPV DNA and diffuse p16^{INK4a} expression. Patients were followed-up for a median of 22 months (1-184 months). Neither the sole HPV status nor combined HPV/p16^{INK4a} status was associated with disease free (DFS) or overall survival (OS). Patients with tumors displaying focal p16^{INK4a} expression tended to show a favorable OS compared to diffuse (p=0.25) or no p16^{INK4a} expression (p=0.06). Thus only a small fraction of oral SCC might be induced by HPV, as indicated by HPV DNA and p16^{INK4a} positivity. No association with prognosis could be observed for the combined HPV/p16^{INK4a} status; however definite conclusions would require a larger sample size and prospective study design. The observed trend towards a better OS for patients with tumors displaying focal p16^{INK4a} expression might be related to successful senescence induction which may lead to p16^{INK4a} overexpression. This hypothesis could be supported by the observation that p16^{INK4a}-expressing cells in these focally p16^{INK4a}-expressing tumors never co-expressed proliferation marker Ki-67, indicating successful cell cycle arrest. In contrast, p16^{INK4a}-expressing cells in diffusely p16^{INK4a}-positive tumors obviously lost cell cycle control as indicated by co-expression of Ki-67.

FC 10-13

HPV INFECTION IN SQUAMOUS CELL CARCINOMAS ARISING FROM DIFFERENT MUCOSAL SITES OF THE HEAD AND NECK REGION. DIFFERENT SIGNIFICANCE OF P16 IMMUNOHISTOCHEMISTRY.**Bussu F, Delogu G, Gallus R, Graziani C, Sali M, Santangelo R, Galli J, Almadori G, Vellone V, Valentini V, Paludetti G***Università Cattolica del Sacro Cuore, Roma*

Objectives. Head and neck SCC are an extremely heterogeneous group of tumors both under a molecular (1, 2) and under a clinical point of view (3). In this context, if the role of high risk HPV in oropharyngeal carcinogenesis is established (4-6), evidence about its significance in the other head and neck sites is definitely lacking.

Our purpose is to evaluate the incidence of HPV infection in SCC from different subsites and the reliability of p16 overexpression at immunohistochemistry as a diagnostic tool for HPV infection in head and neck SCCs.

Methods. We enrolled 125 patients with squamous cell carcinomas from different sites in the head and neck. We evaluated HPV infection by HPV DNA and RNA detection. We performed immunohistochemistry for p16.

21 patients resulted high-risk HPV positive both at DNA and RNA detection (concordance between the methods 100%). 17 of these patients were affected by oropharyngeal SCC, 2 by oral SCC, 2 by supraglottic SCC. 46 patients were p16 positive at immunohistochemistry. IHC for p16 correlated with HPV infection only in oropharynx (p=0.01), not in the other sites in the head and neck.

Conclusions. The present study confirms the relevance of HPV infection as a risk factor for oropharyngeal SCC (4). Our data shows that p16 immunohistochemistry is absolutely unreliable for the diagnosis of HPV infection in the most frequent head and neck SCCs, and namely oral and laryngeal, with maybe the exception of the marginal larynx and in particular of the lingual aspect of the epiglottis. On the other hand in the oropharynx p16 IHC shows a good but not absolute correlation with HPV infection.

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PROGNOSTIC FACTORS INCLUDING ROLE OF HPV IN EARLY OROPHARYNGEAL CARCINOMAS**G. Psychogios, K. Mantsopoulos, M. Kapsreiter, F. Waldfahrer, Zenk, H. Iro,***Department of Otorhinolaryngology, Head and Neck Surgery, Friedrich Alexander University of Erlangen-Nuremberg*

Background: Nonsurgical therapies, which utilize combinations of chemotherapy and radiotherapy, have become popular treatments for early oropharyngeal carcinomas (OPC). The aim of this study is to analyze the outcome of primary definitive surgical management of patients with limited OPC and assess the influence of various prognostic factors in survival.

Methods: A retrospective study was conducted between 1980 and 2007. 266 surgically treated patients with pT1-2pN0-1 stage were included. The endpoints for the analysis were disease specific survival (DSS) and local control (LC) with respect to tumor subsite, T and N classification, status of margins, tumor depth, type of treatment and HPV infection.

Results: Overall five-year DSS was 88.7% and LC 93.3%. The univariate analysis showed a significant difference in DSS between pT1 and pT2 OPC (DSS 94.0% vs. 81.2%, $p=0.008$) and patients with tumor depth greater than 5 mm (DSS 94.5% vs. 78.9%, $p=0.031$). No difference could be found as to N-stage, marginal status, p16 (HPV) status, type of treatment and adjuvant radiotherapy. Multivariate analysis showed that T-stage (HR=2.49, 95%-CI=1.24-4.97, $p=0.01$) and tumor depth (HR=2.88, 95%-CI=1.06-7.87, $p=0.039$) were independent predictors of a reduced DSS.

Conclusion: Primary surgical treatment is a very effective therapy of limited OPC independent of p16 status and surgical technique used. Patients with pT2 status and tumor depth of more than 5 mm show a significantly worse survival rate and should be considered for adjuvant radiotherapy.

HPV PREVALENCE AND RELATION TO TOBACCO AND ALCOHOL IN HNSCC IN NORTH-EAST ITALY**Del Mistro A¹, Baboci L¹, Trevisan R¹, P. Boscolo-Rizzo², R. Fuson², G. Spinato³, G. Tirelli⁴, M.C. Da Mosto².***1 Istituto Oncologico Veneto, IOV - IRCCS, Immunology and Molecular Oncology Unit, Padova;**2 Otolaryngology Clinic, University of Padua School of Medicine, Treviso Regional Hospital, Treviso;**3 Otolaryngology Unit, Mirano Hospital; 4 Otolaryngology Unit, Trieste Hospital; Italy.*

Objectives. To evaluate HPV type-specific prevalence in HNSCC, correlate it with tobacco and alcohol consumption, and with clinical outcome.

Methods. Cases: previously untreated consecutive patients with HNSCC enrolled prospectively; 77 during 2003-2006, and 115 after 2008 (enrolment still ongoing). Fresh tumour samples (and adjacent mucosa for the cases collected after 2008) are analyzed for HPV sequences by PCR with MY09/MY11 primers. Typing is accomplished by restriction fragment length polymorphism analysis and by HPV 16 type-specific primers. Overall, 192 cases (152 males, 40 females; median age 69,7 years) have been analyzed so far; tumour site: oral cavity 50, oropharynx 45, hypopharynx 20, larynx 77; 67 patients were tobacco+alcohol users, 47 tobacco only users, 18 alcohol only users, 60 non-tobacco/non-alcohol users (this group comprises never users, former users, and occasional users). HPV16 sequences were detected in 18 (9.4%) cases; 12 patients were non-drinker/non-smokers, 5 were smokers only, 1 was smoker+drinker ($P=0.006$, Chi-square test). Prevalence was highest (12/45, 27%) for oropharyngeal cancers (OPC), and increased over time: 18% (4/22) before 2006 versus 35% (8/23) after 2008. After a median follow-up of 21,1 months (range 1-102), 52 patients (27%) are dead, and median overall survival is 61,4 months (IC 95%: 52,9-69,9). HPV-positive/non-smoker/non-drinker cases showed higher Disease Specific Survival (DSS at 3-years) than HPV-negative cases ($P=0.03$, log-rank test).

Conclusions. Among our study population, HPV appears to be correlated to HNSCC independently of tobacco and alcohol. A sharp increase in the proportion of HPV-associated OPC was found from cases occurred during 2003-2006 and cases occurred after 2008. Non-smoker/never-drinker patients with HPV-positive tumours showed higher DSS than patients with HPV-negative cancers.

FC 10-16

THE EXPRESSION PROFILE AND DIAGNOSTIC IMPLICATION OF HPV AND P16 IN SQUAMOUS CELL CARCINOMA (SCC) IN MULTIPLE ORGANS

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Objectives: p16 is a cell cycle regulatory protein keeping pRb in an unphosphorylated state and maintaining its tumor suppressor activity. Following integration of the human papilloma (HPV) viral DNA into host genome, the viral E7 protein binds to and degrades pRb resulting in p16 upregulation. We study the p16 expression in SCC in multiple sites for its diagnostic utility. We also examined the presence of high risk HPV 16 DNA by in situ hybridization (ISH).

Methods: A total 56 primary SCC were retrieved and H&E slides were reviewed. HPV 16 ISH and p16 immunostain were performed on paraffin sections (Ventana 1:200). p16 expression scoring was based on proportion of positive cells: negative 0: <10%, weak 1+:10-30%, moderate 2+: 50-75%, strong 3+: 75-100%. Nuclear or combined nuclear and cytoplasmic stains were specific. ISH was scored as positive or negative based on nuclear positivity. Of 25 cases of lung SCC, 40% were positive for p16. All cases from the anogenital area, 40% of skin SCC, 27.3% of head/neck SCC were positive for p16. All esophagus cases were negative for p16. HPV 16 was detected in 4% of lung SCC, 37.5% of anogenital SCC and 27.3% of head/neck SCC. There was no detection in all esophageal and skin SCC.

SCC site, p16, and HPV 16 expression

| SCC site | Number of cases | P16 immuno score | | | | HPV16 ISH | |
|------------|-----------------|------------------|----|----|----|-----------|----------|
| | | 0 | 1+ | 2+ | 3+ | Positive | Negative |
| Lung | 25 | 15 | 1 | 5 | 4 | 1 | 24 |
| Anogenital | 8 | 0 | 0 | 0 | 8 | 3 | 5 |
| Skin | 7 | 5 | 1 | 0 | 1 | 0 | 7 |
| Esophagus | 5 | 5 | 0 | 0 | 0 | 0 | 5 |
| Head/Neck | 11 | 8 | 0 | 0 | 3 | 3 | 8 |

Conclusions: 1) p16 protein and HPV 16 DNA expression profiles are not always consistent in lung, anogenital and head and neck SCC. More pathways or more HPV species might be involved with the tumorigenesis. 2) Majority of pulmonary SCC is negative for HPV 16 DNA; a positive HPV 16 case should be considered as a metastatic from anogenital or head and neck primaries. 3) SCC arising from skin and esophagus are not related to HPV 16 pathogenesis. 4) HPV 16 ISH can be valuable in our daily practice

P 1-1

DISTRIBUTION OF HPV GENOTYPES IN WOMEN WITH >CIN2 LESIONS IN MADRID.

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OBJECTIVES: HPV 16 and 18 are considered the most prevalent high risk genotypes (HR-HPV) in cervical cancer worldwide. To know the most prevalent HR-HPV in different geographical areas is important to design diagnostic tests and to ascertain the benefits of vaccine implementation in each country. Limited data are available regarding the distribution of HR-HPV genotypes in Spanish women with a histological confirmed >CIN2 cervical lesion. The goal of this study was to investigate whether genotypes 16 and 18 (target of screening tests and vaccine) are the most prevalent in a total of 308 women with histological confirmed >ASCUS lesions who attended our Gynaecology Department for opportunistic screening of HPV infection.

METHODS: Cervical samples were taken in a PreservCyt vial (Cytoc Corporation, Boxborough, MA) and sent to the Pathology Department for cytological study. Only women with available > ASCUS cytology results confirmed with histology were included in the study. All samples were investigated with polymerase chain reaction (PCR) (Linear Array HPV Genotyping Test, Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instructions.

CONCLUSIONS: The histological results were as follows: 18 (5.84%) normal, 1 (0.32%) ASCUS, 136 (44.16) CIN1, 85 (27.6%) CIN2, 60 (19.48%) CIN3 and 8 (2.6%) cervical cancer. One or more HR-HPV were detected in 280 (90.91%) of the samples, 15 (4.87%) presented a LR-HPV and in 13 (4.22%) no HPV was found. In > CIN3 samples (Fig 1) HPV 16 was the most prevalent (41 CIN3 and 4 cancer) followed by HPV 31 (13 CIN3), HPV 33 (6 CIN3), HPV 51(4 CIN3 plus 1 cancer) and HPV 58(4 cancer). HPV 18 was only present in 1 CIN3 sample. Our results confirm that type specific HR-HPV screening assays and protocols in our country should focus on HPV 16. HPV 18 and HPV 45 were not as prevalent as in other countries not only in preneoplastic lesions but even in cervical cancers. Epidemiological studies are necessary because the trends of HR-HPV distribution in >CIN3 lesions may change with the implementation of vaccines.

PREVALENCE OF HPV INFECTION AMONG MEN ATTENDING STI CLINICS IN ENGLAND

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Objectives: To describe HPV type-specific epidemiology in men at high risk of HPV infection.

Relatively few data are available about type-specific epidemiology of HPV infections in men, particularly according to socio-demographic and behavioural factors. This is partly due to the lack of suitable opportunistic genital samples from men. Evaluation of HPV vaccination of females in the UK will importantly include determination of the impact of herd immunity on HPV infections in men (both heterosexual and MSM).

Methods: Two studies that collected urine samples from men attending STI clinics were conducted from 2008-2010.

One study (group 1) collected residual urine samples from 957 men (85% heterosexual, 15% MSM) attending STI clinics. Another study (group 2) collected urine from 87 men who reported multiple sexual partners or a diagnosis of genital warts within the previous 6 months.

All samples were anonymous and unlinked from any personal identifiers. Samples were genotyped for 13 high-risk and 7 other HPV types (including types 6 and 11) using Bio-Plex® (Luminex xMAP®) suspension array technology.

Conclusions: Amongst urine samples from group 1, one or more high-risk HPV type was detected in 12.6% (95% CI 10.6% to 14.9%) and HPV 16 and/or 18 in 4.6% (95% CI 3.4% to 6.1%). Overall high-risk HPV prevalence was not associated with sexual orientation although there were differences in age-group specific prevalence between MSM and heterosexual men. There was a higher prevalence of high-risk HPV infection amongst young (<25 years old) heterosexual men than young MSM although prevalence steadily increased with increasing age for MSM. The detected high-risk HPV prevalence in group 2 was 16.1% (95% CI 9.1% to 25.5%) for one or more high-risk type and 4.6% (95% CI 1.3% to 11.4%) positive for HPV 16 and/or 18. High-risk HPV was detected more often in urine from men who were uncircumcised (22.8% vs. 4.2%).

A recent diagnosis of genital warts was associated with higher detection of HPV types 6 and/or 11 (group 2: 26.9% vs. 5.5%).

Other studies have shown that urine can be used for epidemiological analyses of HPV. This epidemiology is expected to change due to high uptake of HPV vaccination among young women: we expect to see a reduction in HPV 16/18 prevalence amongst younger, heterosexual men in coming years.

MIXED LESIONS (SQUAMOUS INTRAEPITHELIAL NEOPLASIA AND ADENOCARCINOMA IN SITU) OF THE CERVIX ARE INDUCED BY A SINGLE HPV GENOTYPE

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Objectives: The initial step in cervical carcinogenesis is infection with human papillomavirus (HPV) high-risk genotypes. Transforming infections of stem/reserve cells will give rise to squamous cell carcinoma or adenocarcinoma via the precursor lesions squamous intraepithelial neoplasia and adenocarcinoma in situ. It is assumed that there are two types of stem/reserve cells: One population differentiates exclusively to endocervical cells and another population has a divergent potential to develop into squamous and glandular epithelium. It is not well known, if independent lesions of squamous and glandular neoplasias at separate locations are due to infections with a single HPV- genotype or induced by multiple HPV-genotypes.

Methods: We examined 6 cervical cone specimens containing both squamous intraepithelial neoplasia and adenocarcinoma in situ. Formalin fixed and paraffin embedded material was evaluated for the presence of HPV 16 and HPV 18 with real time PCR.

Results: In 3 cone specimens both squamous intraepithelial neoplasia and adenocarcinoma in situ were positive for HPV 18, in 2 cone specimens HPV 16 was the single genotype. In one cone specimen both intraepithelial lesions were negative for HPV 16 and HPV 18. None of the investigated cases showed co-infection with HPV 16 and HPV 18.

Conclusions: Our preliminary results indicate that a single HPV genotype induces squamous intraepithelial neoplasia and adenocarcinoma in situ in mixed lesions, either by infecting both stem/reserve cell types which then independently give rise to squamous and glandular neoplasias at different locations or by infecting reserve cells with the potential of divergent differentiation.

P 1-4

PREVALENCE OF HUMAN PAPILLOMAVIRUS IN GREEK PATIENTS WITH HEAD AND NECK CANCERS**Tsimplaki E.¹, Laskaris S.², Kouvousi M.¹, Xesfyngi D.³, Panotopoulou E.¹**

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Objective: An outbreak of human papillomavirus (HPV) - associated head and neck squamous cell carcinomas (HNSCCs) has been observed over the last years.

Greek data on HPV prevalence in HNSCCs are limited. The objective of this study was to identify HPV genotypes in Greek patients with HNSCCs.

Methods: The study included 74 biopsies collected from patients with HNSCCs (45 from the oral cavity, 5 from the oropharynx and 24 from the larynx). Samples were tested for HPV DNA using PapilloCheck HPV genotyping assay.

Conclusions: The overall prevalence of HPV infection was 12.1% (9/74). HPV DNA was detected in 8.9% (4/45) of cancers of the oral cavity, 40% (2/5) of the oropharynx and 12.5% (3/24) of the larynx. HPV6 was found in one case of cancer of the larynx, and of the oral cavity (tongue), HPV16 in one case of cancer of the oropharynx (tonsil) and in two cases of the oral cavity (tongue and floor of the mouth), HPV18 in one case of cancer of the oropharynx and of the oral cavity (tongue) and HPV51 in two cases of cancer of the larynx.

The results of this early stage study show that head and neck squamous cell carcinomas are associated with HPV infection, confirming the international data.

P 1-5

PREVALENCE OF MULTIPLE HPV INFECTIONS IN A COHORT OF GREEK WOMEN WITH REGARDS TO AGE AND CYTOLOGY**Argyri E.¹, Tsimplaki E.¹, Tsiaousi I.¹, Daskalopoulou D.², Magiakos G.³, Panotopoulou E.¹**

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Objective: Multiple infections may constitute a risk factor for development of cytologic abnormalities according to the international literature. In this study we present the prevalence of multiple HPV infections in correlation with age and cervical cytology.

Methods: Cervical smears obtained from 3170 patients, were analysed for HPV DNA typing using PapilloCheck HPV genotyping assay. Cytological findings were classified according to the Bethesda classification system.

Conclusions: Women were 14-70 years old and divided according to their age in three groups: 14-25 (858 women), 26-46 (1760 women) and 47-70 (552 women). Normal cytology was diagnosed in 70% of the samples, ASCUS was found in 1.7% of our population, LSIL in 25.9% and HSIL was identified in 2.5%.

Multiple HPV infection with either low risk or high risk HPV types was found in 12.9% (410/3170) of all samples and 39.1% (410/1049) of the HPV infected samples. The prevalence of HPV multiple infection was higher in the 14-25 years age group and reduced in older ages. Multiple HPV positivity was significantly more common in LSIL (279/821, 34%) or HSIL (30/78, 38.5%) than in ASCUS (5/53, 9.4%) or Normal (95/2218, 4.3%) ($p < 0.001$).

Among women with multiple infections, 66.8% were infected with two types; the most common combination was of 16 with 51, 56 with 66 and 6 with 42. There were 22.6% of infected women with three HPV types. There were also cases with 4 HPV types (8.3%), 5 HPV types (1.4%) and 6 HPV types (1%).

GENOTYPIC PROFILE OF THE HUMAN PAPILLOMAVIRUS IN RELATION TO CERVICAL CANCER IN WOMEN OF KINSHASA, D.R. CONGO

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Objectives: Cervical cancer has been related to cervical infection by the Human Papillomavirus (HPV) whose genotypic profile may vary between different populations. The aim of this study was to determine subtypes of HPV present in Congolese women with a positive smear suspect for dysplasia.

Methods: 586 smears were collected during regular gynecological consultations in the HPGRK and Ngaliema Hospital of Kinshasa. The HIV status was largely unknown in most women. A comparative cytologic analysis has been performed on conventional smears and on monolayers prepared for ThinPrep diagnosis. In case of dysplastic lesions the subtypes of HPV have been determined with PCR (INNO-LIPA).

Conclusions: 10 % of patients had suspect lesions of which 80 % could be classified as ASCUS and ASC-H, another 15 % were high grade lesions, containing 3 % and 12 % as CIN2 and CIN3/CIS respectively. In 5 % an invasive carcinoma could be diagnosed. The genotypes of HPV found in patients presenting these lesions were: for ASCUS and ASC-H: 16, 35, 42, 45, 51, 52, 66, for CIN3: 16, 33, 66, for CIS: 16, and for invasive cancer : 16, 59. Type 16 was only found in 6% of patients with suspect lesions. Type 18 was only found in one case. Multitype infection was present in 19 % of suspect lesions.

These results demonstrate that the oncogenic subtypes 16 and 18 of the HPV which dominate the profile in European and American populations have been found to be less frequent in women of Kinshasa. These findings are consistent with results found in Congolese HIV+ women (1) and women in Mombasa, Kenya (2).

The presence of other oncogenic HPV subtypes has to be taken into consideration when vaccination against the major subtypes 16 and 18 will be generalized.

(1. Ali-Risasi et al. Human papillomavirus genotype profile in Kinshasa, Democratic Republic of Congo : implications for vaccination. *Med Trop* 2008, 68, 617-620 ; 2. De Vuyst et al. The prevalence of human papillomavirus infection in Mombasa, Kenya. *Cancer Causes Control* 2010, 21, 2309-2313)

MOLECULAR EPIDEMIOLOGY OF HPV AND CHLAMYDIA TRACHOMATIS INFECTIONS IN A COHORT OF YOUNG WOMEN IN NORTHERN ITALY

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Objectives: HPV and Chlamydia trachomatis are the causes of two of the most common STIs, affecting adolescent/young people in industrialized countries. HPVs are double-stranded DNA viruses. More than 40 HPV genotypes can infect the genital area: HR-HPVs (e.g. HPV types 16 and 18) can cause cervical cancers and LR-HPVs (e.g. HPV types 6 and 11) can cause genital warts. Chlamydia trachomatis is an obligate intracellular bacterium and infection with this pathogen has also been demonstrated to be a risk factor for the acquisition of HPV infection. This study aimed at studying the molecular epidemiology of HPV and Chlamydia trachomatis infections in sexually active young women aged 13-24 years in northern Italy and evaluating the HPV/Chlamydia trachomatis co-infection.

Methods: 563 cervical brush samples were collected from sexually active females aged 13-24 years (median age 19 years), between January 2009-December 2011. All samples were analyzed to identify the presence of HPV and Chlamydia trachomatis infections using PCR assay amplifying an ORF L1 segment (450bp) and a cryptic plasmid segment (150bp), respectively. All HPV-DNA positive samples were subjected to viral typing analyses using a restriction fragment length polymorphisms (RFLP) assay.

Conclusions: The prevalence of HPV infection in women aged 13-24 years was 22.7%. In particular, the HPV prevalence was 19.3% in women aged 13-18 years and 26% in women aged 19-24 years. Over 64% of the detected infections were attributable to HR-clade genotypes. The most prevalent genotypes were HPV-16 (20% of HPV positive women), -31 (13.3%), and -52 (12.5%). The prevalence of Chlamydia trachomatis infections was 5.7%. In particular, the Chlamydia trachomatis prevalence was 7.3% in women aged 13-18 years and 4.2% in those of 19-24 years. Three percent of young women had HPV/Chlamydia trachomatis co-infection. The prevalence of Chlamydia trachomatis infection was higher among HPV-DNA positive women than among HPV-negative ones (13.2% vs. 3.4%, $p < 0.001$), thus suggesting that persons with an ongoing STI are more likely to acquire other STIs.

P 1-8

THE PREVALENCE AND GENOTYPE OF HUMAN PAPILLOMAVIRUS ON CERVICAL SAMPLES FROM AN IRISH FEMALE POPULATION WITH EXTERNAL GENITAL WARTS

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Objective: The aim of this study was to determine the cervical genotype profile of females who presented to an STI Clinic with external genital warts; and to determine the potential vaccine coverage prior to the uptake of the HPV vaccines.

Methods: 61 cervical scrapings were taken from females aged 18 – 35 years who had external genital warts or a history of external genital warts (EGW). The resulting 50 samples that were positive for HPV-DNA were subjected to genotype identification. 46 of these samples had detectable genotypes by LIPA analysis and most (78%, 36/46) had multiple Low Risk (LR) and High Risk (HR) genotypes on the cervix. 25 of these samples (54%) had more than 1 HR genotype. Of the 36 patients who had any HR genotypes, 18 (50%) were identified to have the most oncogenic HPV genotypes, namely 16 and 18. Three of these samples had both 16 and 18 on the cervix.

Conclusion: The presence of multiple HR genotypes on the majority of cervical samples from a self-referred population of females with EGW is presented. This study is of importance since persistent HR-HPV is the necessary risk factor in the development of precancerous and cancerous lesions of the cervix. Gardasil, the quadrivalent HPV vaccine would have been useful in the prevention of 28% (13/46) of these infections.

P 2-1

MONITORING THE EARLY IMPACT OF YOUNG GIRLS QUADRIVALENT HPV VACCINATION ON GENITAL WARTS IN BELGIUM.

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Objective : To monitor the population impact on genital warts (GW) 4 years after the introduction of the quadrivalent human papillomavirus (qHPV) vaccine in Belgium.

Methods : A retrospective observational study was performed on a healthcare insurance (MLOZ) database. Imiquimod prescriptions were used as a surrogate marker for GW based on specific and controlled prescription of this drug for this indication in Belgium. The number of GW was described by age-group, gender and over time between 2003 and March 2011. The number of individuals vaccinated with qHPV vaccine was described from the same database since its introduction in 2007. GW cumulative incidence estimates were compared between women that were vaccinated with qHPV vaccine and those who were not vaccinated (or vaccinated with the bivalent vaccine which does not protect against GW) from 2007 onwards. Those analyses were restricted to age-groups of women likely to have been vaccinated with qHPV vaccine.

From 2007 onwards, 55 193 women aged 16-20 year-old were retrieved, of whom 13 117 were vaccinated with qHPV vaccine. Within this age-group, 435 GW cases were observed, 423 in the control group (non vaccinated with qHPV vaccine) and 12 in the group vaccinated with qHPV vaccine which is expected to prevent about 90% of the genital warts (those related to HPV 6 & HPV 11 types). The cumulative incidence estimates of GW were significantly lower among women that were vaccinated with qHPV vaccine compared to those that were not: 0.12% (0.07-0.23) vs 0.93% (0.85-1.03), $p < 0.0001$. By comparing the 2 groups of women aged 16 to 20 years over a 4 year period, those non-vaccinated with qHPV vaccine were estimated to have 8 times more risk to get GW than those vaccinated with qHPV vaccine.

Conclusions : This first impact evaluation study suggests that a marked reduction of GW and associated resources use in Belgium may be achievable through qHPV vaccination.

ASSOCIATION BETWEEN HUMAN PAPILLOMAVIRUS (HPV) VACCINATION AND MIGRAINE**van 't Klooster TM¹, Kemmeren JM¹, van der Maas NAT¹, Sturkenboom M², de Melker HE¹***1. Centre for Infectious Disease Control, National Institute for Public Health and the Environment (RIVM), The Netherlands**2. Department of Medical Informatics, Erasmus Medical Centre Rotterdam, The Netherlands*

Objectives: In the Netherlands, the bivalent HPV-vaccine was introduced for girls in 2009. A catch-up campaign was organized for 13-16-year-old girls in 2009 and in 2010 routine vaccination of 12-year-old girls is included in the national immunization program (NIP). Enhanced vaccine safety surveillance carried out after introduction, showed a rise in the number of reports of migraine. In 2009 and 2010, the reporting rates of migraine were 0.09 and 0.12 per 10,000 administered doses, respectively. Validation of this signal is important to maintain trust in the NIP.

Methods: Migraine cases (certain and probable) were retrospectively selected from an electronic database of medical records from Dutch General Practitioners (Interdisciplinary Processing of Clinical Information (IPCI), Erasmus MC Rotterdam) of the years 2005/2006 (pre-vaccination) and 2009/2010. Age and gender specific incidences will be calculated in periods before and after introduction of HPV-vaccination. Cases in 2009/2010 will be linked with the vaccination registry (Praeventis, RIVM) to determine HPV vaccination status. Using self-controlled case series (SCCS) analysis a possible association between HPV vaccination and the occurrence of migraine will be studied. Furthermore, the incidences in vaccinated and non-vaccinated migraine cases will be compared.

Conclusions: For women in the pre-vaccination period, the minimum (certain cases) and maximum (certain and probable cases) incidence of migraine was 28 and 43 per 10,000 women, respectively. Menstruation related migraine concerned yearly 3 per 10,000 women yearly. For men, the minimum pre-vaccination incidence of migraine was 11 per 10,000 yearly compared to a maximum incidence of 15 per 10,000 yearly. Age-specific incidences for the pre-vaccination period were minimal 28 and maximal 40 per 10,000 for 10-14-year-old girls, and minimal 38 and maximal 59 per 10,000 for 15-19-year-old girls. Post-vaccination data will be presented at the meeting. To our knowledge, this study is the first in the Netherlands that examine the association between HPV-vaccination and migraine by linking medical records and vaccination data. Linkage studies using electronic databases are an important tool to validate signals emerging from passive surveillance systems.

ACCEPTABILITY OF HPV VACCINATION AMONG UNDERSERVED MOTHERS FOR THEIR DAUGHTER: ANALYSIS BY EDUCATION LEVEL**Haesebaert J.¹, Kalecinski J.², Lutringer-Magnin D.¹, Barone G.¹, Borne H.³, Jacquard AC.⁴, Régnier V.², Leocmach Y.³, Vanhems P.⁵, Chauvin F.², Lasset C.¹***1. Université Lyon 1, CNRS UMR 5558, Centre Léon Bérard, Lyon, France.**2. Institut de Cancérologie de la Loire, Université Jean Monnet de Saint-Étienne, IFR 143, Saint-Étienne, France.**3. Centre hospitalier des Quatre-Villes, 92310 Sèvres, France 4. Sanofi-Pasteur MSD, Lyon, France.**5. Université Lyon 1, CNRS UMR 5558, Hospices Civils de Lyon, Lyon.*

Objective: To assess acceptability of HPV vaccine among mothers and identify its determinants according to education level.

Method: Mothers of 14-18 year-old (yo) daughters were selected from a series of 1478 18-65 yo women from the Rhône-Alpes region recruited by general practitioners to complete a self-administered questionnaire on cervical cancer (CC) prevention. Education level was classified as low - LEL (lower secondary), medium - MEL (upper secondary) or high - HEL (tertiary). Eighteen LEL mothers were interviewed by a sociologist.

Conclusion: A total of 188 mothers of 14-18 yo daughters were analyzed: 99 LEL, 54 MEL and 35 HEL. Compared with HEL and MEL, LEL mothers had less professional activity (88%, 89% and 74% for HEL, MEL et LEL respectively, $p=0.044$), lower socio-professional status (0%, 4% and 16% were workers, $p<0.001$), less consistent gynaecological follow-up (73.5%, 70.4% and 66.8% annually, $p=0.314$) and lower compliance to Pap Smear (PS) screening (PS in the past 3 years: 91.4%, 92.6% and 82.5%, $p=0.144$). PS. I would not put the details if ns to be more focus on parameters on which variations are seen They less frequently mentioned HPV as the cause of CC (77.1%, 38.9% and 19.2%, $p<0.001$) or knew the role of PS (74.3%, 72.2% and 57.6%, $p=0.084$). HPV vaccine acceptance was higher in LEL (60.4%) and MEL mothers (68.6%) than in HEL mothers (46,8%) ($p=0.227$). Newness of the vaccine and fear of side effects were the main barriers: respectively 71%, 56% and 42% of reluctant mothers. Some interviewed LEL mothers feared that the vaccine would affect fertility. Others stated that their daughter was not concerned as she should have no sex before marriage. Favourable opinion for HPV vaccination of LEL mothers was associated with younger age (OR = 6.28 [1.71-23.14], $p=0.003$), hepatitis B vaccination (OR = 2.31 [0.88-6.07], $p=0.090$), pneumococcus vaccination of her child (OR = 2.33 [0.76-7.09], $p=0.140$) and history of abnormal PS result (OR = 5.96 [0.71-49.85], $p=0.083$). Although LEL mothers are less compliant with gynaecological and PS follow-up and have limited knowledge on CC, their acceptance of HPV vaccine is good and even better than among HED mothers. Information on vaccine safety is required to avoid misconceptions of LEL mothers and improve acceptance of HEL mothers.

HPV VACCINATION AND CERVICAL CANCER PREVENTION AMONG GIRLS AND WOMEN FROM 14 TO 23 YEARS IN FRANCE: REVIEW OF PRACTICES FROM A SURVEY REALISED AMONG GPs.

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Introduction: The HPV vaccination is recommended in France since 2007 for all girls aged 14 with a catch up programme for girls aged between 15 and 23 if sexual activity had begun less than 1 year previously. We studied the practices of HPV vaccination and pap smear screening (PSS) in 14-23 years old girls/women of Rhône-Alpes region (RA).

Method: Between June and August 2009, 113 voluntary general practitioners (GPs) issued from a representative sample of GP of RA completed a questionnaire on HPV vaccination and/or on PSS practices for all their patients aged from 14 to 23.

Results:

• **Vaccination:** A complete HPV vaccination was performed in 231/502 (46%) of girls : 34/132 (26.5%) of 14-16 years old (yo) girls, 77/188 (41%) of 17-20 yo girls and 20/182 (11%) of 21-23 yo girls. The GP was involved in the decision to vaccinate in 77.3% and who did the vaccination in 98.2%. The median age at the first injection was 17 yo. The median time between the first and the second injection was 2 months [1-10] and 4 months [2-16] between the second and the third one. The compliance to the 3 doses was about 85% and side effects (whatever the severity) at 12.2%. Among the 268 (53.4%) unvaccinated adolescents, 59% were "off target" (sex life > 1 year) and 11.9% had refused the vaccination (20% among 17-20 years). For 6.7% of them, it was the parents who refused the vaccination (27.5% among 14-16 years). However, 25.7% (75% of 14-16 yo girls) had planned to be vaccinated.

• **Pap smear screening:** 27.1% of 17-20 years old girls and 75.3% of 21-23 had already had a PSS with a median age at the first pap at 18 years-old. Practitioners who had realised it was the GP for 52.9% of 17-20 years old girls and gynaecologist for 65% of 21-24 years.

Discussion: Two years after recommendation, HPV vaccination was mainly done in 17-20 yo girls by the GPs. Compliance with the immunization schedule and the immediate tolerance were good. PSS was common from the age of 20 in the target population of vaccination.

THE VACCINE AND CERVICAL CANCER SCREEN PROJECT - FIRST INTERIM ANALYSIS

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Objectives: The aim of this ongoing project is to assess if cervical cancer screening can be successfully linked with cervical cancer vaccination in a population of unscreened women.

Methods: Five primary schools in the south west part of Tshwane in South Africa were included. At the information event questionnaires were conducted with the parents of female learners, followed by information given to learners and their parents about cervical cancer, screening and vaccination. Learners aged 9 years and above whose parents consented were subsequently vaccinated using either the bivalent or quadrivalent available vaccines.

Screening for cervical cancer by means of tampon testing for high risk HPV was provided to interested female parents. Women who tested positive for high risk HPV were followed up at the gynaecologic oncology clinic with colposcopy and biopsy and treatment as per clinic guidelines.

A follow-up questionnaire was conducted telephonically with all participants after six months.

The study was approved by the ethics committee of the University of Pretoria. Consent to conduct the study was obtained from the National Departments of Health and Education.

Results: There were 779 eligible girls in the five schools. Three hundred and seventeen (40.7%) female parents or guardians participated and attended the information event and answered the questionnaires. Of the non attending parents, 380 (82.2%) provided consent for vaccination of their daughters while 93 (11.9%) parents opted out. Eighty two (10.5%) parents did not respond.

Of the 317 female parents/guardians who attended the information event, 105 (27.48%) returned their self-screening specimens of which 34 (32.4%) tested positive for high risk HPV. These mothers were mostly unscreened in the previous 5 years.

A total of 544 (69.32%) girls were eligible for vaccination of which 541 (99.45%) were vaccinated at least twice and 480 (88.23%) received all three vaccines.

Of the 380 girls who were recruited before July of one year, 370 (97.37%) received all three vaccines.

Conclusions: Uptake of cervical cancer screening was 27% in an unscreened population with a high prevalence of HIV and therefore at high risk for cervical cancer. Of the screened women, 34% tested positive for high risk HPV. Vaccination uptake was 70% of which 90% received all three vaccinations and 99% received two vaccinations.

Cervical cancer vaccination is feasible in the school model in this metropolitan area and if combined with cervical cancer screening opportunities the uptake is acceptable.

POTENTIAL CLINICAL AND ECONOMIC IMPACT OF CROSS-PROTECTION INDUCED BY HPV-VACCINES IN THE CONTEXT OF THE ITALIAN REGIONS

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Objectives: The oncogenic HPV strains 16, 18, 31, 33 and 45 are associated with the development of more than 80% of cases of cervical cancer (CC). In clinical trials, HPV-16/18 vaccine has demonstrated high protective efficacy against HPV 16 and 18 and have shown evidence of cross-protective activity against HPV 31, 33 and 45, while the HPV 16/18/6/11 vaccine has shown cross protection against HPV-31. HPV vaccination for CC prevention was implemented in all Italian regions for adolescent girls. The purpose of this study is to determine the potential clinical and economic impact associated with the use of HPV vaccines in all the Italian regions.

Method: A steady state model was developed, which calculates the HPV-related diseases that occur in the time period of 1 year in the whole population in a steady state condition. The potential difference between the two vaccines has been evaluated in terms of benign (warts) and malignant (cancer and pre-cancerous) lesions potentially avoidable and related costs avoided, adapting the analysis to the context of all the Italian regions and from the Regional Health Service perspective. The vaccine efficacy (VE) also considers each vaccine's cross-protection data based on published studies, and assumes a life-long persistent protection. Price parity and vaccination coverage of 85% were assumed for both vaccines.

Conclusion: The analysis shows that, compared to the quadrivalent vaccine, the additional advantage, resulting from the cross-protection exerted by bivalent vaccine, allows to avoid a greater number of lesions, both low (ASCUS, Genital Warts and CIN1) and high grade (CIN2/3) and cases of CC. Indeed, the bivalent vaccine would avoid, for all regions, an average of 24 CIN1, 89 CIN2 and 3 and 14 CC per year, while the quadrivalent vaccine would avoid an average of 600 genital warts per year. The additional saving offered by the bivalent vaccine would be € 115.116/year on average (range from € 5.077 of Valle d'Aosta, to € 396.000 of Lombardia). Analysis results demonstrate that the bivalent vaccine, also due to the cross-protection towards HPV 31, 33 and 45, contributes substantially to reduce the costs for the regional health service.

HPV DETECTION IN URINE USING CONSENSUS AND GENOTYPING ASSAYS

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Objective: To demonstrate the technical feasibility of HPV detection in urine using a high throughput HPV test - the rT HPV Assay (Abbott).

Methods: A total of 80 urine samples were obtained from a sexual health and well-being clinic and tested by two molecular HPV assays, the rT HPV Assay and the Innolipa HPV Genotyping Test (Innogenetics). A technical comparison of both the consensus result and type specific result (for HPV 16 and 18) was performed. Of the 80 samples, only 4 were invalid by the rT HPV Assay as evidenced by non-amplification of the internal control. When confining analysis to the 14 types detected by both assays, the Innolipa and rT HPV assay detected HPV in 55.3% and 42.1% of samples respectively, with an overall concordance of 73.7%. When focussing on specific detection of HPV 16, 25% & 13% and were positive by the Innolipa and rT HPV assay respectively with an overall concordance of 82.9%. Regarding HPV 18, 10.5% and 6.6% were positive by the Innolipa and rT HPV assay respectively with an overall concordance of 81.6%

Conclusions: As HPV testing becomes increasingly integrated into cervical disease management programmes, it is important to interrogate the potential of non-invasive bio-specimens (such as urine) for HPV testing. To our knowledge, this is the first study to apply the rT HPV Assay to urine samples, which we have shown to be technically feasible. This technical comparison paves the way for a future clinical study where results are correlated to pathology findings.

P 3-2

COMPARISON OF THE REALSTAR® LR HPV DNA ASSAY DETECTING LR-HVP-TYPES 6, 11, 42, 43 AND 44 USING COBAS® Z 480 SYSTEM WITH HYBRID CAPTURE® 2 TEST

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Aims: The cobas® z 480 system (Roche Diagnostics) was recently approved by the U.S. Food and Drug Administration (FDA). This test identifies HPV16 and HPV18 separately as well as a pool of 11 hr-hpv genotypes (HPV31, -33, -35, -39, -45, -51, -52, -56, -58, -59, -68) and HPV66. We compared the altona LR hpv test (RealStar® LR HPV DNA, altona DIAGNOSTICS) using the cobas® z 480 system (Roche Diagnostics) with the hybrid capture® 2 test (hc2t, Qiagen) assay for the detection of low risk hpv dna.

Methods: Randomly, 57 specimens positive and 37 specimens negative for the low risk hc2t assay were tested with the altona LR assay. In case of discordant results the linear array (LA) test was done using 50µl of the dna eluate. The preparation of the master mix for the altona LR and LA in each test was according to the manufacturer's instructions. The hc2t contains probes for hpv genotypes 6, 11, 42, 43 and 44 for a pooled result. The altona LR test captures the same hpv types, but stratifies them in two combined results, namely for hpv 6, 11 and hpv 42, 43, 44. Additionally, the altona LR test carries a β-globin quality control. The LA test individually identifies 37 hpv types (6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51, 52, 53, 54, 55, 56, 58, 59, 61, 62, 64, 66, 67, 68, 69, 70, 71, 72, 73, 81, 82, 83, 84, IS39 and CP6108).

Results: In 94 specimens with hc2t results overall agreement with the altona LR test was 86.2%. Altona LR test and hc2t had conflicting results in thirteen specimens. Eleven specimens were lr hpv positive and altona LR negative. One of these specimens was invalid with LA. In six cases LA confirmed hpv infections with multiple hpv types other than 6, 11, 41, 42 or 43. In one case LA verified a lr hpv infection with hpv type 42. Besides, LA test confirmed an hpv infection with hpv type 53 in one and hpv infection with hpv type CP6108 in the other sample. On the other hand, two lr hpv hc2t-negative samples were positive with the altona LR test. In one specimen the LA test verified an hpv infection with types 84 and CP6108. The other one was negative with the LA test.

Conclusions: Altona LR and hc2t results for the detection of lr-hpv had a concordance of 86.2% respectively discordance in 13.2% and showed therefore a good agreement. The specific characteristic of the assays may be the reason for the discrepancies between the two tests.

P 3-3

EFFECT OF GLACIAL ACETIC ACID PRE-TREATMENT ON THE MOLECULAR DETECTION OF HUMAN PAPILLOMAVIRUS

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Objective: Cytology laboratories treat bloody liquid based cytology (LBC) specimens with glacial acetic acid (GAA) in order to lyse red blood cells and enhance assessment. The principle objective of this study was to determine the impact of GAA treatment of LBC specimens on the molecular detection of HPV.

Methods: 300 ThinPrep® PreservCyt® LBC paired specimens, pre-GAA-treatment and post-GAA-treatment, were collected at the Pathology Department, Royal Infirmary of Edinburgh between March – April 2011. Once cytological grading was reported, 150 paired specimens were selected to include a range of cytological normal and abnormal specimens. Testing was performed on these paired specimens using two commercial HPV tests, the signal amplification Qiagen Hybrid Capture® (HC2) High-Risk (HR) HPV DNA test® and the target amplification Abbott rHPV assay. In addition, ThinPrep® PreservCyt® solution specimens were prepared and spiked with CaSki cell line material and tested with HC2 to determine the effect of GAA treatment with increasing blood concentrations.

Results: Agreement between the pre-GAA-treatment and post-GAA-treatment specimens were assessed in terms of positive/negative status and via the semi quantitative measures of the assays RLU (HC2) and Ct value (Abbott rHPV). Statistical analysis was performed using the McNemar's test and Bland and Altman plots.

HC2 test agreement between untreated and treated specimens was 137/150 (91%) and there was no evidence of a difference in the distribution of discordant results at cutoff of 1 ($p=0.405$) and cutoff of 2 ($p=0.564$), indicating that GAA treatment did not effect the test positivity. Abbott rHPV test agreement between untreated and treated specimens was also high - 133/139 (95.7%), with no evidence of significant difference in positivity ($p=0.414$). Bland and Altman plots analysing paired samples indicated that treated specimens had a lower RLU values and higher Ct values compared with untreated specimens but this did not translate into statistical significance.

Conclusion: In this study with two HPV screening tests, GAA pre-treatment of LBC samples had little effect on subsequent HPV test results. Use of GAA prior to HPV testing should however be validated for any HPV test and all LBC collection media being used in particular diagnostic settings.

DISTRIBUTION OF FIVE HIGH-RISK HUMAN PAPILLOMAVIRUS GENOTYPES AMONG WOMEN IN MONTENEGRO**Vujosevic D¹, Vuksanovic V^{1,2}, Zdravle M¹, Jovanovic M³***1 Center for Medical Microbiology, Institute of Public Health, Podgorica, Montenegro**2 Medical faculty, University of Montenegro, Podgorica, Montenegro 3 Primary Health Care Center Podgorica, Montenegro*

Objective: To analyze the distribution of five the most common identified high-risk human papillomavirus (HPV) genotypes (16, 18, 31, 33 and 45) in cervical samples of Montenegrin women.

Methods: A total of 434 cervical samples, taken from the same number of women with significant clinical signs on the cervix, were included in the study. All specimens were tested for E6/E7 mRNA using a commercially available NucliSENS EasyQ HPV test (Biomerieux, France) at the Department of Molecular Diagnostics of the Institute of Public Health, Montenegro.

Results: Infection caused by HPV was found in 17.5% of women. 13.6% of studied women (86.8% of the HPV infected women) were infected by one HPV genotype, while 2.1% (13.2% of the HPV infected women) had mixed infection. Half of all positive results belong to HPV 16 genotype. HPV 45 was found in 19.7%, followed by HPV 31 with 13.2% and HPV 33 with 11.8%, while HPV genotype 18 was found in a lower percentage (5.3%) of women in our study. The highest frequency of HPV genotypes with similar results were found in the groups of women in the fourth decade (39.7%) and third decade (38.23%) of life. In the category of 40-49 years, the HPV positivity was 22.1%, in the other categories (<20 and >50 years) there were no positive cases. HPV genotype 16 with the highest oncogenic potential was most prevalent in women aged 30 to 39 years, while HPV 18, HPV 31 and HPV 45 were the most common among women aged 20-29 years and decreasing with ages. HPV 33 showed equal presence for women aged 20-39 years, but its presence among women of older age was significantly lower.

Conclusions: The highest distribution of high-risk HPV genotypes (77.93%) is among women aged 20-39 years, with HPV 16 as the most predominant genotype. The results indicate that detection of viral E6/E7 mRNA, in combination with HPV typing, may have significant potential in identifying women who may be at an increased risk for developing cervical dysplasia. These findings require further evaluation and should be complemented by cytological and histopathological analysis.

CORRELATIONS BETWEEN CERVICAL CYTOLOGY AND HUMAN PAPILLOMAVIRUS DETECTION BY CERVISTA™ HR HPV TEST**Dr. VAN DEN HEULE B, Dr. VANDE WALLE H, BISKRI L and GALAND M***Labo CMP Pathology, Brussels, Belgium*

Objectives - The detection and early treatment of precancerous cervical lesions prevent a more serious pathology. Persistent infection with high-risk (HR) human Papillomavirus (HPV) is the main cause of the cervical cancer (1). Both the presence of the HR HPV deoxyribonucleic acid (DNA) and an ambiguous cytological result (atypical squamous cells of undetermined significance, ASC-US) in women could result in a cervical intraepithelial neoplasia (CIN) 2 or 3. Therefore, identification of women with both ASC-US cytology and HR HPV infection is essential to determine those who must be managed or more aggressively treated. The aim of this study was to validate, in our lab and for the Belgian accreditation body, the FDA-approved Cervista™ HR HPV Test for detection of DNA of the 14 HR-types which cause most cervical cancers.

Methods – Labo CMP Pathology is a private lab performing morphological analyses from recurrent patients examined around Brussels and in other areas in Belgium. All cervical cytology testing was performed from ThinPrep® vials, stratified by category and considered as reference. Cytology was compared with the Cervista™ HR HPV Test using the Invader® chemistry: a fluorescent signal amplification for detection of specific DNA based on enzymatic cleavage. Both repeatability (n=18) and reproducibility (n=6) were tested in triplicate by intra- and inter-assays, respectively. For negative test (n=6), women were chosen based on the absence for intraepithelial lesions or malignancies for at least a decade; for positive test (n=5), patients with high-grade intraepithelial lesion were selected and a histological exam was performed to confirm women with ≥ CIN 2. In addition, 118 ASC-US subjects were tested for HPV.

Conclusions – Negative and positive samples for cytology were negative and positive by Cervista™ HR HPV Test, respectively. HPV prevalence was 33.90% in women with ASC-US, which is consistent with the literature (2). All ≥ CIN 2 cases were HPV positive. Acceptable repeatability and reproducibility of the Cervista™ HR HPV Test were demonstrated in our lab, with accurate correlations between cervical cytology and HPV detection. We currently use the Cervista™ HR HPV Test for routine detection of 14 high-risk HPV types.

1. Meijer et al., 2006 2. Cuvelier et al., 2009

P 3-6

HOLOGIC CERVISTA HIGH RISK HPV, AS A TRIAGE SCREENING TOOL IN THE DUTCH NATIONAL CERVICAL SCREENING.

Experience from a Peripheral Hospital

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Objectives: In the Netherlands, human papillomavirus (HPV) DNA testing is used in the National Screening Program as a triage of ASCUS/LSIL cytology for colposcopy. This study presents the results of a comparison between the HCII and Cervista HPV HR HPV tests in a screening population as well as our experience of HPV triage with Cervista HPV HR in a well-organized national screening program.

Method: For the head to head comparison 84 patient samples were included, 57 ASCUS/LSIL triage from the national screening, 14 CIN2+, 12 NILM and 1x PreservCyt.

Women 30 years and older in the Dutch National screening program were enrolled. Pap samples were collected from all subjects using the Rovers broom-like collection device and placed into ThinPrep PreservCyt Collection Medium. Cytology was performed using the ThinPrep system and the residual samples were tested using the FDA-approved Hologic Cervista HPV HR assay and Qiagen HCII assay. All testing was performed in a Dutch Peripheral Hospital, Bethesda Hoogeveen, NL

Results: The comparison study showed a 98% [82/84] concordance between Cervista and HCII results. Both HPV tests demonstrated 100% detection of CIN2+ (12 CIN2 and 2 CIN3 lesions). One case of the NILM group was positive in the Cervista but negative in HC2 test.

Experience in our day-to-day practice showed that 78.3%(N=383) of ASCUS/LSIL triage samples were negative using the Cervista HPV HR test. (The Dutch guidelines refer that at least 70% of the total test woman needs to be negative, www.pathology.nl)

Conclusion: In this head-to-head study, Cervista was highly concordant with HCII in all cytological categories examined. We found that screening for High Risk HPV with the Cervista HPV test is successful in the Dutch National Screening Program.

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DETECTION OF GENETIC ABNORMALITIES USING HPV FISH TECHNIQUE AND SMART TECHNOLOGIES IN CARCINOMA OF THE UTERINE CERVIX

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Objectives: Genetic abnormalities play an important role in tumor transformation etiology. Characteristic marker of malignant tumors is chromosomal instability, either numeric or structural, which has a prognostic and predictive impact in many tumor diseases. In our group of patients, we studied specific chromosomal changes in regions 3q26 (*hTERC* gene) and 8q24 (MYCC gene), which can predict progressive premalignant lesions and cervical carcinomas.

Methods: Chromosomal changes were detected by two methods: HPV-interphase fluorescent *in situ* hybridization (HPV-FISH) and array-comparative genome hybridization (array-CGH).

Results: Using HPV-FISH, we assessed correlation of incidence of genetic abnormalities with clinical and cytological tumor stages. In the group of 11 patients with cervical carcinoma, HPV- positive cells were detected in ten (91%) patients and chromosomal abnormalities were found in nine (82%). Gain of *hTERC* was found in nine (82%) and gain of MYCC was detected in five (45%) of the patients. Gain of *hTERC* was detected in all patients with IIIB tumor stage and all patients positive for lymph-vascular space invasion.

We performed a comprehensive analysis of tumor genome in 13 patients using high density oligonucleotide DNA microarrays. The most frequent change was expected duplication of long arm of chromosome 3 (in six of the 13 patients examined). The second most frequent aberration was not duplication 8q (4/13), but duplication of long arm of chromosome 20 (5/13), followed by duplication of 20p (4/13) and 13q deletions (4/13).

Conclusions: Gain of *hTERC* and MYCC could be used as suitable genetic marker to predict progression of cervical carcinoma. Patients with positive *hTERC* and/or MYCC gene amplification could profit from intensive dispensarization and more aggressive therapy.

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MARKED DIFFERENCES IN HPV GENOTYPING

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Objectives. The aim of the study was to compare 2 different HPV genotyping methods.

Methods. HPV genotyping was the final test in the following stepwise protocol: cytological screening - if ASCUS or follow-up of highgrade cervical lesion → HPV testing by Abbott RealTime High Risk HPV test - if high-risk-positive result but other genotype than HPV 16 or HPV 18 → HPV genotyping by INNO-Lipa HPV Genotyping Extra (Innogenetics) and Full Spectrum PCR HPV Amplification and Detection/Genotyping System by Lab2Lab Diagnostic Service (GenoID). The comparison of genotyping methods was performed on 189 clinical and 20 external quality samples.

Conclusions. Only in 40.2% of the clinical samples the results between 2 genotyping methods were fully concordant. Clinical samples with single infection show more concordance than samples with multiple infections, representing 30.2% and 10% of concordant samples respectively. In 13.2% of the samples the genotyping results were fully discordant. Among these fully discordant results, GenoID-method found 4.8% of the samples negative for HPV and in another 4.8% of samples the method did not detect high risk genotype. Discordance in the detection of multiple infections was observed: in 21.7% of samples were INNO-Lipa detected multiple infections, GenoID-method observed only one HPV genotype. If Abbott-test is considered as reference in the current study for high-risk HPV presence, GenoID-method was false negative for HPV 16 in 17.4% and for HPV 18 in 50% of tested clinical samples. As compared to Abbott-test, INNO-Lipa gave 4.3% false negative results for HPV 16 and 16.7% false positive results for HPV 18. Comparing detection of HPV 45 between genotyping methods, discordance was found in 8 clinical samples. In all these samples, GenoID-method gave negative result for HPV 45 and in 6/8 samples the result by GenoID-method for high-risk HPV was also negative. Based on the results of external quality samples, GenoID-method was not able to detect HPV 18 independent on the viral load of the sample. Summarising, major differences were found in genotyping results according to the method used. Our findings accentuate the importance of method validation prior to its clinical use.

CLINICAL PERFORMANCES OF BIO-RAD DX HR-HPV ASSAY FOR DETECTION OF HIGH-GRADE CERVICAL INTRAEPITHELIAL NEOPLASIA IN WOMEN WITH ABNORMAL CYTOLOGY.

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Objectives: Clinical performances of Bio-Rad Dx HR-HPV Assay was evaluated in comparison with the Hybrid capture 2 (HC2) for the detection of cervical intraepithelial neoplasia 2 or worse (CIN3+).

Methods: Cervical specimens were collected from 700 Women (mean age 34 years) who were referred for colposcopy between may and july 2010. For each included patient, three specimens were collected. For 35.3% patients, the first sampling was done with 3ml Bio-Rad Dx Collection System than 20 ml Hologic device and the STM/ HC2 device. For 64.7% patients, the first sampling was STM/ HC2 device followed by the 3ml Bio-Rad Dx Collection System and the 20 ml Hologic device.

Dx HR-HPV test was performed on Bio-Rad Dx Collection System and Hologic device. HC2 test was performed on STM/ HC2 device. INNO-LIPA TM HPV Genotyping Extra test was used as the main test for resolving discordances between Dx HR-HPV and HC2 test. Clinical performances of Bio-Rad Dx HR-HPV Assay were evaluated against colposcopy and histology results. A consensus histology result and colposcopy served as a gold standard for determining the presence of disease. In the absence of colposcopically visible lesions, no biopsy performed equated to the absence of disease.

Results: Of the 700 cases, 24 were diagnosed with histological results of CIN2 among which 40 CIN3+. 62 had CIN1, 135 with no CIN and 439 with a negative colposcopy and no biopsy performed.

The overall positivity rate of Dx HR-HPV assay was respectively 69.3% and 65.1% for Dx Collection system and Hologic device and 66,0 % of HC2.

Bio-Rad Dx HR-HPV Assay had a clinical sensitivity of 89.1% and 90.6% respectively using the Bio-Rad and Hologic device. This compared with 92.2% with HC2

Similar specificity, PPV and NPV values were also observed for the two tests.

Among 3 CIN2 and 4 CIN3+ cases not detected by Dx HR-HPV Assay, 5 were HC2 negative and two were HC2 positive (Ratio >= 1), confirmed negative with INNOLIPA HR-HPV genotyping test.

Conclusions: Bio-Rad Dx HR-HPV Assay showed equivalent clinical performances for the detection of CIN2+ when compared with HC2. The clinical performances are equivalent when using Bio-Rad or Hologic Device.

P 3-10

PREVALENCE OF HIGH-RISK HPV GENOTYPES AMONG WOMEN IN ALGIERS, ALGERIA**Bahoura N¹, Oudafal B², Benkeroubi I², Seghier M¹.***(1) Laboratoire virus et cancer, service de virologie humaine, Institut Pasteur d'Algérie.**(2) Faculté des Sciences Biologiques, Université de Saad Dahleb, Blida.*

Objectives: The aim of this study was to examine the high risk Human Papillomavirus prevalence and distribution in cervical smears in a sample of Algerian women undergoing cervical sample testing.

Methods: A total of 668 cervical swab samples were collected for HPV DNA assay either by Hybrid capture assay (Digene) or by Polymerase Chain Reaction (Roche). The positive samples were analyzed using the Linear Array HPV Genotyping Test (Roche Diagnostics, Mannheim, Germany) detecting 37 HPV types. Also, each specimen collected was accompanied with detailed questionnaire data.

Conclusions: Our data show that HR-HPV is found in 5.08% of Algerian women, regardless of age, stage of the lesion. Genotypes 16, 58, 31, and 52 are the most prevalent HR-HPV.

P 3-11

EFFECT OF PROTEINASE K TREATMENT OF SUREPATH L-PAP SAMPLES ON DETECTION OF HPV mRNA**Chernesky M.^{1*}, Gilchrist J.¹, Jang D.¹, Toor R.¹, Schroeder A.², and Dockter J.²***1 St. Joseph's Healthcare/McMaster University, Hamilton, ON, Canada**2 Gen-Probe Incorporated, San Diego, CA, USA*

Objectives: SurePath™ (SP) L-Pap is a collection medium for cervical cytology. Storage of SP L-Pap may have a negative effect on the recovery and detection of high risk human papillomavirus (HR HPV) mRNA due to formalin-induced nucleic acid cross-linking. Mock-infected SP samples treated with Proteinase K (ProK) increases the detection of HPV mRNA. Our aim was to determine retrospectively, the effects of ProK digestion on HR HPV E6/E7 mRNA detection from SP specimens following storage at varying times and temperatures.

Methods: SP and PreservCyt (PC) L-Pap samples were collected from 426 women attending a colposcopy clinic. Within 48 hours, 1mL was transferred to an APTIMA® tube and tested for the presence of HR HPV mRNA in an APTIMA® HPV (AHPV) assay (Gen-Probe Inc.). Residual SP samples were held at 4°C for varying time intervals (1-7 months) before being aliquoted for use in one of three ProK treatment strategies: (A) 426 samples following storage at 4°C; (B) 234 samples 4°C storage and 10 days at RT; (C) 220 samples 4°C storage and 3 days at 30°C. One mL of sample was treated with 100uL ProK and a second 1mL as an undigested control, heated for 2 hours at 65°C, then tested. APTIMA HPV testing agreement and Kappa statistic calculations were made between ProK-treated and untreated SP samples with an untreated PreservCyt sample. Sensitivity and specificity of each testing strategy was calculated for detection of CIN2+ pathology.

Conclusions: Initial agreement between SP and PC was 94.8% (k 0.88). After storage at 4°C (A), agreement with original PC results was 86.9% without- and 93.2% with ProK (B), 75.6% without- and 89.7% with ProK after 10 days at 22°C (C), 88.7% without- and 95.2% with ProK after 3 days at 30°C. Percent sensitivity of AHPV for detection of 22 CIN2+ cases was 95.6% for both PC and the original SP and for strategy A was 72.7 without- and 100 with ProK; for strategy B, 57.9% without- and 85.0 with ProK and for strategy C, 78.6 without- and 90.9 with ProK. Each ProK treatment strategy increased the sensitivity of detection to approximate original testing of PC and SP. The number of SP samples positive for HR HPV mRNA decreased during storage. Due to a substantial increase in positive samples with ProK treatment, this would be a useful strategy to enhance HR HPV results from stored SP samples.

EXPERIENCE OF AN ITALIAN SCREENING PROGRAM ON HPV-DNA TESTING IN THE FOLLOW-UP OF PATIENTS TREATED FOR HIGH GRADE CIN.

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Objectives: persistent infection by high-risk human papilloma virus (HR-HPV) is a predictive factor for residual/recurrent cervical intraepithelial neoplasia (CIN) in patients with a treated CIN2-3 (1).

Aim of this study is to evaluate the efficacy of HPV-DNA testing to detect residual/recurrent disease after loop electrosurgical excision procedure (LEEP) in patients diagnosed with high grade intraepithelial lesion, verifying its potential impact on an organized screening program.

Methods: 148 patients underwent LEEP: 60 were CIN2 and 112 CIN3.

They were followed, as indicated in the regional screening protocol of Emilia-Romagna, with cytology, HPV-DNA testing and colposcopy at 6th month (2).

Then patients were followed with cytology and colposcopy after 12 months if the three tests were negative and every 6 months for three times if one or more tests were positive.

Residual or recurrent CIN2+ were histologically proved.

75/148 patients (50,7%) had negative HPV testing at sixth month (28 CIN2 and 73 CIN3 as initial diagnosis). No residual/recurrent CIN2+ were identified during the follow up.

73/148 (49,3%) had positive HPV testing at sixth month (32 CIN2 and 39 CIN3). 8 high grade CIN were found during the follow-up: 1 CIN2 and 3 CIN3 at 6th month, 1 CIN2 and 2 CIN3 at 12th month, 1 CIN3 at 18th month.

Conclusions: this study shows the high sensibility and negative predictive value of HPV-DNA testing. Indeed, all the high-grade residual or recurrent lesions were in the positive HPV-DNA group at 6th month, being either cytology and colposcopy negative or positive.

The results justify a new follow-up protocol with two different approaches based on the HPV-DNA testing at 6th month. The negative HPV-DNA women could be followed after one year with HPV testing and cytology. The positive HPV-DNA women could undergo cytology and colposcopy every 6 months for two years.

A new protocol based on HPV-DNA testing reduces patient controls and improves organization and quality of the screening program.

THE PERFORMANCE OF APTIMA HPV AND APTIMA HPV 16 18/45 GENOTYPE ASSAY

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Objectives: HPV testing has been shown as a sensitive and specific method for the selection of women at risk in primary screening for cervical cancer. However, the test used for this purpose has to have a very good clinical sensitivity and specificity. Furthermore, different techniques for the management of high-risk (HR) HPV positive women in primary screening are a matter of debate. One of the possible approaches might be HPV typing for the most oncogenic types. In this study we compared the performance of new E6/E7 HPV mRNA based test for detection of HPV 16, 18 and/or 45.

Methods: Women (n=72, mean age 28.6 years, range 18-45 years) referred to Center of Gynecological Oncological Prevention for abnormal cytological findings were examined by an expert colposcopy. A cytological smear for cytology analysis and a sample for HPV detection (PreservCyt) were collected; a punch biopsy was taken for histological analysis. All samples were analyzed for HR HPV DNA by Hybrid Capture 2 (HC2, Qiagen, Inc.) and for HR HPV mRNA by the APTIMA HPV (AHPV, Gen-Probe Incorporated) Assay. Furthermore, samples were tested with the APTIMA HPV 16 18/45 Genotype Assay (AHPV-GT), which allows for additional typing of HPV 16, 18 and/or 45. The type specificity was confirmed by the Linear Array (LA, Roche Diagnostics) test.

Conclusions: Overall agreement between HR HC2 and AHPV tests was substantial (92%, Kappa value= 0.6566). The sensitivity for the detection of CIN3+ was identical for AHPV, HR HC2 and cytology (ASC-US+) (98%), for CIN2+ 96%, 98% and 95% for cytology, HR HC2 and AHPV. LA detected 42 samples positive for HPV 16, 5 for HPV 18 and 2 for HPV 45. AHPV-GT detected HPV 16 in 39 of samples and HPV 18/45 in 5 of the 7 samples, and also detected HPV 18/45 in 2 additional samples negative for HPV 18 and 45 on LA.

P 3-14

CLINICAL UTILITY OF HPV TESTING IN LSIL – A REVISION**Saldanha C; Sousa C; Gonçalves V***LAP-Laboratório de Anatomia Patológica, Lda. Porto, Portugal*

Objectives: Evaluate new generation DNA and RNA tests, like the Roche COBAS HPV, the Abbott Realtime HR HPV, Cervista™ and the Aptima HPV (in the new Panther platform), comparing it with Hybrid Capture 2. The high prevalence of HPV limits the usefulness of HPV DNA testing in deciding how to manage LSIL. Within the scope of this study the Aptima HPV GT will be performed as a second, follow-up test that is performed on samples positive for AHPV.

Currently approx 22000 samples per year of liquid based cytology (PreservCyt) are processed at our laboratory, of which approx 430 are LSIL cases. Currently at LAP, HPV testing is performed in women with ASCUS PAP results or in co-testing primary screening. Usually, HPV testing is not done for women with a Pap test diagnosis of LSIL or HSIL unless in the cases of co-testing. A potential better specificity of the new DNA and RNA tests, comparing to HC2, due to the lack of cross-reactivity with low risk types could be (or not) sufficient to be useful in LSIL triage.

Methods: A maximum of 100 consecutive PreservCyt samples diagnosed with LSIL will be selected from the routine screening at LAP. Samples will be processed using the protocol determined by the respective supplier. All results will be compared with routine cytology and histology data generated for that sample. Information regarding the evaluation sample, patient information and data regarding other clinical samples results relating to that patient will be taken from the laboratory database to allow the clinical significance of the results to be assessed. All data stored for the evaluation will be anonymised.

The gold standard is histologically confirmed cervical intraepithelial neoplasia (CIN) grade 2+ or 3+. Sensitivity and specificity of each of the HPV screening tests will be calculated based on disease defined as CIN 2+.

The genotyping assays will be correlated with each other based on the HPV types detected in each assay.

Conclusions: At the present time, only the Abbott Realtime HR HPV test was performed with all samples and we are still collecting data from the other tests. The positivity rate for the Abbott RealTime HR HPV test is 69%. To evaluate this result is important to know the Hybrid Capture 2 results, however, we can say that we expect to obtain higher positivity rates in that test.

P 3-15

HPV DNA TESTING AND TYPING IN A COHORT OF 450 WOMEN IN GREECE**Chrysagi A, Trigoni C, Chrysagi D, Charvalos E, the IASO Group***IASO Maternity Hospital, Kifissias Av. 37-39, 151 23 Maroussi, Athens Greece*

Objectives: Prevention programs for cervical intraepithelial neoplasia, which are mostly based on cytological examination have achieved a 22% reduction in incidence and mortality of invasive cervical cancer but still with limitations due to low sensitivity and the false negative rates reported by numerous studies. HPV detection and typing seems to be an important test enhancing diagnosis and follow-up of patients. The aim of this study was to evaluate the prevalence of HPV high- and low-risk human papilloma virus in a cohort of women by using molecular detection and typing.

Methods- Results: Four hundred and fifty women, aged from 19 to 50 years, have been tested for the presence of HPV in cervical samples, by using the Linear Array method (ROCHE Molecular Diagnostics). The method includes the detection of 13 high-risk and 24 low-risk HPV types. The study was carried out during two years. When colposcopy was positive and the test negative, cross checking was performed by home-made PCR and RFLPs.

Out of 450 cervical samples, 254 have been found positive for HPV. High-risk HPV types (HR-HPV) were detected in 55 women, Low-risk HPV types (LR-HPV) in 78 and High+Low risk in 121 women samples. Among the high-risk-only HPV group, 32 shared DNA sequences of the 16 and 5 shared the 18 subtypes. Typing of the mixed group of subtypes detected in 50 samples the G16, in 13 samples the G18, in 30 the G31, in 12 the G33 and in 16 samples the G45 respectively. Co-infection with two strains, either high- and/or low risk, was detected in 52 samples, with three genotypes in 40 samples and with four or more genotypes in 15 samples. Three samples with colposcopy HSIL findings but negative for high-risk strains were retested by PCR. One was positive and two had different low-risk HPV types.

Conclusions: This was a pilot study accomplished in our laboratory. By using the linear array method we detected high- and low-risk HPV types in women. The most prevalent HPV types were the low-risk ones independently of colposcopy findings. Interestingly, in most of the cases, high- and low-risk types have been detected in the same sample. This typing profile seems to be the most prevalent. We aim in the upcoming future to co-evaluate clinical, cytological and colposcopy findings with the results of Linear Array Method, performed in our laboratory, in the diagnosis of HSIL and also to correlate any immunohistochemical patterns (e.g. p16 and Ki-67 expression) with HPV status in cervical biopsies or cervical conization material performed after a diagnosis of CIN and/or HPV.

CERVICAL LIQUID-BASED CYTOLOGY IN A REFERENCED AMBULATORY OF LOW-RESOURCE POPULATION IN SÃO PAULO COUNTY. ASSOCIATION WITH VAIN AND VIN. DATA FROM RODEO STUDY.

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Objectives: To introduce SurePath liquid-based cytology (LBC) in routine of women submitted to the gynaecological examination at Hospital das Clínicas of Faculty of Medicine, São Paulo State University

Methods: This analysis is part of a cross-sectional study carried out at a large public hospital attending predominantly low-resource population in São Paulo County. This is part of RODEO study that intends to evaluate the performance of LBC, manually screened, or screened by automation. The rationale of this study was to introduce LBC test for women referred to gynecology examination for different reasons (previous abnormal PapTest, follow up of treated cervical lesion, ecc) as well as to women examined for routine screening proposals, and compare the cytological results to the biopsy diagnosis. This study was supported by BD-Brazil.

Conclusions: From 1866 patients, 1423 (76,3%) cases were negative and 443 (23,7%) positive for any cellular alteration. Age of patients ranged from 12 to 86 years. 798 patients were from high risk ambulatory and 1068 from regular screening ambulatory. Abnormal cytology results were observed in 31% of the patients in the first group and 17.4% in the second. ASC-US was found in 13% of the cases, 0.32% presented ASC-H, 0.58% presented AGC, 7.49% presented LSIL and 2.25% presented HSIL. 337 patients were biopsied. We found 7 cases of invasive carcinoma (5 Adenocarcinomas- 1 adenocarcinoma, 1 adenocarcinoma of the cervix, 3 endometrial adenocarcinoma and 2 squamous cells carcinoma of the cervix). In the ASC-US group we were able to confirm by histology 6 cases of CIN 2, one case of CIN 3, 2 cases of VAIN 2, 1 case of Vulvar Intraepithelial Neoplasia (VIN), 3 and 3 invasive cancers. In the LSIL group we found 10 cases of CIN 2 and 2 cases of VAIN 3. In the HSIL group we were able to find 1 case of squamous invasive carcinoma, 9 cases of CIN 2, 4 cases of CIN 3, 1 case of VAIN 2 and one case of VIN 3. We also found a slightly increased prevalence of histologically confirmed VAIN cases (1.3%). We found LBC was feasible to recognize cervical lesions and also other gynecological malignancies.

BARRIERS TO CERVICAL SCREENING IN WOMEN WHO HAVE EXPERIENCED SEXUAL ABUSE: AN EXPLORATORY STUDY

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Objectives: Childhood sexual abuse is known to be a risk factor for cervical cancer, probably because of early exposure to human papillomavirus (HPV). Women who have been abused may be less likely to attend for cervical screening, which is concerning given their high-risk status. This study aimed to explore screening participation and perceived barriers among women who have been sexually abused.

Methods: A short, web-based survey was developed, asking for basic demographic information, cervical screening history and type of abuse experienced. Two open questions asked about barriers to cervical screening attendance, and suggested improvements to the service. A link to the survey was placed on the website of NAPAC, the National Association for People Abused in Childhood. Women were also invited to participate in a discussion group. Data were collected between June and October 2010.

Conclusions: 159 women aged 18-64 years began completing the survey. 78% had taken part in cervical screening but only 34% had been screened in accordance with the NHS Cervical Screening Programme recommendations. The main barriers to screening that emerged were: 1) negative attitudes to self, including stigma, shame, embarrassment and not being 'normal'; 2) relating to power and control, including vulnerability; 3) sexual victimisation and violation; 4) issues around trust and safety; 5) communication; 6) the impact on coping strategies adopted to deal with the effects of the abuse;. Women had a variety of suggestions for improving screening. These included suggestions around abuse disclosure, sharing control and enabling the women, communication and the procedure itself. Further research is needed to see whether the findings hold true in a more representative sample, and to develop ways to improve screening access for this vulnerable group of women.

PROBLEMS OF, AND PROSPECTS FOR, CERVICAL SMEAR SCREENING IN NIGERIA**Aliyu Samuel Abdulumumuni,***Department of Pathology, University of Abuja Teaching Hospital, Abuja, Nigeria*

Cervical cancer represents a tremendous burden on patients, families and societies; its impact on maternal health is worrisome killing one African woman every 10 minute. However, the low level of awareness, inadequate funding, high prevalence of acquired immune deficiency syndrome and the relative unavailability of trained cytologists for cervical smear screening has clearly undermined the prospects for improved service delivery. With a population of 140 million, Nigeria probably only has about 1,000 histo-scientists and 100 histopathologists with most of these professionals located in tertiary health facilities located in major cities. The annual per capita income in Nigeria is estimated at US\$300. The cost for cervical Pap smear examination is not subsidized in Nigeria and has increased from US\$2-6 to US\$17. The emphasis is on taking a look at the situation in Nigeria while giving a broad yet detailed knowledge of prevalent reasons for failure of cervical smear implementation. Successful interventions exist in Nigeria to bring a credible system of assess to essential cervical smear screening services. Included are the community directed approach and the need to increase westernization of the indigenous population by the improvement of education and other social service facilities. Nigeria is a diverse country, and in order to enhance the prospects for cervical smear screening, we must take a regional approach that builds on the culture, strengths and challenges of each of the six geo-political zones.

DENSITY AND PHENOTYPE OF LOCAL IMMUNE CELLS IN VULVAR CANCERS OF THE ANTERIOR FOURCHETTE IN ASSOCIATION WITH THE HPV/P16 STATUS**Reuschenbach M¹, Roos J², Rickert V¹, Sauer M¹, Baldus SE³, Schnürch HG², Petry KU, on behalf of the German Study Group for Colposcopy, von Knebel Doeberitz M¹, Hampl M⁵**

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Objectives: Squamous cell cancers (SCC) of the vulvar anterior fourchette increase in their incidence and occur primarily in rather young women. Our previous analyses indicated that approximately 25% of these tumors are HPV-induced (HPV DNA and p16^{INK4a}-positive) and the remaining tumors either show strong p53 overexpression, which is potentially due to p53 mutations or are negative for HPV/p16^{INK4a} and p53. As it is known that the density and phenotype of tumor infiltrating and surrounding immune cells may influence tumor development and control, we here aimed at characterization of immune cells in vulvar SCC of the anterior fourchette in association with the HPV/p16^{INK4a} and p53 status.

Methods: A total of 105 vulvar SCC located at the anterior fourchette were analyzed by immunohistochemistry for CD3 as pan-T cell marker and Foxp3 as marker for regulatory T cells. The tumors have been previously analyzed for high-risk HPV-DNA by GP5+6+ PCR and Luminex genotyping and p16^{INK4a} and p53 expression by immunohistochemistry.

Conclusions: Intra-tumoral CD3 infiltration was more dense in tumors from younger women ($p=0.006$), tumors surrounded by usual type VIN (0.252) and tumors positive for HPV DNA and diffuse p16^{INK4a} expression ($p=0.010$). The opposite was observed for intra-tumoral Foxp3 infiltration, which was strongest in tumors associated with lichenoid skin disease ($p<0.001$), in more advanced stages ($p=0.005$) and p53-overexpressing tumors ($p=0.144$).

The results indicate that the density and phenotype of local immune cells is associated with the type of vulvar SCC given by HPV/p16^{INK4a} and p53. As shown in other cancer types, both, the density of the immune cells as well as the HPV/p16^{INK4a} status of the tumors may have prognostic relevance, their combined analysis in association with the course of the disease and therapy response might be important also for vulvar cancer patients.

PROGNOSTICATION OF VULVAR CANCER BASED ON P14ARF AND HPV STATUS: MOLECULAR ASSESMENT OF TRANSCRIPT AND PROTEIN

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Objective: To determine the prognostic role of p14^{ARF} in vulvar squamous cell carcinoma (VSCC).

Methods: Immunohistochemistry (IHC) for p14^{ARF} and p53 and fluorescent in situ hybridization (FISH) for *TP53* were performed in 139 cases. HPV genotyping by hybridization method was employed in 100 cases. qRT-PCR for p14^{ARF} and p53 transcript assessment was performed in 16 cases. All results were correlated with clinical-pathological variables.

Conclusions: IHC analysis found p14^{ARF} and p53 positivity in 16,4% and 53% cases respectively. p14^{ARF} expression presented significant association with shorter cancer specific survival ($p=0.04$), disease-free survival (0.02), presence of perineural invasion ($p=0.037$), vascular invasion ($p=0.047$) and lymph node metastasis ($p=0.031$). Also, p14^{ARF}-positive HPV-negative cases presented the shortest cancer specific survival ($p=0.03$) and disease-free survival ($p=0.04$). HPV infection was detected in 32.8% of the cases and HPV 16 was the most prevalent type. Viral infection was more prevalent in those poorly differentiated tumors ($p=0.032$). qRT-PCR demonstrated that *CDKN2A* (p14^{ARF}) was more expressed in tumor samples compared with paired non-cancerous samples ($p<0,001$). *TP53* transcript, on the other hand, was more expressed in normal samples ($p<0,001$). FISH demonstrated 4 cases with deleted *TP53* (6,3%). p14^{ARF} represents important marker of poor prognosis in VSCC. p53 and HPV infection did not represent prognostic importance. Further research assessments of p14^{ARF} may bring important contribution due to its relation with poor outcome and tumor aggressiveness. Mainly due to p14^{ARF}'s relation with lymph node metastasis, the IHC evaluation of this marker appears crucial to establish prognosis and to help determining the most suitable surgical procedure.

HUMAN PAPILLOMAVIRUS (HPV) INFECTION IN ORAL CAVITY OF HIV-POSITIVE SUBJECTS

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Objectives: This study aims at assessing the prevalence of Human Papillomavirus (HPV) infection and viral genotypes affecting the oral cavity of HIV-positive subjects.

Methods: HPV testing was performed on 111 oral-pharyngeal swabs belonging to a population of HIV-positive people. Among these, 83.8% (93/111) were male and 16.2% (18/111) female. The median age was 43 years (IQR:38-49). DNA was extracted using a commercial kit (*NucliSENS[®] miniMAG[®]*, bioMérieux bv, France). HPV-DNA was detected through a specific nested-PCR assay amplifying a 150bp segment of ORF L1 using the two primer pairs MY09/MY11 and GP5+/GP6+. The PCR products of approximately 150bp from the revised L1 consensus PCR assays underwent genotyping through automated DNA sequencing. All statistical analyses were performed using OpenEPI software, version 2.3.1.

Conclusions: The overall prevalence of HPV infection among subjects analyzed was 27.9% (31/111; 95%CI:20.2-36.8%). The median age of HPV positive people was 45.5 years (IQR:36-49.5), 80.6% (25/31; 95%CI:64-91.8%) were male and 19.4% (6/31; 95%CI:8.2-36%) were female. Amplified DNA from 25 positive samples underwent DNA sequencing and 16 distinct HPV genotypes were detected, 7 of which belonging to the mucosal HR-HPV types (HPV-16,-31,-33,-35,-56,-58 and -59) and 8 to the mucosal LR-HPV types (HPV-6,-32,-40,-61,-62,-67,-70 and -72). HPV-6,-32 and -58 were the prevalent mucosal genotypes, each identified in 3/31 (9.7%, 95%CI:2.5-24.1%) samples. Two oral-pharyngeal swabs (6.4%) showed the cutaneous genotype HPV-7. These data confirm HPV detection in oral mucosa of HIV-positive subjects. The presence of viral genotypes in this site may be associated with transient infections, similar to those affecting the genital level, or persistent ones, with an increasing risk of progression to symptomatic clinical forms. Further investigations are needed to clarify the ways of HPV acquisition. Long-term longitudinal studies could also clarify the natural history of HPV infection in the oral cavity of HIV-positive subjects.

P 6-2

RECURRENT GENOMIC ALTERATIONS AND HIGH RISK HPV WITH IMPACT ON PROGNOSIS IN OROPHARYNGEAL SQUAMOUS CELL CARCINOMA

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Objectives: The whole picture of chromosomal alterations in oropharyngeal cancer and their prognostic implications have not been well studied. We aimed to identify chromosomal changes and high risk HPV infection associated with prognosis in oropharyngeal cancers.

Methods: For this purpose, we analyzed copy number alterations in the discovery set of 32 oropharyngeal cancers using oligoarraycomparative genomic hybridization and validated the recurrently altered regions (RARs). High risk HPV in situ hybridization was performed to detect HPV infection.

Conclusions: The positive rate of high risk HPV in situ hybridization was 28.1% (9/32). A total of 10 RARs were defined in the discovery set. Among them, gains on 16p11.2 and 17q12 showed significant associations with high risk HPV infection ($P=0.019$ and $P=0.019$, respectively). There was statistical significant association between gains on 16p11.2 and 17q12 with high risk HPV positivity and the disease-specific survival in patients with oropharyngeal cancer ($p=0.001$). Our findings will help to elucidate molecular mechanisms underlying tumorigenesis of oropharyngeal cancer with high risk HPV infection and to develop clinical tool for predicting prognosis of oropharyngeal cancer.

P 7-1

COST-EFFECTIVENESS OF SHORT-TIME HIGH-RISK HPV TESTING BY SELF-SAMPLING IN SWEDISH POPULATION BASED SCREENING FOR CERVICAL CANCER

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Objectives: Human papillomavirus (HPV) testing is not used for primary cervical cancer screening in Sweden and the cost-effectiveness is unclear. Our objective was, from a societal perspective, to evaluate the cost-effectiveness of testing for high risk (HR)-HPV using self-sampling of vaginal fluid in Swedish population based cervical cancer screening program.

Methods: We used a Markov model to simulate a cohort of women between the age 23 until 85 and estimate outcomes associated with the following screening strategies: 1) Short-time repeat high-risk HPV testing by self-sampling of vaginal fluid (HPVIR method) followed by colposcopy with biopsy after two consequent HR-HPV positive tests; 2) HR- HPV testing as a reflex test on women with minor cytological abnormalities; 3) Conventional cytology followed by colposcopy with biopsy including a repeat cytological investigation in all women with any sign of atypia; 4) Repeat Pap smear on women with atypical squamous cells of undetermined significance (ASCUS), low-grade squamous intraepithelial lesion (LSIL) followed by colposcopy with biopsy on women with two consequent ASCUS-LSIL tests. We used country specific data on cervical cancer risk and mortality from population based registries, HR-HPV screening clinical trials and cost data from the Swedish health care service. Outcome measures included quality-adjusted life years (QALYs), lifetime risk of cervical cancer, disease related death and incremental cost-effectiveness ratios (ICERs). Sensitivity analysis was performed varying key parameters over a significant range of values to identify the cost-effective screening strategy.

Conclusions: Base case result showed that screening with HPV self-sampling between ages 23-60 in 3-5 years intervals slightly increased quality adjusted life expectancy with a lower average life time cost of € 788 to € 1 030 compared to the three alternative screening strategies which all were dominated. In conclusion, it is indicated that screening with HPV self-sampling of vaginal fluid is cost-effective for Swedish population-based cervical cancer screening program with potential to improve health benefits at a lower cost than HPV reflex, Conventional cytology and Repeat Pap smear.

BURDEN OF DISEASE DUE TO NON-CERVICAL HPV-RELATED CANCERS IN SPAIN**Castellsagué X¹, Cerdán J², Cortés J³, Hurtado P⁴, Morillo C⁵, Quer M⁶, Salinas J⁷***(1) Cancer Epidemiology Research Program, Institut Català d'Oncologia, L'Hospitalet de Llobregat, Spain**(2) Department of Digestive Surgery, Hospital Clínico San Carlos. Madrid, Spain**(3) Senior Consultant on Gynaecologic Oncology, Private Practice, Palma de Mallorca, Spain**(4) IMS, Barcelona, Spain (5) Medical Department Sanofi Pasteur MSD, Spain**(6) Department of Otolaryngology, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain**(7) Department of Urology, Hospital Clínico San Carlos, Madrid, Spain*

Objectives: To estimate the burden of non-cervical HPV-related cancers in Spain in 2009 and the cost of treatment of these cancers from both the National Health System and social perspectives during five years' follow up period.

Methods: Data needs were identified by conducting a bibliographic search on management of pathologies, epidemiologic data and direct and indirect costs of oral cavity and pharyngeal, anal, vulvar, vaginal and penis cancer. Cases attributable to HPV-16 and HPV-18 were calculated by means of the fractions attributable to each type, using estimates with greater evidence in the medical literature and data obtained from interviews with scientists in the field. Cost of non-cervical HPV-related cancers included direct and indirect costs. Direct costs were added to the monetary value of diagnosis, hospitalization and surgery, ambulatory treatment, including chemo and radiotherapy, and follow up costs, together with recurrence treatment. Indirect costs included potential years of work life lost (PYWLL).

Conclusions: Between 30-75% of new annual cases can be directly attributable to HPV-16/18. Direct costs were higher in advanced stages of cancer, with the exception of anal cancer. Indirect costs were 55-75% of the total cost depending on the type of cancer. Total costs of non-cervical HPV-related cancers were estimated at 355,748,285€. The fraction attributable to HPV was 137,751,910€ and the fraction attributable to HPV-16/18 was 56,758,762€. There was the difficulty in the estimation of the underlying indirect costs as well as the intangible costs of these cancers concerning quality of life and psychological impact on patients and their families. These tumors could be of interest in Public Health and primary prevention strategies. Currently established vaccination programs for the prevention of cervical cancer may have an eventual impact in the reduction of the incidence and burden of non-cervical HPV-related cancers.

A PRELIMINARY HEALTH AND ECONOMIC ANALYSIS OF VACCINATING BOYS AND GIRLS WITH QUADRIVALENT HUMAN PAPILLOMAVIRUS VACCINE IN FRANCE.**Bresse X¹, LARGERON N¹, ROZE S², MARTY R².***1: Sanofi Pasteur MSD, Lyon, France**2: HEVA, Lyon, France.*

Objectives: The burden of Human Papillomavirus (HPV)-related cancers in men is rising in Europe, and represents around 30% of the overall HPV-related burden in both genders). Vaccinating girls has an indirect protective impact on males but does not eliminate the entire HPV burden. Study objectives were to have a first estimate of the additional health and economic benefits in France of vaccinating males and females as compared to females-only.

Methods: A previously described model was adapted to estimate the epidemiological and economical impact of vaccinating boys and girls with a quadrivalent-HPV6/11/16/18 vaccine on HPV-related diseases (anal, penile, head and neck, vaginal, vulvar, cervical cancers and genital warts). Disease reductions due to vaccination were derived from a US dynamic transmission model. French epidemiological, economical and coverage inputs came from published literature. A 3% discount rate was used. The analysis estimated the incremental economical and clinical benefits of adding a cohort of 14 years old boys to the current vaccination program, i.e. girls aged 14 to 23. Sensitivity analyses were performed to handle uncertainty related to the vaccine's duration of protection, long term cumulative vaccine coverage rates and discount rates.

Conclusions: In France, girls-only vaccination would result in a reduction of 64% in all males HPV-related cancers and 61% of genital warts in males (vs. screening only, at steady state; 100 years, when maximum vaccination effect is reached). Adding a cohort of boys would increase this result to 86% for cancers and 87% for genital warts. Vs. girls-only vaccination, vaccinating boys and girls enables to further reduce by 62% the remaining cancer burden in males: 614 additional HPV-related cancers cases. 13,787 additional genital warts cases would be avoided annually (-65%). Thanks to indirect protection (herd-immunity), vaccinating boys would allow a further reduction of female cancer cases (-214, -39%). Over the study period, 17.2% additional direct medical costs would be saved (-309M€). Discount rate was the most impactful parameter. This first analysis showed that vaccinating boys in addition to girls would have the potential to reduce significantly HPV disease burden in males, and help decreasing the remaining burden in females.

P 7-4

A PRELIMINARY HEALTH AND ECONOMIC ANALYSIS OF VACCINATING BOYS AND GIRLS WITH QUADRIVALENT HUMAN PAPILLOMAVIRUS VACCINE IN SWITZERLAND.

Bresse X¹, Largeron N¹, Roze S², Marty R².

1: Sanofi Pasteur MSD, Lyon, France 2: HEVA, Lyon, France.

Objectives: The burden of HPV-related cancers in men is rising in Europe and represents around 30% of the overall HPV-related burden in both genders. Vaccinating girls has an indirect protective impact on males but does not eliminate the entire HPV burden. Study objectives were to have a first estimate of the additional health and economic benefits in Switzerland of vaccinating males and females as compared to females-only.

Methods: A previously described model was adapted to estimate the epidemiological and economical impact of vaccinating boys and girls with a quadrivalent-HPV6/11/16/18 vaccine on HPV-related diseases (anal, penile, head and neck, vaginal, vulvar, cervical cancers and genital warts). Disease reductions due to vaccination were derived from a US dynamic transmission model. Swiss epidemiological and economical data were used. Theoretical vaccine coverage rates were estimated based on data available from two cantons. A 3% discount rate was used. The analysis estimated the economical and clinical benefits of adding a cohort of boys aged 12 years old to the current vaccination program, i.e. girls aged 11 to 26. Sensitivity analyses were performed to handle uncertainty related to the vaccine's duration of protection, cumulative vaccine coverage rates and discount rates.

Conclusions: Girls-only vaccination would result in a 62% reduction of all males HPV-related cancers and 61% of genital warts in males (vs. screening only, at steady state; 100 years, when maximum vaccination effect is reached). Adding a cohort of boys would increase this result to 83% for cancers and 84% for genital warts. Vs. girls-only vaccination, vaccinating boys and girls enables to further reduce by 57% the remaining cancer burden in males: 53 additional HPV-related cancers cases. 625 additional genital warts cases would be avoided annually (-60%). Thanks to herd-immunity, vaccinating boys would allow a further reduction of female cancer cases (-23, -34%). Over the study period, 18% additional direct medical discounted costs would be saved (-32M€). This first analysis showed that vaccinating boys and girls would have the potential to reduce significantly HPV burden of disease in males, and help decreasing the remaining burden in females. Robust coverage data will be needed in the future to fine tune analyses.

P 7-5

A COMPARATIVE COST-CONSEQUENCE ANALYSIS OF BIVALENT AND QUADRIVALENT HUMAN PAPILLOMAVIRUS VACCINES IN FRANCE.

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1: Sanofi Pasteur MSD, Lyon, France 2: HEVA, Lyon, France.

Objectives: Two Human Papillomavirus (HPV) vaccines (bivalent HPV 16/18 and quadrivalent HPV 6/11/16/18) are currently available in France with differences in terms of disease protection approved in license. Study objectives were to compare the impact of HPV vaccines on disease and cost reductions within different scenarios, in France.

Methods: A model previously described was adapted to the French setting. The impact of vaccinating girls aged 14 to 23 years with the quadrivalent vaccine (QV) was compared to the one with the bivalent vaccine (BV). The respective clinical endpoints reductions and direct medical costs saved were estimated in 8 scenarios to account for differences in licensure indications, proven and/or expected benefits on non cervical HPV-related diseases and efficacy against non-vaccine HPV types. Additional sensitivity analyses were performed to handle uncertainty related to vaccine duration of protection, health-care costs and discount rates. Results are presented in a steady-state situation (at 100 years) for epidemiological reductions and over the study period for health costs saved.

Conclusions: Scenario analyses considering clinical endpoints included in license estimated that both vaccines would allow avoiding annually between 1,336 and 1,770 cervical cancer (CC) cases (HPV 16/18-related). In addition the QV would enable to avoid between 638 and 831 vaginal, vulvar and anal cancers as well as from 53,400 to 72,000 genital warts for both genders. Additional CC cases avoided thanks to cross-protection were estimated between 1 and 174 with the QV and from 2 to 284 with the BV. Scenarios including all cancers estimated that between 1,774 and 2,382 head and neck and penile cancer cases (HPV 16/18-related) would be avoided annually. Only the QV would help reducing the burden of recurrent respiratory papillomatosis (HPV 6/11-related). Over the study period, the QV and the BV would allow savings from 820M€ to 1,603M€ and from 285M€ to 935M€, respectively. Economical added value of cross-protection appears minor compared to genital warts prevention: 50% of costs saved thanks to genital warts prevention versus only 4% savings due to cross-protective effects. Any decision regarding HPV vaccine choice should incorporate the full impact of each vaccine on HPV diseases beyond cervical cancer.

CERVICAL CANCER IN CROATIA: THE IMPORTANCE OF PUBLIC EDUCATION CAMPAIGNS FOR CERVICAL CANCER PREVENTION

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In Croatia, invasive cervical cancer (ICC) continues to be the eighth most common malignancy in women. In 2009, there were 369 newly detected ICC cases and 114 related deaths (age-standardised rates for incidence -14.5/100 000 and mortality - 3.9/100 000). 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 2012, 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.

ATTITUDE TOWARD HUMAN PAPILLOMAVIRUS VACCINATION AMONG COLLEGE STUDENTS

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Objectives: In Japan, more than 2,500 women die of cervical cancer every year. Death from cervical cancer could be preventable with cancer screening and human papillomavirus (HPV) vaccination. HPV-16/18 vaccine has been approved and used in Japan since 2009. It would be best for young girls to receive HPV vaccination before their first intercourse, around pre- or early adolescence. We aimed to elucidate how young women think about receiving HPV vaccination.

Methods: The investigation was conducted at Yokohama National University and Yokohama City University as a transitional research. Newly enrolled female students were recruited to participate in this study. The students who gave consent filled out the questionnaire to assess their attitude toward HPV vaccination. A total of 644 students participated, and 630 participants completed the questionnaire. The mean age of participants was 18.7 years (range=18-46 years, SD=2.62, median=18 years). A total of 102 participants (16.2%) had experienced their first intercourse at the mean age of 17.0±2.02 years.

Conclusions: A total of 312 participants (49.5%) recognized that HPV vaccine was approved and being used in Japan, but only 34 participants (5.4%) had received HPV vaccination. The mean age of participants at the time of their vaccination was 17.8±0.77 years. 134 participants (21.3%) mentioned that they were too young to receive HPV vaccination, and 514 participants (81.6%) would consider HPV vaccination in the future. Today, half of 18-year-old girls have sexual experience in Japan, so it is important to promote knowledge about HPV vaccination among young girls.

P 8-3

ASSESSMENT OF PROGRAMS FOR CERVICAL CANCER PREVENTION ADMINISTERED BY LOCAL GOVERNMENTS AND LOCAL COMMUNITIES IN KANAGAWA PREFECTURE, JAPAN

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Objectives: The increasing incidence of cervical cancer (CC) among young women is a serious social problem in Japan. This study assessed the effectiveness of programs for CC prevention administered by local governments and local communities in Kanagawa prefecture. The programs aim at achieving a high prevalence of HPV vaccination in girls aged 13-17 years and improving the low participation in the Pap test.

Methods: This study was partly subsidized by the Ministry of Health, Labor and Welfare. During the first year of a 3-year sequential research plan related to CC prevention, the basic data were provided by local governments including a capital Yokohama City and the Yokohama City University Hospital, and analyzed.

Results: Among 33 local governments, the "call-recall" approach system to increase CC screening uptake differed considerably, and the participation in CC screening varied between 15% and 35%. However, although the HPV vaccination programs for young girls had begun in 2011 in Yokohama City, it was functioning so well that the vaccination prevalence had reached about 70%. On the other hand, it was revealed that the uptake of CC screening was 51.6% even among medical associates who had catch-up HPV vaccination in our hospital, and they wanted more information about CC screening and how it was administered before they would decide to have it.

Conclusions: Some different strategies are required to achieve the effective CC prevention among women who were not included in the HPV vaccination program in Japan. Our future research plan includes the concept that the social network system might play a crucial role in encouraging women to undergo CC screening.

P 8-4

ATTITUDES TOWARDS HPV TESTING AND SELF-SAMPLING IN THE CERVICAL SCREENING CONTEXT: A QUALITATIVE STUDY OF HINDU WOMEN IN LONDON

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Objectives: Previous studies have highlighted particular concerns regarding HPV testing and self-sampling among Asian women with regard to doing a test themselves and having a test for a sexually transmitted virus that causes cervical cancer (HPV). The objective of this study was to explore further the perceptions of Hindu women regarding cervical screening, HPV testing and HPV self-sampling.

Methods: Women were invited to complete surveys at an open 'house' week-end event at the Shri Swaminarayan Hindu Temple in Neasden. This Temple is attended by a large proportion of the Hindu community in London and the South East. The survey included questions related to age, ethnicity, screening history etc. Women were also invited to participate in discussion groups. Purposive sampling and snowballing techniques were used to recruit to the discussion groups. The study was approved by the UCL Research Ethics Committee.

Two focus groups of 5 and 7 women aged between 31 and 66 years of age were held with a third group planned. They started with a general discussion about cervical cancer and HPV. Women were then given information about HPV and HPV testing. The discussion then moved on to issues around these topics. Finally they were shown two self-sampling devices, the Digene and the Evalyn self-samplers with the corresponding instructions for use.

The focus groups were recorded and transcribed verbatim and analysed using Framework Analysis with the data summarised under thematic headings.

Conclusions: Initial analysis shows 135 women filled in the survey, age range recorded was 14-85 years (mean age 38.5 years). 12% of those between 25 and 65 years said that they had never had a smear test, 10.5% were last screened more than 5 years ago.

The data analysis is not yet fully complete, final results will be presented.

MULTICENTER STUDY OF CINII/III IN THE VALENCIAN COMMUNITY (VC) (SPAIN) IN 2008: EPIDEMIOLOGICAL, CYTOLOGICAL AND HPV, COLPOSCOPICAL, TREATMENT AND FOLLOW-UP DATA AT 6 AND 12 MONTHS

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Gynaecological departments of 1 Fundación IVO, 2 Hospital de la Ribera, 3 Hospital Arnau de Vilanova. Valencia, Spain

Objective : This working group was concerned to obtain for their study, the data of cases of CINII/III diagnosed in 2008 in the Public Network Hospital of the VC (Spain)

Material and methods Database was sent to all public hospitals in the CV. Information was collected on each centre (population dependent), diagnostic cytology/histology, HPV typing, type and biopsy of cone, and controls at 6/12 months. Participation rate of hospitals: 50.09% of the CV. Pap screening rate: 9.69%; pathological cytology rate: 6.78%. There were 431 cases of CIN II / III: 411 valid for the study.

Cytological reports that ended with a diagnosis of CIN II(+): 20% LSIL(82% CH2- positive HR, 28% positive PCR HPV16), 67.5% HSIL(94% CH 2-positive HR, 43% positive PCR VPV16), 7.5% ASC-US (94% CH 2-positive HR, 23% positive PCR HPV16) ASC.H 5% (75% CH 2-positive HR, 56% positive PCR HPV16).

Colposcopy: 302/411colposcopies meet minimum descriptive requirements: 262(87%) are satisfactories. 21(7%) Normal Colposcopy, 112(37%) MinorChanges and 169(56%) Major Changes.

Treatment: Cone/Loop on 369/411, 35 Cones cold, in 7 cases was not known the type of cone. 83(20.67%) cases of affected margins: treatment was performed in 50.3% of them and on 39 occasions, hysterectomy.

Follow-up: 335 cases followed for 6 months post-treatment, 61(18.2%) pathological cytology, 93 colposcopies, 23 biopsies, 10(2.9%) positive. 284 cases followed up at 12 months post-treatment, 32(11.2%) pathological cytology, colposcopy 95, 19 biopsies, 9(3%) positive.

Conclusions 929,322 women of 20 to 65 years dependent population in 13 hospitals belonging to the public network of hospitals in the Valencian Community, is reflected in this project. Pap screening rate was surprisingly low (86,280/929,322 = 9.69%). 8% of abnormal Pap smears ended with a final diagnosis of CIN II +. HPV16: most prevalent virus with cytological diagnosis of HSIL / ASC-H. Major changes (56%) lower than expected. Percentage of affected margins acceptable. High number of hysterectomies in cases of involvement of margins. Good data on persistent/residual disease. 431 CIN II - III / 929,322 women (411 valid cases for study) represent an incidence of 46.37 / 100,000, only on Public Health

THE QIASYMPHONY® DSP AXPH DNA SYSTEM*: FULLY AUTOMATED EXTRACTION OF SUREPATH™ SAMPLES FOR USE IN RCS-DRIVEN HYBRID CAPTURE® 2

Kupfer, C.¹, Lindebaum, K.¹, Marion Schmidt.¹, Lee, Y.¹, Giles, J.², Chendvankar, R.², Gupta, V.², Londono, M.², Chaudhry, M.², Bank, A.², Wallace, J.², Garvin, L.² Cullen, A.², and Sprenger-Haussels, M.¹

¹ QIAGEN GmbH, Hilden, Germany and ² QIAGEN INC, Gaithersburg, MD, USA

Objective: In Europe, the *digene*® HC2 High-Risk HPV DNA Test (HC2 HPV test) is validated for use with cervical specimens collected in SurePath liquid based cytology media (LBC). The approved HC2 specimen conversion protocol requires labor intensive centrifugation and manual decanting to process samples. QIAGEN has developed and validated an automated LBC extraction procedure based on pH-driven anion exchange sample preparation chemistry (AXpH), automated on the QIASymphony SP (QSSP) platform. This system is available as a Clinical Sample Concentrator and is CE-IVD certified. This study provides results from verification performed on a system consisting of AXpH chemistry and protocol, automated on the QSSP, and the HC2 HPV test, automated on the Rapid Capture® System (RCS).

Methods: To compare the approved manual HC2 method with the investigational automated system, specimens were processed with both methods and analyzed using the HC2 HPV test on the RCS. Testing was conducted using either individual, residual, de-identified, clinical SurePath™ samples, pools of residual, de-identified, clinical SurePath™ samples, or cell culture spiked into SurePath medium. Linearity was verified using different HPV positive cell cultures in SurePath media. We verified intermediate precision between instruments and days of the system under investigation. Cross-contamination testing on the QSSP was verified for 5 consecutive days. Further studies were conducted with NILM and ASC-US SurePath samples.

Conclusion: We successfully evaluated the performance characteristics for SurePath specimens processed on the QSSP/RCS HC2 system for linearity, repeatability, cross-contamination, and intermediate precision between instruments, between days, and between instruments and days. Workflow comparison of the reference method with the investigational method using clinical specimens gave an agreement of 94% for the ASC-US population. The modified automated workflow increases process control and reduces the number of repetitive motions associated with the manual workflow.

*CE-IVD marked for in vitro diagnostic use in Europe.

HPV FOCUS 2010 - EVALUATION OF GENITAL DISEASES RELATED TO HPV IN PORTUGUESE WOMEN.

Moutinho JM, Da Silva DP, Moutinho JA, Cunha MJ, Gomes A.

Objective: Assessing the impact of genital disease associated with human papillomavirus (HPV), through data collection regarding its diagnosis, therapeutic approach and follow-up of pre-cancerous lesions of the cervix and genital warts diagnosed in private clinical practice Gynecology / Obstetrics in Portugal.

Methods: This evaluation was performed through a questionnaire involving a total of 118 gynecologists who, from October 2010 to June 2011, collected a minimum number of cases by type of diagnosis (5 cases of histological HPV lesions even with no dysplasia, 5 cases of external genital lesions, 5 cases of cervical dysplasia CIN 1, 5 cases of pre-cancerous lesions of the cervix (CIN 2/3)). These cases were diagnosed in the 12 months prior to the beginning of the data collection. All cases of lesions, with or without dysplasia, of the cervix with HPV positive but without histological confirmation and vulvar papillomatosis were excluded.

Results: Of the 983 diagnosed cases, 813 were considered (83%) for analysis, far above what is usual in studies with similar methodological design, which allowed us to conclude the key trends with statistical validation. On average, there was a predominance of cases between 32 and 36 years (although the virus infects women of all ages, even the older age groups), with onset of sexual activity to 18/19 years who had, on average, two partners and one or two pregnancies/births, and the age of the 1st pregnancy between 25 and 27 years old. More than half of these patients had never smoked and one in eight women has a history of HPV-associated disease.

In total, 16% were diagnosed with external genital lesions, 20% with HPV lesions, 38% with a CIN1 and 26% with a CIN2/3. It was observed that 42% of lesions CIN2/3 occurred in women with an average of 36 years old (Minimum 18 years - Maximum 71 years), who smoke 13 cigarettes/day, and already had two births. Out of these 42%, 6% had a diagnosis of STI (sexually transmitted infection) in the last 24 months and on 36% HPV was detected, which revealed positive in 83% of these cases. 85% of women with lesions CIN 2/3 have been treated, in most cases through Excisional method [Electrosurgery (62%) and laser (25%)], 12% were referred and 2% did not receive any treatment. In 6% of the women with CIN 1 lesions, a STI was diagnosed in the last 24 months and on 49% HPV was detected, which revealed positive in 80% of cases. 68% of women were treated; of these, 33% through Excisional method [Electrosurgery (27%) and Laser (6%)] and 67% by destructive method [Laser (29%), cryotherapy (24%) and Electrocoagulation (14%)], but only 5% were referred and 27% untreated. The average age of these women is 33 years old (Minimum 18 years - Maximum 63 years).

Women with External Genital Lesions were 32 years old on average (Minimum 15 years - Maximum 73 years), 14% had a diagnosis of STI in the last 24 months and in 23% HPV was detected, which was positive in 57% of cases. 93% of these women have been treated, in most cases, the destructive method [chemical (54%) and laser (25%)], but only 1% were referred and 6% untreated.

Regarding women with HPV Histological lesions, the mean age was 33 years old (Minimum 16 years - Maximum 54 years), on 10% a STI was diagnosed in the last 24 months and 43% had HPV detection, revealing positive on 71% of cases. 52% of these women have been treated, in most cases using the destructive method [Electrocoagulation (38%) and Laser (34%)], but only 4% were referred and 44% untreated.

Conclusions: Regardless of the degree of lesion, most women are treated, ie, they receive the corresponding treatment. The method of treatment which is applied corresponds to what is determined to be standard of care for the respective degree of severity. Nevertheless, 12% of women with HPV Histological lesions and 33% of women with CIN 1 were treated with the Excisional method, which seems excessive

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| R. Konno | Japan | SS 8-5 | R. Sankaranarayanan | France | MSS 3-7 | C. White | Ireland | MSS 13-6 |
| Z. Kruzicova | Czech Republic | FC 4-10 | R. Sankaranarayanan | France | SEM 1-15 | C. Whitehead | USA | FC 8-7 |
| E. Kudela | Slovak Republic | FC 5-6 | R. Sankaranarayanan | France | SS 1-5 | U. Wieland | Germany | SS 17-6 |
| C. Lacey | UK | SEM 3-2 | R. Sankaranarayanan | France | SS 11-5 | A. Wikström | Sweden | SS 12-1 |
| C. Lacey | UK | SS 7-4 | P. Sasieni | UK | FC 8-1 | T. Wright | USA | MSS 6-1 |
| C. Lajer | Denmark | FC 10-7 | P. Sasieni | UK | SS 18-1 | K. Youens | USA | FC 2-5 |
| G. Lallas | Greece | FC 9-4 | M. Sauer | Germany | SS 13-6 | L.C. Zeferino | Brazil | GC 1-10 |
| N. Largeron | France | SS 7-6 | A. Schache | UK | FC 10-8 | J. Zhai | USA | FC 10-16 |
| G. Lazdane | Denmark | WS 1-1 | C. Schellenbacher | Austria | MSS 2-4 | P. Zubor | Slovak Republic | WS 1-3 |
| G. Lazdane | Denmark | MSS 4-8 | C. Schellenbacher | Austria | SS 12-7 | P. Zubor | Slovak Republic | FC 4-6 |
| G. Lazdane | Denmark | SS 11-2 | R. Schulze-Rath | Germany | W 1-5 | | | |
| J.L. Lefebvre | France | SEM 1-1 | R. Schulze-Rath | Germany | SS 12-3 | | | |