SEM 1-1

CONSIDERATIONS FOR PRIMARY AND SECONDARY PREVENTION OF HPV-DRIVEN HEAD AND NECK CANCER

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Prevention of HPV-associated cancers can take two forms—one through prevention of infection via prophylactic HPV vaccination, and one through interruption of disease progression through early identification (i.e.: screening) and treatment. Primary prevention via vaccination seems promising, as a proof-of-principal study demonstrated high vaccine efficacy against one-time detection of oral HPV16/18 infection. In addition to the direct benefit of vaccination, indirect protection from reduced genital HPV infection should also reduce oral HPV exposure at the individual level. Yet, for the current unvaccinated cohorts who will bear the burden of HPV-driven oropharyngeal cancers for the foreseeable future, no secondary prevention opportunities exist, as the field has not yet validated any screening methods for any of the non-cervical HPV driven cancers. Serum HPV16 E6 antibody data suggest that this test might one day be able to detect many of the at-risk patients prior to development of HPV-driven oropharyngeal cancer. For any biomarker that proves valid and reliable, transitioning into clinical practice will require additional research focused on 1) diagnostics, 2) effective intervention, and 3) observed reductions in cancer mortality.

SEM 1-3

COMMON BEHAVIORAL QUESTIONS ABOUT ORAL HPV ACQUISITION AND TRANSMISSION

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HPV-positive OSCCs (HPV-OSCC) are associated with oral HPV infection and sexual behavior. Many patients have questions about when, how and why they became infected with HPV, and whether they will transmit HPV to others. Questions about present and future relationships often arise. Some spouses worry about their own risk of HPV infection and related cancers. This talk will review frequently asked questions by patients with HPV-OSCC and their families. We will discuss what answers to these questions are known, and when unknown what the current data suggests. HPV confers a unique demographic and prognostic profile upon patients with OSCC. This talk discusses the personal and social implications of this diagnosis and behavioral information and answers that can be helpful when counseling patients regarding this diagnosis.

CS 1-1

GAIN IN PROTECTION OF CERVICAL CANCER WITH HPV SCREENING. NEW EVIDENCES

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Objectives. Directly estimating the relative efficacy of HPV- vs. cytology-based screening, its modifiers and the duration of protection against invasive cervical cancer with HPV.

Methods. Over 175,000 women aged 20 to 64 years had been randomly assigned to HPV- or cytology-based screening in four individually randomised trials Sweden, the Netherlands, England and Italy. They were followed-up (median follow-up of 6.5 years for a total of over 1,2 million person-years) to identify invasive cervical carcinomas (ICC) by linkage with screening, pathology and cancer registries, with blind review of histological specimens or reports. Cumulative incidence and study-adjusted (HPV vs. cytology arms) ICC incidence rate ratios (RRs) were computed.

Conclusions. When considering all the period from recruitment to end of follow-up the risk of ICC was significantly lower in women screened by HPV then with cytology without heterogeneity between studies. ICC detection was similar between arms during the first 2.5 years of follow-up but was significantly lower in the HPV arm thereafter. Among women test-negative at entry the cumulative ICC incidence 5.5 years after a negative HPV test was about half that observed 3.5 years a normal cytology directly showing that with HPV testing prolonged screening intervals are safe. RRs were similar for cancer stage 1A and >1A but lower for adenocarcinoma than for squamous-cell carcinoma. The lowest RR was observed for women aged 30-34 years. The biopsy RR (HPV vs. cytology) was significantly increased in NTCC (all HPV positive women directly referred to colposcopy) but not significantly in the other studies that triaged HPV-positive women. Large-scale randomized trials find that HPV-based screening provides greater protection against ICC than cytology. Data support start of HPV-based screening with triage from age 30 and prolongation of screening intervals.

CS 1-2

RISK BASED SCREENING STRATEGIES: HOW TO APPLY?

CJLM.Meijer, H.Berkhof, MCG Dijkstra, D.Rijkaart, FJ.van Kemenade and PJF.Snijders

Screening by hrHPV testing is more sensitive but less specific for the detection of high grade cervical lesions (CIN2,CIN3, carcinoma or CIN2+) than cytology. Clinical validation of a hrHPV test helps to limit the detection of transient HPV infections not associated with cervical lesions. If all women with a clinically validated hrHPV pos smear are referred for colposcopy guided biopsy by a gynaecologist two to three times more women are referred for colposcopy, leading to an excessive increase in costs. To limit the number of colposcopies triage of hrHPV pos women is necessary. Triage can be done by cytology, HPV genotyping, p16/Ki67 dual staining and by methylationmarkers. From these triage tools cytology, and HPV genotyping have been best investigated. For screening the balance between the screening burden and safety is important. The screening burden is the chance of having CIN2+ and need to have repeat tests and can be expressed as positive predictive value (PPV) of a screening strategy; the safety of a screening strategy can be measured as the negative predictive value (NPV). Optimal screening strategies are characterised by NPV ≥ 98% for CIN2+ and a PPV of at least 20%. Based on data from two longitudinal population based screening studies in The Netherlands (POBASCAM and VUSA-Screen study) we discuss which triage strategies have the best combination of PPV and NPV and are most suitable for screening. Currently hrHPV-DNA testing followed by triage with cytology at baseline and at 6 or 12 months seems to be a useful strategy to manage HPV-positive women in countries where the quality of cytology is high and the screening interval is long (5 years). The introduction of HPV16 and HPV18 genotyping as a triage tool in a given country depends on the quality of cytology and the minimum PPV for CIN3+ referral acceptable for physicians and health decision makers. Dependent from the available resources p16/KI67 dual staining on cervical smears may replace cytology and recent data suggest that cytology as triage tool may even be replaced by methylation markers. However the use of these new triage tools need confirmation in more studies.

CS 1-4

HPV TESTING: ALONE VS CO-TESTING

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Co-testing with both HPV and cytology is currently recommended in the United States for women aged 30y or over, while primary screening with HPV alone - possibly followed by cytology triage is more favoured in Europe. While co-testing is certain to detect more disease because of the added sensitivity of doing two tests rather than just one, it will also lead to lower specificity. In particular the problem of how to manage HPV negative women with ASCUS cytology occurs with co-testing - but not for primary screening. These women appear to be at minimal increased risk of high grade CIN. A review of the current data where both tests have been done will be provided, and issues related to appropriate management algorithms will be discussed.

CS 1-5

WHICH TEST FOR WHICH SCREENING? COCKTAIL (POOLED) HPV DNA ASSAY

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Objectives: To discuss the performance needs of primary HPV screening tests currently considered for introduction into organised, population-based cervical screening program.

Methods: Randomised HPV screening in the research setting was started in Sweden in 1997. The major bottleneck for actual implementation of primary HPV screening has been i) costs and ii) logistics. The formal procedure for implementing a new screening modality with increased budget was complicated to the extent that we finally considered it realistic to implement HPV based primary screening only if it could be done within the existing budget of the organised screening program (i.e. if costs for HPV testing could be offset by reduced costs for cytology and lengthened screening intervals). This was possible only if HPV testing could be organised in a central, high-volume laboratory. It was not possible to do this with full HPV typing. High-volume testing required automation and that all testing should be completed in a single step.
Thus, the tests eligible in the formal purchasing were only those that either used cocktail HR-HPV testing or only provided a limited HPV typing (16/18/otherHR) with the partial typing completed in the screening step. Introduction of HPV-based screening started in the population-based organised screening program for cervical cancer in the greater Stockholm County, Sweden in January 2012 for women aged 56-60 years. In the HPV screening arm (new policy), HPV-positive women are triaged by cytology. In the old policy, there is primary cytology with HPV triaging for ASCUS and LSIL smears. Screening is gradually being extended to all women aged 30 and older. 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.

Results: During the first year, >20000 invitations was sent, with only positive feedback. The population HPV prevalence was 5.2% and there was thus a 95% decline in number of cytologies performed. There was a 4-fold decrease in ASCUS smears in the HPV screening arm, but the numbers of HSIL smears were similar in both arms.

Implications and Impact: Primary HPV screening using a pooled HR-HPV DNA test is feasible.

CS 1-6

WHICH TEST FOR WHICH SCREENING - HPV GENOTYPING ASSAYS

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Objectives: A number of different HPV genotyping assays are commercially available and genotyping has been incorporated into both the 2012 ACS/ASCCP/ASCP cervical cancer screening guidelines as well as the 2013 ASCCP Consensus Management Guidelines. This lecture will discuss which genotypes should be tested for, the different HPV genotyping assays that are commercially available, and how genotyping can be utilized to improve clinical care.

Methods: In the U.S. three HPV genotyping assays are FDA approved and more are under development. In Europe and Asia additional genotyping assays are available. Some of these assays identify HPV 16 and HPV 18. Others also identify HPV 45 and some identify multiple additional genotypes. Although it is widely recognized that HPV 16 is the single most important hrHPV genotype and that all genotyping assays should include HPV 16, it is unclear which other genotypes these assays should identify in order to provide the optimal combination of sensitivity and specificity in a given clinical setting. It also is unclear whether the optimal combination of genotypes will vary in different geographical locations such as in SouthEast Asia. In order to determine the optimal combination of genotypes for a given clinical setting, large databases are required of the prevalence of each of the 14 hrHPV types in women with and without biopsy-confirmed CIN 3+. In addition, standards need to be developed for the clinical performance of genotyping assays so that a woman with a given genotype identified by any of the assays has the same risk of CIN3+.

Conclusions: HPV genotyping is becoming increasingly used in clinical care. Additional studies are needed to determine the specific combinations of genotypes that are need in given clinical settings to provide the optimal combination of sensitivity and specificity.

CS 1-7

WHICH HPV ASSAYS FULFILL MINIMAL REQUIREMENTS FOR CERVICAL CANCER SCREENING?

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Background: Randomised trials have demonstrated that HPV-based screening using the HC2 assay or the GP5+/6+ PCR with EIA identification of 14 high-risk HPV types is more effective in reducing the incidence of cervical cancer than cytology-based screening (Arbyn, Vaccine 2012). Experts have defined cross-sectional equivalency criteria (Meijer, Int J Cancer, 2009) allowing claims for other HPV DNA assays for use in primary screening.

Methods: The candidate test should demonstrate non-inferior sensitivity and specificity compared to HC2 or GP5+/6+ PCR, with lower 95% confidence interval bounds of ?0.90 and ?0.98, respectively. A representative set of samples (minimally 60 CIN2+ cases, 800 ≤ CIN1 cases) derived from a population-based screening cohort should be selected [4]. Moreover, a high reproducibility (lower confidence bound ≥ 87%) should be reached.
A systematic search of published peer-reviewed references was performed using PUBMED and Embase, completed with citations of the Meijer guideline, using www. scopus.com

Results: The absolute sensitivity and specificity for CIN2+, the p value assessing non-inferiority compared to the reference tests and reproducibility parameters are shown for five HPV tests in tables 1 and 2.

Table 1. Sensitivity and specificity of hrHPV assays and non-inferiority compared to the HC2 and GP5+/6+ PCR for detection of CIN2+.

Evaluated assay	Study	Absolute Sensitivity	Specificity	Reference assay	Non inferiority test p-sens	p-spec
Abbott RT	Carozzi, 2011	96.4%	92.3%	HC2	0.004	0.009
hrHPV test	Poljak, 2011	100.0%	93.3%	HC2	0.011	0.000
	Hesselink, 2013	95.6%	92.0%	GP5+/6+ PCR	0.028	0.000
Cobas-4800	Heideman, 2011	90.0%	94.6%	HC2	0.022	0.001
	Lloveras, 2013	98.3%	86.2%	HC2	0.009	0.001
Papillocheck	Hesselink, 2010	95.8%	96.7%	GP5+/6+ PCR	0.004	0.007
qPCR(E6/E7)	Depuydt, 2012	93.6%	95.6%	HC2	0.000	0.000
APTIMA	Heideman, 2013	95.5%	94.5%	GP5+/6+ PCR	0.067	0.000

CS 1-8

THE USE OF careHPV™ TEST IN RURAL AND REMOTE BRAZILIAN AREAS

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Objective. Cervical cancer is the second most common cancer among Brazilian women. High-risk (hr) human papillomavirus (HPV) persistence is the primary cause of cervical neoplasia. Early detection of hr-HPV is important for identifying women at risk for developing cervical lesions. Approximately 85% of new cases of cervical cancer worldwide and 50% of the total cervical cancer deaths occur in developing countries. careHPV was evaluated in women from various Brazilian regions.

Methods. 5,079 women aged 18–85 years were enrolled in an opportunistic cervical cancer screening program and were randomized into self-vaginal or health professional-guided cervical sampling groups. 2,000 women were admitted to the Barretos Cancer Hospital Prevention Ambulatory (BCH) and 3,079 on Mobile Unit (MU), in 2012. In the ambulatory, random collections of self-sampling or sampling collected by a health care professional were tested. On MU, collection was performed by health professional. The Qiagen careHPV™ test was performed on all samples.

Conclusions. Positive hr-HPV results were obtained in 12.3% (245/2,000). In the first group (BHC), it was used the Chi-square test (X^2) to compare frequencies between the collection group, finding 13.5% to self-sampled and 11.0% to health professional, did not differ in their rates of detection of hr-HPV. Therefore, HPV DNA testing in self-sampled vaginal cells is an alternative to primary screening in low-resource settings. Considering the cytological samples, 36.6% of women classified as ASC-US+ in BCH were positive to hr-HPV test; 78.8% of LSIL and 75.0% to HSIL. Analyzing the MU 8.5% of women classified as NILM were hr-HPV positive; 39.2% of ASC-US+; 56.4% LSIL and 100% HSIL.

Study Supported by Qiagen and CNPq - Process nº 573799/2008-3.

CS 1-10

P16/KI-67 DUAL-STAINED CYTOLOGY AS A TRIAGE MARKER IN WOMEN WITH BORDERLINE/MILD DYSKARYOTIC CERVICAL CYTOLOGY SMEARS

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Background: The objective of this study was to analyze the clinical performance of detecting high-grade lesions with the p16/Ki-67 dual-stained cytology test (CINtec PLUS) in a cohort of women with borderline or mild dyskaryotic (BMD) cervical cytological smears. Methods: Conventional Pap cytological smears from 255 women with BMD previously enrolled into the VUSA-Screen study were de-stained and subsequently subjected to p16/Ki-67 dual staining. In the VUSA-screen study women with BMD at baseline and a positive high-risk Human papillomavirus (hrHPV) test were directly referred to colposcopy. Women with BMD at baseline and a negative hrHPV test were tested by Pap cytology at 6 and 18 months and referred, if Pap cytology was abnormal at one of these occasions. Long-term follow-up data were available for this retrospective analysis.

Results: p16/Ki-67 Dual-staining showed a sensitivity of 100.0% (95% confidence interval (CI): 87.2-100%), a negative predictive value (NPV) of 100.0% (95% CI: 97.0-100%), and a specificity of 64.4% (95%CI: 57.2-71.2%) in detecting CIN3+. In contrast, hrHPV demonstrated a somewhat lower sensitivity of 96.3% (81.0-99.9%), a NPV of 99.1% (95%CI: 95.1-100%), and a significantly lower specificity of 57.6% (95% CI: 50.2-64.7%; p=0.042) in detecting CIN3+lesions. With hrHPV triage at baseline, a significant higher referral rate was obtained (49.1% vs. 43.6%). At CIN2+ level, the results were not significantly different. During the 5-year follow-up, no CIN3+lesions were found in women who were hrHPV positive, but p16/Ki-67 dual-stained cytology negative. However, the cumulative 5-year CIN2 risk of BMD positive, p16/Ki-67 negative women was 4.3% (95% CI: 1.0-7.6%).

Conclusions: Women with BMD can be safely triaged by p16/Ki-67 dual-stained cytology. Compared to HPV triage testing this results in less referrals and a comparable high sensitivity for CIN2/3+, but with a higher specificity. Because of the cumulative 5-year CIN2 risk of BMD positive, p16/Ki-67 negative women of 4.3%, we advise these women to have a follow-up visit within 2 years.

CS 2-2

LONG-TERM REGISTRY-BASED FOLLOW-UP

Joakim Dillner for the HPV Vaccine Nordic Follow-Up Team

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

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

Results: There were no cases of CIN 2+ observed in the GHN population irrespective of HPV type. There were seven (7) cases of CIN 1 observed with follow-up time of 1,088.6 person-years regardless of HPV type in the GHN population. The incidence rate for this endpoint was 0.6 (95% CI: 0.3, 1.3) per 100 person-years at risk. The incidence rates for CIN 1 related to any of the 10 non-vaccine HPV types and not related to any of the 14 assay-identified HPV types were 0.4 (95% CI: 0.1, 0.9) and 0.2 (95% CI: 0.0, 0.7) per 100 person-years at risk, respectively. Data will be presented from an extended follow-up time not available at the time of abstract submission.

Conclusions: HPV-type replacement did not occur at any appreciable level. HPV type replacement will continue to be assessed and further analyses will be performed at two-year intervals.

CS 2-3

HPV VACCINATION AND GENDER EQUITY: DIRECT AND INDIRECT BENEFITS OF VACCINATION OF MALES

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The HPV vaccine is the first vaccine mandated for only one gender. Yet men have equally high HPV rates and are as likely to transmit the infection to their partners. Reducing HPV prevalence among men would decrease transmission of HPV to women. Although gender distinction may be justified based on the available evidence of the vaccine efficacy, lack of gender equity is an ethical problem. It is not fair that young women are vaccinated within vaccination programs, but young men are not. It is not meaningful to leave men to rely on herd immunity only. Even though health economists have demonstrated that male vaccination provides only a small added benefit, this approach is not sound. Although such approach may be feasible from the public health point of view it certainly is unfair or unjustified from the individual point of view. Studies suggest that HPV vaccination for males is generally well accepted by young men, as well as parents and healthcare providers. Vaccination of males is safe and no vaccine- related serious adverse events had been reported in clinical trials. In October 2011 the Advisory Committee on Immunization Practices (ACIP) in the US recommended routine use of quadrivalent HPV vaccine (HPV4; Gardasil, Merck) in males aged 11- 12 years. ACIP also recommended catch- up vaccination for males aged 13- 21. So far, Australia clearly leads the way on HPV vaccination in boys.

CS 2-5

COST-EFFECTIVENESS OF HPV VACCINATION: NEW EVIDENCE

Van de Velde N1

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Objectives: Modeling studies agreed that the vaccination of pre-adolescent girls against HPV is likely to be cost-effective in developed countries. Based on these predictions, many countries have introduced HPV vaccination into their national immunization programs. While most countries target pre-adolescent girls, the vaccination strategies implemented vary depending on health care systems and infrastructure. Cost-effectiveness models are useful to assess and compare differences in implementation strategies. In this review of recent cost-effectiveness studies, the objective is to gather new evidence on the impact of cross-protection, non-cervical diseases, older women vaccination, male vaccination and 2-dose vaccination on the cost-effectiveness of HPV vaccination in developed and developing countries.

Methods: We searched PUBMED for English-language articles published between 2007 and 2013. Search terms included combinations and close variations of "HPV", "vaccination" and "cost-effectiveness". From the 849 articles found, we selected original studies reporting cost-effectiveness predictions for HPV vaccination, providing basic information on demography and sexual behaviors, HPV epidemiology, model assumptions and parameters, costs and outcomes included, and following the methodological framework of cost-effectiveness analyses.

Conclusions: Including lifelong cross-protection effects and non-cervical HPV-related diseases in models increases the cost-effectiveness of HPV vaccination. Vaccinating women up to 19-26 years of age in catch-up programs has produced conflicting results in cost-effectiveness studies. Some countries have now recommended gender-neutral vaccination based on various criteria. In terms of cost-effectiveness alone, male vaccination is generally found not cost-effective compared to female-only strategies when coverage in girls is high. A 2-dose schedule would increase cost-effectiveness ratios but more data on the duration of protection is needed before it can be recommended. Finally, there is an increase in the number of studies in developing countries and the high incidence of cervical cancer combined with low vaccine prices offered to GAVI-eligible countries are likely to make HPV vaccination below WHO cost-effectiveness thresholds in many low-income countries.

CS 2-6

GAVI HPV VACCINE PROGRAMME

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Objectives: 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" .
The GAVI Board in November 2011 made a decision to add HPV to GAVI's portfolio of supported vaccines for the poorest countries in the world.

Methods: According with the Board request, two pathways have been developed to introduce the HPV vaccine in low income countries. The first one is a national programme, accessible to countries demonstrating experience in delivering complete multi-dose series to adolescent population reaching at least 50% of the target vaccination cohort in an average size district. In case countries may not be ready to apply for support of a national roll out, a second option can help to make an informed decision and gather appropriate information to support a national plan: countries can apply for support for a demonstration project. Today 18 countries among the 68 countries eligible for HPV support had or are having a pilot project of HPV vaccine.

Conclusion: The first round of application was opened in summer 2012. Fifteen countries applied for the HPV Demonstration project and two countries applied for the HPV National programme. "On World Cancer Day 2013, GAVI announced the first countries to get support for HPV vaccines through demonstration programmes: Ghana, Kenya, Madagascar, Malawi, Niger, Sierra Leone, Tanzania and Lao PDR" , countries will introduce the HPV vaccine in 2013, Tanzania in 2014. One country has also been approved for the national programme, Rwanda, who will be supported by GAVI starting in 2014. The next round of application will be in summer 2013.

CS 3-1

APPLYING THE CONCEPT OF EQUAL RISK=EQUAL MANAGEMENT TO CERVICAL COLPOSCOPY AND BIOPSY

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There is an ongoing debate on optimal colposcopy-biopsy practice in cervical cancer screening. Current practice ranges from taking single biopsies from the worst appearing lesion to routinely taking multiple random biopsies independent of colposcopy impression. In the NCI-Biopsy Study, the incremental sensitivity and yield of HSIL for taking multiple directed biopsies during colposcopy was quantified. In the overall population, sensitivities for detecting HSIL were 60.6% (95% CI: 54.8%-66.6%) from a single biopsy; 85.6% (95% CIN: 80.3%-90.2%) after two biopsies and 95.6% (95% CI: 91.3%-99.2%) after three biopsies. Stratification of the population by colposcopy impression, cytology, and HPV status allowed estimating the risk of disease in all strata and the incremental yield for taking multiple directed biopsies. The highest proportion of HSIL was observed for women with a high grade colposcopic impression, HSIL cytology, and HPV16 positivity. In these subgroups, additional biopsies had the highest absolute yield of finding additional HSIL. These analyses allow identifying risk-based colposcopy-biopsy protocols to minimize disease misclassification in cervical cancer screening and management.

CS 3-2

ACCURACY OF COLOPOSCOPY-DATA FROM THE FUTURE TRIAL

Stoler M

The paradigm of colposcopic examination after abnormal cervical cytology is still considered the standard for evaluation of cervical neoplasia. Despite colposcopy being a subjective test, its purpose is to identify disease, obtain representative specimens for histology, and direct patient management. However this paradigm must account for the following facts:

- There is significant error in colposcopic assessment.
- Colposcopy with or without single "target" biopsy is an imperfect diagnostic gold standard because of its inherent pitfalls in disease ascertainment.
- Even with specialized training and experience, colposcopic biopsy misses 26% – 42% of prevalent CIN 2+.
- Grouping CIN2 with CIN3 improves performance as biopsied CIN2 often has CIN3 associated with it on definitive excision.
- The most significant factors influencing accuracy are the size of the lesion, the number of biopsies taken and the HPV type.
- Biopsy induced regression also seems to have an effect on natural history, particularly for small lesions.
- Endocervical curettage has a minimal impact on colposcopic sensitivity.
- These data should be taken into account in planning therapy especially for patients in whom the biopsy is less severe than the referral cytology.
- Taking more biopsies is the only compensating strategy.

Stoler MH, Vichnin MD, Ferenczy A, Ferris DG, Perez G, Paavonen J, et al. The accuracy of colposcopic biopsy: analyses from the placebo arm of the Gardasil clinical trials. Int J Cancer. 2011;128(6):1354-62.

CS 3-3

CIN 2: REALITY OR MYTH?

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The natural history of HPV begins with entry of the virion into a basal cell. As the cell matures through its normal differentiation process, the early proteins, E6 and E7 are expressed and in turn enhance cellular proliferation starting in the basal cell layer. Other changes induced by the abnormal cellular proliferation induced by the early proteins include nuclear atypia and multi-nucleation. When these changes are mild and limited to basal cell layer, it is referred to as CIN 1. CIN 2 and 3 lesions are characterized by undifferentiated cells including the characteristic loss of tissue organization and cellular polarity, abnormal mitotic figures and nuclear crowding. CIN 2 has been defined when those abnormal cells extend past the lower third of the epithelium and CIN 3 if found though-out the epithelium. Clearly, there is such a stage since it would require some time for the entire epithelium to be replaced with abnormal cells but the question remains is it a meaningful stage (i.e. is it just a bad case of CIN 1, or is it already on its way to becoming a CIN 3).

Older studies of CIN 2 revealed a regression rate ranging from 34% to 54% with progression rates to CIN 3+ ranging from 20-30%. Hence, these high progression rates resulted in immediate treatment of CIN 2 as well as CIN 3. Several studies using panels of pathologist began to find that CIN 2 was not easily reproduced with pathologist disagreeing approximately 50% of the time. Disagreement rates for CIN 1 and 3 also exist but in general are more consistent. This resulted in pathologists frequently combining diagnosis into a "CIN 2/3." Some researchers took advantage that adolescents often are noncompliant and found when they were able to examine adolescents with CIN 2 that many of the lesions appeared to have regressed. A natural history of CIN 2 in 13-24 year olds found that 50% of HPV 16 CIN 2 regressed and 80% of non16 HR HPV regressed. Interestingly, rate of regression did not differ if there was agreement between pathologist or not. Risk for persistence in this population included HPV persistence, oral contraceptive use and young age of first intercourse. Molecular studies show evidence that CIN 2 may be distinct including differences in CD4 concentration and cell markers such as cytokeratin 2 and Actin alpha. Some advocate using p16 to confirm a CIN 2 lesion, however, there is no evidence to suggest that p16CIN 2 will progress similar to CIN 3. In conclusion, biomarkers are needed to distinguish our current definition for CIN 2 from CIN 3 so as to not overtreat women with a lesion that is destined to regress.

CS 3-4

VALUE OF RANDOM CERVICAL BIOPSIES AT COLPOSCOPY

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Objectives: Determine the increase the yield of cervical intraepithelial neoplasia (CIN) 3 or cancer (CIN 3+) from random cervical biopsy in quadrants without visible lesions.
Methods: Within two cross-sectional trials comparing cervical cancer screening tests conducted between 6/1999 and 4/2001 in Shanxi Province, China, we reviewed 1,383 colposcopic examinations on women with cervical cytology of Atypical Squamous Cells of Uncertain Significance with positive High-Risk Human Papillomavirus or cytology of Low Grade Squamous Lesion or worse. We then reviewed 4,932 colposcopic examinations performed in the SCPMG-Fontana colposcopy clinics between 1/2007 and 12/2009. In both reviews, at colposcopy, the cervix was divided into quadrants by lines from 12 to 6 and 3 to 9 o'clock. Each quadrant had biopsy of colposcopically detected lesions (those with colposcopic impression of Human Papillomavirus, CIN, or Cancer) or random biopsy at the squamocolumnar junction if the colposcopic impression in that quadrant was normal. In the first trial the sensitivity for CIN 3+ of colposcopically directed biopsy was 63.5% (141/222). Among seven physicians, the sensitivity of colposcopically directed biopsy for CIN 3+ varied from 28.6% to 92.9% ($p<.001$). The yield of CIN 3+ per colposcopically directed biopsy (19.9%, 240/1,205) was 7.8 times higher than that of the yield of CIN 3+ per random biopsy (2.5%, 109/4,304, $p<.001$). The yield of CIN 3+ increased from 10.2% for colposcopically directed biopsy to 11.9% with the addition of one random biopsy (10.2% vs. 11.9%, $p<.001$) to 13.0% with the addition of two random biopsies (11.9% vs. 13.0%, $p<.001$) to 13.9% with the addition of three random biopsies (13.0% vs. 13.9%, $p<.001$) to 14.3% with the addition of four random biopsies (13.9% vs. 14.3%, $p=.03$). In the second review, we estimated that 51 of 295 (17.2%) women with CIN 3+ were diagnosed by random cervical biopsy.
Conclusions: Random cervical biopsy in cervical quadrants without visible lesions increases the yield of CIN 3+. The low yield of CIN 3+ per random biopsy would be a greater problem if the random biopsies were difficult to perform, particularly morbid, or difficult to interpret. If the biopsies are done with a 2-mm bronchoscopy biopsy instrument, they are easy to perform, do not cause much discomfort, are large enough to diagnose CIN (the average thickness of CIN 2 and 3 is 290 μm5), and easy to interpret.

CS 3-6

NON-ABLATIVE TREATMENT OF CIN/VIN

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Historically, treatment for high grade CIN and VIN has been primarily extirpative. While in most with CIN and VIN who are treated with surgical procedures the disease does not return, extirpative treatments do not target the underlying reason for developing these precancerous lesions, persistent HPV infection. Also, there is growing evidence of a direct association between surgical removal of CIN and preterm birth in addition to other poor obstetrical outcomes. Surgery for VIN can cause a number of acute morbidities as well as having the potential for poor cosmetic outcomes.

There is a growing understanding of potential targets for the treatment of CIN and VIN. Many newly designed therapies are designed to boost local and/or systemic immune responses targeting HPV infection and HPV infected cells, inducing regression. These therapies include topical therapy (direct antivirals or immunomodulators), therapeutic vaccines, photodynamic therapy, or combinations of these therapies. Surrogates for response are still being developed in parallel to clinical pathways for treatments.

The design of studies in HPV associated in situ carcinomas need to balance the natural history of progression and regression of disease, with the time required to achieve a response. Given the natural regression of some high grade CIN and even VIN lesions, there is a need for a control arm helps to determine the effect size of the agent in a studied cohort. A control arm also takes into account the potential for any given study that might recruit a skewed mostly older or mostly younger subject population, where natural regression rates might differ. Colposcopic endpoints potentially can be problematic given the variable performance of identifying all high grade disease as well as the potential for microscopic foci of remaining disease that would only be identified with a comprehensive pathological assessment of a cervical conization. Toxicities for topical treatments require careful assessment of the keratinized surfaces of the vulva and perianus as well as the non-keratinized surfaces of the cervix for mucosal or skin toxicity with chronic use. Lastly, the comparisons of the treatments with surgical treatments requires pharmacoeconomic assessments that take into account treatment characteristics such as: the magnitude of effect, treatment cost, and patient tolerability and compliance.

CS 3-8

QUALITY CONTROL IN COLPOSCOPY

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Quality control is mandatory in a cervical cancer screening programme, including colposcopy. Nowadays, we often hear that blind four-quadrant biopsies, after an abnormal Pap test, give better results than colposcopic evaluation.

Lack of quality control can explain the poor 50% of correlation between colposcopy and blind multiple biopsies. In Canada, quality control in colposcopy was initialed as early as 1973 and over the years, colposcopic impression mirrored the referral cytology diagnosis in 90% of the cases.

Quality control should cover the following: training of colposcopists, indications for referral, number of new consultations annually, types of treatments, correlation between colposcopy impression and histopathology results. When a colposcopist does not meet the standard, training sessions should be mandatory.

Since HPV vaccination programmes are in place, there will be fewer references for high grade lesions. Quality control will be more important.

CS 4-1

EFFECTS OF PERSISTENCE AND HPV TYPE ON THE LONG-TERM RISK OF CIN3+ FOLLOWING HPV INFECTION: 10 YEAR FOLLOW-UP OF WOMEN IN THE ARTISTIC TRIAL

Peto J[1], **Gilham C** [1], **Sargent A** [2], **Kitchener H** [3] **on behalf of the ARTISTIC trial study group**

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Objectives: To evaluate the role of persistent HPV infection and HPV type in predicting the long-term risk of CIN3+.

Methods and results: (Follow-up to April 2009. Results updated to Dec 2012 will be presented.) 24,502 women who participated in the ARTISTIC trial were flagged through the Office of National Statistics for notification of cancer registrations (including CIN3 and cervical cancer) and deaths, giving almost complete follow-up for 10 years following HPV testing at recruitment in 2001-2003.
HPV genotyping was done on all samples testing positive by Hybrid-Capture II (HC2) by any of three assays (Line Blot, Linear Array (Roche Diagnostics), or Papillocheck (Greiner Bio-one)). Persistent infection was defined as the same HPV type at entry and at round 2 approximately 3 years later (30-48 months after entry).
The cumulative CIN3+ rate 7 years after entry in women who were HC2- at entry was only 0.1% (21/20,478). CIN3 rates 3 years later in women who were HC2+ at entry and were retested at round 2 are shown below.

Status at round 2	CIN3+ rate 3 years later
Persistent HPV16	18%
Persistent other HR-HPV	7%
New HPV16	5%
New other HR-HPV	2%

Conclusions: The risk is low 3 years later in women who have acquired a new infection (5% for HPV16, 2% for other HR-HPVs), including those who previously had a different HPV type that has cleared. A 6-year screening interval, with retesting 3 years later when HPV is detected (possibly earlier for HPV16), would be safe and cost-effective. A 10-year screening interval would also be safe but might require a shorter retesting interval.

CS 4-2

ACCURACY OF 5-TYPE MRNA-TESTING IN THE TRIAGE OF WOMEN WITH MINOR ABNORMAL CERVICAL CYTOLOGY

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Objectives: High-risk HPV (hrHPV) DNA detection is generally accepted to triage women with a cytological diagnosis of atypical squamous cells of undetermined significance (ASC-US). However, no consensus has been reached on the optimal management of low-grade squamous intraepithelial lesions (LSIL).

Methods: In a meta-analysis, the diagnostic accuracy of NASBA-based detection of mRNA of five hrHPV types (PreTect HPV-Proofer, NucliSENS EasyQ) to detect grade two cervical intraepithelial neoplasia or worse (CIN2+) and CIN3+ is assessed in women with ASC-US and LSIL. Results are compared with Hybrid Capture-2 (HC2), which detects DNA of 13 hrHPV types. A bivariate random-effect model, incorporating the intrinsic correlation between true- and false-positivity rates was used for meta-analytical pooling.

Conclusions: Considering underlying CIN2+, the pooled absolute sensitivity was 75% and 76% in triage of ASC-US and LSIL, respectively. The pooled absolute specificity to exclude CIN2+ was 78% and 74% in women with ASC-US and LSIL, respectively. Comparing the mRNA assays with Hybrid Capture-2 (HC2) demonstrates that mRNA testing is substantially more specific than HC2, but less sensitive. Hence, HPV assays detecting mRNA of 5 hrHPV types may reduce over-diagnosis of women with minor cytological abnormalities. However, given the lower sensitivity, women with a negative mRNA test cannot be considered as free of CIN2+ and require further surveillance.

CS 4-3

CLINICAL VALUE OF HPV TESTING

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47 experts representing 23 professional societies, national and international health organizations, and federal agencies met in Bethesda, MD, September 14-15, 2012, to revisit and revise the 2006 American Society for Colposcopy and Cervical Pathology (ASCCP) Consensus Guidelines. The primary goal was to provide revised evidence-based
consensus guidelines for managing women with abnormal cervical cancer screening tests, cervical intraepithelial neoplasia (CIN) and adenocarcinoma in situ (AIS). Five working groups were created for this effort, and many of the new recommendations are based on the principle of "similar management of similar risk." These new recommendations are also based on data from almost 1.4 million women in the Kaiser Permanente Northern California Medical Care Plan. The objective of this presentation is to review new recommendations in this updated guideline.

CS 4-4

EARLIER DETECTION OF HIGH-GRADE CIN AND OVERDIAGNOSIS WITH HPV VS. CYTOLOGY.

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(1)CPO, Torino, Italy (2) ASL Reggio Emilia, Italy (3) S.Croce Hospital, Trento, Italy (4)Venetian Cancer Registry, Padova, Italy (5) Regione Emilia-Romagna, Bologna Italy (6) ISPO, Florence, Italy (7) Queen Mary University, London UK.

Objectives. Estimating the difference in the time of detection of high-grade CIN with HPV-based screening and with cytology. Defining if the high-grade CIN detected by HPV-based screening have a different probability of regressing spontaneously than those detected by cytology.

Methods. The follow-up of the NTCC RCT was updated to spring 2012. The cumulative incidence of CIN2, CIN3 or invasive cancer (CIN2+) in each arm was computed by the Kaplan-Meier method. Data were censored 12 months after the last screening test. Differences in overdiagnosis between HPV- and cytology-detected hg-CIN were estimated by comparing the cumulative incidence of CIN2+ at the end of observation. The differences in time of detection of hg-CIN by HPV and cytology was estimated by computing, for each value of the cumulative incidence (CI) of CIN2 the difference between the time needed to reach that value of CI with HPV and the time needed to reach the same level of CI with cytology. The same estimates were repeated by age at recruitment (25-34 and 35-60) and study phase (first with co-testing and second with standalone HPV in the experimental arm).

Conclusions. After 7-9 years of follow up (according to phase) there was no or little difference between arms in the cumulative incidence of CIN2+, suggesting that overdiagnosis of spontaneously regressive high-grade CIN is similar with HPV and cytology. This was true also among women aged 25-34 years at recruitment. Among women aged 35-60 years at recruitment HPV detected about 30% of hg-CIN >3 to 5 years before cytology and about 20% 1.5 to 3 years in advance while there was no difference (-1 to +1 month) in about 30%. Gains with HPV were larger at younger age.

CS 5-1

EFFECTS OF QUADRIVALENT HPV VACCINE IMMUNISATION IN INDIVIDUAL RRP PATIENTS

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Objective: to investigate and link clinical and immunological parameters in a cohort of recurrent respiratory papillomatosis (RRP) patients including 4 patients who received the quadrivalent HPV vaccine due to very frequent recurrences or a personal wish.
RRP is a chronic and sometimes life threatening disease of the larynx that is associated in >90% with low-risk HPV6 and 11. Juvenile and adult forms are known and current standard treatment is surgical ablation to prevent suffocation by obstructed airways. However, this treatment rarely cures the disease. Gardasil is a prophylactic vaccine against HPV types 6, 11, 16, and 18 which was initially approved for prevention of cervical cancer and genital warts in 2006.

Methods: IRB approval was obtained to investigate RRP-patients. Four patients between age 5 and 64 with persistent and recurrent papillomatosis were vaccinated with Gardasil in conjunction with surgical ablation of RRP. Per individual treatment intervals and extent of surgery were recorded as well as clinical symptoms. Peripheral blood was analysed for immune responses to HPV 6, 11, 16, and 18 L1 antigens as well as E6/E7 antigens. Serologic responses were investigated by Luminex-based multiplexed serology and ex vivo antigen specific memory CD4 T cell quantitation of cytokine (IFN-gamma, IL-2) and CD154 cellular responses was done by flow cytometry.

Results: Despite multiple invasive therapies patients with RRP had a comparably low T cell immune response as healthy controls towards HPV antigens. The vaccinees developed or boosted antibody responses to L1 antigens. During the course of vaccination a significant CD4 T cell response developed in three out of four vaccinated RRP patients. All patients had reduced regrowth of papillomas and treatment intervals increased. Vaccination of RRP patients with quadrivalent HPV vaccine after surgical removal of lesions showed no adverse effects.

Conclusion: Although designed as a prophylactic vaccine there may be a beneficial effect of quadrivalent HPV vaccine application also in patients with established RRP in combination with surgery.

CS 5-2

POTENTIAL MECHANISMS OF POST-EXPOSURE PROPHYLAXIS

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Objective: To describe and discuss observations of beneficial effects of HPV prophylactic vaccination in HPV infected patients.

Methods: Prophylactic HPV vaccines have shown close to 100% efficacy for prevention of dysplasia in women naïve to the respective vaccine HPV type. Efficacy in women already infected by HPV is considerably reduced. Overall, analyses from the large clinical trials showed no benefit of vaccination in persons currently infected or carrying dysplasia. In contrast, reduction of new disease in patients having had a conization has been described for both vaccines. There have been occasional reports, however, describing some benefit in patients with current infection and in certain diseases. This concerns patients with recurrent respiratory papillomatosis, recurrent genital warts, or even patients with immune defects. We have investigated immune responses in individual patients showing a benefit after HPV vaccination. Mechanisms involved in these post-exposure effects of vaccination are not well defined. The antigen L1 is generally not expressed in detectable amounts in basal epithelial cells and therefore not abundantly available as a T cell antigen. However, L1 specific CD4 T helper 1 cells are abundantly induced by HPV vaccines. L1-specific CD8 cytotoxic T cells have only scarcely been detected. Virus neutralizing antibodies are regarded as the main effector mechanism of prophylactic HPV vaccination. Despite multiple invasive treatments patients with persistent disease have rarely mounted measurable immune responses or responses are weak. Following vaccination immunity is boosted and stronger immune responses can be detected. In patients they could contribute to abrogate intraepithelial reinfection and spread or recurrence of the disease.

Conclusion: Several immune mechanisms could contribute to the observed clinical benefit of prophylactic HPV vaccination in patients. These observations need to be systematically investigated.

CS 5-3

PREVENTION OF RECURRENT HIGH-GRADE ANAL DYSPLASIA AND ANAL CONDYLOMA WITH QUADRIVALENT HPV VACCINE IN OLDER MEN

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Background: The quadrivalent human papillomavirus vaccine (qHPV) is FDA-approved for use in males 9 to 26 years old to prevent anogenital condyloma and high-grade anal dysplasia (HSIL). The objective of this study is to determine if qHPV is effective at preventing both recurrent and new onset anal condyloma and HSIL among men who have sex with men (MSM) aged 26 years and older.

Methods: A nonconcurrent cohort study evaluated patients both with and without history of anal condyloma or treated HSIL to determine rates of disease in vaccinated versus unvaccinated patients. HIV- patients were offered off-label qHPV irrespective of age and HPV related disease since June 2006. Most had to pay out-of-pocket for qHPV. Patients with a prior history of condyloma had to be recurrence free for >12 months.

Results: Of 202 patients (mean age 40) with treated HSIL (88 vaccinated), over 340.4 person-years follow-up, 12 (13.6%) vaccinated and 35 (30.7%) unvaccinated patients had recurrent HSIL. Multivariate hazards ratio analysis showed that testing positive for oncogenic HPV genotypes within eight months of study entry was associated with increased risk of recurrent HSIL at two years following study entry (HR 4.06, 95% CI 1.58 to 10.40, p-value = 0.004) and qHPV was associated with decreased risk of recurrent HSIL (HR 0.50, 95% CI 0.26 to 0.98, p-value = 0.04) and for those positive of oncogenic HPV (HR 0.47, 95% CI 0.22 to 1.00, p-value = 0.05). Of 313 patients in the condyloma group (mean age 42) with 37% vaccinated, over 773.6 person-years follow-up, condyloma developed in 10 (8.6%) vaccinated (incidence of 3.7 per 100 person-years) and 37 (18.8%) unvaccinated patients (incidence 7.3 per 100 person-years) (p=0.05). Multivariable hazards ratio showed that qHPV was associated with decreased risk of anal condyloma (HR 0.45; 95% CI 0.22-0.92; p=0.03). History of anal condyloma was associated with increased risk of anal condyloma development (HR 2.28; 95% CI 1.28-4.05; p=0.005) and absence of oncogenic HPV infection was associated with decreased risk of anal condyloma development (HR 0.36; 95% CI 0.15-0.87; p=0.02).

Conclusion: QHPV significantly reduces condyloma and HSIL among older MSM and may be an effective post-treatment adjuvant form of therapy. A randomized controlled trial is needed to confirm these results.

CS 5-4

REDUCTION OF RECURRENT DISEASE AFTER CONIZATION

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Objective: To evaluate the benefit of vaccination of women with conization

Methods: Review of available data

Conclusions: In the phase III trials of the quadrivalent and bivalent vaccine 1350 and 454 women respectively underwent conization after randomization and vaccination since both studies included HPV positive women. Analyzing the incidence of subsequent disease in the vaccinated and the placebo cohort a 48.1% or 42.6% reduction of subsequent CIN 1+ and 64.9% or 88.2% reduction of CIN 2+ was demonstrated for the quadrivalent vaccine or the bivalent vaccine, respectively. In addition a 63% reduction of genital warts after conization was demonstrated in women vaccinated with the quadrivalent vaccine. All these women were vaccinated before conization.

A recent trial comparing 737 women with or without vaccination with the quadrivalent vaccine immediately after conization demonstrated a reduction in the recurrence rate of 2.5% vs. 7.2%.

Available data are consistent and suggest a substantial clinical benefit from vaccination for women undergoing cervical surgery. Two conizations are associated with a tenfold risk of preterm delivery; this risk may be substantially reduced and has to be taken into account for evaluation of the cost-effectiveness.

CS 5-5

HPV TYPES IN RECURRENT DISEASES

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Objective: Although an enormous number of HPV related publications have appeared in recent years, our understanding of factors which determine spontaneous clearance of infection, risk of persistent infection or which modulate the risk of recurrent disease is far from complete. There is no indication that vaccination have a significant role in the clearance rate of HPV infection (1). On the other hand, some studies reported reduced incidence of recurrent disease in the cervix (2) and anus (3) when vaccination was performed before or in association with treatment. The high recurrence rate of genital warts is mainly considered to be caused by persistent infection of the same HPV type. Recurrent CIN 2+ is less frequent, but reported in the range of 5-25 % of cases. A more complete investigation of HPV types in primary and secondary disease will be helpful in our understanding and also to which extent secondary prophylaxis by vaccination may be of benefit in selected indications.

Methods: Recent publications with information of HPV types in primary and secondary treatment specimens have been searched. In all, few studies and limited information was available. The largest so far was only presented as an abstract (4).

Conclusion: Based mainly on a specific study of HPV types in primary and secondary conus specimens >90 % of recurrent CIN2+ lesions are caused by the same HPV type as in the primary lesion. This finding is in some contrast to what have been previously published based on small numbers. To what extent recurrent disease is caused by persistent local infection, reactivation of latent infection, reinfection by partner or auotinfection from a different locus remains to be elucidated. Therefore, evidence based advice on potential value of vaccination at time of treatment needs further specific clinical trials.

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CS 6-1

SCREENING FOR SPECIAL POPULATIONS (DES, HIV, HISTORY OF CIN2+)

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Objectives: The 2012 the American Cancer Society, American Society for Colposcopy and Cervical Pathology, and the American Society for Clinical Pathology screening guidelines specifically address women in the general population. They do not address women at increased risk for cervical disease such as women with in utero DES exposure, immunosuppressed women such as those who are infected with HIV, and women with a history of treatment for CIN2+. Guidelines for screening these higher risk women have been made by a variety of groups and will be discussed.

Methods: The daughters of DES mothers have 40 times the risk of clear cell adenocarcinoma of the cervix and vagina as women in the general population and 2.2 times the risk of CIN. Risk of clear cell adenocarcinoma remains until the 40s and many of the tumors do not appear to be caused by HPV. Therefore routine screening is not adequate for this population. NCI recommends annual cervical cytology examinations with extensive sampling of the vagina (sometimes referred to as the 4 quadrant approach to cytology as long as the woman is healthy and likely to benefit from the early detection of cervical cancer. Similarly, HIV infected women have an elevated risk of cervical cancer and CIN2+ compared to women in the general population. Therefore the CDC recommends that HIV infected women be screened twice with cervical cytology in the first year after being diagnosed with HIV. If both cytology specimens are negative, they should have annual cytological screening thereafter. It should be noted, however, that large follow-up studies in the U.S. have found a low risk of CIN2+ in HIV infected women who are both hrHPV and cytology negative which suggests it may be safe to extend screening interval in adequately treated HIV-infected women when cotesting is used. Similarly, women who have been treated for CIN2+ remain at increased for CIN2+ for 20 years after treatment. Therefore the American College of Obstetrics and Gynecology recommends annual screening for at least 20 years. However the new 2013 ASCCP Consensus Guidelines recommend cotesting at 12 and 24 months after treatment. If both cotests are negative, women should be retested in 3 years. Routine screening is then recommended for 20 years after treatment, even if the woman is over the age of 65 years.

Conclusions: Women at elevated risk for CIN 2+ require more intensive screening than women in the general population.

CS 6-2

SCREENING OLDER WOMEN FOR CERVICAL CANCER

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Objectives: HPV infection occurs in all ages. The prevalence of HPV infection does not decrease below 5-10% in any population even as the woman ages past menopause. The time lag from infection to cancer has produced two distinct age peaks of cervical cancer with the latter among peri- and post-menopausal women.

Methods: Evidence based screening indicates that HPV testing as a primary screen is extremely sensitive but lacks specificity. Cytology as a primary screen presents high specificity with mediocre sensitivity. Using these two technologies in triage provides recommended screening guidelines for older women.

Conclusions: Several appropriate guidelines for cervical cancer screening among older women have been developed. Each has its merits and weaknesses.

CS 6-3

SCREENING OF VACCINATED WOMEN

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HPV vaccines have shown high efficacy in the prevention of type specific HPV infections and all subsequent endpoints. Natural history studies have also documented that HPV 16 related CIN lesions are amongst the first to occur after infection and have a higher visibility in cytology and colposcopy. Therefore it is expected that HPV 16 and 18 vaccinated cohorts will show a significant reduction in the rates of CIN1+ lesions in the order of 50 to 60%. With reduced prevalence of the underlying lesions, cytology based screening assays will dramatically reduce their positive predictive value, thus reducing the validity of the screening effort. Furthermore, quality control of a cytology based screening program will suffer because of the scarcity of relevant lesions. HPV based screening programs amongst vaccinated cohorts should contribute by identifying non vaccine covered HPV infections and more importantly by displaying the full benefits of the increased output in testing at a high sensitivity level afforded by the existing automatic testing platforms.

In countries where HPV vaccination is population based and centrally controlled, the protocols offered to vaccinated women should be based on HPV tests and could facilitate the transition from opportunistic to organized, population based screening programs.

CS 6-4

SCREENING FOLLOWING HYSTERECTOMY: IS IT RELEVANT?

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There is no standardised follow-up after hysterectomy for women treated for CIN. Vaginal intraepithelial neoplasia (VaIN) is an uncommon pre-malignant condition of the vaginal epithelium compared to cervical intra-epithelial neoplasia (CIN) and the incidence has been reported as 0.2 per 100,000 women and account for 0.4% of the lower genital tract intraepithelial disease. The incidence of VaIN following hysterectomy for CIN3, who completed 10 years of follow-up, is 0.91%. The life-time risk of malignant transformation from VaIN to invasive vaginal carcinoma has been reported as 9-10%.

Conventionally vault smears are used to detect vaginal intra-epithelial post-hysterectomy for CIN. HPV DNA testing may be more effective at detecting persistent VaIN than conventional cytology. Debate continues if vaginal vault smears need to be followed up on a long term basis following hysterectomy for high grade CIN or cervical cancer due to the increased risk of primary VaIN lesion. There is no indication for screening following hysterectomy for benign disease as the risk of vaginal cancer is so rare.

CS 6-5

DIRECTIONS FOR RESEARCH IN SCREENING

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Are best practices linked to robust outcomes data? The answer to this question is a resounding 'no'. Only cytology, with its 70-year history, has been unequivocally linked to enduring reductions in cervical cancer incidence and mortality in resource-rich countries.
On the other hand, all subsequent technologies applied to cervical cancer screening have been evaluated via study designs that provide more credible evidence concerning their performance relative to cytology and overall efficacy in detecting cervical cancer precursors.
Although the value of HPV testing in primary screening is now widely accepted much remains to be understood before policymakers can gain full confidence in its implementation in cervical cancer prevention. Many questions remain unanswered. Is HPV testing followed by cytologic triage a better approach than HPV plus Pap cytology cotesting? If HPV testing is adopted for women ages 30 and older what screening options should be recommended for younger women? Is VIA a solution for low-resource settings, either alone or as triage for low-cost HPV primary screening? Is self-sampling a solution to expand the coverage and bring equity to screening? Algorithm management versus risk stratification: what is most suitable for guidelines? What is the role of HPV viral load as clinical tool? Is there a role for genotyping in screening or triage? What is the role of cytology-based staining for prognostic markers of lesion progression? How should we educate healthcare providers and patients concerning HPV testing results? What will be the impact of HPV vaccination on screening performance?
These questions remain promising avenues for new research on cervical cancer prevention.

CS 7-1

BIOMARKERS FOR TRIAGE OF HPV-POSITIVE WOMEN

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Infections with carcinogenic human papillomavirus types are a necessary cause of cervical cancer. Large randomized screening trials have demonstrated that HPV testing can be efficiently used for primary screening of cervical cancers. Women who test negative for HPV have a very low risk of cervical cancer and do not need screening for several years. Women who test positive for HPV are at increased risk of cervical precancer and cancer and require further evaluation to decide who should be referred to colposcopy (triage). Current triage options include cervical cytology and testing for HPV16/18. Several other biomarkers are different stages of evaluation, including p16/Ki-67 cytology, HPV E6/E7 mRNA assays, and viral and host methylation markers. The presentation will summarize the current state-of-the-art of triage markers for cervical cancer screening and will discuss new technologies that are in development.

CS 7-3

HPV GENOTYPING INTEGRATED IN SCREENING: MANAGEMENT BASED ON CIN 3+ RISK

Stoler M

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 genoptype specific risk vs. prevalence is debatable and will potentially incur algorithmic complexity as a trade-off with clinical utility.

CS 7-5

REDUCTION OF NUMBER OF COLPOSCOPY REFERRALS THROUGH THE USE OF SCREENING TESTS AND VACCINATION STATUS

Johannes Berkhof, Maaike Dijkstra, Margot Uijterwaal, Peter Snijders, Chris Meijer.

Objectives

To control the number of colposcopy referrals among HPV positive women, several triage strategies have been identified that are feasible and offer a good balance between safety and colposcopy burden. We focus on triage strategies with cytology and genotyping possibly combined with repeat cytology and/or repeat HPV testing. We will not only examine the impact of triage testing on the direct colposcopy referral rate but also investigate whether the number of colposcopy referrals can be further reduced by extending the screening interval. To assess whether extending the screening interval after negative triage is safe, long-term CIN3+ risks will be estimated.

Methods

Follow-up data of two Dutch screening trials (POBASCAM and VUSASCREEN) over multiple screening rounds 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 protective effect of different triage strategies against CIN3+ over multiple screening rounds will be shown. Separate analyses for HPV16/18-negative women will be shown to illustrate the anticipated long-term CIN3+ risks and colposcopy burden in vaccinated women.

Conclusion

Women that are HPV-positive at baseline have an elevated long-term CIN3+ risk compared to HPV-negative women, also after negative triage. For instance, in HPV-positive women that are negative for HPV16/18 and negative for cytology at baseline and after six months, long-term CIN3+ risks are higher than in HPV-negative women. This supports the idea of extending the interval only for HPV-negative women. The length of the extended screening interval may require further revision for vaccinated women in order to maintain screening program efficiency.

CS 8-1

RISK OF PREMATURE LABOUR AFTER TREATMENT OF CIN

Professor Maggie Cruickshank

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Preterm delivery remains a significant cause of perinatal morbidity and mortality but there is no single underlying cause. Worldwide data is limited but the rate of preterm delivery is estimated to range from 5% in developed countries to 25% in developing countries. The preterm delivery rate has been relatively stable around 5-10% in developed countries for many years, despite changes in colposcopy practice, with planned delivery accounting for about 50% of cases. Factors associated with spontaneous preterm birth are multiple pregnancy, previous preterm birth, young maternal age, BMI <19, low socio-economic status and cigarette smoking. Cervical incompetence is a rare cause of preterm labour but we cannot ignore the evidence which suggesting that treatment of CIN causes preterm delivery.

The data available can be difficult to interpret and we need to remember that the correlation between preterm delivery and treatment of CIN does not automatically imply that one causes the other. Larger studies have already identified that at least part of the increased relative risk of preterm delivery relates to common causal factors.

Both cervical stenosis and incompetence are well recognized complications of cold knife cone (CKC) biopsy. To conserve reproductive function, treatments of CIN, CGIN and micro invasive cervical cancer have moved to less radical excisional treatments and for CIN per se to excisional or ablative treatment. Since we believe that high grade CIN is a precursor of cervical cancer, we need to be certain that we pose the correct clinical question and take care to evaluate the evidence. For example, the inclusion of cases treated by radical treatments for CGIN and cancer are not relevant to the treatment of CIN. Treatment of CIN necessitates removal of the transformation zone to a depth of 7-8mm either by ablation or excision. There is no clear hypothesis on how the higher temperatures involved in cold coagulation to produce thermal coagulation effects are less disruptive to cervical function than the lower temperatures of radiofrequency electric current to cut tissue or achieve haemostasis in LLETZ or LEEP. It is more plausible, that excessive depth of treatment (a known risk factor) is responsible. Current evidence is limited as the amount of cervical tissue removed is not available in large studies of routinely collected data. If cervical damage is a recognized complication then we need to take the same approach to any surgical complication and improve our training and practice of the treatment at colposcopy if we believe that it is an effective means of reducing the risk of cervical cancer.

CS 8-2

ADENOCARCINOMA IN SITU: DIAGNOSIS, TREATMENT AND FOLLOW UP.

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Adenocarcinoma in situ (AIS) is a precursor of invasive adenocarcinoma of the cervix. It is also linked with HPV, especially HPV type 18. The incidence of AIS is rising since the last 20 years counting from 20% of invasive cervical cancers in the nineties to 35% in 2010. Many factors can explain this change: lower incidence of invasive squamous cell carcinoma, better screening with liquid base-cytology, better referral to colposcopy after "AGC" on cytology, and better pathology reports with the wide use of the LOOP excision which replaces destructive cryotherapy for CIN2-3.

It is well accepted that when a patient has "AGC" or "AIS" on cytology, a colposcopy is mandatory, even if colposcopic lesions induced by atypical glandular cells are more difficult to evaluate and interpret than CIN2-3. In front of a suspected lesion, a biopsy is done and an ECC should also be performed. When a biopsy confirms AIS, an excision surgical procedure (large loop excision or conisation) is the rule. When a suspicion of invasion is suggested, a "classical" conisation should give better pathologic evaluation than a LOOP excision.

The classical treatment of AIS is usually a simple hysterectomy. In the last years, if, on pathology, the margins are free of disease, the patient wants to keep her fertility and is reliable for follow-up, hysterectomy can be avoided. "Skip lesion" is reported in 6% to 16%. Therefore, a close follow-up is mandatory.

CS 8-3

HPV GENOTYPE MAPPING OF HIGH-GRADE CINS IN THREE MORPHOLOGICAL ZONES OF CONE SPECIMENS

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Objectives: To elucidate HPV genotype attribution in different regions of dysplasia, we performed molecular mapping of cervical disease based upon cone specimens. To determine the frequency of individual HPV types among CIN localized or generalized HPV infections spreading into the surrounding tissue we examined the HPV type frequency and distribution of single and multiple HPV types in normal epithelial tissue (NET), marginal zone/collision (≤CIN2) or CIN III/carcinoma in conization specimens due to CIN2 or worse in 65 women.

Methods: Micro-dissected tissue biopsies were taken from paraffin embedded cone specimens. All samples were reviewed by an experienced pathologist to diagnose individual areas with NET, ≤CIN2 and ≥CIN III. The DNA extractions were done by Pico PureTM DNA Extraction Kit and QuickExtractTM formalin-fixed paraffin-embedded (FFPE) DNA Extraction Kit, respectively. Genotype specific detection of the HPV was performed with the CLART® HPV2 microarray; detecting 35 defined high and low risk HPV genotypes.

Conclusion: All specimens were found to contain HPV in one or more of the three zones evaluated. Overall HPV was found in 31% of NET, 53% in the ≤ CIN2 and 67% for ?CIN III. Single HPV infections were found more often than multiple HPV infections, yet 28% of the HPV-positive specimens contained more than one HPV type. For NET, ≤CIN2 and ≥CIN III the frequency of multiple HPV types were 20%, 32% and 33% respectively. The most frequently found high-risk HPV type was HPV-16.
Overall, 1/3 of all evaluated cone specimens showed generalized HPV infections by micro-dissection. Generalized HPV infections also outside the CIN tissue might represent a higher risk of recurrence of disease after conization. These generalized HPV infections may represent early developing, regressing or latent infections, and can be addressed by detailed HPV diagnostics, rather than evaluating the resection border of the cone specimen morphologically.
Consequently, any infections found post conus can be defined as a new infection or recurrence of the CIN causing infection, thereby helping to risk stratify the women for recurrence of disease.

CS 8-4

COLPOSCOPIC EVALUATION OF HSIL CYTOLOGY WITH NEGATIVE HR-HPV TESTS RARELY DIAGNOSES ENDOMETRIAL CANCER

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Objectives: Since 1996, colposcopy records from SCPMG-Fontana have been stored in a FileMaker Pro (FileMaker Pro, Inc. Santa Clara, CA) electronic data base. The hc2 HR-HPV test was introduced in our clinic 8/10/98. During a review of the colposcopy clinic experience in SCPMG-Fontana 2007 to 2009, 240 women with cytology of High Grade Squamous Intraepithelial Lesion (HSIL) were found; 223 of these women had positive high-risk human papillomavirus (HR-HPV) (Hybrid Capture 2® [hc2]) tests while 17 had negative HR-HPV tests. Of these 17 women, 4 (23.5%) had cervical intraepithelial neoplasia (CIN) 3 and 2 (11.7%) had endometrial cancer. We hypothesized that endometrial cancer, which is usually HR-HPV negative, might be common among women with cytology of HSIL and negative HR-HPV tests. The colposcopy data base was reviewed to find women evaluated for cytology of HSIL with negative HR-HPV (hc2) tests; chart review was done to confirm their pathologic diagnosis.

Methods: For the period 8/10/1998 to 4/20/2013, 25,033 colposcopies were performed in the colposcopy clinics in SCPMG-Fontana. Cervical cytology of HSIL was found in 880 (3.5%) of these women. Of the 880 women with cytology of HSIL, 800 had a positive HR-HPV test, 21 had no HR-HPV test, and 59 had a negative HR-HPV test. The 59 women with cytology of HSIL and negative HR-HPV tests had a median age of 38 years [range 21-76]. Of these 59 women; 28 had pathology of negative, HPV, of CIN 1; 5 had CIN 2; 20 had CIN 3; 2 had adenocarcinoma of the cervix; 3 were not evaluated; and only 1 (1.7%) had endometrial cancer (Stage IIIC serous adenocarcinoma with involvement of the cervix and 4 pelvic nodes). The review of colposcopy experience 2007-2009 had a single miscoded diagnosis (coded as Grade 2 endometrial cancer rather than Stage IB1 adenocarcinoma of the cervix).

Conclusions: Endometrial cancer is not common among women with cytology of HSIL and negative HR-HPV tests; in fact, 37.5% of women with cytology of HSIL and negative HR-HPV tests had CIN 3 or cervical cancer. With large electronic data bases, there is a risk of miscoding. Unexpected associations within electronic data bases need to be verified rather than accepted verbatim.

CS 8-5

SEMM DEVICE FOR TREATMENT OF HIGH-GRADE INTRAEPITHELIAL LESIONS OF THE UTERINE CERVIX: EXPERIENCE IN A BRAZILIAN UNIVERSITY HOSPITAL

Colaborative project with IARC/WHO

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Introduction: Cervical cancer is the second most common malignant neoplasia in women. According to National Brazilian Cancer Institute data, about 6.1% of all female deaths from cancer occurred due to this type of cancer in 2010. The true precursor cervical cancer lesions are the High Grade Squamous Intraepithelial Lesion (HSIL), so the key is to detect and treat these lesions in all women. The diagnosis and treatment of HSILs through population screening programs has led in developed countries a 50-80% reduction in deaths from cervical cancer. However, existing screening programs in countries with limited resources have been less successful in reducing rates of cervical cancer due to in part to insufficient coverage of the treatment of women with premalignant cervical intraepithelial lesions detected. Different methods are available for treating these lesions that can be divided into excisional methods and destructive methods. Excisional methods are conizations with high frequency, laser and cold knife conization. Although the structure required to apply these methods in less developed centers are not often suitable to the availability of resources, equipment and trained professionals. Many destructive methods are available like laser, cryotherapy and others. The SEMM's device, may be an useful tool for different countries and regions, like in Brazil. It offers advantage over other methods since it is easy to use, inexpensive and does not require refrigerated gases such as cryotherapy, or any surgical proceeding.

Objectives: By IARC/WHO protocol, we present some data to evaluate the clinical utility, acceptability, efficacy and safety of SEMM's treatment for HSIL of the cervix outpatients in the Hospital de Clínicas de Porto Alegre RS - Brazil. (School Hospital of the Federal University/RS – Brazil).

Methods: We selected healthy women from 18 to 60 years old referred to the clinic of gynecology HCPA by high-grade cytology with an intact uterus and no history of debilitating physical and mental illness and who fit the following criteria: proven by biopsy with endocervical extension for up to a maximum of 1 cm and without invasion of the vaginal canal; squamocolumnar junction fully visible; with no clinical evidence of invasive cervical cancer. Colposcopy, treatment and follow-up of 1 month, 6 months and 1 year after application of SEMM's device were made in those eligible women. During the surveillance period, Colposcopy, cytopathological exam were performed to rule out recurrence of HSIL or progression of the disease. The SEMM's device was applied to the cervix of the uterus for about 60 seconds at a temperature of 100°C to 120°C to destroy the cervix transformation zone (TZ). The outpatient procedure was performed without anesthesia by trained professionals.

Results: In 52 patients enrolled, we may present the following data: The age of the patients are between 18 and 60 years old, with 33% of patients are aged 20-30 years old and 31% between 30 to 40 years old. Almost 70% of patients are married or living with a partner and 28% are single, 38% had education above 10 years and below 10 years remaining. Almost 60% of them have a family income between 1.5 to 3 minimum wage salaries, 6 patients were nulliparous, 11 gave birth only once and the others had 2 or more pregnancies. None was pregnant at the time of treatment. In carrying out the physical gynecological examination, 100% of patients had squamocolumnar junction and visible changes in the cervix after application of acetic acid and iodine solution. All selected patients had biopsy with HSIL result (CIN 2 or CIN 3), and 10% of patients had biopsy CIN 2 and 3 concomitant. The main time of follow-up is one year, no recurrence of HSIL proved by Colposcopy and Pap smear has occurred.

Conclusion: The data analyzed in this project until now suggested efficacy and safety of the SEMM's device for HSIL treatment, in our experience we may refer this device as an useful tool for treatment, specially in countries were surgery may be performed but to prevent patients lost follow-up or progression of disease, this kind of treatment is easy and feasible to be employed during the outpatient care, avoiding the need of a cervical proceeding and their potential complications.

CS 8-6

EVALUATION OF THE CLINICAL PERFORMANCE OF THE COBAS® 4800 HPV TEST IN A COLPOSCOPY REFERRED POPULATION

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Objectives: Testing for human papillomavirus (HPV) is now an important tool in managing women with cervical precancer. The potential value of HPV testing in cervical screening and management has led to the development of a range of HPV detection technologies. In this study the clinical performance of the cobas® 4800 HPV test for detection of high-grade disease in a colposcopy referred population was compared with the gold standard HPV test, Hybrid Capture 2.

Results: ThinPrep cervical smears were collected from 558 women referred to colposcopy with repeat abnormal cytology. Histological confirmed diagnosis was available for 491 patients. Biopsy confirmed CIN 1, CIN 2 and CIN 3 were identified in 29.7%, 22.8% and 20.2% respectively, 23.8% were normal on histology, 3.5% had an inadequate biopsy. The overall agreement between the cobas 4800® HPV test and Hybrid Capture 2 was 92.3% (95% CI 91.7%-92.9%). In women with CIN2+, HPV DNA was detected in 90.0% (95% CI 88.8%-91.3%) and 90.5% (95% CI 89.4%-91.7%) by the cobas® 4800 HPV test and Hybrid Capture 2 respectively. A subset of discordant results (n=23) were tested with Linear Array HPV Genotyping Test (Roche Diagnostics). This identified a small number of Hybrid Capture 2 positive/cobas® 4800 HPV negative which were positive for low-risk HPV types only with HPV 53 most commonly detected. The overall clinical sensitivity and specificity for detection of CIN 2+ was 90.0% (95% CI 88.8-91.3) and 55.5% (95% CI 52.5-58.5) for the cobas 4800® HPV test and 90.5% (95% CI 89.4-91.7) and 50.2% (95% CI 47.2-53.2) for Hybrid Capture 2.

Conclusion: In conclusion, the cobas® 4800 HPV test showed comparable performance to hc2 for detection of CIN 2+.

CS 8-7

SURFACE DISTRIBUTION FREQUENCY AND SEVERITY OF CERVICAL INTRAEPITHELIAL NEOPLASIA IN CHINESE WOMEN

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Objectives: Few data have examined patterns of CIN prevalence, stratified by cervical topographical location, for the development of clinical colposcopy guidelines. Our study aims to determine if the prevalence of histologically proven CIN lesions differs by cervical four-quadrant location or by 12-o'clock surface location of diagnosis.

Methods: We conducted a histopathological study of 19 different clinical studies from 1999 to 2010 by the Cancer Institute, Chinese Academy of Medical Sciences. Participants were enrolled if aged 16 to 65 years, not pregnant, with no history of pelvic surgery or irradiation, and with a positive result on one of various cervical cancer screening tests. After being referred to colposcopy, participants received a minimum of four biopsies, one in each cervical quadrant at the squamocolumnar junction at the 2, 4, 8, and 10 o'clock positions. The four cervical quadrants were labeled clockwise with one being at the upper right-hand corner. Only participants with complete four-quadrant biopsy results and pathological diagnoses were included in analyses.

Conclusions: In total, 44,004 biopsies were performed (34,358 (64.8%) random and 9,646 (18.2%) colposcopically directed). By random biopsy, CIN2+ lesions were more frequently found in cervical quadrants two and three than in quadrants one and four (χ^2 =313.48, p<0.0001). CIN1 lesions were also more likely to be detected in quadrants two and three (directed biopsy χ^2 =69.68, p<0.0001; random biopsy χ^2 =26.91, p<0.0001) than quadrants one and four. CIN 1 lesions were more frequent at the 12 hour clock face position. By directed biopsy, CIN2+ lesions were most likely to occur at the 4 and 7 o'clock positions, and least likely at the 11 o'clock position. By random biopsy, CIN2+ lesions were more likely to occur at the 5, 6, 7, and 12 o'clock positions. Our results suggest specific locations on the cervix may be predisposed to CIN occurrence, possibly secondary to anatomical and embryological causes. Prospective studies should confirm incidence and distribution of CIN on the cervix, which may help guide colposcopists in the early detection of cervical cancer.

CS 8-8

PROPORTION OF EXCISION AND PREGNANCY OUTCOMES AFTER LLETZ FOR CIN

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Objective: To determine how the proportion of the cervical volume/length excised affects cervical regeneration and pregnancy outcomes.

Material & Methods: Design: Prospective observational study.
Setting: University Hospital of Ioannina (from 1-2009)
Population: Women planned to undergo LLETZ for CIN who wish future fertility.
Interventions: The cervical volume&dimensions was calculated with MRI,3D-TVS or 2D-TVS before treatment. The volume&dimensions of the cone was assessed before fixation by a volumetric tube and a ruler; the percentage of excision was computed. Cervical regeneration was estimated by repeat MRI/3DTVS/2D-TVS at 6months.
Outcomes: Cervical regeneration in relation to proportion of excision–Pregnancy outcomes.

Results: A total of 198 women have been recruited (MRI:62, 3D-TVS:101, 2D-TVS:35); 176 completed 6months follow-up. Both the total cervical volume before treatment and the volume of the excised cone varied substantially. The estimated proportion of excision varied significantly between 4.7-41% (median 12.7%). Multivariate linear regression revealed that the proportional deficit at 6 months was determined mainly by the proportion of the excised volume. Subgroup analysis revealed similar findings for each imaging technique. Twenty-three women have conceived following treatment. Nineteen have already delivered, 15 at term, two at 34-16 and 2 at 30-32 weeks of gestation. Both preterm births were observed in women with large proportions of excision. Detailed and updated data on outcomes of the pregnancies will be presented.

Conclusions: Careful assessment of risks and benefits of treatment is essential when deciding to treat women who wish to have future pregnancies. All three imaging modalities appear to be equivalent in cervical volume measurements. Assessment of the cervical volume proportion and length excised might identify those that need further surveillance during future pregnancy.

CS 8-9

OUTCOMES OF WOMEN WITH UNTREATED CIN2 LESIONS: IS THERE A ROLE FOR HPV-RELATED BIOMARKERS?

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Background: A proportion of CIN2 lesions regress spontaneously. Unnecessary treatment may lead to morbidity while expectant management appears to be safe.
Objective : To review outcomes of women with untreated CIN2 lesions and to identify whether HPV-related biomarkers could safely predict the likelihood of regression.

Material & Methods:
Setting: Three University Hospitals; Imperial NHS Trust, London-Ioannina, Greece.
Period: 2009-2011
Population: Young women with histologically proven CIN2 lesions under close surveillance
Interventions: Follow-up data on cytology, colposcopy and histology were retrieved. In a subgroup with CIN2 (40%), an LBC specimen was prospectively obtained prior to colposcopy and tested for HPV typing, E6 & E7 mRNA by NASBA or flow cytometry, $p16^{INK4a}$ and microspectroscopy
Outcomes: Progression, persistence, regression rates at 24 months of follow-up. The sensitivity, specificity, PPV and NPV were calculated for combinations of biomarkers. The gold standard was histology.

Results: Out of 102 women, 29% were treated, 18% defaulted at least once, while 71% regressed spontaneously to low-grade or normal findings at 24 months. There were no cases of invasion. Low-grade cytology or colposcopy, young age, small lesions and HPV subtype other than 16 were related to a high likelihood of regression. HPV DNA test achieved high sensitivity, while the combination of NASBA mRNA and p16 optimal specificity; these could be integrated into a clinical algorithm. Results from a larger cohort will be presented.

Conclusion: A substantial proportion of CIN2 lesions in young women spontaneously regress. Some combinations of biomarkers appear to have significant accuracy in identifying misclassified lesions and in predicting lesions likely to regress. This could allow conservative management for women at low risk and avoidance of unnecessary treatment.

CS 8-10

A PLACEBO-CONTROLLED PHASE 2B STUDY OF HEXAMINOLEVULINATE PHOTODYNAMIC THERAPY IN PATIENTS WITH CIN1 AND CIN2

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Objective: Therapeutics for CIN1-2 are missing or associated with increased risk of prenatal complications. HAL PDT works by targeted photoactivation of porphyrins using red light, offering an easy and safe non-invasive modality preserving normal tissue.

Methods: This was a placebo-controlled phase 2b study performed in 262 patients with CIN1-2 using topical administration of HAL 0.2%, 1% and 5% applied to the cervix by a novel intravaginal device with an integrated light source (Photocure ASA, Oslo). Drug application was performed by the gynecologist, photoactivation automatically controlled and completed within 10 hours. The patient removed the device after completion of treatment. Patients were followed for 3 and 6 months, with an option of rePDT after 3 months. Diagnosis and response to treatment was done by adjudicated central review histology (DCL, IN), central HPV PCR testing (Norchip, Norway) and cytology. Primary end-point was overall response at 3 months.

Conclusion: Of 262 patients treated, 190 patients (103 CIN1 and 87 CIN2) were included in the final analysis. HAL showed a clear dose-response in patients with CIN2, HAL 5% being the preferred dose with a significant response of 95% (18/19 patients) vs 57% (12/21 patients) in the placebo group ($p<0.01$) at 3 months, which was sustainable for 6 months. HAL 5% also showed a robust HPV clearance of 77% (10/13 patients) vs 39% (7/18 patients) after 6 months. Further, in HPV16/18 positive patients, HAL 5% demonstrated a clearance of 83% (5/6 patients) vs 33% (2/6 patients) in the placebo group at 6 months. HAL did not show a significant response in patients with CIN1 due to a high spontaneous regression. Patient acceptability was high with only local, self-limiting mild to moderate side effects reported including vaginal discharge, discomfort and spotting.

The study documented a significant effect in patients with CIN2 as well as promising clearance of HPV infections including the most oncogenic subtypes HPV16/18. A phase 3 program is being planned for HAL PDT of patients with CIN2.

CS 8-11

A RANDOMISED TRIAL OF TOPICAL TREATMENT FOR VULVAL INTRAEPITHELIAL NEOPLASIA – PRELIMINARY CLINICAL RESULTS

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Objectives: Vulval Intraepithelial Neoplasia is a chronic condition that can cause distressing symptoms and progress to vulval malignancy. Surgery has been the mainstay of treatment, but recurrence rates and morbidity are high. A variety of novel therapies have been assessed, with small trials showing widely varying response rates. This study aimed to assess the efficacy of topical imiquimod and cidofovir in a women with VIN3.

Methods: In a multicentre, randomised phase 2 trial, funded by Cancer Research UK (CRUK /06/024), women with biopsy proven VIN 3 were randomised 1:1 to either imiquimod or cidofovir. The treatments were self applied by women, up to 3 times per week for up to 24 weeks. Biopsies were taken for histology and HPV testing within three months of commencing treatment and six weeks following completion. Randomisation was stratified by centre and 3 disease variables: new or recurrent disease, unifocal or multifocal and size of lesion. Primary endpoint was complete histological response.

Conclusions: 181 women were randomised by 29 centres, 90 to Cidofovir and 91 to Imiquimod. The average ages were 48 and 46 years respectively, (range 20-81). Multifocal disease was recorded in 45%, recurrent disease in 45%, with 41% of women with a history of intraepithelial disease at other sites. 59% were current smokers, 23% previous smokers and 18% had never smoked. There was a history of immunocompromise in 5%. Of the 141 pre-treatment samples tested to date, 114 (81%) were HPV 16 positive by HPV 16 E6 PCR and of those 45 (39%) showed evidence of integration, with a negative HPV 16 E2 PCR. Post treatment visits have recently been completed and results for clinical response will be presented.

VIN 3 is commonly associated with HPV and disease at other sites. Alternatives to surgery are eagerly awaited and this multicentre, randomised phase 2 trial will provide the most robust assessment to date of efficacy for topical treatment with imiquimod and cidofovir.

W 1-1

RESULTS OF A WORLWIDE E-LEARNING PROGRAM ON CERVICAL CANCER PREVENTION

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Background: Cervical cancer remains the second most important cancer in women worldwide and the cancer priority in most developing countries. Cervical cancer is largely preventable and if diagnosed and treated at an early stage is a highly curable disease. The advent of HPV vaccines and the impact of HPV technology for cervical cancer screening represent a milestone in our opportunities for prevention. The introduction of a new vaccine targeting women worldwide requires that literally tens of thousands of health professionals and decision makers understand its value and mode of use. A virtual course has been designed to provide such information to health professionals worldwide without costs to the participant and overcoming the limitations of travelling, time availability or language.

Objectives: Create and promote an e-learning educational program on HPV and cervical cancer epidemiology and prevention suitable for a wide audience of health professionals.
Create an international network of professionals qualified as key trainers in cervical cancer prevention in critical countries in the world.
The technological platform and the scientific and pedagogical methodology were provided by e-oncología, the e-learning platform from Catalan Institute of Oncology, (ICO) Barcelona, Spain.

Results: Output was an 18 hours distance course in Spanish, English, French and Russian. The course contents are largely based on the ICO HPV Monograph series. The program was scientifically validated and endorsed by the International Federation of Gynecology and Obstetrics (FIGO), the International Union against Cancer (UICC), the International Atomic Energy Agency (IAEA), the International Agency for Research on Cancer (IARC), and the World Health Organization (WHO), the course is being freely distributed.
Since September 2011 more than 8.000 professionals worldwide have registered to the course, a pool of 32 international tutors have been certified and acted as course professors in their own environment, and 70% of the students have been certified.

Conclusions: E-learning methodology with a tutorial support can be a good and cost affordable solution to the medical education in low income countries. The contents are easily adapted to each country particularities including translation to other languages.

W 1-2

EDUCATING WOMEN AND MEN ABOUT HPV ASSOCIATED CANCER SCREENING

Harper DM.

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Objectives: HPV causes several ano-genital cancers most of which have no precursor screening. Cervical cancer is the exception in that cytology sampling and HPV testing have been proven effective population screening tools.

Methods: Evidence based presentations were created to address both benefits and harms that screening presents, including the phenomenon of over-diagnosis. The limitations as well as potential benefits of HPV vaccination were included as well as the difficulties in implementing behavior changes.

Conclusions: To date over 10000 men and women have participated in educational seminars that discuss the role of screening for HPV associated cancers.

W 1-3

MAKING CLINICAL SENSE OF THE FAUNA OF INCREASINGLY INFORMED PATIENTS

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The Internet has become one of the most widely-used communication media and an almost infinite source of information. With the availability of Web 2.0 software, anyone can set up a professional type of Web site and publish information which is then accessible to all and can be virally multiplied. The problem is therefore no longer finding information but assessing the accuracy of a document retrieved from the Net. Too frequently, a given Web site provides no appropriate documentation regarding the scientific validity nor adequate referencing that support given claims.
And now some sites give more than information, they are proposing medical procedures! In the era of open forum access, videos featured on the YouTube medical school series challenge the monopoly of medical practice. Most of the videos fail to mention anything about the potential complications. Videos created by an orthopedic surgeon to provide practical examples of procedures to medical students «or anyone else who wants to study to be a doctor» include 600 videos in the series and have attracted more than 1,300,000 viewings (http://www.youtube.com/user/surgicalgown). Nowadays, bloggers only need a keyboard to become instead experts and able to attract attention of thousands with titles such as «what do Lysol and Gardasil have in common?».
Then how to cope with the flurry of information and be able to separate bad information from good? Looking for HON certification on the site is a good start. Health on the Net Foundation is a nongovernmental organization. It's code of Conduct (HONcode) for medical and health Web sites addresses one of Internet's main healthcare issues: the reliability and credibility of information. Code of Conduct helps standardize the reliability of medical and health information available on the Web and is free of charge. The certification conducted by HON implies a thorough evaluation of websites according to our HONcode guidelines including a continuous surveillance over the year and a systematic biennial review of HONcode certified websites (http://www.hon.ch/HONcode/Patients/). A practical tool for your patients might also be the website page «Immunization Information on the Internet: Can you trust what you read?» (http://resources.cpha.ca/immunize.ca/data/0288e.pdf).

Are all patients the same when bringing information to your attention? No! The typology of patients is quite diverse and the individual approach will need special attention to avoid conflictual situation. From the monkey patient that know all, to the porcupine conspiracy paranoid, to the gazelle looking indifferent but too shy to express doubts, to the wise horse balancing information, to the snobing giraffe that knows all, to the sly fox trying to find your errors and finally to the fighting pitbull that will not change his mind, each type of patients will need a specific approach.

Here are 5 strategies to attenuate conflicts around web information discussion with your patients: 1) active listening, 2) accept what your patient is saying, 3) acknowledge your patient's mood, 4) avoid confrontation by asking how your patient will resolve the differences between the information you have provided and the one your patient gathered on the net and 5) act as a team and not as the coach.

Information available on the Net is slowly and ineluctably changing the interaction between health professionals and patients especially when it comes to prevention or chronic disease. Patients are getting more engaged in their healthcare as they control the agenda and will not avoid confronting health advice if it does not fit their agenda.

W 1-4

HPV VACCINATION: SO WHAT NOW?

Prof. Dr. Federico Martinón-Torres

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The cumulating real life data supporting the success and safety of HPV vaccines is robust. However the existence of HPV vaccine recommendations, and actual degree of implementation and vaccine coverage in the different countries is variable. Every country should audit their actual achievements in HPV vaccination on the light of the available evidence in order to improve coverage. School-based, opt-out systems should be favoured, clearly stating that "opting-out" is an "active" decision with important health consequences as compared to the negligible risks of vaccination. Information -clear, concise and tailored to the audience- and education remain essential, not only of parents and vaccinees, but media, authorities and more importantly, health-care providers. While public acceptance of these vaccines is dependent on several factors, it has repeatedly been shown that active recommendation by a healthcare professional is key to vaccine uptake, clearly influencing vaccinee final decision. The lack of actual commitment and proactivity towards HPV vaccination in an important fraction of healthcare professionals contributes to explain the missed opportunity that the suboptimal HPV coverage reached in some countries means. Antivaccine groups can explain a part of the failure of HPV vaccination campaigns, but this can be counteracted with solid information, skilled communication and the coordinated action from HCP, authorities and media. Vaccination should be indeed considered a children right and thus, appropriately guaranteed. Establishment of integrated action plans to manage any eventual crisis related to the vaccines and involving all stakeholders should be encouraged.
Right now, information and education of both lay public and healthcare providers are key for the improvement in HPV vaccine coverage. Also, a better coordination of the different involved stakeholders is mandatory. HPV vaccines have fulfilled their expectations so far. We now have the obligation to ensure that we actually achieve their full potential.

W 1-6

CERVICAL CANCER-FREE COALITION: MOVING FROM EVIDENCE TO ACTION

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Objective: Through research, policy and advocacy, we have the potential to significantly reduce cervical cancer mortality. Globally, cervical cancer causes over 275,000 deaths annually, with most deaths occurring in less developed countries. Cervical Cancer-Free Coalition (CCFC) is a multi-year initiative driving public health efforts at the local level to reduce cervical cancer.

Methods: Initial activities were conducted in the United States in eight Cervical Cancer-Free America (CCFA) partner states. In May 2013, CCFC was launched to address cervical cancer prevention on a global scale. CCFC partner countries engage in multi-disciplinary activities which address four key challenges to the reduction of cervical cancer: i) HPV infection; ii) lack of screening; iii) screening errors, and iv) lack of follow-up care.

Conclusions: CCFC aims to foster the sharing of lessons learned from implementation programs at the country-level on HPV vaccination, screening and continuity to treatment in order to prevent cervical cancer. CCFA initiatives include, among others, a social media campaign in Kentucky that reached 641,000 individuals in the first month; an educational intervention of over 300 people about attitudes toward HPV vaccination at a Black Expo Health Fair in Indiana; an adolescent vaccination intervention that reached over 130,000 youth in Alabama; and a national policy evaluation of state cancer control plans in the United States to identify opportunities for state cervical cancer planning and program improvements. In March 2011, over 100 people attended a national launch meeting in Washington, D.C., resulting in concrete recommendations for policy, screening, and vaccination in the United States that were published in Cancer Causes and Control. CCFC is interested in identifying nationally-led coalitions to prevent cervical cancer country by country, utilizing the state partner model. Web-based sharing of lessons learned on implementation projects and policy initiatives for cervical cancer prevention on a global scale will allow the exchange of information regarding local country challenges and opportunities, while developing sustainable national programs to prevent cervical cancer based on local need and health care infrastructure.

W 2-1

AN OVERVIEW OF CERVICAL CANCER PREVENTION IN JAPAN

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Screening: Opportunistic screening for cervical cancer (CC) started in the late 1950s in Japan. In 1982, the Health and Medical Service Law for the Aged was passed and an organized CC screening program was implemented nationally. While coverage was not sufficient, the age-adjusted mortality rate of CC fell from 21.3 in 1960 to 5.3 in 1993. In 1998, the law changed and national funding for screening was stopped, leaving responsibility with local governments. As a result by 2004 organized screening coverage had fallen to 14% and total coverage including opportunistic screening was reported to be around 24%. From 2009, free screening coupons have been given to women aged 20, 25, 30, 35, and 40yrs, but have made little difference with annual screening uptake in 2010 said to be around 30% and $>$20% in women $<$29yrs. Consequently incidence of and mortality from CC in young Japanese women is increasing.

HPV Vaccination: The bivalent vaccine was licensed in October 2009 and available for use in December of the same year. The quadrivalent vaccine was licensed in July 2011. From November of the same year, national government agreed to pay 50% of the vaccine cost if local government paid the remaining 50%. Most (but not all) local governments accepted this offer and free vaccination became available for girls aged 12-16yrs. Initial uptake rates were promising, with over 70% of the target population vaccinated in the first year of the program. From April 2013, the vaccine was formally included in the Japanese National Vaccination Program for girls aged 12-16yrs. However, in June 2013, due to reports of adverse events, especially CRPS, the Japanese government partially suspended the HPV vaccination program. Local governments were told they could not actively promote the HPV vaccine, but at the same time health professionals were told they should continue to offer parents seeking the vaccine full support, and facilitate vaccination. Consequently confusion ensued and vaccination rates have dropped dramatically. A Vaccine Adverse Reactions Review Committee was established and will decide whether to once again promote the vaccine or remove it from the national vaccine program.

W 2-3

PARENTAL ATTITUDES TOWARDS HPV VACCINE IN JAPAN

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To better understand how to achieve high uptake rates of human papillomavirus (HPV) vaccination in Japan, two cross-sectional surveys and three studies took place between July and December 2010.

Study 1: A total of 2192 mothers of girls aged 11–14yrs were surveyed and 862 responses analyzed. Overall, 93% of mothers would accept the vaccine for their daughter if free, but only 1.5% would pay the minimum recommended price. Acceptance was higher in mothers who had heard of HPV vaccine (adjusted odds ratio, aOR=2.58, confidence interval, CI:1.47–4.53), and who believed susceptibility to (aOR =2.30, CI:1.34–3.92) and severity of (aOR=3.73, CI:1.41–9.88) HPV to be high. Recommendations from a doctor (aOR=12.60, CI:7.06–21.48) and local health board (aOR=27.80, CI:13.88–55.86) were also positively associated with increased HPV vaccine acceptance. Concerns about side effects (aOR=0.03, CI:0.01–0.08) and not participating in routine cervical screening emerged as barriers to vaccination (aOR=0.49, CI:0.27–0.91).

Study 2: A randomization study within a cross-sectional study of 3,471 mothers (1586 from a city with 55% screening uptake; H-City, and 1885 from a city with 8% screening uptake; L-City, of girls aged 11-14yrs took place to look at the influence of screening history on vaccine acceptance and the effect of an educational intervention. In total 1032 questionnaires (30%) were used in the final analysis. Both screening uptake and vaccine acceptance was higher in H-City, (50% vs 65%, $p<0.0001$) and (84% vs 90%, $P=0.04$), respectively. While the main barrier to HPV vaccine acceptance was safety in H-City (OR=0.28; 95%CI:0.12-0.53), in L-City it was mother's screening history (OR=0.27; 95%CI:0.12-0.61). However, providing an educational intervention significantly increased vaccine acceptability ($P=0.03$) in L-City to levels similar to those of H-City.

Study 3: We looked at attitudes of 27 fathers (16 single; 11 "married") who took part in studies 1 and 2 to see whether marital status affected vaccine acceptance. In all 93% were willing to vaccinate their daughter if free, with no statistical difference according to marital status. However, single fathers were significantly more likely to pay for the vaccine ($p=0.005$), when vaccination came at a cost. Discussing the sexual nature of HPV and having to take a child to the gynecologist were given as barriers to vaccination in single fathers.

W 2-3

PCAF CONDUCTS A SHINE CAMPAIGN FOR PUBLICIZING CURRENT KNOWLEDGE ABOUT CERVICAL CANCER

Kikuko Sakuragi

Vice Representative Director, general incorporatied association PCAF

PCAF stands for People's Campaign Against Female caner. PCAF comprises general citizens and physicians and it communicates with people in Hokkaido to provide them with accurate and essential information about cervical cancer. Japanese people were very unaware of cervical cancer when we started PCAF in 2007. They did not know that cervical cancer was increasing rapidly in young women and it became the most common cancer in young women. They feel ashamed to talk about cancer of female reproductive organs. We set our mission to deliver necessary information about cervical cancer to young women. So far, Japanese Government has not conducted any educational campaign to increase public awareness of prevention of cervical cancer. Under such conditions, we are conducting a Shine campaign to help young women comprehend causes and methods of prevention of cervical cancer through brochure, radio, newspaper, lecture at schools, and open lectures for citizen. We consider the low acceptance rate to cervical cancer screening is the most serious problem to be solved as soon as possible. The reason for not responding to invitation is "it is embarrassing", "wish for female doctors", and "not having enough time to get screened" and so on. Therefore, another mission of PCAF is to create user-friendly environment for cervical cancer screening.

W 2-4

HPV TESTING IN CERVICAL CANCER SCREENING AND FUNCTION-PRESERVING SURGERY IN CERVICAL CANCER TREATMENT

Noriaki Sakuragi

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Representative Director, general incorporatied association PCAF

The number of cases of cervical cancer in Japan is rapidly increasing among women <40 yrs old, accounting for 24.1% of all cervical cancer cases in 2011. Peak incidence of cervical cancer in Japan occurs in women aged 35-39 years old. I would like to discuss briefly the problems of low response rate to cervical cancer screening in Japan and the prevention of deterioration of quality of life (QOL) of patients after treatment.

The response rate to cervical cancer screening as of 2010 was only 23.9%. We would be able to think about some schemes to increase the response rate: 1) a free coupon for cervical cancer screening issued by local governments to women aged 20, 25, 30, 35, and 40, which started in 2009, 2) some local government introduced a model project of call/recall system and reported its effectiveness, and 3) a more user-friendly screening system to which women would likely to respond positively. We are very much interested in introducing self-sampling HPV test to increase the response rate to cervical cancer screening. Women with a positive result will be invited to formal cytological examination. We are planning to conduct a clinical study to validate the effectiveness of self-sample HPV testing followed by cytology in collaboration with Hokkaido University, Hokkaido Cancer Society, and a city with a population of around 120,000 people.

Many of women with cervical cancer are treated with radical hysterectomy. To prevent deterioration of physiological functions related to radical hysterectomy, we need to preserve ovarian function, vaginal function, bladder function and prevent lower leg edema. We shift the position of ovaries outside the pelvis at the level of the lower pole of kidneys; the vaginal stump is not suture-closed but left open and covered softly with peritoneum, which enhances neo-vagina formation; we preserve pelvic autonomic nerves comprising parasympathetic and sympathetic nerves, which are essential for urinary bladder for urinary collection and voiding; we leave the most distal part of lymph nodes along the exernal iliac arteries in situ and this simple procedure is effective for prevention of lower leg edema.

W 2-5

ASPARADISE — PATIENT SUPPORT GROUP FOR GYNECOLOGIC CANCER SUVIVORS

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Asparadise is the Hokkaido support group for women with gynecological cancer. Founded in 2004, it offers support and friendship to women affected by cervical, uterine and ovarian cancer.

The foundation was prompted by the publication of a gynecological cancer guide book in 2004 which I co-authored as a science journalist. Many requests came from readers for a place where gynecological cancers survivors could meet locally.

Our activities started in Sapporo, but have now expanded to other areas, and there are currently almost 600 registered members, including doctors and nurses.

Group activities center around meetings where patients and their families share their feelings and information. They are held every 2 to 3 months in public facilities or hospitals. Topics such as treatments, life after cancer, and communicating with doctors are casually discussed over tea and biscuits. Patients use the opportunity to try and ease their anxiety by discussing their daily hardships with those who have faced similar experiences and often leave the meetings feeling encourage or relieved.

For patient education, we hold lectures and workshops. We invite medical specialists to talk about treatments and care. Many gynecological cancer survivors experience serious post-treatment aftereffects, such as lymphedema, urinary disorders and menopausal symptoms, which not only prevent them from returning to a normal everyday life, but also result in psychological pain caused by the fear they may never recover. We tackle these problems together at self-care workshops and meetings.

Gynecological cancers result in the removal of organs which are an important part of a woman's sexuality. In addition, cervical cancer patients have to face prejudices around the fact that HPV is a virus that is transmitted sexually. Survivors of cervical cancer face three stigmas: cancer, loss of reproductive organs, and sexually transmitted virus infection. We try to get rid of such stigma. Although we know vaccination and regular cervical screening is important, we don't want phrases, such as: "If only you had been vaccinated/screened, then you wouldn't have ended up like this" to be used. This is to protect survivors of gynecological cancers from being referred to in a negative context. We ask for your support for survivors who live with such adversities.

W 2-6

ACTIVITIES OF THE JAPANESE EXPERT BOARD FOR THE ERADICATION OF CERVICAL CANCER TARGETING NIP AND HPV SCREENING

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The cervical cancer screening program was enacted as a national program in 1982 in Japan. The age-adjusted mortality rate of cervical carcinoma fell from 21.3 per 100,000 in 1960 to 5.3 per 100,000 in 1993. But, recent low screening coverage (approximately 24%) has caused an increase in the incidence (10,000) and mortality (3,500) of cervical cancer, especially in young women. This is due to insufficient education about cervical cancer and screening among the general public and limited financial support by the national government. The Japanese national immunization program (NIP) is based on an individual (clinic-based) program, and while routine vaccination is free, non-routine vaccines are basically self-pay. In November, 2008, we established the Japanese Expert Board for the Eradication of Cervical Cancer with the goal of obtaining national funding for HPV vaccination as a routine vaccine and higher coverage of more accurate screening, because educating policy makers and health professionals is important. Members include Clinicians, Virologists, Nurses, Midwives, Cytotechnologists, Epidemiologists, Cancer survivors, Patient support groups, and Cancer Organization. We have presented medical and economic evidence and made policy recommendations for the innovation of cervical cancer prevention in Japan. In December, 2009, one of the HPV vaccines was licensed and became available first as a private (non-routine) vaccine. In November, 2010, the national government and regional governments provided a tentative budget each providing 50% of the cost, and an interim nationwide HPV vaccination program was started. In April, 2013, the NIP was consequently revised and the HPV vaccine was included as one of the routine vaccines. Also, a national pilot program of cervical screening with HPV testing is planning to start in this year. However, since some politicians made an anti-HPV vaccine movement group, unfortunately the Japan government has concluded that, while the HPV vaccine should remain in our routine immunization program, for the meantime, it should not be proactively recommended since this June until the adverse events related to persistent pain (including CRPS: complex of regional pain syndrome) following HPV vaccination are further investigated and becomes clearer. We are now facing a crisis regarding the HPV vaccine in the NIP.

W 2-7

FOR JAPANESE GIRLS FACING THE RISK OF CERVICAL CANCER

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On June 14th the Japanese government withdrew its recommendation for HPV vaccine while analyses are conducted about adverse events(CRPS).These adverse events were reported by newspapers TV and weekly magazines this spring in Japan.Owing to this policy we fear Japanese young women will face an increased risk of cervical cancer after several years.

In 2010 the Japanese government provide funding for the HPV vaccine. Girls aged 12 to 16 year could get free vaccination because national and local government shared the cost of the vaccine. This spring, the Japanese government also included the HPV vaccine in its National Vaccination Program. In the first cohort of vaccinated girls, coverage is estimated at about 70%. And in this cohort adverse events were reported.

How does the policy of the Japanese government affect the health of young women?
The Japan cancer society (JCS) plans to do research on how this policy affects young women's health.

Japanese woman are recommended to have cervical cancer screening from the age of 20. The JCS is the largest cancer screening organization in japan. We carry out cervical cancer screening in around 1, 300,000 women per year. In the next several years we are going to interview medical examinees about inoculation of the HPV vaccine, such as,"Did you get HPV vaccine or not?"

And we will check the results of cervical cancer screening for the next several years.
We expect the discovery rate of CIN2-≥3 to be down in these several years.
But we fear HPV vaccine coverage in young women will also be down because of the Japanese government's withdrawing of its recommendation to inoculate HPV vaccine in 2013. And the discovery rate of CIN2-3 will increase again.

We must strengthen activities of cervical cancer prevention aimed at young women with low vaccination coverage as a result of this policy. We think it is a very important matter for save young women from cervical cancer in Japan.

W 2-8

SURVEY OF ATTITUDES TOWARD CERVICAL CANCER SCREENING

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Background and objectives: The number of new cervical cancer cases, including carcinoma in situ, and death in Japan is reported to be 17000 and 2500, respectively. However, the actual number is estimated to be over 20000 and 3500, respectively. On the other hand, the coverage of cervical cancer screening remains between 20 and 30 %, even with the presence of introductory vouchers for free screening. The issues are serious, especially in the young generation. Social, psychological, and other factors have been mentioned as barriers to cervical cancer screening in many studies, but there has been limited research presenting such evidence in Japan. We also face the problem of a lack of a national screening registry and quality check surveillance. My current plan is to conducttwo surveys of attitudes toward cervical cancer screening in collaboration with the Japan Cancer Society, targeting young Japanese women, to find out what kind of factors affect them adversely.

Methods: We plan to do 2 web-based surveys in the summer of 2013. The intended participants in one survey are 1000 young mothers under the mid-20s, and the other one will focus on 500-600 young women in their 20s and 30s, who are employed. Questions will include the following items: whether or not they know about cervical cancer and its screening, whether or not they go to see an obstetrics and gynecology specialist regularly, whether or not they have received cervical cancer screening, the reasons for not having received any, how they feel about cervical cancer screening services, their smoking history, educational background, income, etc. We will also ask them about HPV vaccination and cervical cancer. Because some adverse events related to HPV vaccine, such as chronic pains, have been reported, the Ministry of Health, Labour and Welfare decided to waive the recommendation on HPV vaccination in June 2013.

Outcomes: We will report the outcomes of our surveys in a session at EUROGIN WACC 2013.

W 2-9

LOCAL ACTIVITIES IN YOKOHAMA CITY

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Objectives: This study aimed to assesse the effectiveness of programs for the prevention of cervical cancer (CC) administered by local governments and communities in the capital city of Kanagawa Prefecture.

Methods: To assess the effectiveness of such program, we examined the following: outcomes of HPV vaccination program targeting girls aged 13-17 years in Yokohama City; attitudes concerning HPV vaccination among new female students in two universities, and attitudes toward CC screening in women belonging to a university hospital-based community who received catch-up HPV vaccinations in our hospital.

Results: The HPV vaccination program for young girls started in Yokohama in 2011, achieving a high prevalence above 70% among those in the target age range. However, the HPV vaccination prevalence of the newly enrolled university students was only 5% in 2011 and 13.5% in 2012. In addition, the uptake of CC screening within two years was about 45% even among medical associates who had received catch-up HPV vaccinations in our hospital. About half of the women who had never had CC screening before HPV vaccination had their first Pap test after the vaccination.

Conclusions : Our interim data reveals the need for improved CC prevention strategies for both young girls involved in the HPV vaccination program and young adult women who are excluded from the program due to age.

OC 1-1

METHYLATION OF THE HPV16 UPSTREAM REGULATORY REGION IN RELATION TO HPV PHYSICAL STATUS IN TONSILLAR AND ANAL CANCER

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Objectives: A substantial proportion of tonsillar and anal carcinomas are induced by human papillomavirus (HPV) oncogene overexpression. Previous analyses showed sustained upregulation of HPV oncogene expression in cervical cancers in association with reduced expression of the HPV early gene E2, which may occur following disruption of the E2 open reading frame upon viral integration into the host genome or by distinct methylation of the HPV upstream regulatory region (URR). The methylation patterns of the URR in anal and tonsillar cancers in association with integration status have not been analyzed yet, leaving their contribution to HPV oncogene expression unclear.

Methods: HPV16 +, $p16^{INK4a}$ + FFPE samples of 24 tonsillar and 44 anal cancers were microdissected. Methylation of 10 CpG sites in the URR (including 2 CpGs in E2 binding site 1 (E2BS) and 5 CpGs in E2BS 2, 3 and 4) was quantitatively analyzed by pyrosequencing. HPV physical status was determined by amplification of the E2 gene.

Conclusions: Eight out of 24 tonsillar samples showed integrated HPV16 status (E2-negative) and 16/ 24 presented the episomal or mixed (E2-positive) form. The percentage of methylation-positive CpGs (methylation >10%) varied from 67 to 100% for all CpGs. Median methylation level of the HPV16 E2BSs was low (</= 30%) in tonsillar samples with integrated HPV status while high methylation was found in the majority of tonsillar lesions with the episomal or mixed form. Low methylation of E2BS 2 was observed in all tonsillar carcinomas, irrespective of HPV16 physical status and in 24 of the currently analyzed 25 anal carcinomas. Methylation of distinct E2BSs appears to be related to HPV physical status in tonsillar cancers in analogy to the uterine cervix. Identifying regulatory mechanisms for HPV oncogene expression may impact on subsequent development of diagnostic or therapeutic techniques in tonsillar and anal cancers.

OC 1-2

INTEGRATION AND METHYLATION STATUS OF E2-BINDING SITES IN HPV 16 IN VULVAR AND VAGINAL CARCINOMA.

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Objectives: Integration of the viral genome has been proposed being an event preceding the transmission from premalignant lesions to carcinoma. However, studies on cervical carcinoma have shown that occasionally the viral genome is kept in episomal state and no, or only partial, integration occurs.

We have studied an alternative method for tumor development, where we hypothesize that methylation of E2-binding sites could deregulate E6 and E7 expression, thereby stopping E2 executing its regulatory role. E2BS3 and 4 are two E2- binding sites to p97, the early promoter of E6 and E7 that potentially could be essential for oncogene regulation.

Methods: Integration status was measured with realtime PCR for HPV 16 E2 and E6. E6 is present in both episomal and integrated viral genome, whereas E2 is deleted upon integration. Results were given as copy number in 20 ng DNA. Integration status was calculated by dividing copy numbers of E2 with E6.

Levels of methylation for E2BS2 and 3 were performed with pyrosequencing. Tumor DNA was bisulfite treated and primers specifically designed for PCR. Pyrosequencing of 5 positions in E2BS3 and 4 (31, 37, 43, 52, 58) were performed and results divided into low (0-10%), medium (10-50%) and high (50-100%) scores.

A total of 57 tumors were analyzed (31 vulvar squamous carcinomas and 26 vaginal carcinomas), collected between 1978-2008. All were previously genotyped for HPV 16.

Conclusions: 11 (19%) of the tumors were integrated (no E2 detected), 14 (25%) were episomal and 32 (56%) were evaluated as a mix of the two. Of the integrated tumors, 10 of 11 showed low methylation levels (0-10%) and one tumor had a medium methylation of 17 %. Most vaginal and vulvar tumors showed low methylation levels, only 6 (11%) tumors showed a high degree of methylation and 5 (9%) had medium degree of methylation. High degree methylated tumors were either in episomal or in mixed episomal/integrated state.

We conclude that only 11% of the tumors are integrated and that high methylation is seen in part of the tumors that are not integrated. Our results indicates that methylation of E2-binding sites could be an alternate mechanism for tumor development.

OC 1-3

METHYLATION OF VIRAL AND HOST GENES IN HPV-16 ASSOCIATED CERVICAL LESIONS

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Objectives: Methylation of viral and host genes could be used as a potential biomarker for risk of cervical cancer among women with HPV-positive cervical lesions. We examined the methylation status of selected sites in HPV-16 and human genes in women with HPV-16 positive cervical intraepithelial neoplasia (CIN) and invasive cancer.

Methods: DNA was extracted from exfoliated cervical cell samples in women harboring HPV-16 positive invasive cancer, CIN3, CIN2, or negative for intraepithelial lesions or malignancy (NILM) (N=25 each). Following bisulfite treatment, methylation of CpG sites 6367 and 6389 in the HPV-16 L1 gene and the human genes EPB41L3 and LMX1 was quantified by pyrosequencing. For all sites, there were significant trends in methylation levels increasing monotonically with lesion severity ($p<0.0001$). Unconditional logistic regression was used to assess the association between methylation and different contrasts of lesion grade severity. Odds ratios (ORs) were highest when comparing cancer with NILM (range: 53.8-infinity, lower 95% confidence bound for all 6 sites: 9.8). The methylation sites that were most independently discriminatory of a higher lesion severity were HPV-16 L1 site 6367 and host gene LMX1. LMX1 had the best discriminating ability for CIN3+ compared to NILM (OR=6.43, 95%CI: 1.6-26.2). Receiver operating characteristic (ROC) curves were used to analyze the diagnostic utility of methylation level for the different sites, as well as that of a composite score that included only the most independently predictive ones. The ROC Area Under the Curve (AUC) were: 0.908 (CIN2+ vs NILM), 0.645 (CIN3 vs. CIN2), and 0.936 (Cancer vs. CIN3).

Conclusions: Our results indicated that high methylation levels in viral and host genes are very common among precancerous and cancer lesions and can provide independent prognostic ability. Methylation of host gene LMX1 and of the viral HPV16 sites showed the most promising ability to distinguish among precancerous lesions and to identify propensity for invasion.

OC 1-4

DISCOVERY OF NEW METHYLATION MARKERS TO IMPROVE EARLY DETECTION OF HIGH-GRADE CERVICAL INTRAEPITHELIAL NEOPLASIA (CIN2/3)

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Objectives: DNA promoter methylation is a frequently observed early event in cervical carcinogenesis. Identification of methylation markers that allow discrimination between normal cervical epithelium and high-grade cervical intraepithelial neoplasia (CIN2+) might improve current population-based screening programs for cervical cancer. In this study, we aimed to identify new CIN2+ specific markers using genome-wide methylation screening to improve the sensitivity and specificity for detection of CIN2+ by DNA methylation analysis in cervical scrapings.

Methods and results: Methylated DNA of 20 normal cervices and 18 CIN2/3 lesions was enriched using MBD2 antibodies followed by genome-wide sequencing. After the statistical analysis of the promoter/exon1 region, 177 regions comprising 163 genes showed differential methylation between normal and CIN2/3 lesions. Internal validation using methylation specific PCR (MSP) showed that for 15 out of 19 markers MSP results were significantly related with the amount of sequencing reads. External validation using MSP on a new patient cohort of 18 normal cervices, 19 CIN2/3 lesions and 13 cervical cancers showed that for 2 out of 3 promising markers high methylation was found in CIN2/3 lesions (>84%) and in cervical cancers (>92%) and almost no methylation in normal cervices. Subsequently, diagnostic evaluation of both markers with QMSP on cervical scrapings was performed. Frequency and relative level of DNA methylation were significantly different between normal and cancer samples ($p<0.001$). In cervical scrapings from patients referred with an abnormal Pap smear, frequency and relative level of DNA methylation was significantly different between low-grade CIN lesions and CIN2+ lesions ($p<0.001$) In addition, ROC analysis showed that the methylation level was discriminative between low-grade CIN lesions and CIN2+ ($p<0.001$).

Conclusion: Using innovative technologies, a genome-wide search for epigenetic changes in (pre)malignant cervical lesions was conducted. Evaluation of identified markers for CIN2+ in cervical scrapings showed interesting candidates for early detection of high-grade CIN. This project is funded by the Dutch Cancer Society (RUG 2009-4577).

OC 1-5

HPV MANIPULATION OF THE SUMO PATHWAY

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Human Papillomaviruses (HPVs) infect stratified epithelium and are the causative agents of cervical cancer, the second most common cause of cancer-related death in women.

Objectives: A critical aspect that still persists in the HPV field is the selection of very sensitive and specific HPV diagnostic assays. Here we provide evidence that proteins involved in the Small Ubiquitin-like Modifier (SUMO) pathway, in particular Ubc9, are affected by HPV E6 and E7 expression and thus could play a major role in the diagnosis of oncogenic HPV infections.
SUMOylation is a post-translational modi?cation that regulates fundamental proteins function. It requires the covalent attachment of a member of the SUMO family to speci?c proteins by E1 activating enzyme (SAE1/SAE2), E2 conjugating enzyme (Ubc9), several SUMO E3 ligases, and SUMO cleaving enzymes. In humans SUMO1, SUMO2 and SUMO3 are ubiquitously expressed. SUMO1 shares approximately 45% sequence identity with SUMO2/3, whereas SUMO2 and SUMO3 are about 96% identical to each other, thus, are often referred to as SUMO2/3.

Methods: We will discuss both in vitro and in vivo data showing that that the expression of crucial proteins in the SUMO pathway is upregulated in high-risk human papillomavirus (HPV) positive lesions, in a manner dependent on HPV oncogene expression.

Conclusions: Our results indicate that HPV 16 extensively modifies the SUMO pathway, candidating its modulation as an effective way to block the persistence of HPV infection, replication and tumor growth in the host.
Furthermore, UBC9 is a promising diagnostic marker for HPV infections.

OC 1-6

CLINICAL VALIDATION OF P164INKA/KI-67 FOR THE USE IN ROTINELY STAINED SMEARS

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Objectives: In Germany, cervical cancer screening is regulated by the German Federal Ministry of Health and Social Security. It is available for all women from the age of 20 on the basis of the Papanicolaou (PAP) smear. $p16^{4INKa}$/Ki-67 testing (Roche Diagnostics Germany) is reimbursed in cervical cancer screening in case of LSIL (PAP 3d) or HSIL (PAP 3d) and ASCUS-H (PAP 3) lesions. We evaluated the histological outcome of $p16^{4INKa}$/Ki-67 positive and negative specimens. It is reported that $p16^{4INKa}$/Ki-67 positive lesion are more likely to progress than negative ones.

Methods: Between September 2010 and April 2013 we collected data from 3.464 women who were tested for $p16^{4INKa}$/Ki-67. To date, data of 1.558 women is available. The full data set will be available at the time of the conference. Baseline Pap status was measured at enrollment. Cervical smears were classified according to the Second Munich Nomenclature (1989). The results were converted to the nearest equivalent in the Bethesda system.

Conclusions: 765 (49.10%) of the 1.558 samples were positive for $p16^{4INKa}$/Ki-67. To date, histology confirmed 120 CIN 3 (15.68%), 44 CIN 2 (5.75%), 29 CIN 1 (3.79%) lesions and two ACIS within the $p16^{4INKa}$/Ki-67 positive group. Nine $p16^{4INKa}$/Ki-67 positive specimen demonstrated no malignancy. Cytology proved in 37 cases a progression to HSIL (Pap IVa). Within in the group of 793 (50.90%) samples that were $p16^{4INKa}$/Ki-67 negative histology confirmed 13 CIN 3 (1.64%), 15 CIN 2 (1.89%), 16 CIN 1 (2.02%) lesions. 17 $p16^{4INKa}$/Ki-67 negative specimen demonstrated no malignancy. 46 histologically verified CIN 3 lesions arose in women who were younger ≤30 years. 17 of these CIN 3 lesions arose in women who were younger ≤25 years. Starting cervical cancer screening at the age of 20 years remains important as this could have prevented the development of a CIN 3 lesion. Our data suggest that adding $p16^{4INKa}$/Ki-67 testing in conjunction with cytology could help to identify women with an underlying cervical lesion who have an elevated risk of developing severe cervical lesions.

OC 1-7

DETECTION AND QUANTIFICATION OF HPV E7 ONCOPROTEINS IN CERVICAL SMEARS

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on behalf of the PIPAVIR consortium (www.pipavir.com)

Objectives: 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 mainly on cytological analyses (Pap smear), which suffer from frequent false-positive and false-negative results. Our finding that E7 oncoproteins are expressed continously 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.,

Methods: 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.

Conclusion: Our results suggest that the detection and quantification of E7 proteins in cervical smears is feasible and provides a promising alternative solution for the reliable detection of HPV-driven precancerous lesions.

OC 1-8

DEVELOPMENT OF A ROBUST IMMUNOFLUORESCENCE TECHNIQUE TO DETECT HPV E4 PROTEIN IN LIQUID BASED CYTOLOGY (LBC) SAMPLES

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Objectives: The low sensitivity of cervical cytology for detection of high grade lesions necessitates exploration of alternative screening strategies. While detection of HPV DNA boasts high diagnostic sensitivity, identification of clinically significant infection will require additional biomarker detection. We hypothesise that prediction of disease severity following HPV infection will be possible by the detection of the HPV late protein E4 and a surrogate marker of viral early proteins, mini chromosome maintenance protein (MCM). Loss of E4 may be considered a marker of clinically significant disease.

Methods : We developed and optimised a novel immunofluorescence assay for the detection of E4 and MCM in liquid based cytology (LBC) samples. We trialled different methods of sample collection, storage and preparation to optimise detection of E4 and MCM using fluorescent microscopy on ThinPrep slides prepared from LBC samples. Performance of the assay when applied to clinical samples (both archived obtained from the Scottish HPV Archive and prospective collected from women attending colposcopy clinics) will be presented.

Conclusions: This is the first study we are aware of to show that identification of MCM and HPV E4 by immunofluorescence is technically possible in clinical samples prepared using the ThinPrep system. Clinical data will provide further insight into how combined detection of a host proliferation marker and a marker of late viral gene expression can enhance the risk-stratification of HPV infection and associated disease.

OC 1-9

GENETIC VARIABILITY IN THE MAJOR CAPSID L1 PROTEIN OF HUMAN PAPILLOMAVIRUS TYPE 16 (HPV16) IN THE NETHERLANDS

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Objectives: Intratypic molecular variants of HPV type 16 and -18 are known to occur and are distributed differently within the five continents. In the Netherlands, a bivalent vaccine, composed of recombinant L1 proteins from HPV16 and -18, is used to prevent cervical cancer since 2009. Long term vaccination with L1 proteins, could lead to changes in HPV16 and 18 virus population. In order to be able to detect these changes, knowledge of the genetic diversity of L1 gene in HPV16 and -18 viruses circulating in the Netherlands at the start of vaccination is required.

Methods: Samples were obtained from swabs collected in 2009 and 2011 within the PASSYON (PApillomavirus Surveillance among STI clinic Youngsters) study among Dutch 16- to 24-year old male and female attendees of the sexually transmitted infection (STI) clinics. HPV DNA detection and genotyping was performed previously using the PCR-based reverse line blotting (SPF10-LiPA system version 1, DDL Diagnostic Laboratory). The entire L1 gene was amplified in 3 overlapping PCR products and sequenced by classic Sanger sequencing in 213 HPV16 positive samples from women. Sequences were aligned using Bionumerics and compared with HPV16 European German reference sequence AF536179.

Conclusions: Sequencing of the entire HPV16 L1 gene revealed 95 SNPs (68 silent and 27 non-silent mutations) in all samples. The majority of the HPV16 isolates (198/213, 93%) clustered with the European/Asian types and 16/213 (7%) with the African variants. The most common L1 sequence found was detected in 31% of the samples and was very similar to the reference strain differing in only two positions with silent mutations. The majority of the non-silent mutations (17/27, 63%) was located in sequences encoding alpha helix, beta sheet or surface loops, in particular in the immunodominant FG loop, and may influence the protein secondary structure. Taken together, this study provides unique pre-vaccination data on the genetic variation of the L1 gene of HPV16 viruses circulating in the Netherlands among adolescents and young adults.

OC 1-10

PROTEOMIC ANALYSIS OF THE CERVICOVAGINAL FLUID LEADS TO IDENTIFICATION OF BIOMARKERS FOR CERVIX CANCER

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Objectives: Cervicovaginal fluid (CVF) is composed of secretions originating from organs that are part of the female genital tract, including vagina, cervix, endometrium and ovaries. We therefore postulate that CVF contains a wealth of information concerning the status of all female genital organs, including the HPV infection status of the ectocervix. A differential proteomics study on CVF was therefore performed, with the aim to find CVF biomarkers that correlate with progression towards cervix cancer.

Methods: Six CVF samples from healthy women and six samples from precancerous women were run over a 2D-LC-MS/MS proteomics platform and quantified by spectral counting. After comparison, we identified one protein that was present and absent in all CVF samples originating from precancerous and healthy women, respectively ('qualitative biomarker'). In addition, we also found four proteins that showed a marked up- or downregulation in one of the two conditions (at least 3-fold; 'quantitative' biomarker). ELISA experiments on 2x9 samples from other healthy and precancerous women confirmed a clear difference in average concentration of the above mentioned 'qualitative' biomarker. Moreover, samples originating from woman infected with low-risk HPV types 6 or 11, also showed an augmented concentration of this protein. The marker also followed the appearance or the clearance of the virus in longitudinal samples, taken from the same individual at consecutive time points.

Conclusions: These results show that CVF is a body fluid that contains biomarkers which can be used for follow-up of precancerous women. Since the fluid is easy obtainable by the practitioner or even by the patient herself, the biomarker could be used to optimize current screening programs, eventually making use of a self-diagnosis test.

OC 1-11

MIR-125B EXPRESSION IN HPV INFECTION AND CERVICAL CANCER DEVELOPMENT: A POTENTIAL BIOMARKER

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Objectives: Clinicians are still demanding for predictive/prognostic biomarkers for HPV infection and cervical lesions progression. Recent studies suggested microRNAs as possible biomarkers of HPV-associated cancers, and therefore we aimed to characterize miR-125b expression in cervical samples.

Methods: miR-125b expression was determined by qRT-PCR methodology in 110 women with different cervical lesions: normal epithelium with (n=20) and without (n=29) HPV infection: LSIL (n=28); HSIL (n=21) and CIS/ICC (n=12).
Conclusions: We observed a two-fold increased miR-125b expression among normal cases with HPV infection ($2^{-\Delta\Delta Ct}$=2.11; p=0.038). Data also showed a trend to down-regulation of miR-25b in LSIL and HSIL cases and a significant decreased expression in women with either CIS or ICC ($2^{-\Delta\Delta Ct}$=0.75; $2^{-\Delta\Delta Ct}$=0.78; $2^{-\Delta\Delta Ct}$=0.33 and $2^{-\Delta\Delta Ct}$=0.23, respectively) and its levels were able to predict CIS/ICC with 75% sensitivity and 69% specificity. This is the first study to characterize the expression of miR-125b in cervical samples. Results start by demonstrating that miR-125b expression is increased in normal cervix infected by HPV, while the relative expression seems to decrease significantly as lesions progress. These data suggest that miR-125b levels could be used as potential predictive/prognostic biomarkers of HPV infection and cervical cancer.

OC 2-1

INCIDENCE OF POTENTIALLY HPV RELATED CANCER IN GERMANY - AN ANALYSIS USING NATIONWIDE CANCER REGISTRY DATA.

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Objectives. A recent dynamic model on time trend analyses in cervical cancer incidence and mortality predicted a substantial reduction of both rates within the next decades in Germany [1]. Since 2007, HPV vaccination is recommended for girls aged 12-17 years. This might additionally reduce the burden of HPV related disease other than cervical cancer. We estimated the epidemiologic cancer burden of penile, vulva, vaginal, anal and oropharyngeal (OP) (including base of the tongue, tonsils) squamous cell carcinoma (SCC) in the German population before and shortly after introduction of HPV vaccines.
Methods. Analyses were performed on German cancer registry data (2005-2009) from 9 federal states (covering approximately 50% of the German population) with a >90% degree of completeness of registration. Selection of cancer sites were based on established evidence of the potential role of HPV in carcinogenesis in non-cervical cancer sites (ICD-10) with squamous cell origin (ICD-O-3) [2].
Conclusions. From an estimated 46 067 new cases of non-cervical anogenital and OP cancers registered within 5 years, 85.9% (n=39 547) were considered potentially HPV related. OP SCC contributed 18 975 new cases, 78.1% by men. The majority was diagnosed at the age of 50-69 years, with an incidence rate (IR) of 20.1 per 100 000, while the age-standardized incidence rate (ASIR) was 6.2 per 100 000 in men and 3.8 per 100 000 in the general population (all ages). Vulva SCC posed the largest burden in women with 10 139 new cases in 5 years with a median age of 71 years (IR women ≥70years: 16.6 per 100 000). Even though not every SCC can be attributed to HPV (estimated 38% of OP; 88% of anus; 43% of vulva; 70% of vagina; 50% of penis cancer [3]), SCC from other sites than the cervix contribute substantially to the burden of potentially preventable cancer cases.
[1] Horn, J. et al. Vaccine (2013) 31:2372-2380; [2] IARC Volume 100B (2009). Lyon, France; [3] De Martel, C. et al., Lancet Oncology (2012) 13(6):607-15.

OC 2-2

TRENDS IN INCIDENCE OF CERVICAL CANCER FROM 1985 TO 2009 IN KANAGAWA, JAPAN

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Objective: To investigate trends in incidence of cervical cancer among Japanese females in Kanagawa Prefecture, Japan.

Methods: From among a total population of about 9.06 million over a 24-year period (1985-2009), newly diagnosed cervical cancer cases reported to the Cancer Registry of Kanagawa Prefecture were analyzed. Based on age, patients were classified into the following two groups: 15-49 years old and 50 years and older. Chronological changes in age-specific incidence rates were then compared between the two groups.

Conclusions: A total of 15248 females were diagnosed with invasive cervical cancer (10699 cases) and carcinoma in situ (4549 cases) during the period covered by this study. The age-specific incidence rates of cervical cancer, including both invasive cervical cancer and carcinoma in situ, per 100,000 females in the 15-49 year-old group increased from 13.7 (1985-1989) to 19.2 (2005-2009); however, these rates decreased from 31.4 to 18.2 in the 50 years and older group. Furthermore, the peak of the distribution of age-specific incidence rates has shifted from over 70 years old to between 30 and 40 years old during the same period.

OC 2-3

COMMUNITY-BASED INTERVENTIONS AND TEMPORAL CHANGES IN THE CHARACTERISTICS OF BLACK CERVICAL CANCER CASES IN A LOW-RESOURCE SETTING

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Objectives: The Alabama Black Belt (southern United States) comprises 17 low-resource, historically underserved counties. Black females in some of these counties have cervical cancer incidence and mortality rates comparable to rates in developing nations. During the past 15 years, several community-based interventions including the Deep South Network for Cancer Control, the Deep South Cancer Navigation Network, and the Alabama Breast and Cervical Cancer Early Detection Program have aimed to reduce the burden of cervical cancer among Black females in the Alabama Black Belt by promoting education, screening, and local access to care. The aim of our analysis was to assess whether interventions targeting Black females in the Alabama Black Belt were accompanied by temporal changes in the characteristics of cervical cancer cases referred to our comprehensive cancer center (University of Alabama at Birmingham Health System [UABHS]).

Methods: We used data from the UABHS Tumor Registry. We selected all Black females who resided in Black Belt counties when diagnosed with cervical cancer during the period 1980 – 1984 (n=53) or the period 2005 – 2009 (n=38). We assessed temporal changes in age at diagnosis, stage, and histologic subtype of cervical cancer between these periods for Black females in the Alabama Black Belt. We used Wilcoxon rank sum test or Fisher's exact test, as indicated, to assess changes in characteristics between the time periods.

Conclusions: The median age at diagnosis was largely unchanged between the study periods (53 to 54 years; P=0.49). Late-stage (stage IV metastatic disease) diagnosis decreased from 6.8% to 0% (P<0.001), whereas the percentage of adenocarcinomas increased from 1.9% to 16% (P=0.04). Our results suggest that community-based interventions targeting Black females in the Alabama Black Belt may be altering the pattern of referral to the UABHS. Community-based interventions may be useful in other low-resource settings

OC 2-4

YOUNGER AGE DISTRIBUTION OF CERVICAL CANCER INCIDENCE AMONG SURVIVORS OF PEDIATRIC AND YOUNG ADULT CANCERS IN THE UNITED STATES

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Objective: We explored whether the age distribution of cervical cancer incidence differs between females in the general population and survivors of pediatric and young adult (PAYA) cancers for potential insight about the epidemiology of cervical cancer among PAYA cancer survivors.

Methods: We used data from 9 population-based registries of the United States Surveillance, Epidemiology, and End Results program collected between 1973 and 2010 to assemble two cohorts for comparison: 1) a cohort of females diagnosed with cervical cancer subsequent to a prior diagnosis with any cancer between the ages of 0 and 29 years (i.e. PAYA cancer survivors; n=46), and 2) a cohort of females for whom cervical cancer was the primary diagnosis (i.e. the general population; n=40,968). We compared the overall, race-, and stage-specific median age at diagnosis for both cohorts. We tested the hypothesis that the median age at diagnosis was equivalent in both cohorts using the Wilcoxon rank sum test with a significance threshold of ·=0.05.

Conclusions: The median age at diagnosis of cervical cancer overall for PAYA cancer survivors and the general population was 33 years and 48 years, respectively (P<0.001). The differences in median ages at diagnosis between PAYA cancer survivors and the general population persisted regardless of race or stage (e.g. localized disease: PAYA cancer survivors = 32 years, general population = 42 years; P<0.001). Our results suggest that PAYA cancer survivors are considerably younger when diagnosed with cervical cancer than females in the general population. Given the comparability of cervical cancer screening rates between these populations and the persistent differences in age at diagnosis regardless of race or stage, our results support the interpretation that PAYA cancer survivors experience more rapid progression to invasive cervical cancer than other females.

OC 2-5

INCREASED AGE AND RACE-SPECIFIC INCIDENCE OF CERVICAL CANCER AFTER CORRECTION FOR HYSTERECTOMY IN THE UNITED STATES FROM 2000 TO 2009

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Objectives: Invasive cervical cancer is thought to decline in women over 65 years old, the age at which cessation of routine cervical cancer screening is recommended. However, national cervical cancer incidence rates do not account for the high prevalence of hysterectomy in the US.

Methods: Using data on the prevalence of hysterectomy from the Behavioral Risk Factor Surveillance System (BRFSS), we estimated hysterectomy-corrected age-standardized and age-specific incidence rates of cervical cancer from the Surveillance, Epidemiology, and End Results (SEER) 18 registry in the United States from 2000-2009. Trends in corrected cervical cancer incidence across age were analyzed using Joinpoint regression.

Conclusions: Unlike the relative decline in uncorrected rates, corrected rates continue to increase after age 35-39 but at a slower rate than in 20-24 years ($APC_{CORRECTED}$=6.36). The highest corrected incidence was among 65-69 year old women, with a rate of 26.4 cases per 100,000 women as opposed to the highest uncorrected rate of 15.4 cases per 100,000 aged 40-44 years. Correction for hysterectomy had the largest impact on older, black women given their high prevalence of hysterectomy. In summary, correction for hysterectomy resulted in higher age-specific cervical cancer incidence rates, a shift in the peak incidence to older women, and an increase in the disparity in cervical cancer incidence between black and white women. Given the high and non-declining rate of cervical cancer in women over the age of 60-65 years, when women are eligible to exit screening, risk and screening guidelines for cervical cancer may need to be reconsidered.

OC 2-6

INCIDENCE TRENDS OF CERVICAL CANCER AND HIGH-GRADE CERVICAL INTRAEPITHELIAL NEOPLASIA IN DENMARK, 1997–2011

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Objectives: A declining incidence of cervical cancer (CC) has been observed in several developed countries often coinciding with the onset of screening programs. The majority of this decline has been due to a decline in squamous cell carcinoma (SCC) incidence. In contrast, the incidence of cervical adenocarcinoma (AC) has been observed to increase especially among young women in some countries including Denmark. In this study we investigated the incidence of cervical cancer in Denmark with focus on the subtypes SCC and AC during 1997–2011 where the coverage of the screening programme has been high. Furthermore, we estimated the incidence of carcinoma in situ (CIS), and adenocarcinoma in situ (AIS) in the same period.

Methods: Using two nationwide registries, the Danish Cancer Registry and the Danish Pathology Data Bank, we estimated age-specific and age-standardized incidence rates and estimated annual percentage change (EAPC) for CC, SCC, AC, CIS, and AIS.

Results: The incidence of CC, SCC and CIS significantly decreased from 11.9 to 11.1 per 100,000 women-years from 1997–1998 to 2011 (EAPC: –1.4% (95% CI: –2.4;–0.3)), from 9.4 to 7.8 per 100,000 women-years (EAPC: –2.2% (95% CI: –3.2;–1.2)) and 40.2 to 29.9 per 100,000 women-years (EAPC: –2.5% (95% CI: –4.2;–1.0)), respectively. In contrast, the incidence of AC, and AIS, significantly increased from 2.2 to 3.1 per 100,000 women-years (EAPC: 1.6% (95% CI: 0.1;3.1)), and 3.6 to 10.7 per 100,000 women-years (EAPC: 7.4% (95% CI: 5.3;9.0)), respectively, in the same period.

Conclusions: The incidence of CC, SCC and CIS decreased significantly during the study period, primarily due to a decreased incidence in women 45 years and older, while the incidence of AC, and AIS significantly increased, due to an increased incidence in women 44 years and younger.

OC 2-7

MORTALITY OF NON-PARTICIPANTS IN CERVICAL CANCER SCREENING

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Objectives: The evaluation of the effect of screening programs can potentially be biased by differences in the health profile of participants and non-participants. Determinants of cervical screening participation are well-known and often associated with poorer health status, but the resulting impact they have on the mortality has hardly been studied. Our aim was to quantify the relative mortality of non-participants vs. participants.

Methods: Data were retrieved from the Danish Pathology Data Bank, the National Health Service Register and the National Patient Register for screening participation, and from the Civil Registration System and Cause of Death Register for vital status and deaths. Screening participation was first evaluated over 4 years, corresponding to one screening round taking place in 1990-1993, allowing for some delay in attendance. Then, 2 screening rounds (1990-1997) were taken into account. Women were followed until 2010. We computed hazard ratios (HR) from Cox models to compare the risk of death in non-participants to the risk in participants. Sensitivity analyses were performed to assess the impact of age at participation, calendar period of the screening rounds evaluated, intensity of screening, length of follow-up, and cause of death.

Conclusions: Non-participants in cervical screening have a substantially higher mortality than participants. After 1 screening round, the HR for premature death in non-participants was 1.61 (95% CI: 1.59-1.63) over a 17-year follow-up. After 2 rounds, women who never participated had a HR of 2.09 (95% CI: 2.05-2.14) compared to those screened regularly, whereas those participating irregularly had a HR of 1.54 (95% CI: 1.51-1.57). Higher HRs were found in younger age groups, where coverage rates were higher. The excess risk of death was not higher in the first years following screening evaluation, suggesting that acute illness was not a major reason for women's non-participation. Being screened more than recommended by the guidelines was not associated with lower mortality compared to being screened in line with the recommendations. The risk of death by cancer was moderately increased as compared to e.g. cardiovascular disease. These results could help interpret future epidemiological studies on the effect of cervical cancer screening, and help identify women at risk for a premature death.

OC 2-8

LOWER SOCIOECONOMIC STATUS GROUPS HAVE AN INCREASED RISK OF CERVICAL CANCER REGARDLESS OF SCREEN COVERAGE

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Objectives: **Women with lower socioeconomic status (SES) participate less often in screening programs and are more often** diagnosed with cervical cancer. In the present study we determined whether differences in background risk contribute to the increased risk of developing cervical cancer in women with lower SES.

Methods: We analysed data from first smears only, thus eliminating the effect of an incomplete screen history. Retrospective data were obtained from the Dutch nationwide network and registry of histo- and cytopathology (PALGA). We identified all women who had had their first smear, within or outside the national screening program, between 2000 and the first quarter of 2009. Based on the outcome of these smears and the direct follow-up, women were subdivided into those with cervical cancer and those with other diagnoses or a normal first smear. Multiple logistic regression analyses were performed to assess the effect of SES (low, intermediate, high) on the occurrence of cervical cancer, unadjusted and adjusted for age and purpose of the smear (i.e. within / outside the screening program).

Conclusions: We included 717,042 women with a first smear in the study, 1,355 women (0.2%) were diagnosed with cervical cancer. Women with lower SES were significantly older and more often had cervical cancer compared to other women. In addition, their first smears were more often performed outside the screening program (all: $p<0.001$).
Compared to women with high SES, the risk of cervical cancer was increased in women with low SES (Odds Ratio (OR) 1.64 (95% CI 1.38 - 1.95)) unadjusted, adjusted for age (OR 1.50 (95% CI 1.26 - 1.78)) and adjusted for age and purpose of the smear (OR 1.49 (95% CI 1.26 - 1.78)). In women with intermediate SES the risk adjusted for age and purpose of the smear was also increased (OR 1.22 (95% CI 1.04 - 1.42) compared to that of high SES women, but the increase was less pronounced.
When eliminating screen coverage, women with a low SES still have a 50% higher risk to be diagnosed with cervical cancer than women with high SES. This indicates that women with low SES have an increased risk of developing cervical cancer through a combination of insufficient screening participation and an increased background risk.

OC 2-9

CERVICAL CANCER SCREENING IN WOMEN AGES 50-65: IMPACT ON CANCER RISK AFTER AGE 65

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Objective: The Swedish organized cervical screening program stops inviting women after age 60. In recent years, approximately one third of cervical cancer cases are diagnosed in women aged above 65. This study aims to do a population based evaluation of the effectiveness of the Swedish cervical cancer screening program for women above age 65, and to identify women at high risk of developing cervical cancer after age 65.

Method: A nested case-control study was conducted as a part of Swedish national audit project for cervical cancer prevention. Cases were women diagnosed with invasive cervical cancer after 65 years of age during 2002-2010, retrieved from the National Cancer Register. Controls were matched on birth-year and place of residence, from the Total Population Register. Women's screening information during ages 50-65 was retrieved from the National Quality Register for Cervical Cancer Prevention. Cancer risks were compared by attendance patterns and cytological diagnoses, using conditional and unconditional logistic regression models.

Conclusion: 393 invasive cervical cancer cases after 65 years of age with screening information back to age 50 were identified, along with 9,986 eligible matched controls. Compared to women who attended cervical screening during both ages 50-55 and 56-60 and only had negative Pap smears, there was an increased risk for women who were absent from screening in these age intervals (OR=3.31, 95%CI=2.40, 4.55), were absent at age 56-60 (OR=1.84, 95%CI=1.26, 2.67), or had a positive Pap smear at age 50-60 (OR=2.80, 95%CI=1.52, 5.18). Having at least one negative Pap smear between ages 61 and 65 conferred a lower cancer risk among women who were absent from screening at age 50-60 (OR= 0.42, 95%CI=0.22, 0.81), and also among women who had two negative Pap smears at ages 50-60 (OR=0.70, 95%CI=0.50, 0.98). Cervical cytological screening between ages 50 and 60 is effective for preventing invasive cervical cancer after age 65, although an additional Pap smear after age 60 may further lower the risk. Women who are absent from screening or have any abnormality at age 50-60 should be targeted for additional screening after 60 years of age.

OC 2-10

CERVICAL CANCER IN ASSOCIATION WITH PREGNANCY

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Objective: The objective of the study was to evaluate all cervical cancer cases diagnosed during pregnancy or within 6 months after parturition during 16 years (1993–2008) in the Western region of Sweden and to compare the outcome with a previous study from the same region (1973-1992).

Methods: The study is based on data from different registries and medical records.
Incidence, diagnostic measures, treatment, outcome of the disease were recorded and compared to the previous study.

Conclusions: Cervical cancer was diagnosed in 47 women (15.6/100 000 deliveries). Only 16 women had abnormal vaginal bleeding and/or discharge. The other 31 were asymptomatic and diagnosed by abnormal cervical smear or clinical signs at vaginal examination. Notably, 9 women had ASCUS as presenting cervical atypia. Among all women 22 had stage IA cancer, 17 stage IB1, 6 stage IB2 and 2 stage IIA cancer. Cancer was diagnosed in the 1st trimester in two women, in the 2nd trimester in 14, in the 3rd trimester in five and post-partum in 26 women. Twenty women underwent cesarean section due to cancer, combined with the Wertheim-Meigs procedure in six. Sixteen of the women having stage IA cancer underwent conization as final treatment. Six women died of the disease. All these women were insufficiently screened. Neither the incidence nor mortality were significantly altered compared to the previous study, but the proportion of stage IA cancer was significantly increased ($p < 0.001$) whereas the incidence of stage IB and ?IIA was unchanged. Thus, it can be concluded that early detection of cervical cytological atypia and proper follow-up during pregnancy lead to the detection of an increased proportion of stage I cancer, thereby avoiding radical operative procedures and will possibly reduce the mortality in the future.

OC 2-11

NATURAL HISTORY OF CIN2/3 IN AN HPV THERAPEUTIC VACCINE STUDY

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Objectives: Literature data regarding the natural history of CIN2/3 followed for a short course of time is limited. The course of CIN2/3 over 6 to 8 months in a placebo population enrolled in a therapeutic vaccine trial of tipapkinogen sovacivec was analyzed.

Methods: Patients with biopsy confirmed CIN2 or CIN3 were randomized 2:1 to receive active treatment or placebo followed by interim colposcopy +/- biopsy at Month 3 and conization at Month 6. HPV response was defined as clearance of baseline HPV genotypes. Efficacy was assessed based on central pathology review (CPR) of conization samples in the modified intent to treat population (mITT) (patients with CPR confirmed CIN2/3 at entry who received at least 1 study injection).

Results: The study enrolled 206 patients with 70 placebo patients, 63 of whom were in the mITT population. Fifty eight placebo patients (30 CIN2, 28 CIN3) underwent conization up to Day 270. Of the 30 CIN2 placebo patients, 6 (20%) had histologic resolution (no CIN), 3 (10%) had CIN1, 11 (37%) remained CIN2, 7 (23%) had CIN3, and 3 (10%) had adenocarcinoma in situ (ACIS). Of the 28 CIN3 patients, 4 (14%) had histologic response (no CIN or CIN1), 5 (18%) had CIN2, 18 (64%) remained CIN3, and 2 (7%) had cancer (adenocarcinoma and adenosquamous carcinoma). Of the 6 placebo patients who achieved histologic resolution, 2 (33%) achieved viral clearance, and of the 13 patients who achieved histologic response, 4 (31%) achieved viral clearance.

Conclusions: Despite the known potential therapeutic impact of biopsy, 69% of placebo CIN2/3 patients who had surgical excision persisted or had a worse diagnosis. Since only 22% regressed to < CIN2, it's possible that the study entry biopsy potentially missed prevalent CIN3+ including ACIS or invasive cancer. None of the CIN3 placebo patients had their CIN entirely resolved within 8 months but 30% of women with CIN 2 were found to have regressed to CIN 1 or no CIN.

OC 2-12

ROLE OF HPV IN THE PATHOGENESIS OF VIN3 OF THE ANTERIOR FOURCHETTE

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Objectives: The incidence of preneoplastic and neoplastic lesions located in the anterior fourchette between clitoris and urethra have increased in Germany during the last decade.
On colposcopy, some of these lesions and the sourrounding skin often have the aspect of accompanying lichenoid skin diasease. Others rather look like typical HPV induced condylomatous lesions.

Material and Methods: We analyzed 35 tumor samples from high grade intraepithelial vulvar neoplasia (VIN3) of the anterior fourchette for the expression of HPV DNA with consecutive HPV typing, p53 expression by IHC and a marker for lichenoid skin diasease, ECM 1. ECM 1 is an extracellular matrix protein which has been found to be altered/overexpressed in lichen sclerosis compared to healthy controls (1)

Conclusions : The mean age of women with these lesions was 54 years. The VIN 3 lesions located between clitoris and urethra were HPV positive (type31 and 16) in 46% (17/35), show overexpression of p53 in 42% (15/35) and were ECM 1 positive in 71% (25/35). Women over 50 years (n=24) had more often tumors overexpressing p53, HPV negative (10:1) and ECM positive (19/24). On conventional histology, none of the lesions or sourrounding skin were diagnosed to have lichen sclerosis. Our results suggest that HPV does not play the dominant role in VIN 3 of the anterior fourchette in contrast to VIN 3 of other locations, which are HPV induced in over 90% (own data). The high expression of the marker ECM 1 in two third of theses lesions supports the clinical aspect of an underlying lichenoid skin disease in some of these patients also they have no histologically proven lichen sclerosis.

1 Gambichler et al, JEADV 2012, 26, 207-2012

OC 3-1

PERFORMANCE OF DYNAMIC SPECTRAL IMAGING COLPOSCOPY IN PATIENTS WITH ABNORMAL CYTOLOGY OR A POSITIVE HRHPV-TEST

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Objective: The risk for cervical cancer is lower in women with borderline or mild dyskaryosis (BMD) cytology than with cytology worse than BMD (>BMD). Therefore, it is more difficult to recognise high-grade cervical disease colposcopically in women with BMD smears. One solution is to use reflex hrHPV-testing in case of a BMD smear, referring only the BMD hrHPV positive cases. With this study we aimed to determine the accuracy of Dynamic Spectral Imaging (DSI) in identifying high-grade cervical neoplasia in new colposcopy referrals with BMD versus >BMD cytology and in a population using reflex hrHPV-testing in BMD cases.

Methods: In this sub-study of the DSI colposcope validation trial,(1) cervical lesions were first located and graded using the DSI colposcope as a regular videocolposcope. Subsequently, the DSI map was reviewed and biopsies were taken from all suspected areas, plus one control (random) biopsy.

Conclusion: The cohort included 183 women: 107 with BMD cytology and 59 with >BMD. Of the 107 BMD cases, 83 were hrHPV positive. The results are shown in Tables 1 and 2.

	BMD group (n=107)		**> BMD group (n=59)**	
	Sens (95%CI)	Spec (95%CI)	Sens (95%CI)	Spec (95%CI)
DSI	82% (70–94)	78% (68–88)	77% (65–90)	73% (51–96)
Colposcopist	44% (28–59)	87% (79–95)	64% (49–78)	67% (43–91)

Table 1: Sensitivity and specificity data per referral cytology group.

	Sens (95%CI)	**Spec (95%CI)**
DSI	79% (71–88)	75% (64–86)
Colposcopist	55% (44–66)	81% (72–91)

Table 2: BMD/hrHPV positive, and >BMD combined (n=142)

DSI has a higher sensitivity in the BMD group than conventional colposcopy. The sensitivity of DSI in this group is more or less similar to the sensitivity in the >BMD group, indicating a baseline sensitivity of DSI, irrespective of referral cytology. Also, when BMD cases negative for hrHPV are excluded, inclusion of the DSI map leads to a significant increase in sensitivity compared to conventional colposcopy.

OC 3-2

WOMEN'S PREFERENCES OF DYNAMIC SPECTRAL IMAGING COLPOSCOPY

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Objective : The focus of testing the Dynamic Spectral Imaging (DSI) colposcope has been on the technical characteristics and the clinical performance. However, aspects from a patient's perspective can be just as important. Therefore we designed this study to assess the preferences of women concerning a colposcopic examination with DSI using questionnaires.

Methods: **This study was designed as a sub-study of the DSI validation study, a prospective comparative, multicenter clin**ical trial to assess the clinical performance of DSI colposcopy. All women included in this study were asked to complete two questionnaires, one patient characteristics questionnaire and a patient satisfaction questionnaire.

Conclusions: In the initial study a total of 239 women were included in the intention-to-treat cohort, which included cases who did not necessarily adhere strictly to the clinical protocol, but all underwent DSI colposcopy. Of these, 230 (96.2%) women completed both questionnaires. When assessing the women's preferences for some of the possible uses of DSI colposcopy, a high level of agreement was noted for all potential implementations. In general, women found the additional time DSI colposcopy took acceptable: just 15 women (6.5%) thought the time DSI colposcopy took made them feel uncomfortable. Furthermore, women ranked test accuracy as the most important characteristic, followed by (more) rapid testing and comfort. Quick notification of the results and costs were considered the least important characteristics.
In conclusion, the main finding of this study is that women are willing to accept discomfort in the form of an additional or longer test if there is clinical benefit.

OC 3-3

IMPACT OF ACETIC ACID ON HPV TESTING USING HYBRID CAPTURE 2

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Objectives: HPV testing should not be routinely used in the colposcopy clinic, but could be helpful in planning the follow-up of women with abnormal cytology but normal colposcopy. An HPV sample could be collected during colposcopy if the cervix is normal, before biopsies are obtained. An HPV negative result combined with normal biopsies could enable more rapid dismissal back to regular screening. Before investigating this possibility, the present project verified if the application of acetic acid (AA) impacts HPV test results.

Methods: Participants referred for colposcopy were eligible if immunocompetent, over 18 years old and not pregnant. Half of participants received two consecutive HPV tests (controls). The other half had a first sample collected before and a second sample collected 3 minutes after the application of AA. Samples were tested for HPV DNA with Hybrid Capture 2 (HC2) according to the manufacturer's instructions.

Conclusion: From October 17th, 2012 to January 10th, 2013, a total of 101 women were recruited in two colposcopy clinics. Approximately half of women in each group had a positive result for the first HPV test (46.0% vs 52.9%; p=0.49). One woman in each group had a first sample that tested negative but a second second sample that tested positive, for a concordance rate of 98.0% for both groups between the first and the second test. When the reactivity in relative light units was considered, there was no statistically significant difference between the first and second groups for the difference of ratios between samples (-0.02 vs -0.04; p=0.99), the variation of ratios in percent (42.1% vs 42.4%; p=0.75) and the proportion of women whose 2nd ratio was superior to the 1st one (42.0% vs 39.2%; p=0.78). The results of this study suggest that acetic acid at concentrations of 3 to 5% and sequential cervical sampling do not modify the result of HPV testing by Hybrid Capture 2.

OC 3-4

COLPOSCOPY UTILIZATION FOLLOWING PRIMARY HPV SCREENING WITH CYTOLOGY TRIAGE

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Objectives: The HPV FOCAL trial is evaluating HPV as a primary screen for cervical cancer among women drawn from the British Columbia Cervical Cancer Screening Program (BCCCSP). HPV is given at 4 year intervals (intervention) with colposcopy for those HPV+/LBC+ and retesting at 12 months for those HPV+/LBC- with colposcopy for those persistently HPV+. The comparison LBC arm (control) screens every 2 years with colposcopy for >=LSIL and ASCUS/HPV+. The BCCCSP employs a strategy with ASCUS/LSIL only referred to colposcopy after persistence for 2 years. This report examines the impact of changing the primary screening test and referral criteria on demand for colposcopy services in British Columbia.

Methods: Data from the first 4 years of the FOCAL trial was extracted for the two study arms and for a comparable routine screening population (RSP) from the BCCCSP who had a Pap smear in 2005. Kaplan-Meier analysis was used to compute the likelihood of a colposcopy by time since enrolment up to 4 years.

Conclusions: 9,553 intervention and 9,457 control FOCAL subjects were enrolled and 427,648 RSP women were identified. The cytology findings in the RSP and control groups were similar: proportion NILM (negative) at entry 97.2% and 96.6% respectively, suggesting RSP and trial subjects were comparable. By 6 months, 2.0% of the intervention, 2.0% of the control and 0.8% of RSP group had colposcopy. A further 2.6%, 0.7%, and 0.5% (respectively) had colposcopy in the following 18 months and by 4 years 5.1%, 4.2%, and 2.0% had received colposcopy. Adopting a management plan where individuals with ASCUS/HPV+ and LSIL were immediately referred to colposcopy resulted in an increase of 2.2%. Changing the primary screening test to HPV resulted in a further 0.9% increase. Results varied by age with the largest increases in referral seen in women 25-29 yrs and the smallest increases seen in women 50-65 yrs.
Changing the primary screen from cytology to HPV testing in British Columbia would result in a 2.5 fold increase in referral to colposcopy over 4 years but the majority of this increase derives from the change in referral threshold and not a change in the screening test. The absolute and relative effect was age-specific.

OC 3-5

PUNCH BIOPSIES AND LOOP BIOPSIES TO EXCLUDE MICROINVASIVE CANCER IN EARLY PREGNANCY ARE SAFE PROCEDURES

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Objective: To evaluate whether colposcopically directed biopsies during pregnancy are associated with surgical/obstetric complications.

Methods: 251 pregnant women with atypical cervical cytology in early pregnancy were investigated by colposcopically directed punch biopsies (n =196), colposcopically directed loop-biopsies (n=46), or LEEP-cones (n=9) in pregnancy week 5-26 (mean= 15.7). Postoperative complications were recorded. Gestational length and mode of delivery were recorded and compared to the obstetric outcome of the 54 919 other births in the same geographical area during the study period (March 15, 2001 to June 30 2009).

Results: No postoperative bleeding that needed surgical (diathermy/suture) treatment occurred and the miscarriage rate was low (0.8%). There were no differences in mode of delivery (OR 0.85 for caesarean section (CI 95% 0.60-1.22)), premature birth between the study group and the large control cohort with length of pregnancy 39.70 weeks (CI 95% 39.43-39.97) and 39.73 weeks (CI 95% 39.70-39.75), respectively. Four cancer cases were detected by loop biopsies or cone.

Conclusion: Investigation of atypical cytology before 26th week of pregnancy with biopsy, including loop biopsy, is a safe procedure in regards to surgical complications and obstetrical outcome. It can be recommended in cases where micro invasive cancer cannot be excluded.

OC 3-6

ANALYSIS OF COLPOSCOPIC MULTI-POINT BIOPSY OF 539 PATIENTS DIAGNOSED WITH ASC-H IN CERVICAL CYTOLOGY REPORT AND ONE YEAR FOLLOW-UP

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Objective. To perform cohort study with the results from colposcopic multi-point cervical biopsy and 1-year follow-up of patients with ASC-H in cytology report from September, 2004 to December, 2012 in our hospital.

Methods. All patients diagnosed with ASC-H in cervical cytology report in our hospital were recommended for HPV DNA testing and colposcopy-directed cervical biopsy. The patients were grouped by age, to evaluate the impact of age on the disease outcome, and to analyze the opportunity of HPV infection and pathological findings. Patients without grade II and above lesions of cervical intraepithelial neoplasia (≥ CIN2) were cytologically followed up at 6th and 12th month respectively after the initial testing, and were suggested for HPV detection at the 12th month follow-up. Colposcopy-directed cervical biopsy was performed in patients with reappeared abnormality in cytology and/or HPV test. An endpoint was determined when patient had continuous twice of cytology examination, or was diagnosed with ≥ CIN2 lesions after second colposcopic biopsy, or received diagnostic cervical conization.

Results. There were a total of 539 patients enrolled in this study, with an average age of 39.3 (23-73) years. In the initial colposcopic biopsy, patients with ? CIN2 lesions accounted for 36.9% (199/539), including 190 cases of CIN2/3 and 9 cases of cervical squamous cell carcinoma. In this group of patients, the rate of HPV+ cases was 75.3%; among the HPV- patients, CIN2 detection rate was 2.1%. The negative predictive value of HPVDNA assay was 97.9%. For the 5 age groups (years), < 30, 30-39, 40-49, 50-59, and ≥ 60, the chances for HPV infection were 77.0%, 82.8%, 72.9%, 70.7%, and 65.1%, respectively. And the detection of ≥ CIN2 lesions were 47.1%,44.9%, 35.3%, 21.3%, and 25.6%, respectively. There was 1 case diagnosed as CIN2 in 1 year follow-up .

Conclusions.1. The opportunity for detection of ? CIN2 lesions among patients with ASC-H in cytology report was high. The chance for detection of invasive cervical cancer especially increased among patients older than 30 years.

2.The chance for detection of invasive cervical cancer especially increased with age in HPV+ women, though HPV+ rate was decreased at the same time,

3.the difference was statistically significant between 60 years or older group and other groups. Therefore, we believe that HPV test is valuable for triage management for women older than 60.

OC 3-7

PAIN DURING COLPOSCOPY AND CERVICAL BIOPSIES WITH OR WITHOUT LOCAL ANESTHESIA: PRELIMINARY RESULTS OF A RANDOMIZED TRIAL

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Objective. This study was undertaken to determine whether administration of local anesthesia reduces pain associated with colposcopically directed cervical biopsies.

Methods. Two hundred consecutive women referred with abnormal cytology for colposcopic assessment were recruited to a randomized, prospective trial conducted in the colposcopy clinic at Department of Obstetrics and Gynaecology, Helsinki University Hospital. Based on randomization half of the patients received local anesthesia injected to all four quadrants of the cervix (5ml prilocaine 30 mg/ml with fenylpressin 0.03 IU/ml, Citanest cum Octapressin®) prior to cervical biopsies and curettage. Before the examination patients completed Beck's anxiety inventory (BAI) form and evaluated anticipated pain by using 10-cm visual analog scale (VAS). After injection of local anesthetic, cervical biopsies or endocervical curettage were taken and patients reported their actual pain with VAS. The main outcome was pain VAS with or without local anesthesia.

In both groups anticipated pain (4.98 and 4.49) exceeded the actual pain experienced. BAI scores correlated positively with anticipated pain. The mean BAI score, around seven in both groups, indicates mild anxiety. The mean VAS score for local anesthesia injection was 2.83 (SD 2.46), which was not statistically lower than pain VAS for biopsy (3.46, p=0.10). Injection of local anesthesia reduced pain scores for cervical biopsies (from 3.46 to 0.89, P <0.001) and endocervical curettage (from 3.18 to 1.90, P= 0.002).

Conclusions. This study demonstrates that injection of local anesthesia at colposcopy decreases the pain experienced by patients. Injection of a local anesthesia, however, was almost as painful as biobsy without local anesthesia. Local anesthesia might, however, benefit women with high anxiety scores prior the procedure.

OC 4-1

PERSISTENT HIGH-RISK HUMAN PAPILLOMAVIRUS INFECTION AND ITS ROLE IN RECURRENCE OF CERVICAL INTRAEPITHELIAL NEOPLASIA

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Objectives: To assess the frequency of persistent high-risk human papillomavirus (HPV) infection and the risk of cervical intraepithelial neoplasia (CIN) recurrence in light of absence or presence of HPV infection in patients treated for persistent CIN 1, CIN 2 or worse (CIN 2+). To analyze the risk of CIN recurrence depending on the type of HPV and the character of the infection.

Methods and Results: One hundred patients diagnosed with and treated for persistent CIN 1 and CIN 2+ were analyzed based on cytological, colposcopic, molecular tests (HPV DNA and HPV mRNA) and histopathological data. Inclusion criteria was based on pathomorphological analysis of postoperative material obtained during loop electro-surgical excision procedures and diagnostic sampling of the endocervical canal. Excluded were patients vaccinated against HPV. Ninety-five percent of patients diagnosed with CIN were positive for HPV DNA prior to treatment. The first follow-up, three months post treatment, revealed that twenty-two percent of patients were positive for HPV DNA. Throughout the observation period, twenty-one recurrences of CIN were observed. Fifty-two percent of patients with diagnosed recurrence during the first follow-up visit had persistent viral infection. The most frequent type of HPV infection in patients with recurrence was type 16.

Conclusions: There is a close correlation between persistent high-risk HPV infection and the development of CIN, and consequently the development of cervical cancer. This study shows that persistent HPV infection in patients post treatment of CIN may contribute to recurrence of neoplasia.

OC 4-2

HPV MRNA IS MORE SPECIFIC THAN HPV DNA IN TRIAGE OF WOMEN WITH MINOR CERVICAL LESIONS

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Objectives. In Norway, repeat cytology and HPV testing is used in delayed triage of women with minor cytological lesions. The objective of this study was to evaluate HPV DNA and HPV mRNA testing "head-to-head" in triage of women with repeated ASC-US / LSIL.

Methods. Repeat cytology, HPV DNA testing (Roche Cobas 4800), and HPV mRNA testing (NorChip PreTect HPV-Proofer) were used to follow up 575 women aged 25–69 years with ASC-US / LSIL in primary screening. A total of 206 women (35.8%) were DNA+ and 107 (18.6%) were mRNA+. Repeated ASC-US / LSIL was found in 249 women (43.3%), of whom 120 (48.2%) were DNA+ and 57 (22.9%) were mRNA+. We received biopsies from 75.8% (91/120) of the DNA+ and 73.7% (42/57) of the mRNA+ cases. The positive predictive values for CIN2+ were 22.0% (20/91) for DNA+ and 33.3% (14/42) for mRNA+. Of the 258 women with normal repeat cytology (NILM), 38 (14.7%) were DNA+ and 16 (6.2%) were mRNA+

Conclusions. HPV mRNA is more specific than HPV DNA in triage of women with repeated ASC-US / LSIL. Within 9 months of triage, only 91 of 120 DNA+ women and 42 of 57 mRNA+ women had met for colposcopy and biopsy. The referral rate for colposcopy after repeated ASC-US / LSIL was double for DNA+ relative to mRNA+ cases, winning 6 more cases of CIN2+. The need for follow-ups in NILM patients was more than doubled for DNA+ cases relative to mRNA+ cases. Compliance with required follow-ups for HPV+ NILM cases was low. Compared with the mRNA-test, the use of DNA-tests in triage created additional work for gynaecologists and laboratories, as well as unnecessary psychological stress for the patients. As long as repeated ASC-US / LSIL with negative mRNA are followed by a new cytology after 12 months, very few cases of CIN2+ captured by DNA at triage will be lost. It may be worthwhile considering the trade-off between sensitivity and specificity when designing screening algorithms.

OC 4-3

CLINICAL AND ANALYTICAL EVALUATION OF THE ONCLARITY™ HPV ASSAY IN WOMEN REFERRED FOR COLPOSCOPY

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Objective: The novel BD Onclarity™ HPV assay on the BD ViperT™ LT system (BD Diagnostics, Baltimore, MD), detects E6/E7 DNA from 14 high risk HPV genotypes with individual results on six genotypes (16, 18, 31, 45, 51, 52) and three sets of genotype results (33/58, 56/59/66, 35/39/68). We compared the analytical and clinical performance of this assay to that of Hybrid Capture 2 (HC2; Qiagen, Gaithersburg, MD) ,LINEAR ARRAY (LA; Roche, Pleasanton, CA), and histological outcomes in women referred for colposcopy.

Methods: 274 women from Copenhagen, Denmark, referred for colposcopy with abnormal cytology and/or a positive HPV test, were enrolled between November 2012 and May 2013. Before undergoing a biopsy and ECC, all women had two samples taken: one in BD SurePath™ Preservative Fluid for cytology, HC2, LA and BD Onclarity testing; and another using a BD cervical brush for BD Onclarity HPV testing. The BD Onclarity HPV assay was used to test the SurePath medium aliquoted into BD HPV LBC diluent tubes and on the Cervical brush (CB)diluent. All testing was undertaken according to manufacturers' protocols.

Conclusions: Histology was normal in 93 (34%) women, 59 (22%) women had CIN1, 32 (12%) CIN2, 68 (25%) CIN3, and 2 (<1%) had cervical cancer, whereas 20 (7%) biopsies returned the diagnosis of "CIN not otherwise specified" (NOS). The BD Onclarity HPV assay on CB material detected 68 (97%) out of 70 ≥CIN3 and 99 (97%) out of 102 ≥CIN2; on the LBC material it detected 69 (99%) ≥CIN3 and 100 (99%) ≥CIN2. HC2 detected 68 (97%)≥CIN3 and 100 (98%) ≥CIN2. When the definition of a positive LA test was limited to 14 oncogenic genotypes, LA detected 67 (96%) ≥CIN3 and 97 (95%) ≥CIN2. All HPV assays detected the two cervical cancers found. The specificities for <CIN2 were 20%, 16%, 20% and 20%, for HC2, BD Onclarity CB, BD Onclarity LBC, and LA, respectively. There was a high degree of agreement between assays in terms of positive results; for example, BD Onclarity CB and HC2 agreed in 90% of samples, BD Onclarity CB and LA in 94%, and BD Onclarity LBC and LA in 96%. Overall, the BD Onclarity HPV assay performed well on SurePath LBC and CB media, with clinical sensitivity and specificity matching those of HC2 and LA. This and the ability to report the currently most advanced genotype result makes BD Onclarity a good candidate for use in cervical screening applications.

OC 4-4

EFFECTIVENESS OF TWO STRATEGIES TO TRIAGE EQUIVOCAL AND LOW-GRADE SCREENING RESULTS IN THE NETHERLANDS: CYTOLOGICAL FOLLOW-UP WITH OR WITHOUT HRHPV TESTING

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Background: hrHPV testing has acquired a position in the Dutch cervical screening program in the follow-up of ASC-US (Atypical Squamous Cells of Undetermined Significance) and LSIL (Low-grade squamous intraepithelial lesion) screening results. A triage scheme with or without a hrHPV test have been allowed. The effectiveness of these triage strategies have not been compared yet.

Objective: To study the effectiveness of hrHPV triage by comparison of referral-, cytological follow-up and histological detection rates with cytological triage.

Methods: Data were extracted from PALGA: Dutch Pathology Registry. All ASC-US/LSIL results diagnosed within the framework of the cervical cancer screening program in 2008 were retrieved including gynaecological follow-up resulting in 13,734 cases with 36-48 months of follow-up. The protocol followed for triage was determined as well as cytological follow-up results and most severe histological outcome.

Results: More than half of the cases (56,2%) of ASC-US/LSIL cytology followed a hrHPV triage scheme. During follow-up of these cases 46,8% could be referred early to the regular screening scheme and only 28.6% of the cases had to be followed cytologically. These rates differed significantly from the cytological triage scheme (0.0% and 76.1% respectively). With hrHPV triage a significantly higher rate of persisting ASC-US/LSIL was found. Compliance with the repeat/referral advice was significantly higher with hrHPV triage. These two finding result in a significantly higher pressure on colposcopy clinics. HrHPV triage also showed significantly higher CIN 1 and CIN 2 detection rates as compared to cytological triage (9,5% versus 7.0% and 6.4% versus 4.9% respectively). The higher detection rates of CIN 1 with hrHPV triage was only seen in the youngest age cohort (30-34 years). No differences were found for CIN 3 or more severe lesions.

Conclusions: With hrHPV triage almost half of the cases could be referred back to the regular screening program beforehand and the number of women needing cytological follow-up was strongly reduced but hrHPV triage resulted in a significantly higher pressure on colposcopy clinics. With hrHPV triage equal rates of CIN 3 or more severe are found as compared with cytological triage. However, with hrHPV triage detection rates of CIN 1 and CIN 2 are significantly increased. The adverse side-effects of hrHPV triage could be improved by restriction of hrHPV triage to older age groups or by addition of a second, more specific triage test.

OC 4-5

ROLE OF HUMAN PAPILLOMAVIRUS (HPV) TESTING AND CYTOLOGY IN THE FOLLOW-UP AFTER CONIZATION

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Objectives. Follow-up strategy of women who have undergone conization for high-grade lesions on the cervix uteri is a crucial part of the cervical screening program. This prospective cohort study evaluates performance of testing for high-risk (HR) types of HPV, cytology and co-testing (HPV and cytology) in predicting cervical intraepithelial neoplasia grade 2 or worse (CIN2+) after conization.

Methods. During 2002–2006, cervical specimens from 604 women were collected just before conization, at 6 and 12 month follow-up visits after conization. They were used for cytology examination and Hybrid Capture 2 (HC2) detection of HR HPV. Furthermore the women were passively followed until 2 years after the first follow-up visit by linkage to The Pathology Data Bank.

Results. At the first visit after conization (median time: 3.7 months), 20.7% were HC2 positive and 17.5% had atypical squamous intraepithelial lesion or more severe cytology (ASCUS+). The 2-year incidence of histologically confirmed CIN2+ after conization was 4.5%. ASCUS+ cytology was 77.8% (95% CI, 52.4-93.6) sensitive for detection of CIN2+ after conization. HC2 testing (90.9%; 95% CI, 70.8-98.9) and co-testing (95.2%; 95% CI, 76.2-99.9) were both more sensitive than cytology. Specificity was higher for ASCUS+ cytology (85.9%; 95% CI, 82.0-89.3) compared to HC2 (81.8%; 95% CI, 77.5-85.6) whereas for co-testing it was markedly lower (73.3%; 95% CI, 68.5-77.7).

Conclusions. This study shows that HR HPV testing 3-4 months after conization is valuable in predicting the appearance of CIN2+ within 2 years.

OC 4-6

CHANGES IN HPV SPECIFIC VIRAL LOAD AS PREDICTION OF HISTOLOGY OUTCOME

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Objective: This retrospective study aimed to examine if HPV specific changes in viral load in cervical smears are predictive for the natural evolution of HPV infections.

Methods: A private Belgian cervical cytology database was used to select women with biopsy-proven CIN in 2012, and at least two liquid-based cytology samples before diagnosis of CIN and having a single-type HPV infection. We included the first 80 cases (30 CIN1, 29 CIN2, 20 CIN3 and 1 CIN3+). Before cytology, 18 different qPCRs allowed HPV type specific viral load measurement. Changes in HPV specific load between 2 and 3 measurements were assessed by linear regression. According to the degree of increase/decrease of viral load, 2 processes were considered 1) transient virion producing infections $\pm$0.3 HPV copies/cell/day 2) basal cell transforming infections leading to CIN3+ 0.003 HPV copies/cell/day. For 2 measurements, only slopes between 0.001 to 0.0099 and -0.001 to -0.0099 HPV copies/cell/day were considered as HPV transforming processes. All other slopes $>$0.0099, $<$-0.0099 and $<$0.001 to $>$-0.001 HPV copies/cell/day were defined as virion productive. For 3 measurements only a limited combinations of increasing/decreasing slope occurred. A linear increasing process was considered when $R^2 > 0.9$ (clonal expansion). For $R^2 < 0.9$ we considered that the process we measured was originating from a single transient infection.

Results: For 2 consecutive measurements of HPV specific viral load there was a significant decrease in productive infections from CIN1 to CIN3+ (73%, 59%, 20%, 0%) ($p=0.03$) and a significant increase in the number of transforming infections (27%, 41%, 80%, 100%) ($p=0.026$). For 3 consecutive measurements we ascertained the same trend for productive processes ($p=0.02$) and transforming processes ($p=0.01$). For the first time we could clearly demonstrate regressing lesions with a persistent linear decrease in viral load ($R^2>0.9$ and -0.003 HPV copies/cell/day).

Conclusions: The increase and/or decrease in HPV specific viral load correlated with biopsy-proven diagnosis of CIN in 2, but even better after 3 consecutive measurements. Serial measurements allow to classify HPV driven processes in transient virion productive and basal cell transforming, enabling prediction to high-grade CIN.

OC 4-7

NEW DIAGNOSIS AND TREATMENT OF HPV-ASSOCIATED PREINVASIVE CERVICAL NEOPLASIA IN THE REPRODUCTIVE AGE

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Objective: to improve organ preservation treatment of cervical intraepithelial neoplasia in women of reproductive age by including in the diagnostic process OCT speed modification and use of PDT in the presence of resection margin after elektrokonizatsii cervical signs of HPV infection.

Materials and methods: A total of 32 patients from 19 to 38 y.o. with CIN II-III, cancer in situ of the cervix. After extended colposcopy patients conducted OCT scanning speed zones maximum colposcopic changes. The remoteness of the cervical lesions was fixed in millimeters using a special cervical line, developed at the Department of Obstetrics and Gynecology NizhGMA. Transition boundary doubtful and malignant OCT images in benign designated as the planned ektotservikalny margin of resection. Assesses whether OCT signs PVI beyond the planned resection margin.

Results: HPV WRC was in 20 patients. The first stage of treatment for all patients was implementation elektrokonizatsii cervical scraping the cervical canal. Upon receipt of the results of histology patients were divided into 2 groups. In one group consisted of 17 women with no surgical margins epithelial changes characteristic of HPV infection, of which CIN II - 3, CIN II - 10, cancer in situ - 4. Their treatment is limited to the first stage. And in the group 2 - 15 patients with HPV-associated changes in the resection margin, of whom 7 - MBE changes characteristic of HPV infection, in 5 - CIN I, at 2 - CIN II, at 1 - at the maximum technically feasible verified excision of CIN III. All patients 2 groups to influence the clinical and subclinical forms of IMC, and in the latter case, on nerezetsirovannye CIN lesions, the second step the PDT. Postlechebny monitoring was implemented through OCT colposcopic, cytological, virological studies after complete healing of the cervix. Regression of HPV-associated epithelial changes achieved in all patients. Complete eradication of HPV was achieved in 12 women from the two groups.

Conclusion: The new high-speed OCT modification enables refinement Colposcopic picture and the use of cervical line optimizes the planning area of resection. In addition, demonstrated the efficacy of PDT in the presence of positive HPV-associated epithelial changes after organ resection margin transactions. Obviously, PDT in women of reproductive age after excision of the cervix reduces the likelihood of recurrence of CIN, and ultimately prevents the development of cervical cancer.

OC 5-1

COMPARISON OF FOUR HPV ASSAYS IN A PRIMARY SCREENING POPULATION

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Objectives. The few studies comparing multiple HPV assays in the same women undergoing primary cervical screening aged ≥30 years did not always include follow-up of women with normal cytology and positive HPV tests. In the Danish Horizon/Control study comparing Hybrid Capture 2 (HC2), APTIMA, CLART and cobas HPV assays, all women with positive HPV tests were offered follow up.

Methods. SurePath cytology samples from 2,869 consecutive women aged 30-65 years attending routine cervical screening in Copenhagen between June and August 2011 were tested with the four assays. All testing protocols were agreed upon with the manufacturers prior to the study, and all instrumentation and software were used as supplied by the manufacturers. Women with cytological abnormalities were routinely recommended for repeated testing or were referred for colposcopy. Women with normal cytology and at least one positive HPV result were offered repeated co-testing in 18 months. Women's worst histological diagnosis by May 2013 was retrieved from the Danish national Pathology DataBank.

Conclusions. In this population, 4% of women had abnormal cytology, whereas 12% tested positive on HC2, 16% on cobas, 16% on CLART, and 9% on APTIMA. HC2 and APTIMA assays detected 7% more ≥CIN3 than cytology, and cobas and CLART 13% more. The differences in the detection rates were not significant. On the other hand, the differences in false positive (FP) tests, defined as positive screening tests not followed by a diagnosis of ≥CIN3, were substantial. In total, 3.4% of women had FP primary cytology. Primary screening with HC2 would result in 10.6% FP screening tests, 15.0% with cobas, 14.6% with CLART, and 8.3% with APTIMA. The differences in FP tests were significant in all comparisons of the four assays except when cobas and CLART were compared to each other. When ≥CIN2 was used as the endpoint, the results were similar. Clinical follow-up of women with normal cytology and positive HPV tests is though still on-going, so the detection rates for HPV assays are likely to increase. These preliminary data suggest that the four HPV assays detect similar numbers of ≥CIN3 but lead to strikingly high and variable proportions of women with FP tests.

OC 5-2

EVALUATING THE INTRODUCTION OF PRIMARY HPV DNA SCREENING IN ENGLAND: USING A NOVEL STOCHASTIC MODEL OF HPV DISEASE PROGRESSION

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England is piloting a screening strategy in which the primary assessment is a DNA test for high risk human papillomavirus (HR-HPV); negative test results lead to a recall to screening in 3 -5 years, while positive test results lead to cytological assessment. The HPV primary screening pilot is currently operative at six sentinel sites and will recruit 150,000 women in the first year. The objective of this study was to use a modelling-approach to assess the effectiveness of primary HR-HPV testing compared with existing primary cytology-based cervical screening, in order to inform policy making in England and elsewhere.

A novel stochastic model was developed to characterise the transmission and natural history of HPV infection, in which the incidence of cervical intraepithelial neoplasia and invasive cervical cancer is characterised by the cumulative risk of disease progression as a function of time post-HR-HPV infection, as well as age. The model integrates competing hazards of disease progression and regression and generates distributions of times spent in distinct health / disease states. Heterogeneity between individuals, in terms of rates of HPV acquisition, disease progression and screening adherence, is captured in an accelerated hazard framework. This is the first time such a framework has been used in a mechanistic HPV model; importantly, it allows women with a diverse range of lifetime screening patterns to be represented, and hence captures the potential implications of screening more realistically.

Model calibration was carried out using data from the National Health Service Cervical Screening Programme, cervical cancer registrations, and a large national survey on sexual behaviour. The calibrated model was used to make projections for women undergoing HPV primary screening. Model simulations identify potential advantages when HPV testing is used to select women for cervical cytology and to determine screening-recall strategy.

OC 5-3

COMPARISON OF CYTOLOGY AND HPV GENOTYPING STRATEGIES FOR THE TRIAGE OF HPV-POSITIVE WOMEN: RESULTS FROM THE CCCAST STUDY

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Objective: To compare the performance of cervical cancer screening strategies incorporating cytology and HPV genotyping for the triage of HPV-positive (HPV+) women to detect CIN2+.
Methods: Women from the Canadian Cervical Cancer Screening Trial (CCCaST) testing HPV+ via the Hybrid Capture 2 test at enrollment (n=614) were evaluated according to two screening strategies: 1) Triage using cytology at thresholds of ASC-US or worse (ASC-US+), LSIL or worse (LSIL+), and HSIL or worse (HSIL+); and 2) Triage using HPV genotyping results (via Linear Array PCR) at thresholds of HPV16-positive, HPV16/18-positive, and HPV16/18/45-positive. Estimates of sensitivity and specificity for the detection of CIN2+ were calculated for the various strategies.
Conclusion: Triage of HPV+ women using cytology at an ASC-US+ threshold demonstrated greater sensitivity than thresholds of LSIL+ or HSIL+ (49.3% vs. 34.3% and 29.9%, respectively). However, the specificity of cytology triage at this threshold was less than that for the other thresholds (77.5% vs. 89.0% for LSIL+ and 95.9% for HSIL+). Among triage strategies incorporating HPV genotyping results, a threshold of HPV16/18/45-positive had the highest sensitivity to detect CIN2+ among women testing HPV+ at enrollment (66.2%; 95%CI: 53.7%-77.2%). Comparatively, sensitivity estimates for the triage of HPV16-positive and HPV16/18-positive women were 50.0% (95%CI: 37.6%-62.4%) and 60.3% (95%CI: 47.7%-72.0%), respectively. Specificity estimates ranged from 74.0% for the HPV16/18/45-positive threshold to 85.2% for the HPV16-positive threshold. Overall, triage strategies employing HPV genotyping results (at any threshold) provided greater sensitivity for detection of CIN2+ than did those using cytology.

OC 5-4

ASSESSMENT OF A 5-YEAR CERVICAL SCREENING INTERVAL WITH HPV TESTING AND CYTOLOGY IN JAPAN

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Objectives: We aimed to assess the safety of a 5-year cervical screening interval in a population-based cervical screening program with HPV testing and cytology for detection of ≥CIN2 in Japan.

Methods: From 2005 to 2006, 3,911 women attending a population-based cervical screening program were enrolled in this study with cytology and HPV testing. High-risk HPV testing was performed using HC2 (Qiagen). At screening appointments, HPV testing and Pap smear samples were collected, and at biopsy appointments, colposcopy-directed biopsies were taken. Pathological diagnoses of biopsies were reviewed centrally by independent 3 pathologists. We analyzed the progression of lesions up to ≥CIN2 or worse by histology obtained from a punch biopsy or conization during a 5 year follow-up period.

Conclusions: Among the 3,911 women, 511 were cytology (-) and HPV (-) and 89 women were cytology (-) and HPV (+) at baseline and followed up over 5 years. The cumulative incidence rate of CIN2+ and CIN3 + over 5 years was 1.6% and 1.2 % among women with cytology(-) and HPV(-) at enrollment, respectively. By contrast, the cumulative incidence rate of CIN2+ and CIN3 + over 5 years was 30.0% and 15.2 % among women with cytology(-) and HPV(+) at enrollment, respectively. The difference in CIN2+/3+ incidence rate between those women who were HPV(-) and HPV(+) at baseline was statistically significant ($p<0.001$). For women both HPV and cytology negative, a 5-year screening interval is both safe and reassuring. HPV testing is an improved alternative to cytology alone.

OC 5-5

HPV-DNA TEST REPEAT AFTER ONE YEAR IN WOMEN HPV+ AND PAP-. FIRST RESULTS FROM TWO HPV-BASED CERVICAL SCREENING PROGRAMMES.

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Objectives: The test for DNA of high risk types of human papillomavirus (hrHPV) showed a better performance than pap smear in terms of sensitivity and duration of protection after a negative result. In Italy many pilot studies are ongoing in order to evaluate the feasibility of hrHPV-based (with cytology triage) organized screening programmes.

Methods: The HPV-based pilot programmes of LHU 15 (started in July 2010) and 17 (started in April 2009) involve women aged 25-64 years. Samples are taken for hrHPV test (HC2, Qiagen) and for triage pap smear. Women HPV+/pap+ are sent to colposcopy, those HPV+/pap- repeat HPV test after one year and hence referred to colposcopy in case of hrHPV persistence.
By the end of 2011, 28,080 women had been screened by hrHPV test in the two programmes. At baseline, 2.6% women were sent to colposcopy, and 122 CIN2+ lesions were diagnosed (detection rate 4.4‰).
Overall, 1,043 women tested HPV+/pap-; 880 (84.7%) complied to the invitation to repeat hrHPV test after one year, and 79 (7%) informed the programmes that they had spontaneously undergone a pap test recently. 495 cases tested hrHPV+ (56.3%). 31 CIN2+ cases were diagnosed at colposcopy (detection rate on the population screened by hrHPV = 1.1‰).
In the two programmes the cumulative (baseline + one-year repeat) referral rate to colposcopy was 4.4% and the cumulative detection rate was 5.5‰ (20% of which after one-year repeat).

Conclusions: The two programmes show very similar results. Compliance to one-year repeat was good (85%). hrHPV positivity (56%) was higher than expected, suggesting that the protocol could need to be revised in the future.
A hrHPV-based strategy yields a noteworthy workload for colposcopy even with a triage at the baseline. The current triage test is pap smear, that is affected by cytologists' awareness of positivity to hrHPV. Training is fundamental, because triage pap smears are peculiar in terms of prevalence of abnormalities and subsequent management.

OC 5-6

THE ROLE OF CERVISTA HR HPV TEST IN CERVICAL CANCER SCREENING, A CROSS-SECTIONAL STUDY OF CLINICAL ACCURACY IN MAINLAND CHINA

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Objectives: The type distribution of high-risk (HR) HPV associated with cervical neoplasia is somewhat different in China compared to that of Europe or North America, with the most prevalent types being within the A9 species, including HPV types 16, 31, 33, 52 and 58. This study evaluated several high-grade-cervical-disease screening methods, particularly the detection of A9 HR HPV types in routine screening populations and diagnostic testing in outpatient clinics in mainland China.

Methods: 26283 women aged 20-65 years old were enrolled, 20077 from routine cervical screening programs in 3 major cities, and 6206 outpatient cases from 25 clinical centers across China. A single pap test sample was collected from each, and cytology and Cervista HPV HR testing performed on it. Colposcopy and biopsy were performed if ≥ASC-US/HPV+ only with endpoint being CIN2+. Sensitivity, specificity, positive predictive value, negative predictive value and Youden index were calculated for each screening method and scenario with statistical analysis performed by SPSS 19.0.
372 women (1.9%) in the screening group and 875 women (14.1%) in the outpatient group had CIN2+. In the screening group the Youden Index for a HPV+ result (0.85) was greater than that for ≥ASC-US alone (0.75) and ≥ASC-US/HPV+ (0.64) while the NVP for these 3 strategies were all >99%. In the outpatient group the Youden Index for ≥ASCUS/HPV+ (0.69) was greater than that for ≥ASC-US/HPV A9+ (0.67) and ≥ASC-US only (0.40). Logistic regression showed that ≥ASC-US, HPV+ and HPV A9+ are risk factors for CIN2+, whereas HPV A7+ or HPV A5/A6+ are not.

Conclusions: This is the largest diagnostic study in China for Cervista HR HPV test and demonstrates the Cervista HR HPV test, especially A9 group test, provides high sensitivity, specificity, and good Youden Index in prediction of CIN2+ in Chinese women. This test may be used as an effective first step test for routine screening of cervical cancer.

OC 5-8

EVALUATION OF THE CERVISTA HPV A9 GROUP IN THE TRIAGE OF PATIENTS WITH HIGH-RISK HPV-POSITIVE IN POPULATION BASED CERVICAL CANCER SCREENING FROM SHENCCAST II

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Objective: Shenzhen Cervical Cancer Screening Trial II (SHENCCAST II) was to test three high-risk HPV assays, self and direct cervical sampling,and a newly-upgraded computer-assisted image diagnosis system in a population based screening event. Here we report the evaluation of Cervista HPV A9 group in the triage of patients with High-risk HPV-positive

Methods: 10000 eligible women were sampled through a physician-collecting procedure after a self sampling. Self-sampling was block randomized between the Qiagen brush and the new POI brush and tested with Cervista and MALDI-TOF for HR-HPV. The physician -collected sample were processed for HR-HPV tests and cytology in such an order as Cytology, HC-II, Cervista and MALDI-TOF. Patients positive for high-risk HPV (self or direct) and/or Cytology > ASCUS were recalled for colposcope-directed biopsy plus Endocervical curettage using the POI micro-biopsy protocol of directed and random biopsies.

Results: 8,556 women between the ages of 25-59 who had all screening and diagnostic procedures with no missing data are included in this analysis. The mean age was 38.9 years.≥CIN 3 rates were 1.7% (141/8556). Overall HPV infection rates was 11.1% (950/8556) for Cervista . Among the endocervical Cervista HPV positive, the HPV A5/A6 , A7 and A9 group were 26.5% (227/950), 22.9% (218/950) and 67.8%(644/950), respectively.The HPV A9 group is highly related to high grade cervical lesions (CIN2+ OR = 103.61, CIN3+ OR = 128.059).The sensitivity for≥CIN 3 for Cervista HPV A9 group was 89.3% (92.8, 93.7), The specificities was 93.8%(93.3-94.3). HPV A9 combined cytology has a sensitivity in diagnosis of CIN3+ of 94.3% and a specificity of 92.6%, There is no significant difference compared to Cervista HPV test.

Conclusion: It is useful for Cervista HPV A9 group as a indicator of positive High-risk population distribution.

OC 5-9

HPV GENOTYPING CARCINOGENICITY AND OPTIMIZATIONAL SECONDARY SCREENING AFTER PRIMARY OF HUMAN PAPILLOMAVIRUS

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Objective: To research carcinogenicity of HPV subtypes and explore optimized secondary screening trial after primary screening of HPV.

Methods The Shenzhen Cervical Cancer Screening Trial I & II are population-based cross-sectional cervical cancer screening study conducted in Shenzhen and surrounding area, China. 12,095 women aged 25-59 years were included in the analysis. All women were detected by liquid-based cytology test and high-risk HPV-DNA test with the polymerase chain reaction-based matrix-assisted laser desorption/ionization time-of-flight mass spectrometry assay reported 14 HR-HPV subtypes. Any women with a positive lesion on HPV test or cytology were referred to colposcopy and biopsy.

Results 2133 women with a positive of cytology and HPV testing underwent colposcopy and cervical biopsy. The overall prevalence rate of cervical intraepithelial lesions (CIN) was 13.6%, of which the prevalence rate of the high-grade cervical intraepithelial lesions and worse (≥CIN 2) was 2.4%. The high carcinogenicity subtypes are HPV-16 -58 -18 -31 -33 and -52. HPV-16 is the highest carcinogenic risk to CIN3 and cervical cancer, with 229 times of HPV negative. Primary HPV has 95.5% sensitivity and 89.9% specificity in detecting CIN 3 or cancer. Secondary cervical cytology had a sensitivity and specificity of 83.4% and 95.7%, respectively, at the expense of a loss in sensitivity. Secondary genotyping for HPV- 16 or -18 had a sensitivity and specificity of 54.8% and 98.0%, respectively; Set 6 subtypes of HPV-16, 18, 31, 33, 52, and 58 as a colposcopy triage index has satisfactory sensitivity (93.0%)and specificity (92.8%) ; Set HPV subtypes joint cytology as secondary screening trial, that is : "if HPV16/18 positive, callback underwent colposcopy"; "if HPV positive except 16/18 subtypes , further cytology examination, then only Cyto ≥ASCUS, callback underwent colposcopy" has high sensitivity (91.7%), specificity (94.6%) and low callback rate(6.6%).

Conclusions: Genotyping is efficient if it is part of the primary screening result. HPV 16/18 potentially identifies a high percentage of cancer: " HPV subtypes joint cytology as secondary screening trial " keeps high sensitivity, takes the advantage of carcinogenicity HPV16/18 and high specificity of cytology to make up for low specificity of HPV primary screening . This new trial is a practical optimized screening method.

OC 5-10

COMPARISON BETWEEN CYTOLOGY AND HPV DNA TESTING - USING THE COBAS 4800 SYSTEM, IN THE CONTEXT OF PRIMARY SCREENING FOR CERVICAL CANCER PREVENTION. PRELIMINARY RESULTS FROM A MULTICENTER GREEK STUDY.

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Objectives: The comparison of the performance of Cytology versus HPV DNA testing (using the COBAS 4800 System, Roche®), in the context of primary screening for cervical cancer prevention.

Methods: In women 25-55 years old, who visit the outpatient clinics of OB/GYN Univ. Departments in Greece's largest cities for cervical screening purposes, a liquid-based (ThinPrep®) Pap test is performed. After the cytologic evaluation the material collected is used to detect the DNA of 14 oncogenic HPV types [16 &18 separately, and 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68 as a pool], using the COBAS 4800 System, Roche®.

The objective of cervical cancer screening is to detect lesions ≥CIN2 (CIN2+). In the first round, women found positive, either at Pap test (≥ASCUS) or at HPV DNA test, are subjected to colposcopic evaluation (+/- biopsies, according to the colposcopic findings). If colposcopy proves normal, women are subjected again to HPV DNA and Pap testing in 1 year. At that time, if both tests are negative, reevaluation in 3 years; if one of these tests is positive, colposcopy (+/- biopsy, if needed, as mentioned before).

Results: Up to now we have collected 4.102 samples in the first round (4.4% ≥ASCUS and 6.1% hrHPV positive). Thirty cases with CIN2+ have been histologically detected. In all cases (100%) the HPV DNA test was positive, whereas only in 14 (46.6%) the Pap test was abnormal (≥ASCUS). In addition, 51 cases with CIN1 were histologically detected (88.1% HPV DNA positive and 41.2% with abnormal cytology).

Conclusion: HPV DNA testing using the Cobas 4800 System, Roche® seems to be by far more sensitive than cytology as a method of primary screening for cervical cancer.

OC 5-11

THE BENEFIT-HARM FRONTIER OF DIFFERENT HPV PRIMARY SCREENING STRATEGIES FOR CERVICAL CANCER IN GERMANY

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Objectives: Compared to cytology, HPV testing has the potential to improve the effectiveness due to improved early detection and treatment but it has also a higher risk of overdiagnosis and overtreatment of lesions that may not progress to invasive cancer. Using a benefit-harm frontier (BHF) approach, we systematically compared benefits and harms of different HPV-and cytology-based primary cervical cancer screening strategies in the German health care context.

Methods: A previously validated and published Markov model1 for the German health care context was used to analyze the trade-off between benefits and harms of different screening strategies differing by length of screening interval and test algorithms, including HPV testing alone or in combination with cytology or with cytological triage for HPV-positive women. We used published German clinical and epidemiological data as well as test accuracy data from international meta-analyses. Predicted outcomes included reduction in cervical cancer cases (CC) and deaths and unnecessary treatment (defined as invasive treatments such as conizations of cervical lesions < CIN 3).

Results: Overall, HPV-based screening was more effective than cytology alone, with a relative reduction in cervical cancer incidence of 49%-90% compared to 33%-80% with cytology alone (depending on screening intervals). The incremental gain in effectiveness with HPV screening compared to cytology was higher and incremental increase in harms was lower with extended screening intervals. Based on the BHF, 12 of 17 screening strategies were dominated, including annual cytology, the current recommended standard in Germany. Biennial HPV screening was similarly effective as annual cytology and reduced unnecessary treatment (depending on test and follow-up algorithm). Moving from biennial HPV screening with cytological triage to annual HPV screening alone would result in an incremental harm-benefit ratio of 15-533 unnecessary treatments per additional prevented cervical cancer case (depending on screening adherence rate).

Conclusion: The benefit-harm frontier is a useful tool to demonstrate the trade-off between expected gains and risks of different screening strategies. Based on our analyses, HPV-based cervical cancer screening is more effective than cytology alone, but has a higher risk of overtreatment when used in annual screening. In the German health care context, depending on screening adherence rate biennial or triennial HPV screening for women aged 30 years and older is similarly or more effective as annual cytology alone and with significantly reduced unnecessary treatments.

OC 6-1

TWO DOSES OF QUADRIVALENT HPV VACCINE MIGHT BE SUFFICIENT WHEN VACCINATING PREADOLESCENTS

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Objectives: The need for multiple doses to complete a vaccination course is an important challenge/barrier in both low and high income countries. HPV vaccines are highly immunogenic in preadolescents and longer intervals between doses generally increase antibody level. We aimed to assess the immunogenicity of the quadrivalent HPV vaccine after the first, second and third dose, according to an alternative schedule.

Methods: This randomized clinical trial assessed the immunogenicity of the quadrivalent HPV vaccine in 9-10 year-old girls vaccinated according to 0, 6-month schedule and the effect of a third dose given at month 42 of the study. Antibodies were measured by using a Luminex total IgG assay and titers are presented in Luminex units (LU). 416 subjects were enrolled and 366 participated in both phases of the study (2008 & 2012). Intention-to-treat results are presented.

Conclusions: Six months after the first dose: 94, 100, 99 and 96% of subjects had detectable antibodies to HPV 6, 11, 16 and 18, respectively. GMTs varied from 12 to 71 LU. One month post-second dose, all subjects had detectable antibodies to the four genotypes included in the vaccine. The GMTs were 1154, 4003, 3399, and 931 LU for HPV 6, 11, 16 and 18, respectively (55 to 100-fold increase in GMTs post/pre-second dose, depending on the HPV type). At month 42, 99% of subjects had detectable antibodies to HPV 18 and 100% to HPV 6, 11 and 16. The GMTs were 81, 321, 306, and 52 LU for HPV 6, 11, 16 and 18, respectively. One month post-third dose (month 43), GMTs were slightly higher (1.1-1.8-fold) compared to those observed post-second dose, varying from 1666 LU for HPV 18 to 4552 LU for HPV 16. The results suggest that one dose of quadrivalent HPV vaccine ensures priming. The second dose when administered six months post-first dose induces a strong anamnestic response. A persistence of immunity was observed 36 months after two doses of Gardasil given 6 months apart. The effect of a third dose seems small. A reduction in the number of doses would facilitate the use of HPV vaccines.

OC 6-2

EARLY EFFECT OF THE HPV BIVALENT VACCINE ON HIGH-RISK HPV PREVALENCE AND HIGH-GRADE CERVICAL ABNORMALITIES IN SCOTLAND

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Objectives: a national HPV immunisation programme was initiated in Scotland in 2008 for 12-13 year olds with a 3 year "catch up" for those under the age of 18. In tandem with the national immunisation programme, a programme of longitudinal HPV surveillance was also initiated. Key elements of surveillance were yearly sampling and HPV genotyping of women attending for their first smear and the monitoring of high grade lesion prevalence through interrogation of national databases. As age at screening debut is currently 20 in Scotland, we are now able to determine the impact of a national immunisation programme on rates of HPV infection and HPV associated disease.

Methods: liquid-based cytology (LBC) samples from women attending their first cervical smear were genotyped for HPV and data linkage enabled HPV prevalence to be stratified by immunisation status. In addition, we analysed data from the National Colposcopy Clinical Information and Audit System (NCCIAS), a national colposcopy database which contains, data on referral cytology, interventions and histology results associated with any colposcopy visit.

Conclusions: We have demonstrated that vaccine significantly reduces the prevalence of HPV 16 and 18 in young women attending for screening and has an impact on other types including 31, 33 and 45. While the vaccine was not associated with a reduction in low-grade cervical abnormalities, there was a statistically significant reduction in CIN3 diagnoses associated with vaccination status. This is one of the first studies to show demonstrable impact of the bivalent vaccine on HPV prevalence and associated disease at the population level. These data are very encouraging for countries that have national programmes which have achieved high vaccine uptake.

OC 6-3

LONG-TERM EFFECTIVENESS AND IMMUNOGENICITY OF GARDASIL™ IN THE NORDIC COUNTRIES

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Objectives: The GARDASIL™ long-term follow-up (LTFU) study is an ongoing extension of a pivotal randomized, placebo-controlled, double-blind, 4-year study to investigate the safety, immunogenicity, and effectiveness of quadrivalent Human Papillomavirus vaccine (qHPV) on the incidence of HPV 16/18-related cervical intraepithelial neoplasia (CIN) 2 or worse in 16-to 23-year old women (Protocol 015). The LTFU study is taking place in 4 Nordic countries (Denmark, Iceland, Norway, and Sweden). Here, we analyze the effectiveness and immunogenicity of the vaccine in this population of women up to 9 years after the start of vaccination.

MethodsAll women in the trial are followed through different national registries immunogenicity and effectiveness data as well as safety data such as deaths, cancer, and hospitalizations. Effectiveness and safety analyses started approximately 2 years following completion of Protocol 015 and will occur approximately every 2 years thereafter for 10 years. Cohort 1 included approximately 2,700 subjects who received qHPV vaccine at the start of Protocol 015. Cohort 2 consists of approximately 2,100 subjects who received placebo at the start of Protocol 015 and qHPV vaccine prior to entry into the LTFU. Vaccine effectiveness against HPV 16/18-related CIN 2 or worse was estimated by calculating the expected incidence of CIN 2/3 or worse in an unvaccinated (placebo) cohort using historical registry data. The primary analysis approach was per-protocol. Neutralizing antibody response to HPV 16, 18, 6, 11 was detected by competitive Luminex immunoassay (cLIA) and total IgG response was measured by VLP-specific total IgG Luminex immunoassay (total IgG LIA).

Conclusions In the initial analysis of effectiveness after the first 8 years, there were 1,080 subjects that contributed to the follow-up period out of a total of 2,195 eligible subjects in the per-protocol population in Cohort 1. In these subjects there were no cases of HPV 16/18-related CIN 2 or worse observed. There were also no cases of HPV 6/11/16/18-related CIN, vulvar cancer, and vaginal cancer observed. Total IgG seropositivity to HPV 6, 11, 16 and 18 was 98%, 96%, 100% and 91%, respectively, at 9 years after vaccination.

qHPV vaccine shows a trend of continued protection in women, although there is as yet insufficient data to confirm that protection is maintained. The qHPV vaccine continues to be immunogenic and generally well tolerated up to 9 years following vaccination.

OC 6-4

LONG-TERM EFFECTIVENESS OF QUADRIVALENT HPV VACCINE AGAINST NON-VACCINE HPV TYPES IN MEN

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Objectives: Previous data from quadrivalent HPV vaccine (qHPV; GARDASIL®) protocol 020 have demonstrated the efficacy of the vaccine against external genital lesions (EGL; perianal/perineal/penile intraepithelial neoplasia and condyloma) in men aged 16 to 26 years. Subsequently, an extension was added to the original protocol 020 base study to offer qHPV vaccine to those subjects who were in the original placebo group and to those who received incomplete dose regimens during the base study. In this analysis we report on the cumulative incidence of non-vaccine related EGL

Methods: This report comprises follow-up data accumulated through the data cutoff date of 01-Jun-2012. Out of the 2,966 subjects who completed the protocol 020 base study, 1,805 subjects have participated in the long-term study as of the data cutoff date. Among these subjects, the median follow-up time post-dose 3 as of the data cutoff was 5.8 years in the early vaccination group (EVG) who received qHPV in the base study. Subjects underwent genital exams and HPV sampling from the penis, scrotum, and perineal/perianal area at regular intervals. All lesions were to be biopsied for diagnosis and PCR testing. Analyses were performed in a generally HPV naïve (GHN) analysis population that was negative to vaccine HPV types by serology and to 14 tested HPV types by PCR prior to vaccination.

Conclusions: The cumulative incidence of EGL related to any HPV type from the start of the base study through all visits completed before the cutoff date, in the EVG GHN population was 0.1 per 100 person years at risk (95% CI: 0.1, 0.3). All 7 cases of this endpoint were reported during the base study. There were 5 cases of EGL related to HPV 31/33/35/39/45/51/52/56/58/59 during follow-up; all reported during the base study. There were 29 cases of anal intraepithelial neoplasia related to HPV types 31/33/35/39/45/51/52/56/58/59 among men who have sex with men during follow-up; 22 of these cases had been reported during the base study. In summary,
the incidence of disease related to non-vaccine HPV types remains low during the extension period of protocol 020 and points to a lack of HPV type replacement in men who are vaccinated.

OC 6-5

EFFECTIVENESS OF THE QUADRIVALENT HUMAN PAPILLOMAVIRUS VACCINE AGAINST CERVICAL DYSPLASIA IN MANITOBA, CANADA

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Objectives: We evaluated the effectiveness of the quadrivalent HPV vaccine (QHPV) in protecting against cervical dysplasia using a population-based administrative healthcare database from Manitoba, Canada.

Methods: In this matched retrospective cohort study, females ≥ 15 years old who received the QHPV in Manitoba between September 2006 and April 2010 were identified (n=3,541) using Manitoba's immunization registry, and matched on age and place of residence to three non-vaccinated females (n=9,594).Results of all Pap tests performed before and after enrolment were obtained by linking with the database of CervixCheck, Manitoba's organized cervical cancer screening program. Cox regression analysis was undertaken to estimate the hazard ratios of each of the study outcomes— atypical squamous cells of undetermined significance (ASCUS), low-grade squamous intraepithelial lesions (LSIL), and high-grade SIL (HSIL) —conditional on vaccination status, age group and having an abnormal cytology prior to enrolment. Vaccine effectiveness was calculated as (1- hazard ratio) x 100.

Conclusions: Our findings suggest that the QHPV vaccine appears to be moderately protective against the detection of HSIL, LSIL and ASCUS if it is received at a young age (15 17), with an adjusted vaccine effectiveness of 35% (95%CI: -19–65%), 21% (-0–43%) and -1% (-44–29%), respectively. However, QHPV was less effective among older females (?18), especially those with history of abnormal cytology before vaccination; e.g., QHPV was associated with 23% (-17–48%) reduction in HSIL risk among those ≥18 with no history of abnormal cytology, but there was no evidence of protection among those with such a history (-8% [-59–27%]).These findings affirm the importance of vaccinating females at a young age before any significant exposure to HPV occurs and highlight the need for maintaining organized high-quality screening programs that cover all sexually-active women, even if they were vaccinated.

OC 6-6

DOSE EFFECTIVENESS OF QUADRIVALENT HUMAN PAPILLOMAVIRUS VACCINE: A NATIONAL COHORT STUDY

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Objectives: Assessing dose effectiveness is essential to minimizing vaccination-program costs and increasing mass vaccination feasibility. Currently, a three-dose vaccination schedule is recommended for both quadrivalent and bivalent HPV-vaccines. HPV-types 6/11 are included in the quadrivalent vaccine and cause about 90% of condyloma. Condyloma is the earliest HPV-related disease outcome possible to measure. We examined quadrivalent HPV-vaccine effectiveness against condyloma per vaccine dose.

Methods: An open cohort of all females age 10-24 years living in Sweden (n=1 045 165) were followed between 2006 and 2010 for HPV vaccination and first occurrence of condyloma. Data were collected using the Swedish nationwide population-based health data registers. Incidence rate ratios (IRR) of condyloma were estimated using Poisson regression with vaccine dose as a time-dependent exposure, adjusting for attained age and parental education. IRRs were also stratified on age-at-first-vaccination for ages 10-16 and 17-19 separately. Vaccine effectiveness was calculated as 1-IRR*100%. To account for prevalent infections, models included a three-month buffer-period of delayed case counting.

Conclusions: This study shows that after adjustment for prevalent HPV infections, vaccination with two doses of the quadrivalent vaccine provided substantial disease protection, with 68% effectiveness (95% CI=60-74). Three-dose vaccination provided maximum protection with an effectiveness of 80% (95% CI=77-83). When stratified by age-at-first-vaccination, the initial dose had higher effectiveness among those younger at-first-vaccination, 68% for those 10-16 (95% CI 51-80) compared to 29% (95% CI 8-45) for those 17-19.

OC 6-7

LONG-TERM EXTENSION STUDY OF GARDASIL IN ADOLESCENTS; RESULTS THROUGH MONTH 96

Iversen, O.E. for the protocol 018 investigators

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

Methods: In the base study, 1781 sexually naïve boys and girls were assigned (2:1) to GARDASIL or saline placebo at day 1, months 2 and 6. At the end of the base study (month 30), the placebo group received GARDASIL™ following the same regimen. Those vaccinated with GARDASIL in the base study are the early vaccination group (EVG). Those vaccinated with GARDASIL during months 30-36 are the catch-up vaccination group (CVG). As this extension study does not have a placebo arm, effectiveness was assessed by calculating the incidence of the primary endpoints (HPV6/11/16/18 persistent infection or related disease) and comparing these rates with those from previous phase 3 studies in men and women aged 16-26. The median follow-up time for effectiveness was 6.8 years in EVG and 4.7 years in the CVG.

Conclusions: For each of HPV types 6, 11 and 16, the vaccination-induced anti-HPV response persisted long-term. Depending on HPV type, 88%-97% remained seropositive through Month 96. The lower vaccination-induced anti-HPV 18 response over time observed in V501-018-11 is consistent with the persistence profile observed in other studies in the GARDASIL™ program. No cases of HPV 6/11/16/18-related disease were observed. One serious adverse event was reported between months 72-96 (tonic-clonic movements) and was deemed not related to vaccine.

Vaccine-type anti-HPV 6, 11, 16, and 18 responses generated through administration of GARDASIL™ among preadolescents and adolescents persist over the long-term, in accordance with expectations from previous GARDASIL™ studies. No breakthrough cases of disease related to vaccine HPV types 6, 11, 16, and 18 have been observed among preadolescents and adolescents vaccinated with GARDASIL™.

OC 6-8

IMPACT OF GARDASIL® VACCINATION ON THE INCIDENCE OF GENITAL WARTS IN FRANCE (EFFICAE STUDY)

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Objectives: Genital warts (GW) develop soon after HPV infection and about 90% of GW are caused by HPV 6 and 11. GARDASIL® vaccine has demonstrated high efficacy against GW caused by HPV 6/11/16/18 in clinical trials. The objective of this study was to evaluate the effect of the HPV vaccination program on the incidence of GW in France.

Methods: Two 4-month observation periods before (T0 : Dec 2008 to March 2009) and after (T1 : Dec 2011 to March 2012) the HPV vaccination program was initiated in France were compared as an estimation of impact. The incidence of GW was recorded in each period. A total of 160 gynaecologists participated in T0 and 189 in T1. Incidence of genital herpes (HSV) was used as control. Information on all girls with evidence of GW or primary herpes infection was recorded.

Results: During T0, 39,190 15-26 year-old girls were seen among whom 187 had GW and 166 had HSV. During T1, 45,628 girls were seen (243 with GW and 220 with HSV). The overall annual incidence rate for GW was 399/100,000 during T0 and 438/100,000 during T1 ($p=0.18$). Corresponding figures for genital herpes were 355 and 396/100,000, respectively. The incidence of GW varied according to age with a slight decrease between T0 and T1 in girls younger than 20 years (450 vs 396/100,000) and an increase in girls above 20 (591 vs 856/100,000). Conversely, incidence of genital herpes increased in both age categories.

Conclusion: A trend for a decreased incidence of GW is observed in girls below 20 years, an age group in which vaccine coverage is the highest. These results, together with the observed increase in GW in Europe in general and the increase in genital herpes in our study suggest that this decrease might result from HPV vaccination. A substantial increase in vaccine coverage is therefore necessary to observe a more pronounced decreased incidence of GW in the future.

OC 6-9

EVALUATION OF NEUTRALIZING AND CROSS-NEUTRALIZING ANTIBODIES INDUCED BY HPV PROPHYLACTIC VACCINES: AN INDEPENDENT STUDY

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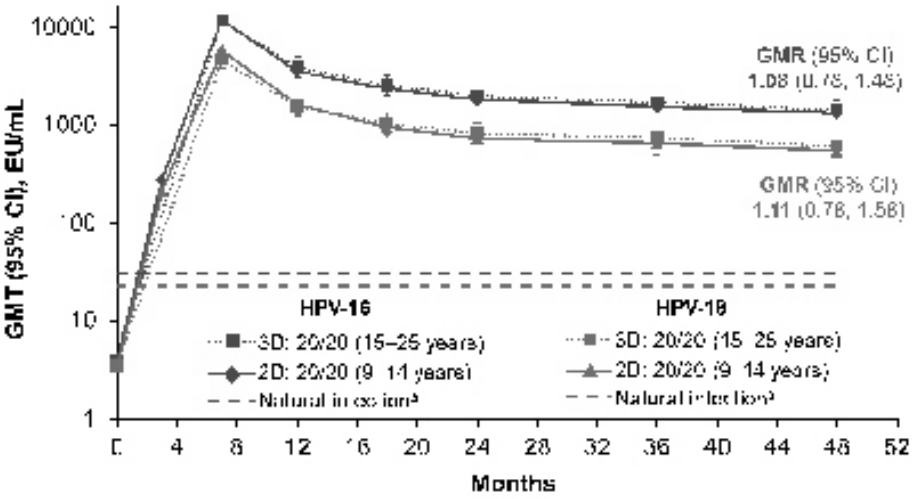
Objectives: Prophylactic bivalent HPV16/18 and quadrivalent HPV6/11/16/18 vaccines have shown protection against HPV16/18-related lesions and against oncogenic non-vaccine HPV31 and HPV45. This is an independent study aiming to compare neutralizing and cross-neutralizing antibody titer induced by both vaccines in a study population of consecutive adolescent girls receiving vaccination.

Methods: HPV type-specific neutralizing antibodies (NAbs) were measured at 1 to 6 months after completion of the third dose of vaccine by using the pseudovirion-based neutralization assay (PBNA) with HPV type 16, 18, 31, 45 pseudovirions. The presence of NAbs was defined by a titer of 1:40 or higher, according to WHO guidelines.

Conclusions: Results were obtained from a group of 160 girls aged 11-13 years vaccinated with Gardasil® ($n=80$) or Cervarix™ ($n=80$). At 1-6 months after vaccination, 100% Gardasil® vaccinees had HPV16 NAbs (GMT ED_{50} 2940 [range 2054:3826]) and 98,7% had HPV18 NAbs (GMT ED_{50} 1027 [range 288:1767]). 100% Cervarix™ vaccinees had HPV16 (GMT ED_{50} 20303 [range 15955:24651] and HPV18 NAbs (GMT ED_{50} 4247 [range -441:8935]). HPV16 and HPV18 NAbs titers were significantly higher ($p<0.0001$) in girls vaccinated with Cervarix™ than in girls vaccinated with Gardasil®. Cross-neutralization assays were performed in a subgroup of 60 vaccinees. HPV31 NAbs were detected in 96.7% of Cervarix™ vaccinees (GMT ED_{50}0 170 [range -1.7:342]) and in 66.7% of Gardasil® vaccinees (GMT ED_{50} 23 [range -206:253]). HPV45 NAbs were detected in 30% of Cervarix™ vaccinees (GMT ED_{50} 4 [range -43:47]) and in 6.7% of Gardasil® vaccinees (GMT ED_{50} 1.3 [range -9.2:12]). In conclusion, both vaccines are highly immunogenic at 1-6 months after vaccination against HPV16/18, with higher NAbs titers induced by Cervarix™. Preliminary results also indicate the induction of cross-NAbs against non-vaccine HPV31/45 was higher for the bivalent vaccine.

Figure 1. Kinetics of antibody response in 3D (15–25 years) and 2D (9–14 years) schedules (M48 ATP cohort for immunogenicity, subjects seronegative at baseline).



HPV 3D, 3-dose schedule (M0, 1, 6); 2D, 2-dose schedule (M0, 6); ATP, according-to-protocol; CI, confidence interval; GMR, geometric mean ratio; GMT, geometric mean titer; M, month. GMR (3D/2D) presented for M48 only. [a]Antibody levels after natural infection in 15–25-year-old subjects from PATRICIA trial (NCT00122681).

HPV-18	M7	309.0 (157.0, 712.0)	532.0 (295.5, 1305.5)	1.0 (1.0, 205.0)
	M12	102.0 (1.0, 385.0)	211.5 (133.0, 541.0)	102.0 (1.0, 218.5)

Units=specific CD4+ T or B cells per respective million of CD4 + T or B Cells; n=smallest number of subjects with available data. All subjects in the ATP cohort for immunogenicity were antiretroviral therapy-naïve at baseline.

A substantially high HPV-16 and -18 specific CD4+ T-cell response in HIV-positive and HIV-negative subjects was observed after HPV-16/18 vaccination, compared with placebo. As expected, no CD8+ T-cell response was observed. For memory B cells, a response was observed in both HIV-positive and HIV-negative vaccinated subjects at M7 and M12, with lower numbers of cells at M12, especially for HPV-18. These findings support previously-reported antibody response data from this study demonstrating that the HPV-16/18 vaccine remains immunogenic in HIV-infected women aged 18–25 years up to 12 months after vaccination.

Study funded by GlaxoSmithKline Biologicals S.A.

OC 6-11

IMMUNE RESPONSE TO THE HPV-16/18 AS04-ADJUVANTED VACCINE ADMINISTERED AS A 2-DOSE OR 3-DOSE SCHEDULE UP TO 4 YEARS AFTER VACCINATION

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Objectives: The licensed 3-dose (3D) schedule of human papillomavirus (HPV)-16/18 AS04-adjuvanted vaccine is highly immunogenic. We evaluated 2-dose (2D) schedules (NCT00541970) and here we present immunogenicity and safety data up to Month (M) 48.

Methods: Healthy, age-stratified females (N=960) were randomised (1:1:1:1) to receive: 20/20 (licensed formulation; 20µg each of HPV-16/18 antigens) at M0,6 (2D); 40/40 (40µg of each antigen) at M0,6 (2D); 40/40 at M0,2 (2D); or 20/20 at M0,1,6 (3D). Seroconversion rates, geometric mean titres (GMT, measured by ELISA) and safety (serious adverse events; SAEs) were assessed and GMT ratios calculated (GMR; 3D/2D). Here, we present results from the licensed formulation in females aged 9–14 years (y) (2D) and 15–25 y (3D).

Conclusions: All initially seronegative subjects seroconverted for HPV-16 and -18; this was maintained up to M48. Responses are shown in Figure 1. GMRs were close to 1 at M48. Kinetics of cross-reactive, non-vaccine HPV-31 and -45 antibodies, phylogenetically related to HPV-16 and -18, were similar for both groups. GMTs (95% confidence interval) for HPV-31 were 195.5 (144.8, 263.9) and 241.7 (166.7, 350.3) for 2D and 3D groups, respectively, and for HPV-45 were 156.6 (114.2, 214.7) and 147.2 (102.0, 212.5) for 2D and 3D groups, respectively. During M0–48, no SAEs were considered to be vaccine-related, and both schedules (2D and 3D) had clinically acceptable safety profiles.

The HPV-16/18 vaccine 2D schedule was immunogenic and generally well-tolerated in girls aged 9–14 y. Cross-reactive and vaccine-induced antibody responses were comparable between the 2D schedule in girls 9–14 y and the 3D schedule in women aged 15–25 y up to 4 y post-vaccination.

This study was funded by GlaxoSmithKline Biologicals SA.

OC 6-12

LONG-TERM EFFECTIVENESS, TOLERABILITY AND IMMUNOGENICITY OF QUADRIVALENT HPV VACCINE IN MEN

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OBJECTIVES: Previous data from quadrivalent HPV vaccine (qHPV; GARDASIL®) protocol 020 have demonstrated the efficacy of the vaccine against external genital lesions (perianal/perineal/penile intraepithelial neoplasia and condyloma) in men aged 16 to 26 years. Subsequently, an extension was added to the original protocol 020 base study to offer qHPV vaccine to those subjects who were in the original placebo group and to those who received incomplete dose regimens during the base study. In this analysis we report the first interim analysis of effectiveness, immunology and safety data from this extension.

METHODS: This report comprises follow-up data accumulated through the data cutoff date of 01-Jun-2012. Out of the 2,966 subjects who completed the protocol 020 base study, 1,805 subjects have participated in the long-term study as of the data cutoff date. Among these subjects, the median follow-up time post-dose 3 as of the data cutoff was 5.8 years in the early vaccination group (EVG) who received qHPV in the base study. Subjects underwent genital exams and HPV sampling from the penis, scrotum, and perineal/perianal area at regular intervals. All lesions were to be biopsied for diagnosis and PCR testing. Yearly serum samples for anti-HPV antibody testing were taken and adverse experiences were catalogued.

CONCLUSIONS: To date, no additional cases of HPV 6/11-related genital warts (GW) or HPV 6/11/16/18-related external genital lesions (EGL) have occurred in the EVG per-protocol extension population. In contrast 3 cases of HPV 6/11-related GW and HPV 6/11/16/18-related EGL developed in the base study. Seropositivity for vaccine HPV types was maintained through month 72. Two serious adverse experiences were reported during the extension period, but neither were deemed related to vaccine.

In summary, data show that administration of qHPV vaccine among young men 16 to 26 years of age is generally well tolerated over the long-term. Anti-HPV 6, 11, 16, and 18 responses generated through administration of qHPV vaccine persist over the long-term, and no additional cases of disease related to vaccine HPV types 6/11/16/18 were observed.

OC 7-1

INFORMATION PROVIDED BY GOOGLE ON HPV VACCINATION: HOW TRUSTWORTHY IS IT?

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Objectives: To assess and analyze the information and recommendations provided by Google Web SearchTM (Google) in relation to web searches on the HPV vaccine, indications for females and males and possible adverse effects.

Methods: Descriptive cross-sectional study of the results of 14 web searches using specific keywords. Comprehensive analysis of results based on general recommendation given (favorable/dissuasive), as well as compliance with pre-established criteria, namely design, content and credibility. Sub-analysis of results according to site category: general information, blog / forum and press.
In the comprehensive analysis of results, 72.2% of websites offer information favorable to HPV vaccination, with varying degrees of content detail, versus 27.8% with highly dissuasive content in relation to HPV vaccination. The most frequent type of site is the blog or forum. The information found is frequently incomplete, poorly structured, and often lacking in updates, bibliography and adequate citations, as well as sound credibility criteria (scientific association accreditation and/or trust mark system).

Conclusions: Google, as a tool which users employ to locate medical information and advice, is not specialized in providing information that is necessarily rigorous or valid from a scientific perspective. Search results and ranking based on Google's generalized algorithms can lead users to poorly grounded opinions and statements, which may impact HPV vaccination perception and subsequent decision making.

OC 7-2

WEB-BASED RECRUITING AND SURVEY ON KNOWLEDGE FOR CERVICAL CANCER PREVENTION AMONG YOUNG JAPANESE WOMEN: A PILOT STUDY

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Objective and Methods: The incidence and mortality of uterine cervical cancer (CC) have been increasing in Japan, particularly in young women. To better understand this increase, we aimed to assess the feasibility of using social networking sites (SNS) to recruit a representative sample of young women living in Kanagawa Prefecture for a knowledge and attitudes study in relation to CC prevention (vaccination and Pap cytology screening). We used methods established in a previous Australian study[1] and compared and contrasted results. From July 2012 to March 2013, advertisements targeting young women living in Kanagawa prefecture aged 16-35 were placed on both a popular SNS (Facebook) and a banner on our homepage to advocate comprehensive CC prevention. Eligible participants received instructions for accessing an online survey by e-mail. The questionnaire detailed demographic variables, plus knowledge of human papillomavirus (HPV) and CC.
Results: During the 10 month survey period, from among 394 women who expressed interests, 264 (67%) returned a signed copy and 243 (62%) completed the online survey. Self-reported Pap smear and HPV vaccination rates were 65% and 12%, respectively.

Conclusion: Results from this study suggest that advertising on an SNS can be a feasible method for recruiting young adult women who use such sites daily. Therefore, we believe this study has implications both for future surveys in Japan on CC prevention and issues related to the sexual and reproductive health, and for comparisons of such data between various populations.

1 Fenner Y, et al. J Med. Internet Res 14, 2012.

OC 7-3

EDUCATION FOR SEXUALL RESPONSIBLE BEHAVIOR AMONG ADOLESCENTS

Doc dr sc Dubravko Lepusiç

HPV is one of the most frequent sexually transmitted diseasees in the world, and according to some authors can be found in over 75% of sexually active women. Given the obligatory role of HPV in the development of cervical neoplasia, a vaccine to immunise against HPV infection would be a valuable strategy for the primary prevention of cervical cancer.

Sexually active adolescents face serious health risks associated with unprotected sexual intercourse including HPV and other sexually transmited diseases as well as unwanted pregnancy. Behaviours particularly relevant to HPV transmission are: early age of sexuall

debut, poor contraceptiv/condom use, multiple sexual partners, certain sexual practices and the use of substances such as alcohol and drugs. Adolescents may not have sufficient cognitive skills to foresee risks in sexual relationships and this may be compounded by unrealistic perceptions of themselves as relatively invurnerable.

Sexually active adolescent females have the highest prevalence rates and are especially vulnerable to the adverse reproductive consequences of infection, including pelvic inflamatory disease, infertility, ectopic pregnancy and maternal and infant morbidity and mortality. Several studies of the etiology of cervical carcinoma suggest that the disease is practically unknown in virgins or persons abstaining from sex. It is pretty rare in some ethnic groups and rather common in women with an early and turbulent onset of sex life with multiple partners.

We want to do something for that young population for education of Human Papillomavirus an sexuall responsible behaviour, so we organized multemedia presentations Name of the project „Knowledge is pleasure".

.Adolescents joind project active, by making their own web sites, scene performances,poems,posters,lectures.... all with sexuall responsible behaviour themas. In appropriate assembly rooms of some Grammar and Secondary School of Zagreb during weekend evenings (Fridays, Saturdays) educational lectures held by medical specialist, were given about sexually transmitted deseases, particularly HPV and sexuall responsivble behaviour andafter the lectures multimedia presentations were held.

Lectures were short (each 20–30 min) accompanied with discussion. Lecture was given in the form of Power Point presentation.

By organizing multimedia presentations the interest of that population to attend would be greater. Concerts and other presentations after the lecture was some kind of bait for that population to be present at the lecture.

By offering free refreshing beverages and media support by radio listened mostly by the young as well as the musical web portal, it has been tried to make popular the whole project.

In all given lectures the lecture rooms were too small to accept all audience interested in lecture.

Questions they made after the lecture were those usual for that age.

They asked about the way of contracting HPV and other STD-s, medical treatment of partners and use of contraceptives and also about vaccina.

Booklets explaining in a popular way the sexually transmitted diseases, way of catching infection and protecting methods were distributed.

Mass media have excellently marked the whole project with almost everyday information. We continued with this project also in the this school year.

CONCLUSIONS: introduce an effective sexual education in schools starting from primary school, develop interdisciplinary cooperation between social and medical sciences, including all experts. Stil the best and most important education comes from a healthy family as the core of our society.

OC 7-4

EFFECT OF EDUCATIONAL INTERVENTION ON HPV VACCINE ACCEPTANCE IN AREAS WITH LOW AND HIGH CERVICAL SCREENING COVERAGE

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Objectives: Studies from Europe and the US have shown that cervical screening history in mothers is a predictor of HPV vaccine acceptance for daughters. We investigated differences in correlates of HPV vaccine acceptance in Japanese women living in areas with "low" and "high" screening uptake, and whether providing an educational intervention could increase vaccine acceptance, particularly in areas with low screening uptake.

Methods: Between October and December 2010, 3,471 mothers, 1,885 in a city with low screening uptake; L-City and 1,586 in a city with high screening uptake; H-City, with girls aged 11-14yrs were randomized to receive a self-administered questionnaire on HPV vaccine acceptance or the questionnaire plus a detailed information sheet on HPV and cervical cancer. A total of 1,095 (31.5%) questionnaires were returned and 1032 used in the final analysis. Apart from marital status-more single mothers in H-city ($p=0.05$) - no statistically significant difference existed in socio-demographics at baseline. As hypothesized both screening uptake and vaccine acceptance was higher in H-City, (50% vs 65%, $p<0.0001$) and (84% vs 90%, $P=0.04$), respectively. While the main barrier to HPV vaccine acceptance was safety in H-City (OR=0.28; 95%CI: 0.12-0.53), in L-City it was mother's screening history (OR=0.27; 95%CI: 0.12-0.61). However, providing an educational intervention significantly increased vaccine acceptability in L-City ($P=0.03$) to levels similar to those in H-City. No similar increase was found in H-City

Conclusions: Cervical cancer disparities may persist or potentially worsen if vaccination coverage is poor in areas with low cervical screening coverage, however providing educational interventions in such areas may help increase vaccine uptake.

OC 7-5

IMPACT OF EDUCATION ON AND ATTITUDES TOWARDS HPV AND VACCINATION, BEFORE AND 6 YEARS AFTER THE INTRODUCTION OF THE VACCINES.

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Objectives: The lifetime risk for acquiring a genital HPV infection is 80% for sexually active people. Mostly, these infections remain subclinical; cervical cancer and anogenital warts are the most common clinical presentations. In 2007, two prophylactic vaccines became available. In 2009 (Flanders, Belgium) and in 2010 (The Netherlands) they were included in the national vaccination programs. The objective of this study is to explore if HPV knowledge, the attitude towards vaccination / HPV vaccination / HPV catch-up vaccination, and (non)-acceptance of the vaccine might be related to gender, or to medical background.

Methods: With an intermission of 6 years (shortly before the introduction, and three years after the inclusion), 715 (2006) and 678 participants (2012) were questioned. Participants were sub-divided in: non-medics, medics, and para-medics.

Conclusions: In general, knowledge on HPV has increased, independent of medical background. The relationship with cervical cancer is well known (> 93% in 2006; > 95% in 2012), just as the facts that an HPV infection might be asymptomatic (> 94% in 2006; > 97% in 2012) and is not treated with antibiotics (> 56% in 2006; > 64% in 2012).
Important reason to accept catch-up vaccination is protection against cervical cancer; reasons to refuse are considering not being at risk and doubting the vaccines' safety.
When we compare 2012 with 2006, these are significant changes:
1) more para- and non-medics, and less medics, would vaccinate all female teenagers; 2) there is an increase to vaccinate daughters and sons; 3) health care providers become the first source of information.
Increased knowledge does not automatically result in an increase of participation in HPV vaccination programs, despite the fact that these programs were regarded as being important. Well-tailored information, without overdosing, is likely to increase participation.

OC 7-6

IMPACT AND ACCEPTABILITY OF FOUR DIFFERENT DECISION-TOOLS FOR HPV VACCINATION AMONG COLLEGE-AGED WOMEN

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Objective: In the U.S., college-aged women have lower rates of HPV vaccination than adolescents. Little is known about how to effectively present HPV vaccine educational materials to this population. We compared 4 different types of decision-tools for HPV vaccination of college-aged women: balanced (i.e. risks and benefits to vaccinating) versus persuasive (benefits only) information, and "fear-provoking images" (pictures of genital warts) versus neutral images (picture of a college-aged woman).

Methods: 140 college-aged women who had not yet received any HPV vaccine doses were randomly assigned to each of the 4 decision tools. After viewing the information, we assessed perceived trustworthiness and understandability of the material, helpfulness in making the HPV decision, HPV vaccination intentions at the time of the intervention, and receipt of HPV vaccine doses 6 months after the intervention. Chi-square tests and linear regression were used to compare categorical and continuous outcomes, respectively.

Conclusions: The mean age of the sample overall was 20 years and 40% were sexually active. Only 3.5% (n=5) had either received or made an appointment to receive the HPV vaccine in the 6 months following the intervention. There were no differences between the tools with respect to impact on vaccination intention or receipt, or perceived trustworthiness or understandability of the material provided. However, a significantly higher proportion of recipients of the fear images indicated they would not recommend the materials to others compared to those who received neutral images (21% vs 8%, p=0.02). Moreover, those receiving fear images had significantly lower scores on the Preparation for Decision Making scale (33.55 vs. 36.18, p=0.03) than those receiving neutral images. In an open-ended response question 18% (n=12) of women in the fear image group indicated that the images were one of the least liked aspects of the materials provided, compared to only 1 woman out of 64 in the neutral image group. From these results we conclude that fear-provoking images related to HPV infection may have a negative impact on college-aged women's perceptions of educational materials.
Future research is needed to determine whether fear-provoking images impact levels of HPV vaccine utilization.

OC 7-7

PARENTAL ACCEPTANCE OF HUMAN PAPILLOMAVIRUS (HPV) VACCINATION FOR DAUGHTERS IN A COUNTRY WITH A HIGH PREVALENCE OF HPV INFECTION

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Objectives: Cervical cancer is the second most common female cancer in Argentina, with non-declining mortality rates, despite opportunistic screening. Human papillomavirus (HPV) vaccination was recently implemented to the national vaccination program, with potential to prevent a major portion of future cervical cancer burden. However, far too little attention has been paid to parental vaccination acceptance following this implementation. Such investigations are urgently needed, particularly from underserved parts of the country with a high burden of the disease. In this context, aims were to investigate acceptance of HPV vaccination, correlates of acceptance as well as perceptions on HPV vaccination among mothers of daughters from a non-metropolitan area of Argentina.

Methods: In all, 180 mothers of 9-15 year old daughters comprised this quantitative cross-sectional questionnaire-based study, conducted at two hospitals in the Mendoza Province. Correlates on acceptance were obtained using multinomial logistic regression models.

Conclusions: Our study showed high theoretical acceptance of HPV vaccination, with 89% of mothers accepting vaccination for their daughters, although acceptance decreased if vaccination was not free (60%). Acceptance was significantly lower among mothers aged 41-45 years compared to younger women ($p=0.02$) and among women who were unsure about vaccine safety in general ($p=0.00$). Also there were extensive misconceptions about the vaccine, which further implies a need for vaccination educational campaigns.

OC 7-8

TRENDS IN HPV VACCINATION ACCEPTANCE BETWEEN 2005 AND 2012 IN GREECE

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OBJECTIVES: To investigate the personal and parental acceptance of HPV vaccination in Greek women as well as the reasons for not accepting HPV vaccination between 2005 and 2012.

METHODS: During 2005 and 2012, 5655 Greek women between 18 and 65 years old were asked to fill a questionnaire answering whether they would accept HPV vaccination for them, and, hypothetically, for their 13 year old daughter or son and if not, for which reason. The survey until 2010 was a part of a large study, the "Lysistrata project" which investigated the knowledge and attitude of Greek women towards cervical cancer screening. The survey continued independently for the following two years.

CONCLUSIONS: Considering that HPV vaccination was introduced in Greece in 2008, the percentage of Greek women that had the intension to get vaccinated against HPV or to accept HPV vaccination for their 13 year old daughter or son was very high for the years 2005 to 2008 – i.e. the years before the introduction of the vaccine - (87-91%, 79-83% and 80-83% respectively). This percentage, at 2009 and 2010 – i.e. immediately after the introduction of the vaccine - was markedly reduced (69-75%, 61-68% and 60-64% respectively). The two following years (2011-2012) we observed a slight increase of that percentage (74-76%, 73-74% and 73% respectively). The main reasons for not accepting HPV vaccination were lack of adequate information and fear of side effects. It is worth mentioning that the lack of information, progressively from 2005 to 2012 became less important as a reason for not accepting HPV vaccination, while the opposite happened concerning fear of side effects. Thus, women, during the past seven years, became more informed, in general, about HPV vaccination, and more afraid of its side effects, meaning that they were probably misinformed and confused.

OC 7-9

NOT THE RIGHT TIME AND NOT ENOUGH EVIDENCE; PARENTS' REASONS TO DECLINE HPV VACCINATION

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Objectives: The aim was to explore parents' reasons to decline HPV vaccination for their daughter aged 10-12 in the Swedish school-based vaccination program.

Methods: This was an explorative interview study with 25 parents who had been offered but not consented to HPV vaccination for their daughter. The interviews were recorded, transcribed verbatim and the transcriptions were analysed with thematic content analysis.

onclusion: Through the interviews five themes emerged revealing the complexity in parents' reasons for not consenting to HPV vaccination: 1. She is just a little girl, 2. Not compatible with our way of life, 3. Not enough adequate information about the vaccine, 4. Scepticism against vaccinations in general and 5. Who can you trust? Parents should be offered more flexibility regarding the time for the vaccination, i.e. a second chance to vaccinate their daughter at an older age and the information should be more transparent and include both benefits and risks about HPV and HPV vaccine. This would benefit both the individual and promote the public health, as parents are the decision makers and have the right to make an informed consent.

OC 7-10

HPV VACCINE ACCEPTABILITY AMONG MOTHERS FROM VARIOUS ETHNIC BACKGROUNDS IN AMSTERDAM, THE NETHERLANDS: A QUALITATIVE STUDY

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Objective: In the Netherlands, HPV vaccination is offered free of charge to all 13-year old girls. Uptake is low (abt. 50%) and lower among girls from some non-Dutch ethnic backgrounds. The incidence of cervical cancer is higher among women from those ethnicities; therefore, increasing the uptake of the HPV vaccination in those groups is a public health priority. We investigated reasons for, and factors influencing (non-)uptake of the HPV vaccination.

Methods: We recruited mothers with a Ghanaian, Moroccan, Surinamese or Turkish background, living in Amsterdam. Per group, 1 or 2 focus groups and 2-5 one-on-one interviews were organized with mothers of daughters who were or will soon be invited for HPV vaccination. Conversations were conducted in the language of choice and covered reasons for (non-) uptake grouped by attitudinal, normative, and control beliefs. Conversations were recorded, translated when necessary, transcribed, and analyzed thematically.

Conclusions: Reasons for non-uptake were: negative outcome expectancies (e.g. the vaccine causing infertility), not being well informed, low perceived risk of HPV infection and cervical cancer, anticipated regret (if the vaccine would have side effects) and distrust. Infertility was viewed as a potential threat to their femininity or respect in the community. Reasons for uptake were: trust, protection of their daughter against cervical cancer, habit, vaccine being an opportunity and not have heard or read about any side effects.
Family and friends were an important source of information in the Moroccan and Turkish group. Surinamese mothers most often consulted their general practitioner. The decision was viewed as a 'women's thing'; therefore the father was often not involved. Most Ghanaian, Moroccan and Turkish mothers asked for support (e.g. from their daughters) to explain the invitation letter. Time and location were not mentioned as a barrier to vaccination uptake.

In conclusion, concerns about possible negative outcomes were prevalent in all groups while sources of information differed between groups. Our findings reveal cultural barriers that may be addressed by education that is tailored to the differential needs, concerns and misperceptions among members of these communities.

OC 7-11

PARENTAL ACCEPTANCE OF HPV VACCINATION OF THEIR SONS IN FOUR EU COUNTRIES

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Objectives: Since 2008, HPV vaccination programmes have been implemented in most EU countries for girls. In its updated guidance on the introduction of HPV vaccines in the EU, the ECDC states that one of the main issues today is whether to include boys in vaccination protocols. Universal coverage would be the most effective strategy to prevent and control HPV-related morbidity in both sexes. Parental acceptance is a key factor for successfully implementing HPV vaccination programmes. We aimed to examine parental attitudes towards HPV vaccination of their sons in four EU countries. The objective was to gain knowledge about acceptability rates and drivers, barriers and socio-cultural factors influencing parental attitudes.

Methods: Following up on a study of parental attitudes toward male HPV vaccination in Denmark, we carried out a larger cross-national study on this issue in France, Germany, Italy and the UK. Our study included a literature study of parental and male acceptability of HPV vaccination as well as popular attitudes towards vaccination, in general. A common questionnaire was developed for the four countries considering country specific health care systems and varying age groups for HPV vaccination. 450 interviews were carried out in each country – by telephone or face-to-face – with parents of one or more sons in an age group eligible for HPV vaccination. The statistical data set was analysed for each country separately and for geographical comparison in a pooled analysis. Relevant cross-examinations were performed to identify main patterns of parental acceptance of HPV vaccination and factors influencing these attitudes.

Conclusion: The overall level of parental acceptance of HPV vaccination of their sons was medium to high (49 – 75%). Main reasons to accept male HPV vaccination were to protect sons directly against cancer and sexually transmitted infection. Main barriers were lack of knowledge of HPV related disease and HPV vaccination as well as fear of side effects. Positive attitudes towards HPV vaccination in general and decision-making related to one's own children differed somewhat. Knowledge of HPV and HPV vaccination and the recommendation of the GP are decisive to parental decision-making. The study results may contribute to inform policy-makers and to improve the adoption of HPV vaccination in girls and boys across Europe.

OC 7-12

UNDERSTANDING THE NEEDS OF AUSTRALIAN DIVERSE POPULATION GROUPS TO INCREASE ADOLESCENT HPV VACCINATION UPTAKE.

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OBJECTIVES: The Australian schoolgirl HPV vaccine program showed disparate uptake, evident within culturally and linguistically diverse (CALD) sub-populations. The implementation strategies overlooked critical social and system ramifications with gendered and culturally non-specific public HPV education, revealing the fundamental complexities in gaining broad acceptance in diverse nations for new adolescent vaccines related to sexually transmitted infections (STIs).

METHODS: A two-phase qualitative cross-cultural study within a socio-ecological framework on Australian CALD parental attitudes toward adolescent HPV vaccination. Parent participants for the phase 1 focus group discussions and phase 2 face-to face interviews were purposively selected according to cultural and linguistic characteristics: Arabic, Turkish, Sri-Lankan, Phillipino, Vietnamese and Indian descendency (n=60) residing in two regions of Victoria where low adolescent HPV vaccine uptake rates had been identified. Recruitment was through local government immunisation services and cultural agencies.

CONCLUSIONS: Interim results indicate the lack of a socio-cultural-impact analysis on HPV vaccine resources prior to its implementation exposed the limitations for CALD populations in cognitive decision making and informed consent, especially for a vaccine linked to sexual debut. Parental needs were diverse and influenced by their emotional, cultural, linguistic and knowledge vulnerabilities. The ethnocentric promotion strategy did not sufficiently address the diverse cultural and linguistic norms or social class determinants of vaccine decision-making. A socio-ecological approach to promotive strategies accommodating cultural and linguistic diversity will optimise delivery and uptake for CALD populations. Intracultural diversity is fundamental to the development of HPV vaccine promotion strategies requiring flexibility, population engagement, self-determination, expanded time frames and differentiated public sexual discourses.(Full results will be reported at the conference).

OC 7-13

THE PSYCHOLOGICAL IMPACT OF CERVICAL INTRAEPITHELIAL NEOPLASIA: A SYSTEMATIC REVIEW

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Objectives: In countries with established cervical screening, treatment for cervical intraepithelial neoplasia (CIN) is a common procedure. It is non-invasive and highly effective. However, pain and bleeding are common, and treatment potentially carries an excess risk of unfavorable obstetric outcomes. As with all cancer screening, there is a possibility of overdiagnosis and overtreatment of CIN. Besides, an excess psychological burden has been described in some studies for women diagnosed with and treated for CIN. We investigated the psychological consequences of CIN diagnosis and treatment in a systematic review.

Method: We searched PubMed using MeSH terms for articles published from January 1990 to February 2013, and perused the reference lists of the retrieved articles. The included studies compared women with a histological diagnosis or treatment of CIN with women having a different outcome in cervical screening (normal or abnormal cytology, cervical cancer). Study selection was undertaken by two researchers independently, and data were abstracted into pre-specified tables. Because the study designs varied substantially, we did not compute summary measures.

Conclusions: From the 5099 retrieved abstracts, 16 studies were included. Four studies were longitudinal following the same women through several steps of screening and the associated follow-up. Most studies used validated questionnaires for various psychological problems, e.g. anxiety, depression, quality of life, psychosocial impact, and sexual impact. Surprisingly, having a diagnosis or being treated for CIN in many studies appeared to have no worse psychological consequences compared to having an abnormal smear which could potentially require a colposcopy. However, a smaller number of studies showed the opposite. No study showed a difference in the psychological outcomes compared to having a cervical cancer diagnosis, but all showed a difference when compared to women with normal screening cytology. In summary, this systematic review suggested that the differences, if any, between having CIN and having an abnormal smear, or any other positive outcome, are small. It therefore indicates that it is not the severity of a lesion or the treatment that has negative psychological consequences for women, but the fact of getting a positive screening outcome of any kind.

OC 8-1

PARALLELS BETWEEN ANAL AND CERVICAL INTRAEPITHELIAL LESIONS- A PROSPECTIVE STUDY

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BACKGROUND: Human papillomavirus (HPV), epitheliotropic, is the most common sexually transmitted disease. In sexually active adults there is an estimated 80% lifetime risk of HPV exposure that can infect anogenital area.
The correlation of HPV infection and the development of cervical intraepithelial neoplasia (CIN) raised the possibility of its involvement in other similar histological tissues, specially the anal canal.

METHODS: A prospective study was conducted in the Colposcopy Unit of the Department of Gynaecology at Hospital de Cascais, from January 2012 to June 2013. The aim of our study was to find predictors of CIN as well as the prevalence of anal dysplasia in women with genital intraepithelial disease. Women with abnormal cervical biopsies high-grade lesions (intraepithelial neoplasia 2 and 3) were selected. A questionnaire was designed in order to evaluate risk factors including smoking habits, coitarche, number of sexual partners in lifetime, anal intercourse, and immunosuppression. At the moment of cervical conisation anal Pap smear was undertaken, in order to screen anal squamous intraepithelial lesions.
Statistical analysis was performed using SPSS, version 20.0.

RESULTS:From a population of 113 women diagnosed with CIN 2+, the mean age was 36,7 +/- 10,5 (SD) years. 57,8% of our population were referred to a Colposcopy appointment due to high grade cervical smear result.
The coitarche range is between 13 and 24 years with an average of 17,2 years. 40,7% of them have had more than 4 sexual partners. 51,3% smoke, the majority since an early age, and despite that, 57,5% are taking combined oral contraceptive.
A considerable number of anal smears were unsatisfactory due to scant cellularity or absence of transformation zone cells. We did found 10% with anal dysplasia.
There was no statistic significance between the group with anal dysplasia and anal intercourse.

CONCLUSIONS: Prevalence data for anal dysplasia is still sparse and therefore screening method, frequency and appropriate treatment need to be clarified. However our study points out that women with multiple risk factors for cervical intraepithelial lesion might benefit from anal intraepithelial dysplasia screening. This procedure would help clinicians to diagnose anal lesions in an earlier stage, decreasing women´s morbidity and mortality. We do believe that this screening is a turning point on the follow up of these patients.

OC 8-2

ANAL HIGH-RISK HPV INCIDENCE AMONG HIV-NEGATIVE AND HIV-POSITIVE MSM

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Objectives: Our aims were to assess the incidence of anal high-risk HPV (hr-HPV) infection among HIV-negative and HIV-positive men who have sex with men (MSM), and to identify determinants for hr-HPV incidence. Specifically, we assessed the effect of HIV infection on anal HPV incidence.

Methods: MSM were recruited at 3 study sites in Amsterdam (the Netherlands) and followed-up semi-annually. Participants completed risk-factor questionnaires, and collected anal self-swabs at baseline, 6, and 12 months. Samples were tested for HPV DNA and genotyped using the SPF_{10}-PCR & DEIA-$LiPA_{25}$ system (DDL), detecting 12 hr-HPV types.

Statistical analyses were performed using multivariable Poisson regression with Generalized Estimating Equations. Multivariable models were adjusted a priori for the following variables measured at baseline: age, smoking, CD4 count and HIV viral load (only in the stratified model for HIV-positive MSM), and the following time-updated variables (regarding the preceding 6 months of follow-up): use of poppers and cannabis, fisting, and number of recent anal sexual partners.

Conclusions: Among 459 HIV-negative and 317 HIV-positive MSM followed for a median of 11.9 months, 620 incident anal hr-HPV infections were detected. The incidence rate (IR) of HPV-16 was 10.8 per 100 person-years (py); the IR of HPV-18 was 7.0/100 py. Although the IR of HPV-16 and HPV-18 were higher in HIV-positive compared to HIV-negative MSM, this was not significant. In the overall multivariable model, HIV-positive individuals were 1.3 times more likely to acquire an incident hr-HPV infection compared to HIV-negative MSM ($P=0.04$). Current smoking and having had more than two recent anal sexual partners were borderline significant in the multivariable model. In analyses stratified by HIV status, no risk factors were significant.
In conclusion, the incidence of anal hr-HPV is very high among MSM, specially among HIV-positive MSM. Further research is necessary on risk factors for incident hr-HPV infections among HIV-negative and HIV-positive MSM.

OC 8-3

SCREENING THE ANAL DYSPLASIA IN HIV-POSITIVE PATIENTS: HOW EFFICIENT IS HIGH-RESOLUTION ANOSCOPY?

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Objective: Human immunodeficiency virus (HIV) and Papillomavirus (HPV) are major co factors of developing anal dysplasia. Annually anal screening is recommended in HIV positive subjects because of a high-risk of anal dysplasia. The high-resolution anoscopy (HRA) has been shown to improve the exploration of the anal mucosa by magnifying the macroscopic changes. It is recommended to patients presenting anal dysplasia on cytology (low or high grade squamous intra-epithelial lesion: LSIL or HSIL, and atypical squamous cell of undetermined significance: ASCUS). Its interest remains under debate in the anal screening of dysplasia. The aim of this study is to evaluate the HRA performances in HIV-positive patients with abnormal cytology, using biopsies on both identified lesions and normal mucosal appearance as a gold standard.

Methods: Between October 2010 and April 2013, 107 HRA has been performed to 70 HIV-positive patients (66(94.3%) men, mean age 47.6 (27-69; SD 10.1)) with dysplasia on cytology. The tinctorial and macroscopic aspects of the anal canal were used to characterize the abnormal areas: acetowhite, iodonegative, mucosal punctuations, warty raised, mosaic pattern, vascular abnormalities and miscellaneous. Biopsies were systematically made on both abnormal and normal areas.

Results: Macroscopic lesions were associated to abnormal cytologies type ASCUS in 14.4%, LSIL in 56.7% and HSIL in 28.4%. Histological anal intra-epithelial neoplasias (AIN) were more frequently observed in association to mucosal punctuations (48.1%), warty raised (55%) and mosaic pattern (50%). No statistical significance was observed in association with vascular abnormalities. Macroscopic lesions were associated to AIN in 34/55 (61.8%) and to high-grade AIN (AIN 2-3) in 21/55 (38.2%). Besides, statistical significance was observed in association between acetowhite, iodonegative areas and AIN, respectively 20/46 (43.5%),18/46 (39.1%), and in both, 11/46 (24%) of AIN 2-3. Nevertheless, AIN lesions were observed in approximately 6/42 (15%) of patients with normal tinctorial aspects, in 17/38 (44.7%) of patients without macroscopic lesion and in 35/107 (32.7%) of normal areas (AIN 2-3 in 18/107 (16.8%)). As compared to histology, sensitivity and specificity of HAR were 34/51 (66.7%) & 10/27 (37%) to detect AIN and 21/51 (41.2%) & 17/27 (63%) to detect AIN 2-3 respectively.

Conclusions: A normal HRA doesn't rule out the presence of AIN. Both abnormal and normal areas have to be biopsied. This technique may be preferred to detect and to treat macroscopic changes rather than to screen dysplasia.

OC 8-4

HIGH PREVALENCE OF HIGH GRADE ANAL INTRAEPITHELIAL NEOPLASIA IN WOMEN LIVING WITH HIV, PRELIMINARY RESULTS OF THE EVVA STUDY

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Women living with HIV (WLHIV) are at increased risk of developing anal intraepithelial neoplasia (AIN) and anal cancer. **Objectives:** The EVVA study (Evaluation of HPV, HIV and AIN in women) was created to quantify the burden of high-grade AIN (AIN-2,3) in WLHIV, in Montreal, Canada.

Methods: EVVA is a prospective cohort study. Data collected include questionnaires, cervical/anal HPV testing and cervical/anal cytology every 6 months for 2 years. A systematic high-resolution anoscopy (HRA) with biopsies of suspicious areas is also performed at baseline and at 2 years for all participants, with additional HRA as clinically indicated. Biopsies of the transformation zone are taken if the HRA is visually normal. We present here the baseline results of the first 100 recruited participants.

Results: The anal cytology results for the baseline visit of 89 participants (11 had unsatisfactory samples) were: 5 high-grade squamous intraepithelial lesions (HSIL) (5.6%, 95% confidence interval (CI) 1.8-12.6), 4 low-grade SIL (LSIL) (4.5%, 95% CI 1.2-11.1), 19 Atypical Squamous Cells of Undetermined Significance (ASCUS) (21.3%, 95% CI 13.4-31.3) and 61 normals (68.5%, 95% CI 57.8-78.0). Histology analyses of the biopsies taken through HRA from 95 participants revealed 17 AIN-2,3 (17.9%, 95% CI 10.8-27.1), 43 AIN1 (45.3%, 95% CI 35.0-55.8), and only 35 without AIN (36.8%, 95% CI 27.2-47.4). Cervical cytology results were: 1 HSIL, 5 LSIL, 14 ASCUS and 80 normals.

Conclusion: Our study revealed that AIN-2,3 is a frequent finding in WLHIV. To our knowledge, this it the highest rate described in published literature. Systematic HRA and blind biopsies might explain the difference. Anal cytology results also largely underestimated the extent of anal disease in WLHIV.

OC 8-5

ANAL HPV INTEGRATION AND IDENTIFICATION OF OTHER DNA VIRUSES IN HIV-UNINFECTED MEN WHO HAVE SEX WITH MEN

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Objectives: Studies on Human Papillomavirus (HPV) integration in anal samples were conducted mainly in high-grade lesions and invasive cancer of HIV-infected individuals. Our goal was to investigate HPV physical status in HIV-negative men who have sex with men (MSM) with a detectable anal HPV infection. In the same time the presence of other circular DNA viruses in the anal region was also explored.

Methods: Study participants were attendees of an STI screening program. HPV physical status was assessed using multiply-primed RCA. HPV16-positive samples were also analyzed using E2/E6 multiplex PCR, qRT-PCR and APOT assay. RCA and virus specific PCR were employed to investigate the presence of other DNA viruses.

Results and conclusions: Anal HPV infection was detected in 76.9% of the 230 enrolled MSM. No high grade lesions were detected and HPV physical status was evaluated in 109 anal samples. Integration was observed in one HPV16-positive sample (0.9%), in which integrate-derived viral transcripts were detected. Sequencing of the amplification products revealed chimeric viral-cellular transcripts with integration in chromosome 14q. The fusion transcript encompassed E7-E1 sequences at the 5?-end, followed by E4 and flanking cellular sequences at the 3'-end. Multiplex PCR and qPCR evidenced the presence of E2 together with E6 amplification, suggesting that E2 gene is partially retained, at least in the region where the specific primers anneal to.
In 22 of the 53 (41.5%) mucosal HPV-negative samples, RCA restriction results indicated the presence of circular DNA viruses. Indeed, cutaneous HPV (4 samples), MCPyV (5 samples) and TTV (4 samples) were detected. In conclusion, anal HPV integration was rarely evidenced in HIV-uninfected MSM with no or mild anal cytological abnormalities, although the integration rate may have been underestimated because of the limitations of the employed assays. Other DNA viruses were detected in the anal samples of these individuals, although the significance of this occurrence needs to be assessed.

OC 8-6

DETECTION OF HIGH-RISK HPV IN MEN WHO HAVE SEX WITH MEN, LIVING WITH HIV: APPLICATION OF AN AUTOMATED REAL-TIME PCR TEST.

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HPV associated anal cancer is more common among MSM, who are HIV infected. However, there is currently no recommended HPV test for men.

Objective: To evaluate the sensitivity and specificity of Real Time High Risk (HR) HPV assay, compared with anal cytology in a sample of Mexican MSM living with HIV.

Methods: Study participants were recruited from Clínica Especializada Condesa at Mexico City. Evaluation of anal cytology was performed on specimens that were collected by rotating a Dacron-tipped swab in the anal canal and that was then placed in liquid-based Thin Prep (Cytyc, Boxborough, Massachusetts, USA). Oncogenic HPV detection by Real Time High Risk (HR) HPV assay (Abbott Laboratories, Abbott Park, Illinois, U.S.A.) was made from an aliquot (700 ul) of cytology specimens. We calculated sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for HPV on Real Time High Risk (HR) HPV assay.

Conclusions: In this study 595 MSM living with HIV were included. Cytology revealed that 68% of men had some form of squamous intraepithelial lesions (SIL), mainly low-grade (65%), while 3% had high-grade HSIL. High risk HPV types were possible to assess in 495 individuals, and HR-HPV types were present in 92% of the patients, of which 39% showed oncogenic types 16 and / or 18, and 33% had at least two HPV types. The HPV test had a high sensitivity (93%) but low specificity (7%) probably due to the relatively low frequency observed of HSIL among subjects and the high prevalence of HR-HPV in MSM. This research showed that Real Time High Risk (HR) HPV assay could be an alternative for identification of HPV in this population type. Although it has been proposed that the identification of HR-HPV should be a routine test in populations with a high probability of developing anal cancer, as MSM and people living with HIV, there is still no accepted diagnostic test. Screening programs for anal cancer may include detecting HR-HPV as a complementary test to cytology, with greater probability of early detection of cases that could become cancer.

OC 9-1

PREVALENCE OF HUMAN PAPILLOMAVIRUS INFECTION IN A KASHMIRI ETHNIC FEMALE POPULATION

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Human papillomavirus (HPV) infection is estimated to be the most common sexually transmitted infection and is one of the causal factors in cervical cancer. Understanding the epidemiology of this infection is an important step toward developing strategies for its prevention.

Objectives: To investigate the HPV prevalence, age distribution, and risk factors for HPV infection in Kashmiri ethnic population.

Methods: We interviewed and obtained cervical specimens from 210 women with normal and abnormal cytomorphology. Specimens were tested for the presence of HPV DNA using MY09/MY11 and GP5+/6+ primer-mediated PCR. With the aim of identifying the HPV types, samples were also subjected to TS-PCR using specific primers for HPV types 16 and 18.The PCR amplification of HPV genomes is a sensitive method that is used for the detection of cervicovaginal HPV. In addition, basic demographic information, sociodemographic characteristics, and sexual behavior were recorded.

Conclusion: HPV was detected in 13.8% of the study population aged 18 to 57 years using PCR. HPV16 (6.6%) was more commonly detected than HPV18 (3.8%). The highest prevalence of HPV infection was seen in women below 27 years old, and then, a new increase was seen higher than the age of 48. In conclusion, our study demonstrated that younger age at marriage, economic status, parity, and dwelling are the major risk factors determining HPV infection.
Key words: Human papillomavirus , pap smear, cervical cancer, PCR.

OC 9-2

HIGH-RISK UROGENITAL HPV INFECTIONS IN PARAMARIBO, SURINAME: PREVALENCE AND RISK FACTORS IN AN ETHNICALLY DIVERSE POPULATION OF WOMEN

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Objectives: Cervical cancer is caused by high-risk (hr) Human Papilloma Virus (HPV) infections. The mortality rate of cervical cancer in Suriname is 8/100.000 per year. HPV vaccination is not yet available in Suriname. We estimated the prevalence of, and determinants for urogenital hr-HPV infections among women from five major ethnic groups in Paramaribo, Suriname in the pre-vaccination era.

Methods: Between July 2009 and February 2010, women aged ≥18 years were recruited at a family planning clinic and sexually transmitted infections (STI) clinic. Vaginal swabs were collected, shipped and tested for HPV DNA and genotyped using the SPF_{10}-PCR & DEIA-$LiPA_{25}$ system (DDL), targeting 25 different genotypes. Logistic regression analysis was used to identify determinants of HPV infection.

Conclusions: 1001 women were included and tested for HPV of whom 584 (58%) were DEIA positive. Of the positive samples 472 (81%) could be typed by LiPA25. hr-HPV types were detected in 303 (30%) of all samples. HPV type 52 was the most prevalent genotype (n=79; 8%). Of the women with hr-HPV, 46 (15%) had only hr-HPV types 16 and/or 18. Adjusted for age the following independent associations with hr-HPV were found; ≥2 recent partners (OR=1.5, 95%CI=1.1-2.2), *Chlamydia trachomatis* co-infection (OR=2.3, 95%CI=1.5-3.5) and ethnic group (OR=2.1, 95%CI=1.4-3.1 for Creole; OR=2.0, 95%CI=1.2-3.3 for Maroon; OR=2.8, 95%CI=1.8-4.4 for Mixed race, all compared to Hindustani).
HPV prevalence in Suriname is high and hr-HPV is not equally distributed among ethnic groups. These data provide a useful baseline to assess possible shifts in HPV genotype prevalence following introduction of vaccination.

OC 9-3

PREVALENCE AND DISTRIBUTION OF HUMAN PAPILLOMAVIRUS TYPES AMONG THAI FEMALES IN BANGKHAYENG, PRATHUMTHANI PROVINCE, THAILAND

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Despite the high incidence of cervical cancer reported from Thailand, large scale population based studies on the HPV prevalence and genotype distribution are rare. Baseline population prevalence data for HPV infection in Thailand would be useful.

Objective : To determine the prevalence and distribution of HPV among Thai females that live in Bangkhayaeng district, Thailand.

Materials and methods : A community-based sample of women aged 20 years and above was recruited from a single district in Thailand during the period February–May 2013. A total of 1217 age-eligible women were recruited to participate in the cervical cancer screening program of Chulabhorn hospital, Bangkok, Thailand. The cervical samples were analyzed for HPV DNA by Linear array (Roche, USA)

Results : Of the 967 eligible women, 12.9% were positive for any HPV DNA The overall prevalence of high- and low- risk HPV types was 6.7% and 8.0%, respectively. Prevalences of high-risk HPV types were highest in females aged 31 to 40 years (9.2%). The most common high risk HPV types detected were HPV-52 (1.3%), HPV-16 (1.1%) and HPV-51 (1.0%). The most common low risk HPV types detected were HPV-72 (1.9%), HPV-62 (1.5%) and HPV-70 (1.3%). According to HPV vaccine; HPV-6 was detected in 0.2%, HPV-11 in 0.0%, and HPV-18 in 0.2% of female participants.

Conclusions : The prevalence of HPV infection from this study is quite lower than other studies in the western countries. Our results suggest that the HPV type in a general screening population from Bangkhayaeng district is different to that reported in other countries. However, the prevalence of HPV vaccine types was relatively low.

OC 9-4

HPV INFECTION IN CERVICAL AND OTHER CANCERS IN SAUDI ARABIA: IMPLICATION FOR PREVENTION AND VACCINATION

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Objectives: HPV is closely associated with cervical cancer that the incidence of this tumor is regarded as a surrogate marker for HPV infection in countries lacking epidemiological studies. HPV is also implicated in subsets of anogenital, oropharyngeal, colorectal and breast cancers. Cervical cancer is the third most common cancer in women worldwide; however, its incidence is low in Saudi Arabian women, suggesting low prevalence to HPV infection due to environmental, cultural and genetic predisposing differences. Evidence of insusceptibility was reviewed from published data investigating HPV prevalence and genotype distribution in Saudi Arabia.

Methods: Data were collected from Saudi Cancer Registry Report and studies published in PubMed (NCBI) using HPV and Saudi Arabia as keywords. The search had returned 17 hits of which only 13 were relevant documents (1993 – 2013). Cervical cancer in Saudi Arabia ranked number 12 between all cancers in females and accounts only for 2.4% of all new cases, despite the lack of national screening programs. Compiled results of about 300 patients showed that 87% of cervical tumors are infected with HPV of which 78% are HPV-16 and 18 genotypes. One study looked for possible association with 9 genetic single nucleotide polymorphisms (SNPs) presumed to predispose to cancer. While significant association was observed for XRCC1 G399A, TP53 G72C showed borderline association only in HPV-positive patients. Deviation from HWE in HPV-positive patients indicated co-selection, hence implicating the combination of HPV and SNPs in cancer predisposition.

Conclusions: Beside the salient difference of having very low incidence of cervical cancer in Saudi Arabia, the involvement of HPV infection is comparable to the rest of the world with HPV-16 and HPV-18 being the two most common genotypes and account together for three-quarters of HPV infection. Genetic predisposition implicated certain SNPs as biomarkers of susceptibility when associated with HPV infection only. Vaccination against HPV would protect three-quarters of cervical cancer patients in Saudi Arabia. However, due to its low incidence (2.1/100,000 women), its involvement in other cancers and a proper cost-effectiveness analysis is required to justify the implementation of a costly national vaccination program. Supported by NSTIP-KACST grant 12-MED2945-20.

OC 9-5

CLUSTERING OF MULTIPLE HPV TYPES ACROSS DIVERSE RISK POPULATIONS IN THE NETHERLANDS; POPULATION- OR TYPE-SPECIFIC?

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Objectives: In view of possible type-replacement upon introduction of HPV vaccination, we aimed to explore whether patterns of multiple HPV infections in populations with varying background infection risks are indicative of type-specific interactions.

Methods: Women (n=3874) of three cross-sectional studies provided a vaginal self-sample, which was tested for 25 HPV genotypes by a sensitive molecular assay (SPF10-LiPA). The number of concurrent HPV infections per woman was studied by Poisson regression. Associations between HPV types were investigated by generalized estimating equations (GEE) analyses.

Results: Prevalence of any HPV type was 14% in a population-based study, 54% in a Chlamydia screening intervention study and 73% in a study among attendees of sexually transmitted infection clinics. Overall, multiple HPV infections were detected in 26% of the women. The number of concurrent HPV infections conformed to an overdispersed Poisson distribution, also after correction for known risk factors. Types differed significantly in their tendency to be involved in a co-infection, but no evidence for particular type-type interactions was found. Moreover, strongest associations were observed in the lowest-risk population and vice versa.

Conclusions: We found no indications of type-specific interactions, but our findings do suggest that clustering differs between HPV types and varies across risk groups

OC 9-6

INTERACTION BETWEEN HPV TYPES IN SQUAMOUS CERVICAL NEOPLASIA

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Objectives: HPV types are generally believed to interact positively. However, a negative interaction effect has been shown between HPV16 and HPV6 [1]. We further investigated interactions between high-risk (HR)-HPV and low risk (LR)-HPV types in CIN3 and squamous cervical cancer (SCC), respectively.

Methods: In two nested case-control studies among women participating to cervical screening, with a first cytologically normal smear, we collected 5783 archival cervical smears from 646 women with CIN3, 473 with SCC, and matched controls. All smears were tested for HPV with RDBH or Luminex methodology. The median study time was 5-8 years.
We found that HPV16/18 infection in the first smear during follow-up was associated with a 17-fold increased risk for future SCC compared to HPV-negative women ($p<0.0001$). Non-16/18 HR-HPV infection in the first smear was associated with a 3-fold increased risk for future SCC ($p<0.0001$). However, having both HPV16/18 and non-16/18 HR-HPV types in the first smear conferred a joint risk of SCC which was lower than the direct multiplicative sum of the individual effects (i.e. OR=9, 95% CI 2-43, instead of an expected OR=51 if no interaction effect was present). We noted a similar interaction effect when analyzing HPV-types present in the last smear, although CI:s were wide. Furthermore, the presence of both LR-HPV and HPV16/18 were associated with a similarly lower-than-expected joint future risk for both CIN3 and SCC, although this was not significant. Estimates for categories HPV16 and non-16 HRHPV, instead of HPV16/18 and non-16/18 HR-HPV, showed similar results. Finally, we found that a woman with HPV1618 + non-16/18 HR-HPV had about half the risk of someone with HPV16/18 only for future SCC, and a woman with HPV16/18 + LR strains had about 1/3 the risk of someone with HPV1618 only for future SCC, although inference again was limited by low power and results for the CIN3 study were mixed.

Conclusions: We find that concurrent infection with both HPV16/18 and other HR-/LR-strains is associated with altered risks for CIN3 and SCC development than infection with HPV16/18 only. Although precision was limited, our findings should be explored further in HPV-based screening.

[1] Arnheim Dahlström et al, Cancer Epidem Biomarkers Prev, 2011.

OC 9-7

TYPE-SPECIFIC ONCOGENIC HPV INFECTION IN HIGH GRADE CERVICAL DISEASE IN NEW ZEALAND

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Objectives: Understanding the prevalence of different human papillomavirus (HPV) genotypes in a population is important for public health policy, including screening policy, in the era of HPV vaccination. In New Zealand HPV vaccination was introduced in 2008. The aim of this study was to estimate the baseline prevalence of oncogenic HPV types in New Zealand women at the start of the vaccination programme. This information will inform ongoing surveillance and evaluation of HPV vaccination effectiveness and guide necessary changes in cervical screening parameters such as the age of screening initiation and interval between screens. A specific objective was to determine whether there were differences in HPV genotypes between indigenous and other ethnic groups in New Zealand.

Methods: Women aged 20-69 years, with cytological high grade lesions were recruited via the National Cervical Screening Programme register. Liquid based cytology specimens were tested for 37 HPV types using linear array genotyping (RochePleasanton, CA). Results were analysed both for all women with high grade cytology and for the subgroup of women in whom a high grade lesion was confirmed histologically. Age specific trends for the relative proportion of HPV 16/18 vs other oncogenic types in CIN2/3 were assessed. Further analyses by Maori vs non Maori are being undertaken.

Conclusions: A total of 594 women with high grade cytology were recruited. Of these 356 (60%) had histological confirmation of CIN2/3 and 6 (1%) had confirmed AIS or glandular dysplasia.
Positivity rates for any oncogenic HPV infection and for HPV 16 and/or 18 within confirmed CIN2/3 – AIS were 95% (95%CI: 92-97%) and 60% (54-65%) respectively; in all women with high grade cytology (ASC-H/HSIL+AGC/AIS) it was 87% (84-89%) and 53% (49-57%) respectively. The most common reported HPV types in women with CIN2/3 were 16 (51%), 52(19%), 31 (17%), 33 (13%) and 18 (12%). Test positivity rates for type 52 appear higher than in comparable other studies. These initial findings provide a baseline for ongoing surveillance and optimising the choice of future HPV vaccine composition. Differences between major ethnic groups in New Zealand will be presented.

OC 9-8

HUMAN PAPILLOMAVIRUS MULTIPLE INFECTIONS: COMPARISON BETWEEN CYTOLOGICAL AND HISTOLOGICAL LESIONS

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Objectives. High risk human papillomavirus (HR-HPV) detection tests have recently assumed an important role in cervical cancer screening, but an open question remains the utility of HPV genotyping tests. Aim of this study was to estimate the correlation between HPV multiple infections and grade of cytological/histological lesions, and the importance of genotyping in patients management.

Methods. 1005 Pap smears (345 normal cases, 411 low grade squamous intraepithelial lesions -L-SIL, 30 high grade SIL - H-SIL, 180 atypical squamous lesions of unknown significance – ASCUS, and 39 cannot exclude high-grade atypical squamous cells - ASC-H) and 652 cervical biopsies (180 without alterations, 280 with cervical intraepithelial neoplasia - CIN1, 132 with CIN2, 52 with CIN3 and 8 with cervical cancer) were tested to detect and genotype HPV by end-point PCR and reverse hybridization (InnoLipa). Multiple infections were confirmed with AMPLIQUALITY HPV-TYPE EXPRESS kit (AB Analitica). Statistical correlations were calculated with chi-square test.

Conclusions. Correlation between HPV and cytological lesions: overall HPV prevalence in Pap smears was 62% and significantly increased with progression of cytological alteration ($p<0.005$). Multiple infections were observed in 31.6% of positive cases, but no significant difference was observed between various grade of alterations. Correlation between HPV and histological lesions: overall HPV prevalence in cervical biopsies was 81%. Data showed a statistically significant correlation ($p<0.005$) between: 1) HPV frequency and grade of lesion; b) HPV multiple infection and grade of alteration: multiple infections were higher in early stages of disease (CIN1: 36% and CIN2: 39%) than in normal cases (8.3%) and CIN3 (7.7%). Statistically significant difference was observed even in cases without HPV 16 and 18 ($p<0.005$). Reported data suggest that detection of multiple infections in early stages of disease is a negative prognostic factor of progression. Therefore HPV genotyping seems to be important not only for epidemiological study and to estimate reinfections in follow-up, but also to identify patients with higher risk of disease progression, even in the absence of HPV 16 and 18.

OC 9-9

ASSOCIATION OF SMOKING WITH HIGH-RISK HPV INFECTION IN WOMEN UNDERGOING CERVICAL CANCER SCREENING IN NORTHERN GREECE

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Objectives: To investigate whether there is an association between tobacco smoking and high-risk Human Papilloma Virus (HPV) infection in a population of women undergoing cervical cancer screening in Northern Greece.

Methods: We recruited 2001 women aged 25-55 years, undergoing Pap smear testing, for cervical cancer screening, between August 2011 and May 2013, at three outpatient Gynecology Clinics, in Thessaloniki, Greece. Cytological evaluation was performed using Liquid-based cytology (THINPREP®). A fraction of each sample was used in order to detect DNA of 14 High Risk HPV types (HR-HPV): HPVs 16 and 18, separately, and HPVs 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68 using the COBAS 4800® system (Roche®). Women answered questions about smoking habits (number of cigarettes per day, years of smoking).

Conclusion: Among 324 HR-HPV positive women (16.2%), only 141 (43.5%) were non-smokers, whereas among 1677 HR-HPV negative women, 961 (57.3%) were non-smokers ($p<0.001$). This association was attributed to the youngest age group of women, aged 25-34 years ($OR=2.6$; $p<0,001$). The same trend was observed concerning HPV 16 positivity ($OR=2.2$, $p=0.005$) and HPV positivity for high risk types other than 16 and 18 ($OR=2.5$, $p<0.001$), but not for HPV 18 positivity and Pap testing abnormality. The intensity of smoking showed no statistically significant association with HR-HPV infection. Nevertheless generally by applying binary logistic regression it occurs that smoking intensity affects the occurrence of positive HPV for any type HPV , HPV 16 and high risk HPV. Combined with age it occurs that females in younger age groups tend to be more frequently positive to HPV.

OC 9-10

PREVALENCE AND DISTRIBUTION OF HPV GENOTYPES IN SQUAMOUS CELL PAPILLOMA OF THE CONJUNCTIVA

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Objectives: Conjunctival squamous cell papillomas are relatively rare benign epithelial tumors that occur in both children and adults presenting with different clinical features and outcomes. In previous studies HPV-DNA has been detected in 5-92% of cases of conjunctival papillomas, with HPV6 and HPV11 being the most frequently detected HPV genotypes, although occasionally other alpha-HPVs like HPV16 were also identified. The aim of this study was to investigate further the prevalence and distribution of HPV genotypes in conjunctival papillomas using highly sensitive methodological approach.

Methods: 31 formalin-fixed, paraffin-embedded tissue specimens of conjunctival papillomas were included and tested for the presence of HPV-DNA using 7 different PCRs, covering four HPV genera and at least 108 different HPV genotypes. Slides were histologically reviewed for the presence of goblet cells, intraepithelial inflammation, koilocytosis and type of growth (papillary versus flat). Papillomas were characterised as either keratinised or non-keratinised with basal cell predominance.

Conclusions: Alpha-HPV genotypes were detected in 19/30 (63.3%) specimens: HPV6 was detected in 12 cases and HPV11 in 7 cases. Beta-HPV genotypes were detected in 5/30 (16.7%) samples, mostly as co-infections with HPV6 or HPV11. Gamma- and mu-HPV-DNA was not detected in any of the samples. Presence of goblet cells and basal cell predominance ($P=0.017$ and 0.004, respectively) but not the growth pattern or the presence of intraepithelial inflammation were significantly associated with HPV6/11 infection. Convincing koilocytosis was present only in two papillomas. In conclusion, HPV6 and HPV11 play an important role in the etiopathogenesis of non-keratinising conjunctival papillomas with basal cell predominance and goblet cells. Etiology of HPV-negative keratinising conjunctival papillomas remains to be determined in further studies.

OC 9-11

TYPE-SPECIFIC TRANSMISSION PROBABILITIES AND THE ROLE OF INFECTION-INDUCED IMMUNITY IN THE SPREAD OF HPV IN THE NETHERLANDS

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Objectives: Infection with the human papillomavirus (HPV) is a necessary cause for developing cervical cancer. There is still much uncertainty regarding HPV transmission and infection-induced immunity, both important factors in predicting the long-term effect and cost-effectiveness of HPV vaccination. Transmission models have been developed, yet they typically estimate the transmission probability per partnership instead of per sexual contact, only the transmission of a few HPV-types, or cannot fully grasp the complexity of the spread of sexually transmitted infections (STI) through sexual networks. We aim to provide more insight in HPV transmission and epidemiology, especially in the process of infection-induced immunity, by investigating assumptions regarding biological and behavioral factors.

Methods: We used the established microsimulation model STDSIM for STI transmission and control, quantified to the Netherlands. Assumptions regarding sexual behavior are validated by modeling Chlamydia in addition to HPV. We fitted the type-specific HPV prevalence of 14 high risk HPV types by evaluating pre-set combinations of transmission probabilities and mean durations of acquired immunity for each type, using a grid search. We then checked which combinations of transmission probabilities and immunity durations predicted the age-specific prevalence patterns of the different types correctly.

Conclusions: Preliminary results show that it is necessary for all HPV-types to assume infection-induced immunity, which has been overlooked in many previous cost-effectiveness models. Transmission probabilities per sexual contact are lower and durations of infection-induced immunity are shorter than expected, and both are dependent on HPV-type. Common assumptions such as attributing an exponential distribution for the infection duration do not seem to be adequate for modeling the natural history of HPV. The results of this study provide new information regarding the natural history of HPV and will improve HPV vaccination (cost-) effectiveness studies.

OC 9-12

PREVALENCE OF GENITAL WARTS IN SPECIALISTS' PRACTICES IN THE PHILIPPINES

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Objectives: This study aimed to estimate the prevalence of genital warts (GW) in physician practices in four regions of the Philippines.

Methods and results: Physicians located in Manila, Luzon, Mindanao and Visayas (including 28 primary care providers [PCP], 43 gynaecologists [OBGYN], 29 dermatologists [DERM], 29 urologists [URO], and 28 infectious disease specialists [IDS]) prospectively recorded daily logs of patients (N=11,179) for a 2-week period to estimate GW prevalence between July 2011 and September 2012. Age, gender and GW diagnosis status (new, resistant, recurrent) of each patient was recorded in the daily logs. Prevalence (number of GW patients/total patients seen by the physician) was obtained for each physician and calculated into a single estimate across all physician types. Overall prevalence estimates (and 95% CI) were weighted by the estimated number of physicians in each specialty and the estimated proportion of total patients attending to each specialty. The overall GW prevalence estimate was 4.8% (95% CI: 4.58-4.98%) for patients between 18-60 years, with 3.4% (95% CI: 3.13-3.65%) in females and 8.0% (95% CI: 7.69-8.31%) in males. Prevalence estimates in PCP practices was 0.33% (95% CI: 0.33-0.33%), 2.1% (95% CI: 2.13-2.14%) in OBGYN, 3.5% (95% CI: 3.47-3.49%) in URO, 2.1% (95% CI: 2.07-2.08%) in DERM and 18.7% (95% CI: 18.7-18.7%) in IDS practices. Of the 337 total male GW cases, 233 (66.7%) were newly diagnosed and 104 (33.22%) were existing. Among the existing cases 61 (57.7%) were recurrent, and 43 (41.3%) were resistant to treatment. Of the 247 total female GW cases, 166 (65.3%) were newly diagnosed and 81(34.7%) were existing cases. Among the existing cases, 61 (75.3%) were recurrent, and 20 (24.6%) were resistant.

Conclusion: In this study, GW is prevalent in the Philippines in both male and female patients. More than 60% of GW cases are newly diagnosed.

OC 9-13

PREVALENCE OF GENITAL WARTS IN HEALTH CARE PROFESSIONAL PRACTICES IN SOUTH AFRICA

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Objectives: To estimate the prevalence of genital warts (GW) in health care professional practices in South Africa.

Methods and results: Health Care Professionals (HCPs) (including 64 general practitioners [GP], 25 HIV specialists [HIV], 33 gynaecologists [OBGYN], 25 dermatologists [DERM], 21 urologists [URO], and 32 primary health care nurses [PHCN]) located in Area One (Eastern Cape and Free State), Area Two (Gauteng), Area Three (KwaZulu-Natal), Area Four (Limpopo and Mpumalanga), and Area Five (Northern Cape, North West, and Western Cape) prospectively recorded daily logs of patients (N=25,028) for a 2-week period to estimate GW prevalence between September 2011 and June 2012. Patient age, gender and GW diagnosis status (new, resistant, recurrent) was recorded in the daily logs. Prevalence (number of GW patients/total patients seen by the HCP) was obtained for each HCP and calculated into a single estimate across all HCP types. Overall prevalence estimates (and 95% CI) were weighted by the estimated number of HCPs in each practice category and the estimated proportion of total patients attending each practice category. Prevalence among GP/PHCN/ HIV was compared to that of DERM/OBGYN/URO practice category, giving final sample sizes of each practice category. The overall GW prevalence estimate was 10.5% (95% CI: 10.1-10.9%) for patients between age 18-60 years, with 8.7% (95% CI: 8.3-9.1%) GW prevalence in females and 15.4% (95% CI: 14.6-16.2%) in males. Combined GW prevalence estimates in GP/HCN/HIV practices was 10.6% (95% CI: 10.2-11%), and 5.1% (95% CI: 2.5-7.7%) in DERM/OBGYN/URO practices. Of the 546 total male GW cases, 302 (55.3%) were newly diagnosed and 244 (44.6%) were existing cases. Of the 897 total female GW cases, 557 (62.0%) were newly diagnosed and 340 (37.9%) were existing cases.

Conclusion: GW is widely prevalent in GP/PHCN/ HIV/ DERM/OBGYN/URO practices in five regions in South Africa. More than 50% of females GW cases and >60% male cases are newly diagnosed.

OC 10-1

ASSESSMENT OF HPV VACCINATION IN GENERAL POPULATION BY COMPARING VACCINATION AND SCREENING DATA IN ALSACE-FRANCE.

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Background: In France, HPV vaccination has been advised and reimbursed for girls aged 14 from 2007 to 2012. Till 2013, guidelines recommend vaccination from 11 to 14. Catching up vaccination was possible up to 23, nowadays up to 19. There is no vaccination program. Since 1994, the EVE association has conducted a cervical cancer screening program in the Alsatian region.

Objectives: To assess impact of HPV vaccination on HPV induced cervical lesions, on screening and on viral ecology and to improve vaccination practice.

Methods: A cohort of all vaccinated girls after June 2009 and leaving in Alsace, is built through health reimbursements of vaccines. Data are validated by answering patients or practitioners especially when vaccination seems not complete. This cohort will, in the future, be compared to the screening program database and to the cancer registries in order to check that vaccinated women take part in screening and to detect all cervical lesions occurring in vaccinated people. Assessment of vaccination's impact will be based on the comparison of CIN2+ proportion found in vaccinated people versus in non-vaccinated ones.

Conclusions: No data are already available on cervical lesions because people in the cohort are not yet 25 years old.
Assessment of vaccination practice shows that medical practitioners essentially vaccinated in the targeted age group (14-15) of the guidelines. Vaccine coverage was on the other hand low, 16.8% at 14.
Based on reimbursed data, complete vaccinations represent 40.1% of all, but in 96.0% of these cases, patients' answers show that all three injections were done.
This cohort will permit an assessment of vaccination's impact in the real life.
First results show that efforts are needed to achieve a higher coverage by medical training and population information.

OC 10-2

MODELLING STRATEGIES FOR CERVICAL CANCER SCREENING: A SYSTEMATIC REVIEW

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Objectives: Diverse recommendations worldwide have been informed by evidence from model-based epidemiological and economic studies assessing the impact of one or several of the multiple technologies available for the prevention of cervical cancer. This is the first review to appraise the findings of published evaluations of cervical cancer screening strategies, in vaccinated and unvaccinated women, and to characterise the modelling approaches used.

Methods: We searched MEDLINE, EMBASE, EconLit,HEED, and The Cochrane Library for peer-reviewed evaluations of the effectiveness and cost-effectiveness of cervical screening technologies, provided the use of mathematical models of HPV infection and/or cervical disease progression. We also included analyses of alternative cervical screening strategies alongside HPV vaccination.

Conclusions: We found 125 articles published up to May 2013. Most of the published evaluations of technologies and/or strategies for cervical cancer screening consist of cost-effectiveness analyses for high-income countries (HIC). The models used are frequently adapted to extend the analysis to alternative technologies and settings. Most analyses are based on static state-transition models simulating cohorts of women over a lifetime. Relatively few studies assessed visual inspection with acid acetic (VIA) or lugol-iodine (VILI), and none were found assessing self-sampling technologies. The use of VIA has been supported in the context of low- or middle- income countries (LMIC), whereas the introduction of liquid-based cytology (LBC) was overall considered cost-effective in HIC. More recently, the introduction of HPV DNA testing and of hrHPV vaccination have been the main subject of analysis irrespective of the setting income level. Several studies support the switch to primary HPV DNA screening (mainly in HIC and for older women), and many indicate vaccination combined with less frequent screening starting at a later age as the most reasonable option, even for LMICs.

OC 10-3

EVALUATING THE COST-EFFECTIVENESS OF HUMAN PAPILLOMAVIRUS (HPV)-BASED CERVICAL CANCER SCREENING STRATEGIES, INCLUDING GENOTYPING FOR HPV 16/18

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Objective: To compare the cost-effectiveness of two cervical cancer (CxCa) screening algorithms to primary screening with a novel HPV test, which identifies genotypes 16/18 individually while simultaneously detecting the other high-risk HPV types.

Methods: A cohort Markov model was developed to compare three CxCa screening strategies: cytology with reflex HPV test (ASCUS triage), HPV-DNA test with reflex cytology (HPV test), and a novel HPV-DNA test with genotyping and reflex cytology (genotyping). Screening began at age 30 with routine screening every 3 years thereafter, and was modeled over a time horizon of 40 years. Sensitivity and specificity for ≥CIN 3 for each strategy was obtained from the ATHENA (Addressing THE Need for Advanced HPV Diagnostics) trial. Trial baseline data were used for the base case, and 1-year follow-up outcomes were estimated for the alternative scenario, assuming diseases deferred at baseline were persistent and detected in the subsequent visit. The direct costs for screening and treatment of CxCa were estimated from a US payer perspective in 2013 US dollars. Costs and quality-adjusted life years (QALYs) were discounted at 3% annually. Extensive sensitivity analyses were conducted.

Results: Using a $50,000/QALY threshold, base case results showed that routine screening with genotyping dominated the HPV strategy, and was cost-effective compared to ASCUS triage at $12,923/QALY gained, driven by earlier diagnosis and treatment of clinically relevant ≥CIN 3 cases. In the 1-year follow-up scenario, genotyping was also cost-effective at $8,677/QALY gained compared to ASCUS triage, and reduced the overall cost by 22% compared to the HPV test due to more efficient use of healthcare resources. Sensitivity analyses showed that the model results were most influenced by test performance, followed by the costs of test used. Genotyping strategy was cost-effective for the majority of the probabilistic simulations.

Conclusions: Routine HPV screening with 16/18 genotyping was a cost-effective alternative compared to other CxCa screening strategies, and resulted in improved protection against CxCa.

OC 10-4

THE LIFETIME RISK OF SCREEN-DETECTED HR-HPV OR ABNORMAL CYTOLOGY

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Objectives: Cytology-based cervical cancer screening programs substantially reduced cervical cancer morbidity and mortality. HPV DNA testing has a higher sensitivity and negative predictive value for the detection of CIN3+ compared to cytology and provides a cost-effective alternative. Lifetime risk estimates of screen-detected hrHPV or abnormal cytology (≥ ASCUS) provide means to compare the burden of screen-positives and to assess the impact of bivalent HPV16/18 vaccination.

Methods: Data collected in two population-based screening trials were used (POBASCAM (PO) and NTCC studies). Conventional cytology and HPV DNA testing were done at two subsequent screening visits. In POBASCAM HPV testing was performed using GP5+/6+ PCR, followed by reverse line blot (RLB) analyses on positive samples. In NTCC the Hybrid Capture type 2 (HC2) hybridization assay was used for HPV testing, followed by genotyping with GP5+/6+ and RLB on HC2 positive samples. Incidence of HPV or ≥ ASCUS was estimated among baseline screen-negative women who attended the second round conform program after 5 years (PO) or 3 years (NTCC). Lifetime risk was estimated from the cumulative incidence and baseline prevalence, substituting screening rounds for age-groups. The projected impact of adolescent HPV16/18 vaccination was estimated by applying published vaccine-efficacy rates to the fraction of vaccine-preventable endpoints at baseline and follow-up screens.

Conclusion: In the absence of vaccination, lifetime risk of a screen-positive result was approximately 1.3 times higher with HPV-based compared to cytology-based screening in both studies (26.2% vs 20.5% in PO and 41.8% vs 32.3% in NTCC). In both studies, lifetime risks of screen-detected HPV or abnormal cytology in vaccinated cohorts were similar - indicating that the impact of vaccination is larger on hrHPV infections than on abnormal cytology. Since the burden of cervical cancer is expected to decline with HPV-based testing and with vaccination – screening guidelines need to be revised in order to reduce the burden of screen-positives and to maintain program efficiency if HPV-based testing is introduced or once vaccinated cohorts reach screening age.

OC 10-5

RISK STRATIFICATION ANALYSIS FOR INFORMED DECISIONS ON SCREENING STRATEGY

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Objectives: Screening tests are used, alone or in combination, to separate the targeted population into those with a high risk of disease and those with a low risk of disease. Usually a group with moderate risk is also defined, and has to be resolved over time in close follow-up. Each screening setting should set its own standards as to acceptable risk among those allowed to return to routine intervals and the required positive predictive value of referral for diagnostic verification. For informed decisions regarding changes to the screening algorithm, the risks of precancerous lesions and cancer after each combination of test-results were estimated in the national cervical cancer screening programme.

Methods: Central registration of all cervical cytology and HPV test results and high coverage of cervical histopathology and treatment data provide a unique opportunity for longitudinal algorithm evaluation in Norway. Around 4 million primary and secondary (risk-group) screening episodes in the period 2000-2009 were identified from the national cervical cytology and HPV registers. Outcome was defined as highest grade cervical histology recorded in the cancer, cervical histopathology, and clinical CIN treatment registers within a 3.5 year follow-up period, corresponding to one complete screening interval with following screen. Cumulative hazard plots and risk of CIN2+, CIN3+ and cancer after each screening test result in primary or secondary screening rounds were used to define the current risk management profile and performance of the screening tests in use. Estimates were corrected for verification bias by adjusting for actual follow-up.

Conclusions: Women with test-combinations with an adjusted 3.5 year CIN3+ risk of up to 10% were returned to routine screening and women with test-combinations carrying a 3.5 year CIN3+ risk of more than 20% were referred for diagnostic verification. Corresponding risk thresholds for invasive cervical cancer were 0.33% and 0.63% respectively. CIN3+ and invasive cancer risks were not always unequivocally correlated. The longitudinal sensitivity of primary cytology for CIN3+ diagnosed in the next 3.5 years was 71% at the threshold of ASCUS+. Corresponding sensitivity of secondary cytology was 83% and sensitivity of secondary HPV testing by Hybrid Capture 2 was 94%. The results of this study are valuable when considering modifications to the screening algorithm.

OC 10-6

CERVICAL CANCER SCREENING BASED ON PRIMARY HIGH-RISK HPV DNATESTING IN ITALY: FIRST AND SECOND ROUND PERFORMANCE INDICATORS

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Objective : High-risk (hr) HPV DNA testing demonstrated superior performance versus cytology screening in reducing cervical cancer and precancer in randomized population-based trials. Abbott RealTime High Risk HPV (RealTime) is a qualitative multiplex-real-time PCR detecting the 13 hrHPV types targeted by Digene Hybrid Capture 2 (HC2) and HPV 66. RealTime distinguishes HPV16 and HPV18 from non-HPV16/18 hrHPVs. The test demonstrated non-inferiority to HC2 and GP5+/5+ DEIA in several validation studies for primary cervical cancer screening following consensus guidelines (Meijer et al. 2009 Int J Cancer 124:516-520).
The aim of this study was to establish clinical performance indicators of hrHPV DNA testing using HC2 in the 1st and RealTime in the 2nd screening round after 3 years in a local cervical cancer screening program.

Methods: Women at 25-64 years of age were invited to attend the local cervical cancer screening program comprising cytology and hrHPV testing. Cervical scrapes for hrHPV testing were sampled with collection devices provided by the suppliers of the respective HPV tests used. Cytology was interpreted for hrHPV positive (+) women only. Women with abnormal cytology (ASC-US+) underwent immediate colposcopy, while hrHPV+ women with normal cytology were referred after 1 year.

Results: hrHPV prevalence in the 1st round (invited/accepted: 24.000/7639 [32.5%]) was 5.6%. 141(34%) of the hrHPV+ women were found with ASCUS+; the referral rate was 1.8%, revealing 20 CIN2+ (PPV 15%). Compliance for 1 year follow-up was 58% (166/286): 90 (54%) women were hrHPV+ and 5 CIN2+ were found. After 3 years, hrHPV prevalence in the cohort (invited/accepted: 23.700/7323 [30.7%]) was 4.58%. ASC-US+ cytology was found in 70 (20%) of the hrHPV+ cases and 1.6% underwent colposcopy with 22 CIN2+ identified (PPV 15%). 1 year follow-up is ongoing.

Conclusion: This study provides the first report on 2nd round performance indicators for hrHPV DNA screening in Italy. The hrHPV DNA-based cervical cancer screening program implemented in Lazio, Italy, demonstrated that both hrHPV tests used led to similar referral rates in both subsequent screening rounds. Further analyses from the 2nd screening round may support future refinement of risk stratification for hrHPV+ women with negative cytology and persistent hrHPV infection based on partial genotyping provided with the Abbott RealTime High Risk HPV test.

OC 10-7

HPV4 VACCINE ADHERENCE IN A SAFETY NET POPULATION WORSENS AS BMI INCREASES

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Objectives: Obese women are less likely to participate in cervical cancer screening than women of normal body mass index (BMI). The aim of this study was to determine whether adherence to HPV4 vaccination in a safety net population was influenced by BMI.

Methods: A historical prospective study used data from a safety net health system from 2006 through 2009 abstracting demographics, and dates of HPV4 vaccination. On-time completion of the triplet series was defined. WHO categories of BMI defined weight classifications.

Results: 1240 females, 10-26 years old, received at least one dose of HPV4; 38% were obese (class I, II and III) and 25% were overweight. Females with normal and overweight BMI received on-time triplet dosing significantly more often than did the obese class II and III females (25% vs. 16%, $p=0.012$). However, across all BMI categories, there were significantly lower proportions of females completing on-time triplet dosing compared to a singleton HPV4 dose, this being most pronounced for the obese class II and III categories, (15% vs. 55%, $p<0.001$ and 16% vs. 55%, $p=0.002$, respectively). Obese females of all classes have a significant 36% less chance of completing the on-time triplet HPV4 series than normal women (OR=0.64, 95% CI: 0.47, 0.88). Hispanic women and those with at least one live birth are also less likely to complete the series (OR=0.26 (0.11, 0.63) and 0.60 (0.52, 0.71) respectively).

Conclusions: Obesity is a risk factor for lack of HPV4 vaccine adherence among women in a safety net population.

OC 10-8

ORGANIZATION AND QUALITY CONTROL IN CERVICAL CANCER PREVENTION: EVIDENCE FROM A EUROPE-WIDE SURVEY

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Objectives: Screening programs in Europe have reduced cervical cancer incidence and mortality but the level of success is variable between countries. Organization of programs is essential for equity and cost-effectiveness; however, there are differences in effectiveness also among organised programs. Similarly, HPV vaccination programs have been recently launched in European countries, but their organisation and levels of success vary. In order to identify the key organizational components that determine effectiveness, we performed a Europe-wide survey on organization and quality control measures as well as associated costs.

Methods: A comprehensive questionnaire was developed through an extensive review of the literature, in particular the 2nd edition of the European guidelines for quality assurance in cervical screening. The survey was sent to program organisers, Ministries of Health, and key experts in 34 EU and EFTA countries. Detailed aspects of program organization, quality control, monitoring, evaluation, and corresponding line-item costs were recorded. Twenty-six out of 34 countries responded.

Conclusions: To our knowledge, this is the first questionnaire of its kind to request detailed information on the actual organization of screening and vaccination programs. The results showed that organized efforts for quality assurance, monitoring and evaluation were carried out to a differing extent and were not standardized, which makes it difficult to compare the cost-effectiveness of different screening and vaccination programs. Most countries found it hard to estimate the costs associated with launching and operating an organized program. The results of this survey can be used as a basis for further development of standardized guidelines on organization and quality control of cervical cancer screening and HPV vaccination programs in Europe.

OC 11-1

EFFECTIVENESS OF MALE CONDOM IN PREVENTION OF CERVICAL NEOPLASIA: SYSTEMATIC REVIEW OF LONGITUDINAL STUDIES

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Objectives. Cross-sectional studies evaluating the relationship between the use of male condom and human papillomavirus (HPV) infections and cervical neoplasia showed inconsistent results, suggesting that little is to be gained in prevention of cervical cancer from using condoms. However, the designs of these studies were inadequate to address the question. Longitudinal studies are more appropriate for the purpose, and we reviewed the existing longitudinal studies in a systematic review.

Methods. We searched PubMed using MeSH terms for articles published until May 2013, and perused the reference lists of the retrieved reviews. No prior systematic review of longitudinal studies could be identified. The included studies compared the incidence, clearance or persistence of HPV infections, or incidence, regression or persistence of untreated cervical neoplasia between women whose male sexual partners used condoms during sexual intercourse and women whose partners did not. Study selection was undertaken by two researchers independently, and data were abstracted into prespecified tables. Because of the variation in the study designs, we did not compute summary measures.

Conclusions. Of the 18 longitudinal studies included in this systematic review, three were designed to evaluate the role of condom use in preventing HPV infections or in regression of cervical neoplasia, and three, additionally, focused specifically on condom use while also evaluating other risk factors for HPV infections. Several of these six studies used elaborate questionnaires to determine the frequency of condom use among their participants, and information on the use was updated in short intervals. All but one of the six studies showed a protective effect of condoms, the exception being a study with poor overall compliance with condom use. Of the remaining 12 longitudinal studies which were not designed to focus specifically on condom use, only one showed a protective effect. In these studies, the measurement of condom use tended to be less accurate than in the studies specifically designed to evaluate the effectiveness of condoms. In summary, the few longitudinal studies designed to evaluate the relationship unequivocally showed that the use of male condom is protective for HPV infections and cervical neoplasia.

OC 11-2

FACTORS ASSOCIATED WITH THE DEVELOPMENT OF MALE EXTERNAL GENITAL LESIONS: THE HIM STUDY

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Objectives: To identify potential factors associated with the risk of developing external genital lesions (EGLs) in men.

Methods: A prospective analysis was conducted within the *HPV Infection in Men (HIM) Study*, an HPV natural history study conducted in the United States, Brazil, and Mexico. Men (n=2,754) were included in this study if they had ≥2 study visits following the introduction of a pathology protocol. Every 6 months (up to 4 years), men completed a risk factor questionnaire and underwent a clinical exam. Visual inspection of the external genital skin was conducted, and exfoliated cells from the normal genital skin and the surface of any EGLs were collected using swabs. All samples underwent HPV genotyping by Linear Array. Visually distinct EGLs were biopsied and subjected to pathological evaluation (e.g., condyloma and penile intraepithelial neoplasia [PeIN]). The cumulative risk of developing an EGL in the first 12 months of follow-up was estimated using the Kaplan-Meier method, and the log-rank test was used to compare risk of EGL development across risk factor groups.

Conclusions: Throughout the follow-up period, 198 men developed a pathologically confirmed, incident EGL. The following factors were significantly associated with an increased risk of EGL development: younger age (p=0.02), inconsistent condom use (p=0.01), *Chlamydia* infection (p=0.01), prior infection with genital HPV 6/11/16/18 (p<0.001–0.01), and baseline HPV 6 or 18 seropositivity (p<0.001, p=0.01). For condylomas and EGLs suggestive of condyloma, the following factors were specifically associated with EGL development: younger age (p=0.03), inconsistent condom use (p=0.01), prior genital HPV 6/11/16/18 infection (p<0.001–0.01), and HPV 6 seropositivity (p=0.02). For PeIN, *Chlamydia* infection (p=0.03) and prior genital HPV 11 or 16 infections (p=0.01, p<0.001) were significantly associated with EGL development. Future analyses will utilize multivariable models to examine independent associations between these factors and the risk of developing EGLs in men.

OC 11-3

HIGH-RISK ANAL HUMAN PAPILLOMAVIRUS (HPV) TYPES IN AUSTRALIAN HOMOSEXUAL MEN - PATTERNS OF INFECTION

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Objectives: HPV causes around 90% of anal cancer, and HPV16 causes 90% of HPV-positive cases. Morbidity and mortality from HPV-related diseases are markedly higher among homosexual men. We describe the prevalence of anal HPV, the potential predictors of anal high-risk (HR) HPV and HPV16, and incidence and clearance of HPV16, in a cohort of Australian homosexual men.

Methods: The Study for the Prevention of Anal Cancer is a three-year prospective study of HIV-negative and positive homosexual men aged ? 35 years. At five study visits, participants complete behavioural questionnaires and undergo anal canal examination. Anal swabs were analysed using liquid-based anal cytology (ThinPrep®). Separate aliquots of samples collected in Thinprep® medium were used for HPV genotyping by Roche Linear Array.

Conclusion: By March 2013, 342 participants (median age 49 years; 28.7% HIV positive) had attended a baseline visit. The vast majority of men (85.8%) had one or more HPV genotypes detected. Almost two thirds had at least one HR genotype (64.4%) and almost a third had HPV16 (30.3%) detected. HR-HPV detection was significantly associated with positive HIV status ($p=0.010$), younger age ($p=0.007$), more lifetime ($p=0.013$) and recent ($p=0.005$) male sexual partners, more receptive anal behaviours in the last 6 months, including unprotected ($p=0.001$) and protected ($p=0.019$) intercourse, rimming ($p<0.001$) and fingering ($p=0.004$. The overall incidence of anal HPV16 infection was 5.6 per 100PY, and was higher among HIV positive participants, (HIV positive 10.00 per 100PY vs HIV negative 3.66 per 100PY, HR 2.74, 95% CI 0.73-10.27). Clearance of HPV16 (30.67 per 100 PY) was similar among HIV positive and negative participants (27.69 vs 32.54 per 100 PY, HR 0.91, 95% CI 0.32-2.57). Thus, HR anal HPV was extremely common in this cohort of homosexual men. Receptive anal sexual practices and recent sexual activity were important predictors of HR-HPV detection.

OC 11-4

HIGH INCIDENCE AND CLEARANCE OF ANAL HIGH-GRADE SQUAMOUS INTRAEPITHELIAL LESIONS (HSIL) IN HOMOSEXUAL MEN

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Objectives: Homosexual men are at increased risk of anal cancer. Screening and treatment of the precursor, HSIL, has been advocated by some, but screening is not recommended in widely-accepted guidelines. We aimed to describe the prevalence, incidence, and clearance rates of anal HSIL, and association with human papillomavirus (HPV) status, in a community-recruited cohort of homosexual men.

Methods: The SPANC study is a three-year prospective study of the natural history of anal HPV infection and cancer precursors in HIV-negative and -positive homosexual men aged ? 35 years. At each visit all men undergo an anal swab for cytology and HPV genotyping (Roche Linear Array), followed by high resolution anoscopy-aided biopsy. Anal HSIL was defined as having either anal intraepithelial neoplasia grade 2/3 on histology and/or HSIL/ASC-H on cytology.

Conclusions: A total of 342 men were recruited by March 2013. Median age was 49 years (range: 35-79) and 28.7% were HIV-positive. At baseline, the prevalence of anal HSIL was 50.0% and 43.9% in the HIV-positive and HIV-negative respectively ($p=0.303$). Among those free of HSIL at baseline, HSIL incidence was 27.8 and 27.6 cases per 100 person-years (PY) among HIV-positive and HIV-negative men, respectively $p=0.920$). Among those had HSIL at baseline, the clearance rate was 41.0 and 42.7 cases per 100 PY in HIV-positive and HIV-negative men, respectively ($p=0.851$). Men who tested HPV16 positive (vs negative) on their anal swabs at baseline were significantly more likely to develop incident HSIL (57.1 vs 23.0 per 100 person-years, $p=0.010$), and less likely to clear prevalent HSIL (17.6 vs. 61.3 per 100 person-years, $p=0.001$). Anal HSIL was highly prevalent in homosexual men. Both incidence and clearance of HSIL were common, and were closely associated with HPV16 status. The high rates of clearance are consistent with the observation that anal HSIL progresses to cancer less commonly than high-grade cervical lesions.

OC 11-5

ANAL AND PENILE LOW-RISK HPV INFECTION IN HIV-NEGATIVE AND HIV-INFECTED MSM

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Objectives: The vast majority of anogenital warts is caused by low-risk HPV (lrHPV). Although generally benign, they represent a considerable disease burden. Our goal was to assess (1) the prevalence of anal and penile lrHPV in HIV-negative and HIV-infected men who have sex with men (MSM), and (2) the association between HIV infection and anal and penile lrHPV.

Methods: MSM aged ≥18 years were recruited from three sites in Amsterdam, the Netherlands. Participants completed a risk factor questionnaire. HPV DNA was analysed in baseline anal and penile shaft self-swabs and genotyped using the SPF_{10}-PCR & DEIA-LiPA25 system (DDL); HPV types 6, 11, 34, 40, 42, 43, 44, 53, 54, 66, 68/73, 70 and 74 were classified as low-risk. Multivariable logistic regression analyses were performed to assess the association between HIV infection and lrHPV.

Conclusions: MSM (n=778) were included in 2010-2011, of whom 317 (41%) were HIV-infected (median CD4+ count of 530 cells/mm^3). The median age was 38 years in HIV-negative and 46 years in HIV-infected MSM (P<0.001). Prevalence of anal lrHPV was 45% (95%CI 41-50%) in HIV-negative and 69% (64-74%) in HIV-infected MSM (P<0.001). HPV-6 was most frequently detected (19% overall; 95%CI 16-22%), and associated with self-reported history of anal warts (OR 2.1; 1.5-3.1). In multivariable analysis (adjusting for age, smoking, number of recent and lifetime sexual partners), HIV infection was significantly associated with anal lrHPV (aOR 2.2; 1.6-3.2). Prevalence of penile lrHPV was 20% (16-23%) in HIV-negative and 37% (31-42%) in HIV-infected MSM (P<0.001). Again, HPV-6 was most frequently detected (6%; 95%CI 4-8%), and was associated with self-reported history of penile warts (OR 2.7; 1.4-5.1). In multivariable analysis (adjusting for age, smoking, circumcision status, number of recent and lifetime sexual partners), HIV infection was significantly associated with penile lrHPV (aOR 2.2; 1.5-3.2). In conclusion, anal and penile lrHPV infections are very common in MSM. HIV infection is a strong and independent determinant for anogenital lrHPV.

OC 11-6

PREVALENCE & CORRELATES OF UNSATISFACTORY ANAL PAP TESTS (UAPT) IN A COMMUNITY-RECRUITED COHORT OF AUSTRALIAN HOMOSEXUAL MEN

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Objectives: Anal Pap tests have been advocated as a screening tool for anal precancerous lesions. We aimed to ascertain prevalence and correlates of UAPT among homosexual men.

Methods: The Study for the Prevention of Anal Cancer is a 3-year prospective study of homosexual men aged ? 35 years in Sydney. At baseline, data were collected and study procedures, including an anal swab for ThinPrep® cytology, were performed.

Conclusions: UAPT initially occurred in 40 (11.7%) of 342 participants (median age 49 years; 28.7% HIV positive) enrolled by end March 2013. There was no difference in UAPT by collecting clinician (p=0.669) or reporting pathologist (p=0.267). UAPT occurred more than twice as often among HIV negative compared with positive men (13.9% vs 6.1%, p=0.048) and were less likely than satisfactory smears to contain transformation zone (TZ) cells (4.6% vs 35.4%, p<0.001). UAPT were more common among men who had never anally douched (p<0.001). Among men who douched, they were associated with soapy water douching (p=0.004), but not with more frequent or more recent douching. Having an UAPT was associated with more anal Pap swab discomfort (p=0.037) and feeling more tense during the exam (p=0.008), but not with haemorrhoids or past anal surgery. STI correlates of UAPT included past anal gonorrhoea (p=0.023) and fewer anal HPV types being detected (p=0.003). Sexual behavioural correlates were: fewer lifetime protected (p-trend=0.013), but not unprotected (p-trend=0.144) receptive anal sex partners and preference for the insertive role in anal intercourse (p-trend=0.055). On multivariate analysis, never douching (p<0.001), soapy water douching (among those who douched) (p=0.003), fewer anal HPV types (p=0.005) and feeling more tense during the exam (p=0.039) remained independently associated with UAPT. All UAPT were repeated within a month and results were: 21 (52.5%) again unsatisfactory, 13 (32.5%) negative, 2 (5.0%) ASCUS/LSIL and 4 (10%) ASC-H/HSIL. Our findings suggest UAPT are more common among men with less receptive anal sexual experience and that the cause of UAPT may be a combination of sampling, behavioural and biological factors.

OC 11-7

SEROCONVERSION AFTER HPV INFECTION AND EFFECT OF ANTIBODIES ON SUBSEQUENT HPV INFECTION IN HIV-NEGATIVE AND HIV-INFECTED MSM

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Objectives: Our aims were to assess 1) type-specific seroconversion following anal or penile HPV infection in HIV-negative and HIV-infected men who have sex with men (MSM), and 2) whether natural antibodies protect against incident type-specific HPV infections.

Methods: MSM were recruited at 3 study sites in Amsterdam (the Netherlands) and followed-up semi-annually. Participants completed a risk-factor questionnaire at baseline. Antibodies against 7 high-risk HPV types in baseline and 12-month serum samples were tested using a fluorescent bead-based multiplex assay; baseline, 6-, and 12-month anal and penile samples were tested for HPV DNA and genotyped using the SPF_{10}-PCR & DEIA-$LiPA_{25}$ system (DDL). Statistical analyses were performed using multivariable logistic and Cox regression.

Conclusions: In 2010-11, 756 MSM (median age 40 years; IQR 35-48) were included; 308 (41%) were HIV-infected. In analyses stratified by HIV status, persistent (aOR 3.7; 95%CI 1.4-9.4) and incident (aOR 1.9; 95%CI 1.1-3.3) anal HPV infections were independently associated with type-specific HPV seroconversion in HIV-negative, but not HIV-infected MSM. Penile HPV infection at any time point was not significantly associated with seroconversion. HPV seropositivity at baseline showed no significant association with incident type-specific anal (aHR 1.2; 95%CI 0.9-1.6) or penile (aHR 0.8; 95%CI 0.6-1.2) HPV infection at 6 or 12 months. Furthermore, in MSM with highest antibody concentrations HPV incidence was not decreased compared to seronegative MSM. In conclusion, a persistent or incident anal (mucosal), but not penile (non-mucosal), high-risk HPV infection was an independent determinant for seroconversion in HIV-negative MSM. Natural antibodies did not protect against subsequent anal or penile HPV infection.

OC 12-1

DETECTION OF HIGH-GRADE CIN BY FOUR HPV ASSAYS IN WOMEN WITH ABNORMAL CYTOLOGY

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Objectives. Few studies compared the sensitivity and specificity for high-grade CIN in women with abnormal cytology using several HPV assays simultaneously. The Danish Horizon study allowed for a comparison between Hybrid Capture 2 (HC2), APTIMA, CLART and cobas HPV assays.

Methods. The SurePath samples from 5,034 consecutive women attending routine cervical screening including follow-up of previous abnormalities in Copenhagen between June and August 2011 were tested with the four assays. All testing protocols were agreed upon with the manufacturers prior to the study, and all instrumentation and software were used as supplied by the manufacturers. Women with cytological abnormalities were routinely recommended for repeated testing or were referred for colposcopy. Their worst histological diagnosis by May 2013 was retrieved from the Danish national Pathology DataBank, using the unique personal identification numbers for linkage.

Conclusions. Of all 367 women with cytological abnormalities, 20 (5%) had no follow-up, 232 (63%) had no high-grade CIN, 33 (9%) had CIN2, 79 (22%) CIN3, and 3 (1%) cervical cancer. HC2 detected 79 (96%) of 82 ≥CIN3 and 111 (97%) of 115 ≥CIN2. Cobas detected 78 ≥CIN3 (sensitivity: 95%) and 110 ≥CIN2 (sensitivity: 96%). When the definition of a positive CLART test was limited to 13 oncogenic genotypes out of the 35 detectable, it detected 76 ≥CIN3 (sensitivity: 93%) and 108 ≥CIN2 (sensitivity: 94%). APTIMA, the only mRNA assay included in the study, detected 71 ≥CIN3 (sensitivity: 87%) and 102 ≥CIN2 (sensitivity: 89%). Among the three cervical cancers, one tested negative on CLART and one on cobas; HC2 and APTIMA were positive in all three. The specificity of the assays for <CIN3 was 22% for HC2, 28% for cobas, 32% for CLART, and 35% for APTIMA. The specificities for <CIN2 were 25%, 31%, 36%, and 39%, respectively. In this referral population where all high-grade CIN were detected by cytology, APTIMA expectedly detected fewer lesions than the three DNA assays, but had slightly higher specificity. The DNA assays detected a similar number of ≥CIN3 and ≥CIN2. HC2 tended to have the lowest specificity, but only significantly so when compared to CLART.

OC 12-3

PERFORMANCE OF HYBRID CAPTURE USING RESIDUAL SUREPATH PELLETS

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Objectives: Although HPV testing using Hybrid Capture 2 (hc2) is widely performed using residual SurePath (SP) pellets, relatively few studies have been published that document the performance of hc2 using SP with a clinical endpoint of biopsy-confirmed CIN2+. This study evaluated the clinical performance characteristics of hc2 using SP pellets for the detection of CIN2+ and directly compared hc2 performed using SP pellets and specimen transport media (STM) in the same women.

Methods: hc2 testing was performed using both the residual SP pellets and a separate STM sample obtained from the cervix of 966 women with ASCUS and 4,728 ≥30 yrs with NILM. Colposcopy with adjudicated review of biopsies was performed for HPV+ (hc2 using STM), ASCUS, and a random subset of NILM/HPV-. Overall, 6.0% of NILM subjects and 47.9% of ASCUS subjects were HPV+ using hc2 and the residual SP pellets. 48 cases of CIN2+ were identified in women with ASCUS and in this group of women the sensitivity, specificity, PPV, and NPV of hc2 using SP pellets were 95.8%, 55.7%, 10.8%, and 99.6%, respectively. In women with ASCUS and NILM, there was a 91.8% and 96.1% overall agreement between hc2 results obtained using SP pellet and STM, respectively.

Conclusions: When performed using residual SP pellets, hc2 testing demonstrates a high sensitivity (95.8%) and NPV (99.6%) for the detection of CIN2+ in women with ASCUS. Moreover, there is good overall agreement between hc2 results when performed using residual SP pellets and STM samples in both women with ASCUS and women ≥30 years with NILM cervical cytology.

OC 12-4

A COMPARISON OF THE PERFORMANCE OF DIFFERENT HPV TESTS IN THINPREP VS SUREPATH

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Objectives. Most studies of HPV testing have been conducted in either ThinPrep mediou or STM. Increasing SurePath is now being used for liquid based cytology and there is a need to evaluate the performance of HPV testing assays in this medium.

Methods. In this study we compared the quantitative performance of Hybrid Capture 2, APTIMA, and the Abbott HPV test in 630 patients referred to a colposcopy clinic because of an abnormal smear. The cytologic abnormalities on which referral was based were: 54 moderate/severe dyskaryosis, 380 mild dyskaryosis, 130 repeat borderline changes, 63 single borderline. Two samples were taken from each woman and stored in -20ml of ThinPrep and 10 ml SurePath. The order in which the collection media were used was randomised (319 SurePath first and 311 ThinPrep first). HC2 results were only available for the second part of the study.

Results. In general HPV positivity and level was lower in SurePath (Table). There were always more SurePath negative discordant pairs (ie T pos/Sneg / vs T-neg /S-pos/) and the slope of the log (1+RLU) or minus Ct values was always less than one. Scatterplots or the paired samples will be shown.

Test	N tested	Positive ThinPrep (%)	Discordant pairs Thin +/Sure- vs -/+,OR (95% CI)	Slope (95% CI) Sure regressed on Thin
HC2	342	83.9	28 vs 8, 3.50 (1.55, 8.89)	0.83 (0.78, 0.89)
Abbott All	630	75.6	35 vs 5, 7.00 (2.73,22.89)	0.77 (0.74, 0.81)
Abbott HPV 16	630	24.8	8 vs 3, 2.67 (0.64, 15.60)	0.79 (0.76, 0.81)
Abbott HPV 18	630	8.6	3 vs 1, 3.00 (0.24, 157.49)	0.77 (0.75, 0.79)
Abbott Other HR	630	54.4	39 vs 10, 3.90 (1.91, 8.76)	0.75 (0.72, 0.78)
APTIMA	613	77.0	49 vs 18, 2.72 (1.56, 4.97)	0.64 (0.59, 0.69)

For the HC2 and APTIMA tests the lower measurements were seen with Surepath primarily when it was the second sample. However this was a between patient comparison so is less reliable. Differences between first and second samples will be reported in detail. The data suggest that a lower positivity cutoff is appropriate when samples are collected in SurePath.
Pathology review is ongoing and correlations with disease status will not be reported at this stage.

OC 12-5

CLINICAL VALIDATION (VALGENT-2) OF THE LMNX GENOTYPING KIT HPV GP TARGETING 14 HRHPVS AND AN INTERNAL CONTROL

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Objectives: Longitudinal randomized controlled trials have demonstrated that cervical cancer screening by high-risk (hr)HPV testing is more effective than by cytology. A difference in carcinogenic potential exists between hrHPV genotypes. hrHPV tests with full genotyping capacity, e.g. the GP5+/6+ PCR-based LMNX Genotyping Kit HPV GP (Diassay), might be useful in clinical practice. We aimed to validate this assay by comparison with the GP5+/6+ EIA (Diassay) as a reference hrHPV test, in accordance with defined international guidelines (1).
Methods: A study panel (VALGENT-2) was composed of continuous cervical swab specimens from a Scottish screening population (n=998), enriched with samples with abnormal cytology, i.e., 100 ASCUS, 100 LSIL and 100 HSIL. DNA was extracted and amplified by GP5+/6+ primers. One aliquot of amplimers was tested for presence of 14 hrHPVs by the EIA. A second aliquot was analyzed by the LMNX Genotyping Kit HPV GP, which contains probes for the same 14 hrHPVs and for a human DNA target as an internal control (2). Clinical sensitivities were determined in the group of women who had histologically diagnosed clinical outcome of CIN2+ (n=91) and CIN3+ (n=46). Clinical specificities were calculated among those women who had two subsequent cytologically negative swabs (n=747).
Conclusions: The LMNX Genotyping Kit HPV GP and EIA were in high agreement (Cohen's kappa= 0.958) for detection of 14 hrHPVs and no significant difference was observed (McNemar's P=0.400). The LMNX Genotyping Kit HPV GP demonstrated a clinical sensitivity of 96.7% for CIN2+ and of 97.8% for CIN3+, and a clinical specificity of 90.4%. This clinical performance was non-inferior to that of the EIA, which demonstrated a clinical sensitivity of 94.5% for CIN2+ and of 97.8% for CIN3+, and a clinical specificity of 90.2%. In a recent meta-analysis (3), the EIA had demonstrated equal sensitivity for CIN2+ (94.5%), but somewhat higher specificity (94.8%). When our analyses were restricted to the women aged 30 years and older (67.8%), clinical specificity increased to a comparable 92.3%. In summary, the LMNX Genotyping Kit HPV GP is clinically validated for primary screening.

1) Meijer et al, 2009, IJC; Guidelines for human papillomavirus DNA test requirements for primary cervical cancer screening in women 30 years and older
2) Caballero et al, 1995, Nucleic Acids Research; Low stringency-PCR (LS-PCR) allows entirely internally standardized DNA quantitation
3) Arbyn et al, 2012,Vaccine; Evidence regarding human papillomavirus testing in secondary prevention of cervical cancer

OC 12-6

TESTING FOR CERVICAL DYSPLASIA BY VAGINAL LAVAGE AND E6 ONCOPROTEIN ASSAY – A PILOT TRIAL

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Introduction: In developed countries, introduction of cytological screening has decreased prevalence of cervical cancer by up to 80% [1]. HPV infection is closely associated with cervical cancer, with HPV types 16 or 18 DNA detectable in 70% or more of all cases [2]. The usage of Delphi Screener vaginal lavages in combination with HC2, DiAssay and PCR-based Multiplex Genotyping (MPG) has been validated as a reliable diagnostic method [3,4]. It could be used for self-sampling in developing countries where acceptable and specific screening methods are needed. We combined vaginal lavage sampling by Delphi Screener with a novel E6 oncoprotein-based assay (OncoE6™ Cervical Test) as a rapid and easy to use diagnostic method.

Material and Methods: In this pilot study we used Delphi Screener to obtain vaginal lavages of patients with invasive cervical cancer in combination with the Arbor Vita test detecting E6 oncoprotein of HPV types 16/18. For comparison, physician collected samples were collected from the cervix by polyester tipped swabs and cytobrushes applied to PreservCyt storage media. Cytobrush collected samples were exemplary genotyped by GP5+/6+ PCR followed by MPG readout.

Results and Conclusions: Complete sample sets were obtained from 15 patients (HPV16 or 18+) and analyzed. Delphi screener lavage, polyester tipped swab and cytobrush samples revealed concordant outcome by the Arbor Vita E6 oncoprotein test. The detected HPV type corresponded with the GP5+/6+ PCR confirmed HPV types. Delphi screener sampling was easy to perform, well accepted, and resulted in sufficient material. The Arbor Vita E6 oncoprotein test was quick (3 hours) and simple to perform by using technically simple methods and devices.
Delphi Screener-collected vaginal lavages are adequate for HPV genotyping and E6 oncoprotein assay. Combining this self-sampling with a HPV 16/18 E6 oncoprotein rapid test to detect women with highest risk for severe dysplasia may allow rapid secondary cancer prevention "on-the-doorstep".

[1] Peto et al., Lancet, 365:249 (2004) [2] Clifford et al., BJC, 89:101 (2003)
[3] Deleré et al., JCM, 49:3519 (2011) [4] Gök et al., BMJ, 11:340 (2010)

OC 12-7

EVALUATION OF THE REALTIME HIGH RISK HPV ASSAY ON SELF-COLLECTED CERVICOVAGINAL LAVAGE SAMPLES

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OBJECTIVE: Self-collection of cervical samples for high-risk human papillomavirus (hrHPV) testing may support improving the participation rate in cervical cancer screening programs among non-responders. The aim of our study was to investigate the feasibility of testing self-collected cervicovaginal lavage samples for the presence of hrHPV with the Abbott RealTime High-Risk HPV Test (RealTi*me*) by comparison to Qiagen/Digene Hybrid Capture 2 (HC2) in matched self-collected versus clinician-collected cervical specimens.

METHODS: Women referred to colposcopy (N = 100) were given a self-sampling device (Delphi Screener) prior to colposcopy to collect cervicovaginal lavage samples; cells were transferred into Cervatec medium (mtm) and lysates were prepared and stored according to the manufacturer´s instructions. Cervical scrape samples were collected in PreservCyt medium by the colposcopist prior to colposcopy on the same day. Disease status was determined by colposcopy and biopsies were taken when lesions were visible from 49 women (14 without disease, 14 CIN1, 11 CIN2, 8 CIN3 and 2 invasive cancers). All specimens were tested with RealTime and HC2.

RESULTS: The overall agreement between RealTime and HC2 in the self-collected and clinician-collected samples was 85% (κ= 0.665) and 83% (κ= 0.620), respectively. The agreement between self-sampled and matching clinician-collected smears was higher with RealTime (93%; κ= 0.849) than with HC2 (89%;κ= 0.741). In the self-collected samples, sensitivity and specificity for CIN2 or worse of RealTime / HC2 were 81.0% (95%-CI 58.1 – 94.6%) / 66.7% (95%-CI 43.0 – 85.4%) [p = 0.25] and 53.6% (95%-CI 33.9 – 74.5%)/ 57.1% (95%-CI 37.2 – 75.5%), respectively. The sensitivity for CIN3 or worse of RealTime and HC2 were 80.0% (95%-CI 44.4 – 97.5 %) and 70.0% (95%-CI 34.8 – 93.3%) [p = 1.0]. The specificity for CIN3 or worse observed with RealTime was 43.6% (95%-CI 27.8 – 60.4%) and 51.3% (95%-CI 34.8 – 67.6%) with HC2.

CONCLUSIONS : High-risk HPV detection in self-collected cervicovaginal lavage samples with the Abbott RealTi*m*e High Risk HPV assay appears to be technically feasible and results from this study indicate least equal quality and performance of the test in comparison to HC2.

OC 12-8

EFFICIENCY AND COST EFFECTIVENESS OF AN URINE TEST FOR HUMAN PAPILLOMAVIRUS (HPV) DETECTION IN A NEW STRATEGY FOR CERVICAL CANCER SCREENING (THE PAPU29 STUDY)

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Objectives: In France, only 55% of women have had a Pap smear for cervical cancer screening due to the lack of organized screening; this rate decreases to 39% in west Brittany. The aim of our study is to evaluate the efficiency and cost of a new strategy based on a rapid HPV-DNA assay in urine.

Methods: The HPV test was performed using 1 mL of urine with the NucliSENS EasyMAG extractor (bioMérieux) and real-time PCR Lightcycler (Roche) systems allowing automated testing of 24 samples in 3 hours (Payan JCM2007). Internal control (DICO, Argene) and SiHa HPV16+ cells were used as controls. Urine samples were obtained between December 2008 and September 2010, from 25-65 years old women who were non-responder to a previous invitation to Pap smear, and living in west Brittany. Among 15,489 invited women, 3897 (25%) were excluded, 701 (4.5%) accepted the Pap smear test, showing abnormal cytology in 20 cases (2%) with 2 precancer CIN2+ lesions; 12,764 denied Pap test (82%) and among them 3115 (25%) sent by mail their urine sample to our lab; 772 (26%) were found HPV-positive and were invited to have a Pap smear; 52 among them had abnormal cytology (7%) with 14 precancer CIN2+ lesions and 1 cervical cancer (1/5000 expected). Avoided cancer accounted to 0.39 and 3.45 in the Pap smear group and the urine HPV group respectively, and costs per screened woman were 93€ and 41€ respectively. A three year follow-up is ongoing, yet showing no abnormal cytology in urine HPV-negative women (n=362). All women with precancer were treated and no relapse was observed.

Conclusion: With about a 6-fold increase of screened women and higher detection of precancer CIN2+ lesions (x7), with a lower cost compared to Pap smear test alone (x2.3), our urine HPV test is well adapted for cervical cancer screening in women from the general population with no Pap smear test over the last 3 years.

Grants from the French Ligue contre le Cancer.

OC 12-9

EVALUATION OF THE CLART® HPV2 MICRO ARRAY ASSAY ON SEMINAL PLASMA SAMPLES

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Objectives. Human papillomavirus (HPV) infection has been demonstrated in the sperm of a large percentage of sexually active males. The detection and genotyping of HPV in semen are of high interest, especially in the field of medically assisted reproduction. Until now, there is no commercial assay validated in sperm. One critical point to check is the potential presence of PCR inhibitors in semen. The aim of this study was to evaluate the sensitivity of the Clart® HPV2 (Genomica) test for the detection of HPV in seminal plasma samples.

Methods. Seminal samples were obtained from men enrolled in a Medically Assisted Procreation program at the Reproductive Unit of the University Hospital of Saint-Etienne. A low positive control was elaborated from a 16/31/71 HPV positive CVF sample and used to infect artificially 10 repetititions of the same seminal plasma either undiluted, or diluted 1:1, 1:3, 1:7 in PBS. The reproducibility of the test was then verified on a panel of 10 semen sampled from 10 different men. To determine the sensitivity threshold, we used serial dilutions of a 1,000 copies/ml quantified HPV16 plasmid (French HPV Reference Center, Pasteur Institute, Paris) to artificially contaminate 10 additional seminal plasmas. Considering the detection of the 3 genotypes and the intensity of the HPV 16 signal in 10 out of 10 undiluted replicates of the same sample, as well as in the 10 different seminal plasmas, our results suggest that seminal plasma samples can be tested without previous dilution for the detection of HPV DNA. In addition, the concentration of 50 copies/ml was detected in all the 10 samples and 10 copies/ml was detected in 6 out of 10. The presence of HPV DNA was then prospectively tested in the seminal of 30 consenting men, in order to evaluate the prevalence of HPV DNA in the male population enrolled in a medically assisted procreation program. A total of 3 samples (10%) were found positive for HPV DNA. The identification of genotypes was as follow: 59/66 (co-infection), 16 and 33 (mono-infections) respectively.

Conclusions. Our data indicate that the Clart® HPV2 micro array assay can be successfully used for the detection and genotyping of HPV in seminal plasma. This could be helpful for routine analysis and in epidemiological survey or physiopathological studies.

OC 12-10

ANALYTICAL COMPARISON OF THE REALTIME HIGH RISK HPV WITH HYBRID CAPTURE II AND GP 5+/6+ HR-HPV PCR ON SUREPATH SPECIMENS

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Objectives Screening for high-risk (hr) Human Papillomavirus (HPV) provides higher sensitivity than cytology for the detection of (pre)malignant cervical lesions. We evaluated the analytical performance of the Abbott RealTime High Risk HPV test (RealTime) with cervical scrapes collected in SurePath liquid-based cytology (LBC) medium by comparison to Hybrid Capture 2 (HC2) and GP 5+/6+ HR-HPV PCR (GP5+/6+), two reference tests with established clinical performance.

Methods RealTime is a qualitative multiplex-real-time PCR detecting the 14 hrHPV types targeted by GP5+/6+, and simultaneous genotyping of HPV16 and HPV18. A human ß-globin Internal Control ensures sample cellularity and controls DNA extraction efficiency and PCR inhibition. A total of 275 cervical scrapes collected in SurePath LBC previously examined by cytology, were selected based on HC2 results (2/3 positives, 1/3 negatives) for analysis by RealTime and GP5+/6+. Analytical sensitivity and specificity were determined based on the concordance of at least 2 out of 3 results. 95% confidence intervals (CI) and kappa values (k) were calculated.

Results Overall concordance of 85.8% was observed between RealTime and both reference tests. The agreement between RealTime and GP5+/6+ (94.2%; k: 0.89) was higher compared to that between RealTim and HC2 (88.4%; k: 0.75) and between both reference tests (89.1%; k: 0.77). Analytical sensitivity of RealTime (94.9%) was comparable to that of HC2 (97.7%) and GP5+/6+ (97.7%). Analytical specificity of RealTime (100%) was comparable to that of GP5+/6+ (97.0%) and significantly higher than that of HC2 (81.0%).

Conclusions The analytical performance of the Abbott RealTime assay was found comparable to that of GP5+/6+ when testing SurePath samples. Both PCR-based hrHPV DNA tests demonstrated analytical sensitivity comparable to that of HC2, while their specificities were superior to that of HC2. SurePath LBC appears to be a reliable medium for the detection of hrHPV in clinical samples with the Abbott RealTime test.

OC 12-11

PERFORMANCE ASSESSMENT OF AUTOMATED HYBRID CAPTURE 2®: QIASYMPHONY® DSP HPV MEDIA KIT & RAPID CAPTURE® SYSTEM

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Objective: The digene HC2 High-Risk HPV DNA Test® (HC2) is used in Europe for screening PreservCyt® (PC) and SurePath (SP) specimens for Human Papillomavirus (HPV). The current established workflow for processing specimens involves manual processing including a number of ergonomically repetitive steps. QIAGEN is currently developing novel workflows for automated extraction of human cervical cells stored in different media. This abstract presents the results from verification studies performed for specimens stored in PC or SP, processed on a fully automated system consisting of the QIAsymphony DSP HPV Media Kit and QIAsymphony SP instrument followed by automated HC2 testing on the Rapid Capture System (RCS).

Methods: For evaluating the novel automated workflow, sample aliquots of cervical cells (cell culture or clinical material) were extracted from PC (3ml) or SP (1ml) specimens using the newly developed QIAsymphony DSP HPV media kit, followed by HC2 testing on the RCS using DNS- or C-scripts, respectively.

Conclusions: QIAsymphony DSP HPV Media Reagent cartridges are sufficient for 352 sample extractions and have an open time of 30 hours within a total usage time of 4 weeks after first use. PC sample extracts generated with the system can be stored up to 5 days at 2-8 °C prior to processing on the RCS. Analysis of Clinical performance using a cohort of 1678 individual clinical PC specimens resembling the intended use population of the assay resulted in an overall agreement of 96,1% compared to manually converted samples run on the RCS (96,0% positive, 96,2% negative agreement). No cross contamination was observed using a checkerboard set-up for testing executed on five consecutive days. Similar data will be presented for specimens stored in SP. The QIAsymphony DSP HPV media extraction, automated on the QIAsymphony SP instrument, provides a highly controlled and precise alternative to the approved manual conversion method reducing valuable technologist hands on time.

The QIAsymphony applications presented here are intended for in vitro diagnostic use in compliance to IVDD 98/79/EC and will be available in Europe during mid/late 2013 (PreservCyt specimens planned for Q3/2013, SurePath (prequot) specimens planned for Q4/2013). This product will not be available in the US.

OC 12-12

HIGHLY SENSITIVE DISCRIMINATION OF HIGH GRADE CERVICAL CYTOLOGY FROM NORMAL CYTOLOGY USING "THE SLIDELESS PAP™" PARAMETERS AND BIOINFORMATICS A PRELIMINARY ANALYSIS

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Objectives: To identify high grade cervical cytology specimens using HPV OncoTect 3Dx reagents and the resulting multiplexed parameters including HPV E6, E7 mRNA, cell cycle DNA, and morphometric parameters measured in suspension without slides.

Methods: The HPV OncoTect 3Dx assay was performed on 20 normal cytology samples and 26 high grade cytology samples (HSIL) on a Millipore FlowSIght instrument to determine which parameters gave the best discrimination of high grade cytology from normal samples. E6, E7 mRNA overexpression, nuclear to cytoplasmic ratio (N/C ratio), nuclear area, post G1% (DNA cell cycle) were determined to be the best discriminators on all samples and used in the bioinformatics approaches described below.

Results: Bioinformatics employing the parameters listed above and using two types of SVM predictions using different kernel functions (one sigmoid, one polynomial) resulted in a general accuracy of 80%, Sensitivity: 87%, and Specificity: 72% in determining which specimens were HSIL in a blinded analysis of normal and HSIL samples. Using N/C ratio, nuclear area, and post G1% alone, we improved the performance of the test to general accuracy: 85%, Sensitivity: 93%, and Specificity: 78%. Using brute-force tuning optimizations and a C_SVC Support Vector Machine running with a Sigmoid-function kernel, with maximum sensitivity optimized resulted in general accuracy: 94%, Sensitivity: 100%, and Specificity: 89%. This was achieved with measurements from a 5-fold cross validation.

Conclusions: Using morphometric and cell cycle measurements on cells in suspension assayed with HPV OncoTect 3Dx, we were able to distinguish high grade cervical cytology samples from normal samples without the use of slides or microscopic review.

OC 12-13

URINARY HPV TESTING AS AN ALTERNATIVE TO THE CERVICAL SMEAR IN CERVICAL CANCER SCREENING: A PILOT STUDY IN MAINE ET LOIRE

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Objectives: High-risk human papillomavirus infection is a necessary factor in the development of cervical cancer. In France, cervical cancer screening currently leans on cytological examination of a vaginal smear for women aged 25 to 65. Unfortunately, the screening coverage is unsatisfactory since it is only 58%. Therefore, it would be interesting to increase this rate. To this purpose, we led a study whose objective was to evaluate the acceptance of a urinary HPV test. Indeed, previous studies have shown that self sampling increased rates of compliance.

Methods: We conducted this study in the department of Maine et Loire. Between November 2012 and January 2013, 5000 letters were sent to women aged 40 to 65. These women had not had a vaginal smear done though they had previously received two invitations in a period of nine months. The purpose of this letter was to propose a new screening method using urine instead of a vaginal smear. The patients had to send their urine sample to the laboratory of virology at the CHU of Angers where they were analysed by PCR. The technique used was a real time PCR commercialised by Abbott (Abbott Real Time High Risk HPV) and requiring the m2000sp for genomic extraction and the m2000rt for amplification. This technique enables the detection of genotype 16, 18 and 12 other high-risk HPV (HPV HR). In the case of a positive result, the patient was contacted by her doctor in order to have a vaginal smear done as soon as possible.

Conclusions: 703 samples were analysed out of 771 samples received. We found high risk HPV in 29 of them. 3 samples were positive with HPV 16 alone and 3 with HPV 16 in association with an HPV HR other than 16 or 18. An HPV other than 16 or 18 non-associated with 16 or 18 was found in 22 samples and in association with an HPV 18 in one case. At the present time, 19 women with a positive result had a vaginal smear done. The results showed 11 normal smears, 3 ASC-US, 2 LSIL and 3 HSIL. Among the 3 HSIL smears, one presented confirmed CIN3 lesion.
Our study shows urinary HPV testing is better accepted by women who refuse a vaginal smear. Moreover, we were able to diagnose high-grade lesions in smears from women who were not regularly followed. Therefore, the urinary HPV test could be an alternative to the usual screening by cervical smear thus extending screening coverage in France.

OC 12-14

EUROPEAN HPV DNA TEST EXTERNAL QUALITY ASSURANCE SCHEME (EHEQAS)

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Objective: To improve the quality of laboratories in HPV detection and typing we run the program of external quality control - EHEQAS.

Methods: EHEQAS was founded in 2006 and by 2013 already 20 laboratories from 7 European countries are participating. Until now 177 samples have been tested in 16 rounds. Any European laboratory performing HPV tests may participate (low or high-throughput, no typing or low- or high-resolution typing, in-house or commercial test). Batches of 5-10 samples are sent from the coordinator to participants 1-2 times per year. Samples are either real patient samples (cervical cell pellets, DNA isolated from cervical cells, biopsies) or prepared from international HPV standards (NIBSC) as standalone dilutions or mixtures with real patient samples. Participants have to report results to the coordinator within 3 weeks. Results are evaluated and consensus results are issued and announced to participants in a confidential way. To test for reproducibility samples, which have reached a high-agreement consensus result, are used in duplicate in the same round and are repeated in different rounds. Linearity is evaluated by different dilutions of the same sample in the same and in different rounds. Marks are awarded to participants based on defined rules that reflect the clinical value of the result (e.g. higher penalty for errors regarding types 16 and 18). Once every 18 months certificates of competence are issued.

Conclusions: 128 positive samples analysed so far in EHEQAS contained more than 30 different HPV types. There is a gradual increase in the number of participants and in the quality of their performance. EHEQAS improves quality with the coordinating team providing feedback to participants on how to improve their methodology. EHEQAS is already assessing the ability of laboratories to detect clinically relevant viral loads for screening (≥12,500 iu/mg) and for the triage of ASCUS or for the follow-up of women treated for cervical lesions (≥5,000 iu/mg). EHEQAS is preparing for ISO 17043 accreditation. In the future EHEQAS will also offer the possibility of HPV viral load measurement.

OC 12-15

COMPARISON OF INNOLIPA HPV GENOTYPING EXTRA AND SEEGENE ANYPLEX™ II HPV28 ASSAYS ON TWO DIFFERENT CERVICAL COLLECTION MEDIA FOR HPV GENOTYPING

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Objectives: HPV genotyping could screen patients with highest risk of cancer development. Furthermore, they might be effective tools for monitoring the effectiveness of HPV vaccination. But genotyping assays have uneven performances. Our study aimed to compare the Innolipa HPV Genotyping Extra (Innogenetics) and the Anyplex™ II HPV28 Detection kit (Seegene) on two types of cervical collection media: cytobrushes (STM, Qiagen) and Easyfix liquid based cytology specimens (Labonord).

Methods: 80 endocervical samples were collected: 55 cervical brushes and 25 liquid based cytology specimens. Samples were routinely tested with the Innolipa assay after extraction with Easymag (Biomérieux). They were secondarily tested with the Seegene assay. Both tests detect the same HPV genotypes except HPV 42 (only Anyplex) and HPV 74 and 69 (only Innolipa). Agreement between both methods was measured by overall agreement and Cohen's kappa (k) statistics.

Conclusions: We analyzed the analytical agreement, defined by a total concordance of all the HPV typed, and the clinical agreement, defined by at least one high-risk HPV found in the sample. 63.64% of analytical concordance was observed with the cytobrushes media, and 80% with the liquid-based specimens (68.75% on average). The discordant results of Anyplex assay can be divided into 3 categories: 52% of extra-HPV type(s) detection, 36% of not-detected HPV type(s), and 12% of results with one extra-HPV type and still one not-detected. Considering the 102 HPV types found in all samples by the Innolipa assay and detectable by Anyplex assay, 12.75% were not detected, including 2 HPV 16. In contrast we observed 28 additional HPV (27,45%) detected by Anyplex assay including 12 samples with HPV 42. Clinical agreement between the assays was high: 92.74% (k 0.82) for cytobrushes specimens and 100% (k 1) for liquid based specimens (k 0.88 for all media). This study shows that both tests are generally well correlated, specifically for the screening of HR HPV. Detailed analysis of the detected genotypes shows that the Anyplex assay seems to have a tendency to overstate multiple infections compared to Innolipa assay.

OC 13-1

ROLE OF NATURALLY-ACQUIRED ANTIBODIES IN NEW HPV INFECTION AND PRE-CANCEROUS LESIONS: ANALYSIS OF THE CONTROL COHORT OF THE PATRICIA STUDY

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Objectives: To evaluate the relationship between the risk of new HPV infection and histopathological lesions (CIN) and changes in naturally-acquired type-specific HPV antibody levels and other determinants.

Methods: In a subgroup of approximately 1500 control subjects from the PATRICIA study (NCT00122681), HPV-16/18 antibody levels were repeatedly measured during a 4-year period. Multivariable Cox regression models were used to assess the relationships between HPV-specific antibody levels (as a time-dependent variable) and other determinants and the risk of new HPV infection and CIN.

Conclusions: There was a decreased risk of HPV-16 new incident infection (n of events=215), 6-month persistent infection (n=112), and atypical squamous cells of undetermined significance (ASC-US+; n=102) in HPV-16 seropositive subjects compared with seronegative subjects (HR=0.74, 0.63 and 0.66, respectively). Other significantly associated determinants were: marital status, smoking, number of sexual partners and Chlamydia infection. Previous HPV-16 infection was the greatest risk factor for HPV-16 ASC-US+, CIN1+ (n=35) and CIN2+ (n=18) (HR=23.3, 62.6 and 105.5, respectively). Risk of HPV-18 incident infection (n=122), 6-month persistent infection (n=46), and ASC-US+ (n=47) was also decreased in HPV-18 seropositive subjects (HR=0.86, 0.56 and 0.62, respectively). HPV-18 CIN1+ and CIN2+ were not analyzed because of the low number of observed events (n=8 and 4, respectively).

Analysis of longitudinal naturally-acquired HPV-16/18 antibody levels showed a decreased risk of new HPV-type specific infection in seropositive subjects, whereas an HPV-type specific previous infection was the major risk factor for CIN1+/CIN2+.

OC 13-2

CLONAL CD4+ T CELLS CROSS-REACT WITH HOMOLOGOUS L1-DERIVED PEPTIDES OF HIGH RISK AND LOW RISK HPV TYPES

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Objectives: HPV vaccines induce strong immunological responses against epitopes of the L1 protein. Broad CD4+ T cell responses are detectable in immunized persons. Clinical cross protection to related non-vaccine HPV types has been observed. We investigated the clonal $CD4^+$ T cell responses to vaccine and non-vaccine HPV type L1 antigens with the aim to characterize cross reactivities[1].

Methods: PBMC from healthy women vaccinated with Cervarix® or Gardasil® were stimulated with 10 synthetic 15mer peptides of the HPV16 and 18 L1 protein, respectively. Peptides were predicted to be HLA-DRB-restricted epitopes for our donors who bear HLA-DRB alleles being common in caucasian population. Single cell cloned T cells, generated from our donor's PBMC, were selected according to CD4, CD8 and CD19 staining and responsiveness to the cognate epitope. Reactive clones were restimulated with homologous peptides of the nearest relative high risk HPV type 31 and 45, respectively and with homologous peptides of the low risk HPV types 6 and 11. T cell activation was determined by expression of CD154, IFN-gamma and TNF-alpha measured by intracellular flow cytometry. Cytokine secretion (IL-4, IL-6, IL-10, TNF-α and IFN-γ) was investigated more detailed by cytometric bead array. Results between vaccine groups were compared. TCR Vβ-chain PCRs prove clonality of T cell lines and give information about TCR differences of clones with distinct cross reactivities.

Conclusions: 70 % of predicted HPV16 and 90 % of the predicted HPV18 epitopes are actually found to be epitopes specifically activating $CD4^+$ T cells of at least one of our donors. $CD4^+$ T cells of HPV vaccinated women stimulated with L1 peptides of high risk HPV types are able to cross react against homologous HPV L1 peptides of related high risk HPV types, but also against homologous peptides of low risk HPV types 6 and 11. We found comparable patterns of cytokine secretion in both vaccine groups but higher amounts of secreted cytokines within the Cervarix® group. Immunological reaction against L1 peptides of non-vaccine HPV types could be supported by activation of $CD4^+$ T cell clones by cross reactive epitopes.

1 The collaboration and financial support by GlaxoSmithKline is gratefully acknowledged.

OC 13-3

CHARACTERIZATION OF THE INFLAMMATORY INFILTRATE IN HUMAN PAPILLOMAVIRUS ASSOCIATED CERVICAL LESIONS

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Objectives: Inflammation may play a dual role in cancer development. Chronic inflammation may be a risk factor generating lesions, reactive nitrogen and oxygen species and causing mutation. Inflammation may also suppress adaptive immune responses participating in tumor cell evasion mechanisms. In patients with cervical cancer, T cell responses are fairly well characterized. However, the interactions between other leukocytes and T cells locally and systemically are still poorly understood, even more regarding lesion progression from low to high grade to cancer.
The objective of this study is to characterize the local inflammatory infiltrate in HPV associated cervical lesions from low grade lesions to cancer and, in parallel, estimate the efficiency of antigen presentation in cells from peripheral blood.

Methods: Enrolled patients were attended in the Gynecological Ambulatory at Hospital das Clinicas, São Paulo, Brazil. In this study, we are collecting cervical biopsies and peripheral blood from 20 patients with lesions correspondent to the categories above mentioned. Biopsies were processed into single cell suspensions that were labeled with antibodies against myeloid and lymphoid cells and analyzed by flow cytometry. We also saved material for histology and HPV typing. PBMCs were isolated from blood for dendritic cell differentiation and allogeneic T cell stimulation.

Conclusions: We have successfully analyzed cervical lesion samples by flow cytometry. In this lesions, we observed the presence of both lymphoid and myeloid populations. Within the samples analyzed until now, we found CD8 and CD4 T cells in 100% and 92%, respectively. We also found CD16+ cells in 85% of the samples and macrophages in most samples, M1 type in 77% of the samples and M2 type in 44% of the samples, however the last one concentrated in high grade lesions.

Financial support: This project is supported by FAPESP grants.

OC 13-4

HPV 16 E2-, E6- AND E7-SPECIFIC T-CELL RESPONSES IN CHILDREN AND THEIR MOTHERS

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Objectives: We analyzd the HPV16-specific CMI responses among the women who developed an incident CIN during 14-year follow-up (FU). HPV16-specific immunity was related to their known oral and genital HPV DNA status and HPV serology during the FU. Of special interest was to assess whether or not their children have developed HPV16-specific CMI reactivity at the same time point. The children have had neither any sexual contact nor were vaccinated for HPV.

Methods: 10 women and 10 children were tested for HPV16-specific T-cell proliferation response from PBMCs. We used overlapping 30-35 mer peptides covering the entire protein E2, E6 and E7.

• HPV16-specific proliferative T cell response was done with ^{3}H-thymidine based proliferation assay. 9 children and only 4 women had responses against both E2 peptide pools. 6 children and 2 women displayed reactivity to E6 and/or E7.

• IL-2, IL-4, IL-5, IL-10, IL-17A, IFN-γ and TNF-α levels were measured with Cytometric bead array system (BD Bioscience) from supernatants isolated at day 6 of the proliferation assay. The cytokine levels of IL-2 ($p=0.023$) and IL-5 ($p=0.028$) induced by all peptide pools, were also higher among children than their mothers. The children of the mothers with incident CIN3 had significantly higher IFN-γ ($p=0.032$) and TNF-α ($p=0.008$) levels than other children.

• CD4+CD25+Foxp3+ regulatory T cell detection was done by stimulating the PBMCs with E2, E6 and E7 peptides for 6 days and analyzes with flow cytometry (BD Bioscience). Foxp3+ subsets were expanded among 3 children and 2 women at least for some peptide pools.

Conclusions: All these children harbored a T-cell response against HPV16 peptides and the responses of the children were broader than those of their mothers ($p=0.0146$). These results indicate that children aging 12 years have already developed HPV16-specific memory T-cells, the responses being most strong among children born to mothers with incident CIN3.

OC 13-5

A NEW BENCHMARK OF THE PRE-VACCINE SEROPREVALENCE OF HPV16 IN THE NETHERLANDS

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Objectives: Serological data might be utilized to monitor the effect of vaccination against human papillomavirus type 16 (HPV16). However, the serological response to infection with HPV is not well understood. Surveillance methods that rely on a cut-off value to classify seropositive and seronegative individuals do not give a satisfactory description of the seroprevalence of HPV16 in the general population prior to vaccination. We set out to provide an alternative estimate of the pre-vaccine seroprevalence of HPV16 based on antibody concentrations.

Methods: HPV-specific serum antibodies were measured in a cross-sectional population-based serological survey performed in the Netherlands in 2006/2007 with 7179 participants. Both men and women between 0 and 79 years of age provided a blood sample, which was analyzed with a VLP-based multiplex immunoassay.
We described the log-concentration of HPV16 antibodies by a mixture model with two components, representing seronegatives and seropositives for HPV16. Both mixture components were assumed to be normally distributed with unknown mean and variance (independent of age). We assumed the log-concentration of 0-10 year olds to inform only the seronegative component and used penalized splines to smooth the seroprevalence over age. We fitted separate models to serological data of men and of women to assess whether HPV16 seroprevalence is gender-specific.

Conclusions: The gender-specific seronegative component densities were similar, but the mean concentration of the seropositive component density was higher for women than for men. This suggests a stronger serological response to HPV16 infection in women as compared to men. Seroprevalence showed a similar increase around age 20 in men and women, but remained increasing in men whereas seroprevalence had a decreasing trend from age 40 onwards in women. Seroprevalence would be largely underestimated if seropositivity were assigned on the basis of a predefined cut-off. Test sensitivity when using the predefined cut-off would be 44% for men and 61% for women.
In conclusion, we provide a new benchmark of the pre-vaccine seroprevalence of HPV16 which could be utilized to monitor the effects of HPV vaccination from future serological surveys.

OC 13-6

IMMUNE CELL INFILTRATION IN RELATION TO P16INK4A EXPRESSION IN CERVICAL INTRAEPITHELIAL NEOPLASIA AND CANCER

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Objectives: The patient's immune system plays a crucial role in the natural course of HPV-induced cervical dysplasia and could contribute to the dynamic process of spontaneous regression and progression. We hypothesize that shifts in density and phenotype of infiltrating T cells are linked to the transforming infection stage as reflected by $p16^{INK4a}$ overexpression.

Methods: In total 71 CIN and cervical carcinoma patients were investigated. Biopsies were stained by immunohistochemistry for $p16^{INK4a}$ expression and for the following T cell phenotypes: CD3 (Pan-T cell marker), Foxp3 (Tregs), CD8 (CTLs), Granzyme B (activated CTLs) and CD3 ζ-chain (T cell signaling and activation). The results obtained for infiltrating T cells were stratified by the $p16^{INK4a}$ expression status of the lesions within the different CIN grades.

Conclusions: The numbers of immune cells infiltrating CIN1 lesions and the adjacent stroma are generally low when compared to higher CIN grades. The comparison between $p16^{INK4a}$-negative and $p16^{INK4a}$-positive CIN1 does not reveal any significant differences in densities of different T cell phenotypes, however the mean counts tended to be higher in $p16^{INK4a}$-negative compared to $p16^{INK4a}$-positive CIN1. True transforming HPV infections in $p16^{INK4a}$-positive CIN2 and CIN3 (high-grade CIN) and carcinomas are characterized by an increased number of different T cell phenotypes compared to the low-grade CIN (CIN1 and $p16^{INK4a}$-negative CIN2) in the lesion and the adjacent stroma. Thus in conclusion it appears that in early lesions (CIN1) there may be already transforming infections ($p16^{INK4a}$-positive), but local immune cells are still not significantly different compared to non-transforming infections ($p16^{INK4a}$-negative), however only in later, $p16^{INK4a}$-positive CIN stages the shift becomes apparent. The data may be important for immunotherapeutic treatment strategies in HPV-associated premalignant lesions.

OC 13-7

NATURALLY OCCURRING FG LOOP VARIANTS OF HPV31: IMPLICATIONS FOR CURRENT AND NEXT GENERATION L1-BASED PROPHYLACTIC VACCINES

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An analysis of available full length HPV31 L1 sequences (n=95) identified two major variant residues within the FG loop compared to the reference sequence: a Thr to Asn at position 274 (T274N) in 28% of sequences and a dual variant containing Thr to Ala at position 267 (T267A) alongside T274N in 49% of sequences. The FG sequence of the reference was present in only a minority of L1 sequences (15%). The epitopes of several neutralising monoclonal antibodies have been mapped to a region encompassing residue 267 and adjacent to 274 suggesting that changes in this region may alter antibody recognition.

Objectives: To evaluate whether L1 proteins with the single or dual FG loop variant residues differ from the L1 protein representing the reference in their ability to elicit type-specific neutralising antibodies (NAb) and in their sensitivity to both type-specific and cross-reactive antibody (Ab) mediated neutralisation.

Methods: HPV31 L1 virus-like particles (VLP) were generated containing the single T274N or dual T267A/T274N variant residues. These VLP were used in animal immunisations alongside VLP based upon the reference L1 sequence to generate type-specific NAb. Cervarix® and Gardasil® vaccinee sera were used as a source of cross-reactive HPV16/HPV31 NAb. The potential of the sera to neutralise HPV31 L1L2 pseudovirions (PsV) containing the single or dual variant residues were evaluated by neutralisation assay, alongside the PsV based upon the reference.

Conclusions: Overall, little difference was observed between the neutralisation potency of type-specific and cross-reactive Ab targeting the single or dual PsV compared to the reference PsV. For example, type-specific Ab raised against the reference L1 VLP (n=7) demonstrated no significant difference in NAb titre against the dual PsV compared to the reference PsV [geometric mean fold-difference 1.09 (95% CI: 0.45-2.62; p 0.612)]. Cross-reactive Ab in HPV vaccinee sera (n=17) did demonstrate borderline significantly higher NAb titres against the dual PsV compared to the reference PsV [1.35 (1.10-1.65; p=0.019)]. Naturally occurring polymorphisms in the FG loop of HPV31 are unlikely to have a major impact on the recognition of NAb elicited by the current or next generation L1 VLP-based vaccines.

OC 13-8

HIGH-RISK HPV51: L1 AND L2 AMINO ACID DIVERSITY AND RECOGNITION BY ANTIBODIES ELICITED BY NATURAL INFECTION

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Background: Persistent infection with high-risk HPV (HRHPV) genotypes is highly associated with the development of cervical cancer. HRHPV genotypes are clustered within the alpha-9 (HPV16,31,33,35,52,58), alpha-7 (HPV18,45,39,59), alpha5 (HPV51) and alpha-6 (HPV56) species groups and are collectively responsible for about 90% of squamous cervical cancer and 94% of adenocarcinoma worldwide. Among the HRHPV genotypes, HPV51 is one of the most understudied and little information is available on the degree of naturally-occurring, intra-type variation and on the extent of viral-induced immune response.

Objectives: The aim of this study was to identify naturally occurring intra-type diversity in the L1 and L2 genes of HPV51 and to address their impact on the natural history of the virus-induced immune response.

Methods: Full length HPV51 L1 and L2 sequences were generated from DNA extracted from genital samples from European women previously tested for the presence of HPV genotypes.
Serum samples from patients harbouring HPV51 DNA were tested for the presence of antibodies against HPV51 using an L1 VLP-ELISA, based upon reference sequence (Genbank M62877).

Conclusions: L2 sequence analysis revealed a novel mutation at T284K, and two stretches of variable amino acids (95–99, 179–186) which differ greatly from the prototype, but are identical to the only other existing L2 sequence from Japan (GQ487712). Two variations from the reference sequence (V264G and G265S) within in the FG loop of L1 were present in the majority of sequences. Despite being infected with the V264G/G265S variant of HPV51, 42% of the sera analysed recognized VLPs based on the prototype sequence. Potential differences in antibody titre between the prototype and variant VLPs and pseudovirus are being investigated.

OC 14-1

PREVALENCE AND RISK FACTORS FOR ORAL HPV INFECTION IN YOUNG AUSTRALIANS

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Objectives: During the last decade there has been a large increase in the incidence of mucosal squamous cell carcinomas of the head and neck (HNSCCs) in younger non-smokers. These are suspected of being caused by infection with human papillomavirus. The prevalence of oral infection in the wider community remains unknown. We sought to determine the prevalence of, and identify risk factors for, oral HPV infection in a young, healthy Australians.

Methods: We recruited 307 students (ages 18 to 35 yrs) from an Australian university. We asked participants to report anonymously about basic demographics, sexual behaviour, illicit drugs, alcohol and tobacco use as well as history of genital infections and abnormal pap smears.
Participants rinsed their mouth for 30 sec with 7 ml saline. DNA was extracted using the QIAamp DNA Mini Kit. All samples were analysed for presence of HPV with GP+ PCR, and tested with ,-globin PCR to ensure DNA quality.

Results: Seven of the 307 (2.3%) students tested positive for oral HPV infection. Six of the 7 oral HPV positive individuals were males ($p=0.008$). One of the 7 oral HPV positive individuals reported to have kissed passionately, but had never engaged in oral sex or had sexual intercourse. Students with oral HPV infection reported having given oral sex to more partners in lifetime and in the last year compared to HPV negative subjects ($p<0.0001$ and 0.036, respectively). The same trend was seen for receiving oral sex (lifetime $p=0.0004$; past year $p<0.0001$). Self-reported past history of sexually transmitted diseases were significantly associated with oral HPV infection (Gonorrhoea $p=0.0003$; HPV $p=0.0007$; Herpes Simplex $p=0.017$ and HIV $p<0.0001$). Individuals with oral HPV infection were more likely to have ever used illicit drugs ($p=0.015$). Number of passionate kissing and sexual intercourse partners, alcohol consumption and smoking were not associated with oral HPV infection.

Conclusions: Oral HPV infection was found to be associated with male gender, giving and receiving oral sex, previous history of sexually transmitted disease and to ever have used illicit drugs.

OC 14-2

PROGNOSTIC VALUE OF HUMAN PAPILLOMAVIRUS AND SQUAMOUS CELL CARCINOMA ANTIGEN IN HEAD AND NECK SQUAMOUS CELL CARCINOMA

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Objectives: To clarify the synergistic influence of human papillomavirus (HPV) status and squamous cell carcinoma antigen (SCCA) mRNA expression on prognosis of head and neck squamous cell carcinoma (HNSCC).

Methods: HPV DNA presence and SCCA1 and SCCA2 mRNA expression were determined by polymerase chain reaction (PCR) and quantitative real-time reverse transcription-PCR, respectively, in 121 patients with primary HNSCC who were receiving curative treatment. HPV DNA was detected in 28.1% (34/121) of HNSCC cases, and only high-risk types (HPV-16, HPV-33, HPV-35, and HPV-58) were observed. Positive HPV status showed a significantly better prognosis than negative HPV status ($P = 0.022$). An elevated SCCA2/SCCA1 mRNA ratio was an independent predictor of disease recurrence ($P = 0.004$). In addition, HPV-negative patients with a high SCCA2/SCCA1 ratio (>0.27) had a significantly lower recurrence-free survival rate than HPV-negative patients with a low SCCA2/SCCA1 ratio ($P < 0.011$).

Conclusions: Our findings revealed that both HPV status and the SCCA2/SCCA1 mRNA ratio are independently associated with prognosis in HNSCC. Patients with both a HPV-negative status and a high SCCA2/SCCA1 ratio may need intensified treatment and rigorous follow-up after treatment because of the high risk of recurrence.

OC 14-3

SMOKELESS TOBACCO INCREASES ANEUPLOIDY IN ORAL HPV 16 E6/E7 TRANSFORMED KERATINOCYTES

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Objectives. This study aimed to evaluate possible synergism between human papillomavirus (HPV) infection and tobacco use increasing the risk of oral cancer.

Methods. We exposed HPV-positive and –negative oral keratinocytes and oral HPV-negative fibroblasts to smokeless tobacco extract (STE) prepared from the Scandinavian (STE1) and US type (STE2) snuff. Flow cytometry was run to determine cell cycle profiles. HPV E6/E7 mRNA expression in HPV-positive oral keratinocytes were assayed using RT-qPCR. The number of aneuploid cells increased from 27% to 80% of which 44% were in S-phase in HPV-positive keratinocytes after STE2 exposure. None of the diploid cells were in S-phase. After STE1 exposure the changes were less than seen after STE2 (27% to 31% of which 34% were in S-phase). No effect of STE exposure was detected on HPV 16 E6/E7 expression in HPV-positive keratinocytes. In oral HPV-negative keratinocytes, the number of aneuploid cells at G2-M stage increased from 3% to 9% and 7% after STE1 and STE2 exposure, respectively. Similarly, in HPV-negative oral fibroblasts, the number of cells at G2-M phase increased from 11% to 21% after STE1 and 29% after STE2 exposure.

Conclusion. The effect of STE differed in the cell lines. In HPV-positive oral keratinocytes, STE2 increased significantly the proportion of aneuploid cells. However, HPV 16 E6/E7 expression remained unchanged. This indicates that tobacco products may enhance the growth advantage of cells with DNA aneuploidy in favor of diploid cells among the hetero cell population. This might be related to HPV 16, which is however not directly associated with simultaneous increase in E6 and E7 mRNA expression.

OC 14-4

EFFECT OF SMOKING HABITS ON HPV STATUS AND SLPI (SECRETORY LEUKOCYTE INHIBITOR) EXPRESSION IN HEAD AND NECK CANCER

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Objectives: Recently, we demonstrated a correlation between SLPI regulation and HPV infection in HNSCC, suggesting that high levels of SLPI correlate with a protective effect against HPV. Thus, SLPI plays a pivotal role in the HPV infection process of mucosa cells. We showed that smoking induces SLPI expression hindering HPV infection.

Methods: Here we collected biopsies from healthy mucosal and HNSCC tissue. Also, healthy mucosa was retrieved from patients without HNSCC; results were correlated with smoking habits. DNA from fresh frozen biopsies (n=74) was used for PCR-based HPV detection and RNA was transcribed into cDNA for SLPI gene expression.
SLPI gene expression in mucosal tissue of non-HNSCC patients was 8 times higher in smokers than in non-smokers. In mucosal tissue of HNSCC patients smoking resulted in a 15 fold increase in SLPI gene expression. The tumor tissues of patients with smoking habits showed 35 times higher SLPI expression. Biopsies of 6 patients were HPV positive, all tonsillar carcinomas (5 non-smokers). All of these patients showed significantly lower SLPI gene expression levels when compared to the averaged gene expression.

Conclusions: The data clearly show a correlation between smoking and SLPI expression supporting our hypothesis that SLPI expression and HPV infections are linked in HNSCCs. We speculate based on our previous data and the data presented here that smoking might be a crucial component up-regulating SLPI expression and consequently reducing HPV infections of HNSCCs.

OC 14-5

HUMAN PAPILLOMAVIRUS AND CYSTIC NECK METASTASIS IN OROPHARYNGEAL CARCINOMA AND UNKNOWN PRIMARY CARCINOMA

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Objectives: To clarify baseline data of HPV prevalence in head and neck squamous cell carcinoma (HNSCC) among Japanese population and evaluate relationship between neck metastasis and HPV status.

Methods: 493 HNSCCs were evaluated by PCR to detect HPV. For analysis of HPV status of lymph nodes, 44 samples from formalin-fixed paraffin-embedded surgically dissected nodes and 53 samples from fresh frozen fine-needle aspirate (FNA) were investigated. Two hundred forty-five metastatic nodes were classified to cystic node or other by review of enhanced CT.

Conclusions: First, 35% of oropharyngeal carcinoma (OPC) was HPV-positive. In contrast, HPV-positive tumors are significantly less frequent in other sites (0%, 12%, 4%, and 4% in oral, nasopharyngeal, hypopharyngeal, and laryngeal carcinomas, respectively). HPV 16 accounts for no less than 91%, while other types of high risk HPV are significantly less frequent. Second, 9 of 28 HNSCCs with dissected nodes were HPV-positive for both primary tumor and metastatic node. Thirteen of 40 HNSCCs with FNAs were HPV-positive for the both. None of the HNSCCs turned out to be HPV-positive in one of either primary tumor or metastatic node. All of the HPV-positive HNSCCs were OPC. As for unknown primary carcinoma with neck metastasis, 5 of 16 (31%) dissected nodes and 5 of 13 (38%) FNAs were HPV-positive. Sixteen of 29 unknown primary carcinoma underwent tonsillectomy concurrently with neck dissection. Six tonsillar primary carcinomas were detected and 3 of those were HPV-positive for both primary tumor and metastatic node. Collectively, it seems likely that unknown primary carcinoma with HPV-positive neck metastasis originates from the oropharynx. Third, cystic lymph node was specific to only OPC and unknown primary carcinoma (7%, 7% and 0% from oropharyngeal, unknown primary and other sites, respectively). Limited to oropharyngeal and unknown primary, 75% of carcinomas with cystic metastasis were HPV-positive (Odd ratio = 6.2; 95% confidence interval = 1.3-44; P = 0.019). In conclusion, PCR analysis of FNA enables the detection of HPV DNA. Unknown primary carcinoma with HPV-positive neck metastasis was very likely to be arising from oropharynx. Cystic metastasis is strong indicator of HPV-positive primary site.

OC 14-6

VIROLOGICAL MARKERS IN HPV-DRIVEN OROPHARYNGEAL SQUAMOUS CELL CARCINOMAS: VIRAL DNA METHYLATION SEEMS TO BE A PROMISING PROGNOSTIC MARKER

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Objective. It is well established that oropharyngeal squamous cell carcinomas (OPSCC) positive for human papillomavirus (HPV) infection constitute a defined subgroup of head & neck tumours, with better prognosis and response to chemo/radiotherapy compared to those negative. However, even among HPV-positive patients there are some with poor outcome, highlighting the necessity for additional markers.

Methods. We analyzed 81 biopsies of OPSCC for HPV genotyping with a sequencing-based HPV DNA test (HPV sign® Genotyping test, Qiagen) to search also the presence of viral intratype variants. We also studied viral DNA methylation frequency in the long control region (LCR) by pyrosequencing for patients with transcriptionally-active HPV16-infection, analyzing it in function of other available clinical/virological data (i.e. viral load and integration). Overall HPV prevalence was 74.1% and HPV16 was confirmed the most prevalent genotype (85.0%, mostly single infections). Interestingly, we detected for the first time HPV16 African variants in 9 cases, pointing out their clinical relevance also out of the anogenital district. Regarding viral DNA methylation, the E2BS1 showed a significantly higher mean methylation frequency compared to E2BS3/4, as previously observed in squamous precancerous lesions of the uterine cervix. On the other hand, even if the mean methylation frequency of the early promoter did not correlate with virological data, we observed for patient with a mean methylation frequency $>10\%$ a tendency to have a worse prognosis.

Conclusions. Taken together, our results highlight both the possible involvement also of HPV16 variants in OPSCC development and the role of viral early promoter methylation as promising prognostic marker.

OC 14-7

STUDIES ON THE PRESENCE OF HUMAN PAPILLOMAVIRUS (HPV) AND P53 EXPRESSION IN NECK METASTASIS WITH UNKNOWN PRIMARY IN CORRELATION TO CLINICAL OUTCOME

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Objectives: To investigate whether HPV-status and p53-expression is correlated to the 3-year overall survival (OS) in patients with unknown primary HNSCC. In some patients with head and neck squamous cell carcinoma (HNSCC) the primary tumor is never found. These patients are treated aggressively due to the poor prognosis for HNSCC in general. HPV-positive oropharyngeal squamous cell carcinoma (OSCC) has a considerably better prognosis than other HNSCCs, thus requiring less radical treatment. It is plausible that an HPV-positive metastasis from an unknown primary HNSCC originated from an HPV-positive OSCC and thereby has a similar better prognosis and that a patient with such a metastasis would benefit from a less aggressive treatment thereby avoiding unnecessary side effects.

Methods: 42 neck metastases with unknown primary were analyzed for the presence of HPV by a bead-based multiplex assay on a MagPix instrument. In addition, expression of $p16^{INK4}$ and total p53 was analyzed by immunohistochemistry. The correlation of overexpression of $p16^{INK4}$ and tumor HPV status was evaluated and the presence of HPV and p53 expression was correlated to the clinical outcome.

Conclusions: Patients with HPV-positive metastases had significantly better 3-year OS compared to patients with HPV-negative metastases (79% vs. 46%, respectively; $p = 0.048$). Low p53 expression was also significantly correlated to a better prognosis with an 86% 3-year OS ($p < 0.001$) as compared to 63% and 8% 3-year OS respectively in patients with intermediary and high expression of p53, indicating mutated p53. The results indicate that HPV-status and expression of mutated p53 should be considered prognostic factors in unknown primary HNSCC and should be studied further for possible use in individualized patient treatment.

OC 14-8

APTIMA HPV TESTING OF FINE NEEDLE ASPIRATES, SALIVA AND ORAL SWABS FROM PATIENTS WITH SQUAMOUS CELL CARCINOMA OF THE HEAD AND NECK

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Objectives: High risk human papillomavirus (HR HPV) causes a substantial proportion of squamous cell carcinomas (SCC) of the head and neck. Many patients present with enlarged cervical lymph nodes. A fine needle aspirate (FNA) of the cervical node can be performed for pathology assessment, leading to investigations to identify the primary site of the SCC. Nucleic acid amplification tests are available to detect DNA or RNA of HR HPV and have been useful in managing patients with genital and anal cancers. This study focuses on determining optimal sampling for the detection of HR HPV in patients with SCC

Methods: From July 2012 to June 2013, 31 patients with cancer of the head and neck determined as SCC by p16-stained tissue biopsy, FNA or clinical evidence, signed consent into the study. FNA, a swab from the base of the tongue and saliva were collected from each patient and tested for mRNA using the APTIMA HPV (AHPV) assay and DNA using the Cervista assay for 14 high risk types and a genotyping assay for types 16, 18 and 45 (Hologic/Gen-Probe). Saliva was collected into OMNIGENE DISCOVER RNA/DNA collection vials (DNA Genotek), the FNA and tongue swab were placed into HPV specimen transport media (samples were collected into PreservCyt and 1ml was tested). Patient ages ranged between 43 and 74 (median 57); 28 were male (90.3%) and tumours were classified as tonsillar (15), base of tongue (9) and other (7).

Conclusions: Of 31 interim patients enrolled, 21 (67.7%) tested positive for AHPV in the FNA and 95.2% (20/21) were type 16. The saliva samples identified 10/31 (32.3%) and the tongue swab was positive in 6/31 (19.4%). One patient was saliva positive/FNA negative. All 21 AHPV-positive patients were DNA positive and 2 patients negative by p16 staining and HPV RNA showed type 16 DNA in FNA. 82% (23/28) of SCC positives were p16 positive and 17.4% (4/23) were HPV negative in all samples and tests. HPV 16 mRNA and DNA are present in FNA from most patients. Saliva collected into OMNIGENE DISCOVER tubes may be of value as a complementary sample to identify infected patients. AHPV testing results could be reported within 48 hours to enable early treatment. HPV testing of FNA and saliva appears promising in this feasibility study.

OC 14-9

HUMAN PAPILLOMAVIRUS (HPV) DNA DETECTION IN DIFFERENT BIOLOGICAL TISSUES OF PATIENTS WITH ORAL LEUKOPLAKIA.

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Oral leukoplakia is considered a pre-malignant lesion for the development of oral squamous cell carcinoma (World Healthy Organization). Several risk factors can be related to this carcinogenesis, including the human papillomavirus (HPV). HPV is the main cause of cervix cancer, and your role in the oral carcinogenesis is still controversial. There are not studies between different biological tissues and the presence of HPV DNA.

Objectives: The aims of this study was to detect the presence of HPV DNA in fresh tissue samples, plasma and oral exfoliated cells extracted from patients with oral leukoplakia (OL), analyzed by nPCR and make a comparison among these different biological materials sources.

Methods: It was performed the extraction of DNA from 37 patients with OL and amplification of the human ,-globin gene was carried out in all samples to confirm the presence and integrity of DNA. Nested PCR assays revealed the presence of HPV DNA in 68.75% of fresh tissue, 50.0% of plasma, 62.5% of saliva, and in 28.1% of oral exfoliated cells extracted from patients with OL.

Conclusions: Based on the current experiment, HPV could potentially be an etiologic co-factor in the pathogenesis of OL.

OC 14-10

IMPACT OF HPV GENOTYPE ON CLINICAL COURSE OF JUVENILE ONSET RECURRENT RESPIRATORY PAPILLOMATOSIS

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Objectives: To compare the clinical courses of children with juvenile onset recurrent respiratory papillomatosis caused by human papillomavirus (HPV) 6 and 11.

Methods: Data was derived from the Canadian National Juvenile Onset Recurrent Respiratory Papillomatosis Database (1994 – present). Inclusion criteria were 5 or more surgical procedures and if their HPV genotype was determined by molecular detection. Data from 28 subjects was included in the analysis (18 with HPV 6, 10 with HPV 11). Difference in age between groups was assessed using Mann-Whitney U tests. Each surgical intervention was plotted over time and groups were analyzed using non-linear regression analysis with comparison of 'best-fit' curves (quadratic for the HPV 6 and cubic for the HPV 11 subtype).

Conclusions: Twenty-eight subjects in the database met inclusion criteria. Median follow-up was 3.39 (HPV 6) and 3.99 (HPV 11) years from the date of the first debulking procedure. Patients with HPV 11 presented earlier than patients with HPV 6 (median age = 1.93 (IQR 0.85-2.47) and 3.06 years (IQR 2.14-5.84), respectively ($p = 0.035$)). The initial frequency of operation did not differ between the two groups on non-linear regression analysis. However, 7 years after the initial debulking procedure, HPV 11 patients demonstrated a persistent or increasing requirement for surgical debulking while HPV 6 patients had a decreasing requirement and earlier remission. Results suggest that subjects infected with HPV 11 have a more aggressive clinical course compared to HPV 6. These findings have important implications for patient counseling and prognosis.

OC 14-11

EXAMINING DISTRESS LEVELS IN PATIENTS WITH OROPHARYNGEAL VERSUS NON-OPROPHARYNGEAL SQUAMOUS CELL CARCINOMAS OF THE HEAD AND NECK

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Objectives: The aim of this study was to examine whether distress levels, common psychological and practical problems differ between oropharyngeal cancer (OPC) and non-OPC head and neck cancer (HNC) patients, as a surrogate marker for HPV status.

Methods & Results: We identified a total of 146 HNC patients (56 OPC, 90 non-OPC) with available clinicopathological and distress screening data treated in Calgary, Alberta from 2005-2007. Measurements including distress-, fatigue- and pain thermometers, the Canadian Problem Checklist and the Psychosocial Screen for Cancer (which measures anxiety and depression) administered at baseline and 3 month follow-up were compared between OPC and non-OPC patients. Linear mixed models were used to examine group, time and group-by-time effects. The OPC group was more likely to be younger ($p=0.05$), Caucasian ($p=0.001$), non-smokers ($p=0.01$), earn a higher income ($p=0.04$), and present with more advanced stage disease ($p<0.0001$). OPC patients were primarily treated with concurrent chemoradiation therapy whereas non-OPC patients were more likely to be treated surgically. OPC patients reported higher pain scores than non-OPC patients both at baseline and at 3-month follow-up. No significant differences were found on other distress variables between the two groups at baseline. At 3 month follow-up, OPC patients reported more fatigue and anxiety. In both groups, scores on the distress thermometer, depression, psychosocial problems, practical problems and total problems decreased significantly, but there were no differences between groups.

Conclusions: Despite a difference in the clinicodemographic characteristics of OPC vs. non-OPC patients, only baseline pain levels, and fatigue and anxiety at 3-months, differed between the two groups. A larger study focusing on pain, fatigue, and anxiety among HNC patients, stratified by HPV-status, may reveal more information on these differences and help guide necessary psychosocial interventions.

OC 15-1

HPV PREVALENCE IN HNSCC IN QUEENSLAND, AUSTRALIA

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Objectives: During the last decade there has been an increase in the incidence of mucosal squamous cell carcinomas of the head and neck (HNSCCs). This increase has been linked to high-risk human papillomavirus (HPV) types. Our objectives for this study were to determine HPV prevalence and p16 expression in Australian HNSCC patients and to compare other risk factors for HPV positivity.

Methods: We extracted DNA from 267 HNSCC patients (aged 18+ yrs) with histologically-confirmed primary SCC of the oropharynx, oral cavity, hypopharynx or larynx diagnosed at a tertiary referral centre between 2004 and 2010. All patients completed a brief, standard risk factor questionnaire, and clinical data were abstracted from medical records. HPV prevalence was determined with GP+ PCR. Expression of p16 was determined by immunohistochemistry (IHC).

Results: Fifty-five of the 267 (21%) HNSCC patients tested positive for HPV, 63 (24%) overexpressed p16, and 44 (16%) were positive for both HPV DNA and p16.
Stratified by tumour site the HPV prevalence was highest for oropharynx (42%), followed by hypopharynx (13%), larynx (9%) and oral cavity (7%; $p<0.0001$). The tumour subsite with the highest HPV prevalence was tonsil (48%).
The HPV prevalence was inversely correlated with age at diagnosis, being highest in the 3 youngest age groups (33% age up to 39, 21% in 40-49 year olds, and 28% in 50-59) and lower at older ages (13% 60-69 years and 7% in 70 years or older; $p=0.017$). Men had higher HPV prevalence (25%) than women (11%; $p=0.021$).
Alcohol consumption and smoking were not associated with HPV DNA prevalence in HNSCC tissue.

Conclusions: HPV prevalence in Australian HNSCC patients was highest in oropharyngeal tumours, males those patients aged younger than 60 years at diagnosis.

OC 15-2

PREVALENCE AND DISTRIBUTION OF ALPHA AND BETA HPVS IN THE NASOPHARYNX

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Objectives: Traditionally, Alphapapillomaviruses (α-PV) are considered as pathogens predominantly found in anogenital lesions, and Betapapillomaviruses (β-PV) and Gammapapillomaviruses (γ-PV) are typically detected in skin and hair follicle specimens, and recently, also at other sites of the human body, such as the anal canal and head and neck region (oral cavity, larynx, oesophagus etc.). The aim of this study was to determine the prevalence and type distribution of HPV in nasopharynx using PCR protocols preferentially detecting α-PVs and β-PVs.

Methods: Stored DNA samples isolated from 175 nasopharyngeal swab specimens, collected from 75 men and 100 women (age range 0-77 years, median age 28.0 years) with suspected whooping cough, referred to routine PCR testing for Bordetella pertussis, were analyzed by general α-PV GP5+/GP6+ PCR and β-PV M^a/H^a nested PCR. HPV types were identified by direct sequencing of PCR products and Blast analysis.

Conclusions: β-HPV DNA was detected in 36/175 (20.6%) nasopharyngeal swab specimens, while no α-HPV DNA was detected in any of the 175 tested specimens. The most common HPV type was HPV38 (detected in 10 samples), followed by HPV12 (4 samples) and HPV36 (3 samples). HPV5, HPV15, HPV24 and HPV120 were detected in two samples each, while HPV47, HPV80, HPV93, HPV105, HPV145, HPV151 and HPV159 were detected in one sample each. In one sample, the presence of more than two HPV related sequences was recognized, thus preventing the exact determination of HPV types. In three samples infection with three putatively novel β-PV types was detected that were most closely related to HPV96, HPV120 and HPV124. To conclude, this study demonstrated a broad spectrum of β-HPV types being present in the nasopharynx. However, the clinical significance of the presence of β-HPV in nasopharynx remains elusive.

OC 15-3

ORAL HPV16 PERSISTENCE AMONG PARTICIPANTS OF THE HPV INFECTION IN MEN (HIM) STUDY

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Objectives: Persistent infection with oral HPV16 is thought to increase the risk of developing a subset of head and neck cancers. The goal of this study was to estimate oral HPV16 persistence among men.

Methods: A prospective analysis was conducted within the *HPV Infection in Men (HIM) Study*, a large, multinational HPV natural history study. Oral rinse/gargle specimens were collected every 6 months for up to 4 years, and oral cells underwent DNA extraction, PCR, and HPV genotyping (Linear Array and INNO-LiPA Extra). Men were included in this study if they reported no history of head/neck cancer and tested positive for oral HPV16 infection at ≥1 study visit. A 6-month persistent oral HPV16 infection was defined as a positive genotyping result at ≥2 consecutive study visits (~6 months apart), a 12-month persistent infection at ≥3 consecutive study visits, etc. An infection was cleared if it was followed by ?2 consecutive HPV16-negative visits.

Conclusions: Of the 1,626 men tested for oral HPV infection, 28 men tested positive for oral HPV16 (prevalence: 0.6% [n=10]; incidence rate: 0.8 per 1,000 person-months [95% CI: 0.5–1.3; n=18]). Men were aged 18¬–66 years (median: 36 years) and were followed for a median of 35.8 months (IQR: 28.2–48.7). Among the 23 men with sufficient data for persistence analyses, the median duration of oral HPV16 infection was 22.4 months (95% CI: 16.2–28.6), with 34.8% (n=8) of oral HPV16 infections lasting <6 months (transient infections) and 65.2% (n=15) persisting ≥6 months. Additional rates of oral HPV16 persistence are as follows: ≥12mo=56.5%, ≥24mo=45.0%, ≥36mo=25.0%, and ≥48mo=14.3%. Only 52.2% (n=12) of men cleared their oral HPV16 infection during the follow-up period. This is one of the largest prospective studies to examine oral HPV16 persistence among adult men in multiple countries. There is no standard or routine screening test available for HPV-related head and neck cancers; however, we are working toward identifying high-risk groups to inform prevention efforts for HPV-related oropharyngeal cancers.

OC 15-4

CYTOPATHOLOGY AND HPV TESTING IN THE DETECTION OF HEAD AND NECK SQUAMOUS CELL CARCINOMA

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Objectives. To evaluate cytological sampling for HPV testing and morphological interpretation as a diagnostic tool for Head and Neck Squamous Cell Carcinoma (HNSCC) detection.

Methods. Samples were collected by cytobrushing in PreservCyt (Hologic) and evaluated by liquid-based cytology for morphology and by the Linear Array HPV Genotyping test (Roche Diagnostics) for HPV infection.

Conclusions. We analyzed 164 cytobrushings from: 74 cancer cases (31 Oral Cavity, OC; 43 Oropharynx, OP); 40 non-malignant lesions (30 OC, 10 OP); 50 healthy mucosae (16 OC, 34 OP). 106 patients also underwent a biopsy. The presence of cytological abnormalities resulted significantly associated with histologically confirmed cancer (OR= 9.18, 95% CI: 3.27-26.49). None of the OC healthy mucosa samples was HPV positive, while 4/30 OC benign lesions (13.3%) and 2/31 OCSCC cases (6.4%) were HPV positive.

We detected HPV infection in 3/34 OP healthy mucosa cases (8.8%), 3/10 OP benign samples (30.0%) and 25/43 OPSCCs (58.1%), 22 of which presented HPV16 genotype alone or as a coinfection (88.0%). Comparing HPV test results on cytological and corresponding histological material (OCSCC= 25 and OPSCC= 27), we found that 47/52 cases were concordant, showing an excellent raw agreement (90.38%) and K value (0.796, p<0.001). We observed that a patient with an HPV positive cytobrush had a nearly 3-fold higher risk of having abnormal cytology, compared to an HPV negative one (OR= 2.81, 95% CI: 1.20-6.70), but this association was significant only for the OP (OR= 4.57, 95% CI: 1.57-13.57).

We also observed that the patients with abnormal cytology and positive HPV test had more than a 6-fold higher risk of having a cancer (OR= 6.47, 95% CI: 1.32-42.96) compared to patients with at least one negative test. Notably, among the 20 cytologically negative OP cases, 7 were HPV positive and 6 out of these 7 were cancer cases. Therefore, HPV positive OP patients with negative cytology had a more than 9-fold increased risk of cancer compared to those presenting negative results to both the analyses.

OC 15-5

THE ICO STUDY ON HPV DETECTION AND GENOTYPE DISTRIBUTION IN HEAD AND NECK CANCERS: RESULTS FROM THE EUROPEAN REGION

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Objectives: To estimate the worldwide HPV/DNA prevalence and type-specific distribution in head and neck cancers (HNC) in Europe.

Methods: Formalin-fixed paraffin embedded HNC tissues including oral cavity, pharynx and larynx, were collected from pathology archives of collaborating centers worldwide. After centralized histopathological evaluation, HPV/DNA detection was performed using SPF-10 PCR/DEIA/LiPA$_{25}$ (version 1). Cellular protein expression of p16, pRb, Cyclin D1 and p53 was performed.

Results: 2,121 HNC cases (571 from the oral cavity, 1,003 from the pharynx, and 547 from the larynx) were retrieved and HPV analyzed from 12 European countries from 1990 onwards. Overall HPV/DNA detection in HNC was 15.2%: 10% in oral cancer, 23.9% in pharyngeal cancer, and 4.8% in laryngeal cancer. Concerning pharyngeal subsites HPV prevalence was 26.1% in oropharynx, 20% in pharynx unspecified, 14.9% in nasopharynx, and 4.8% in hypopharynx. HPV16 was by far the most common type among HPV positive HNC cases (79.3%), followed by HPV33 (3.2%), HPV35 (1.9%), HPV18/51/52 with a prevalence of 1.2% each. Percentage of multiple infections among HPV positive HNC samples was low (1.9%). Results by European regions, sociodemographic and histological information as well as immunohistochemistry results will be presented at the meeting.

Conclusions: HPV DNA detection in European HNC cases varied by subsites, being the highest in the oropahrynx (26.1%) and the lowest in the larynx (4.8%). HPV16 was highly predominant among positive HNC. This information is essential to assess the potential value of current and future HPV vaccines for the prevention of these cancers.

OC 15-6

GENETIC POLYMORPHISMS IN miRNA GENES AND HPV STATUS AS BIOMARKERS FOR RESPONSE IN OROPHARYNGEAL CANCER PATIENTS

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Objectives: To identify non-tumor biomarkers for the prognosis of patients with squamous cell carcinoma of the oropharynx (SCCOP), we evaluated the value of genetic polymorphisms in miRNA genes as additional markers to HPV status for prognosis of survival of SCCOP patients.

Methods: A group of 72 patients with SCCOP treated with radiotherapy or concomitant platinum-based chemoradiotherapy were considered. HPV status was determined in tumor specimens by CISH and p16 immunohistochemistry (IHC). Two polymorphisms in pre-miRNAs (hsa-mir-146a rs2910164, has-mir-196a2 rs11614913), six polymorphisms in miRNA biosynthesis genes (Drosha: rs639174, rs3805500, rs10520985, rs17410035; XPO5: rs2227301, rs699937) and two polymorphisms in miRNA target genes (KRAS: rs61764370; SMC1B: rs3747238) were genotyped by restriction fragment length polymorphism analysis (RFLP) or high resolution melting (HRM) analysis. Overall survival (OS), disease specific survival (DSS) and disease-free survival (DFS) were considered in the analyses (Kaplan-Meier).

Conclusions: In our study population, 20% of the patients were HPV positive following CISH, whereas 30% of the patients were p16 positive. HPV positive patients (CISH) had a better 2-year OS (100% versus 67%, p=0.018) and a better 2-year DSS (100% versus 78%, p=0.071) compared to HPV negative patients. Statistically significant associations with survival were found for T-stage (p=0.019 for OS, p=0.047 for DFS) and alcohol use (p=0.050 for OS, p=0.019 for DSS, p=0.010 for DFS). Moreover, a statistically significant association was found between survival and the Drosha rs3805500 polymorphism (p=0.003 for OS, p=0.046 for DFS). Our findings indicate that genetic polymorphisms in miRNA biosynthesis genes may be additional prognostic markers for SCCOP. This study will be extended in the future.

OC 15-7

IMPROVED SURVIVAL FROM HEAD AND NECK CANCER IN DENMARK 1978-2010. FOCUS ON HUMAN PAPILLOMAVIRUS ASSOCIATED TUMORS.

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Objective: Describe trends in the survival for patients with head and neck cancers (HNCs) by stage, gender and association with human papillomavirus in Denmark between 1978 and 2010.

Methods: Using data from the Danish Cancer Registry (1978-2010), we categorized HNCs into 4 groups (based on the literature) according to their association with human papillomavirus (HPV): highly associated (oropharynx), moderate association (oral cavity), unclear association (larynx), and not associated (''other''). Kaplan- Meier estimate curves for the whole period (1978-2010) were generated according to calendar period and HPV association. In addition, a Cox proportional hazards model was used to identify factors having an impact on the 5 year survival. Hazard ratios (HR) adjusted for gender, age, calendar period, tumor stage and HPV association were computed.

Conclusion: An improved overall survival from HNC through the period 1978-2010 was found. The 5 year survival was highest following laryngeal cancer; however the improvement in survival was most pronounced for the highly HPV-associated group (34.6% in 1978-1989 to 48.6% in 2000-2005) whereas the laryngeal tumors did only show a modest improvement in survival over time (56.6% in 1978-1989 to 59.2% in 2000-2005).
In the Cox regression analysis we found that compared to HNCs not associated to HPV, tumors at highly HPV associated or laryngeal sites had a better 5-year survival after adjusting for age, gender, and tumor stage at the time of diagnosis ((HR=0.80 (95% CI; 0.75-0.86) and HR=0.62 (95% CI; 0.58-0.66) respectively). Furthermore, a better 5-year survival was seen with decreasing age and stage of tumor at diagnosis and female gender for all four groups of head and neck cancer.

OC 15-8

NATURAL HISTORY AND RISK FACTORS OF ORAL HPV INFECTION IN HIV-INFECTED AND HIV-UNINFECTED WOMEN AND MEN

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Background: Oral HPV prevalence is higher in the HIV-infected (HIV+) than HIV-uninfected (HIV-) people, which could be explained by higher incidence and/or reduced clearance of oral HPV.
Methods: Semi-annual oral rinse samples were collected from HIV+ (327 men/433 women) and at-risk HIV- (222 men/244 women) participants in the Multicenter AIDS Cohort Study and the Women's Interagency HIV Study for up to three years. Samples were tested for 37 HPV genotypes using the linear array and line blot hybridization. Newly detected oral HPV type-specific infections were considered incident. An infection was considered cleared at first negative visit. Risk factors were explored using the multivariate Wei-Lin Weissfield method.
Results: Any oral HPV and HPV16 incidence were higher in HIV+ than in HIV- (any type: 22.9 vs. 10.1 infections/1000 person-months, p<0.001; HPV16: 1.1 vs. 0.45 infections/1000 person-months, p=0.07). Incidence increased with HIV seropositivity (aHR=2.2, 95%CI=1.7-3.0), with CD4<200 (aHR=5.0, 95%CI=3.3-7.4) and as the number of recent and lifetime oral sex partners increased (all p-trend<0.01). Clearance of infections within twelve months was high and did not vary by HIV serostatus (clearance of any incident HPV: HIV+ 85% vs. HIV- 86%). Oral HPV clearance was higher in incident than prevalent HPV (aHR=2.9, 95%CI=2.4-3.5) and higher in women than in men (aHR=1.4, 95%CI=1.1-1.9). In men, clearance was higher in those with a younger age and CD4≥200 (both p<0.01), while for women, it was associated only with never using cigarettes (p<0.01). Results were similar when the analysis was restricted to HPV16 or to all oncogenic types and if two sequential negative results were used to define clearance.
Conclusions: The majority of incident and prevalent oral HPV infections in both HIV+ and HIV- adults were not detected by twelve months of follow-up, suggesting rapid clearance. The higher prevalence of oral HPV in HIV+ than HIV- women and men is likely due to a higher rate of acquisition or reactivation of oral HPV.

OC 15-9

INCIDENCE OF HPV-ASSOCIATED TONSILLAR AND BASE-OF-TONGUE CARCINOMAS FROM THE NORTHERN NETHERLANDS OVER A 16 YEAR PERIOD

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Objectives HPV-associated oropharyngeal cancer has been reported at different frequencies in various countries. Because HPV-associated oropharyngeal tumors behave differently from non-HPV-associated tumors, both biologically and clinically these tumors are considered a separate entity. The aim was to analyze HPV incidence in oropharyngeal tumors treated in the northern part of the Netherlands and compare clinical outcome of HPV-associated vs. non-HPV-associated tumors.

Methods Based on data from the Netherlands cancer registry, we collected clinical, pathological, and follow-up data and formalin-fixed paraffin embedded samples of a complete cohort of 196 tonsillar and base-of-tongue oropharyngeal tumors treated in our centre from 1997-2012. HPV testing was performed using an established algorithm of p16 immunohistochemistry, followed by HPV-PCR of the positive cases. HPV-BRISH was performed as extra control of p16 negative cases. p16 staining could be assessed on 193 cases, and was positive in 64/193 (33%). Of these 64 cases 47 (73%) were HPV-GP PCR-positive; 42 cases were HPV16+, 1 HPV18+, 3 HPV33+ and 1 HPV35+. HPV-BRISH showed 34/180 (19%) positivity. HPV-associated tumor incidence increased from 13% between 1997-2004, to 30% between 2005-2012. HPV-positivity was an independent predictor for longer disease-specific survival (HR=0.22; 95%CI:0.10-0.47).

Conclusions In the period 1997-2012, 24% of the tonsillar and base-of-tongue tumors in the Northern Netherlands was high-risk HPV-positive. This incidence is lower than in other regions in the Netherlands (33%) and in Europe (40%). A large increase in incidence was observed in the more recent years. The relatively low incidence might be due to the high percentage of smokers in our region, associated with non-HPV-associated oropharyngeal tumors. HPV-associated oropharyngeal tumors show a significantly longer disease-specific survival.

OC 15-10

E6 ONCOPROTEIN AS A MARKER FOR HPV INDUCED CANCERS OF THE HEAD AND NECK

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Objectives: The incidence of oral pharyngeal cancers is increasing worldwide, with HPV infection becoming a more prominent risk factor, while tobacco and alcohol abuse decrease as the major causes. Oral pharyngeal cancer is debilitating, also due of the harsh chemo and radio therapy based treatments. HPV induced oral pharyngeal cancers appear to respond better to treatment and may hence afford a less severe treatment. It is therefore important to identify markers that allow the assessment of whether or not an oral pharyngeal cancer is driven by HPV oncogenic activities, rather than HPV infection being an innocent bystander. Elevated expression of the viral E6 oncoprotein is necessary for HPV induced cervical oncogenesis, suggesting that E6 could also be an informative biomarker of oral pharyngeal cancer. Applying the OncoE6™ Test (short: E6 Test) technology developed by Arbor Vita Corp., we tested for HPV E6 oncoprotein expression in oral pharyngeal cancer specimens. The E6 Test consists of a simple lateral flow test for detection of elevated levers of E6 oncoprotein of HPV types 16 and 18.

Methods: Fine needle aspirations (FNA) and polyester swab samples of the bottom of the tongue and saliva specimens were collected into OMNIGENE DISCOVER RNA/DNA collection vials (DNA Genotek). 31 patients with biopsy, FNA and/or clinically confirmed cancer were analyzed for HPV status, for p16 histological staining and for E6 oncoprotein expression.

Conclusions: Of 31 oral pharyngeal cancers (28 male and 3 female subjects) 23 (74.2%) were HPV DNA positive by the Cervista test (Hologic / Gen-Probe), and 21 of the HPV DNA positive specimens tested positive for E6/E7 mRNA by the APTIMA test. The E6 Test was positive for 15 FNA specimens (65.2% of Cervista DNA positives; 71.4% of APTIMA E6/E7 mRNA positives). All E6 Test positives were of HPV type 16 and were positive for both the Cervista (DNA) and the APTIMA (E6/E7 mRNA) tests. None of the swabs from the bottom of the tongue and none of the saliva specimens tested positive for E6 oncoprotein. In this study, we demonstrate for the first time E6 oncoprotein expression in oral pharyngeal tumor tissue. We will discuss potential of the E6 oncoprotein as a marker for HPV driven oral pharyngeal cancer, and possible implications for decisions on therapy.

OC 16-1

AN ANALYSIS OF THE COBAS HPV TEST PERFORMANCE IN MULTIPLE US LABORATORIES

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Background: When new tests are being evaluated for clinical use in the U.S., performance of the test is typically assessed by conducting a method correlation with the existing test serving as the reference method. In such a study, evaluation of test performance takes into consideration a laboratory's unique patient population, which serves as the source material. As population characteristics have the potential to vary across geographic regions, compilation of data from several institutions allow for a generalized assessment of test performance and reduction in possible site-specific bias. Several laboratories across the U.S. have evaluated the performance of the cobas® HPV Test, approved by the FDA in April 2011, which provides specific genotyping information for HPV genotypes 16 and 18, while simultaneously reporting the 12 other hrHPV types as a pooled result, all in one run, from one patient sample.

Objective: To compare performance of the cobas® HPV Test (Roche Diagnostics Corporation) to hc2 (Digene) and Cervista™ HR HPV (Hologic) in multiple U.S. laboratories conducting routine HPV cervical cancer screening.

Method: About 1900 cervical specimens collected into ThinPrep® Pap Test™ PreservCyt® Solution from multiple different laboratories were tested using the cobas® HPV Test: over 1,000 of which were also assessed by hc2 and around 800 on the Cervista™ HR HPV test. Each laboratory performed their own testing, comparing the cobas® HPV Test results to their current HPV testing method as part of an in-house evaluation of the cobas®4800 system. Adjudication of about 150 specimens discordant between either cobas and hc2 or between cobas and Cervista was performed utilizing Linear Array (LA; Roche Molecular Systems) in a blinded-fashion.

Results: Across all laboratories, overall agreement between the cobas® HPV Test and hc2 was about 90% with a kappa value of 0.8; positive and negative agreements were about 84% and 96%, respectively. The overall agreement between cobas® HPV Test and Cervista™ HR HPV test was around 86% with a kappa value under 0.7; positive and negative agreements were less than 75% and 93%, respectively. Linear Array data demonstrated that over 60% of hc2(+)/cobas(-) discrepancies resulted from cross-reactivity of the hc2 test to low risk HPV types, of which genotypes 53, 67, and 70 were the most common [insufficient data were available for the HC2(-)/cobas(+) analysis]. Of the Cervista(+)/cobas(-) discrepants on which LA was performed, greater than 50% were HPV-negative by LA and none were confirmed hrHPV-positive by LA. In contrast, about 50% of cobas(+)/Cervista(-) samples were hrHPV-positive by LA. There were 5 cobas HPV18+ and 1 cobas HPV16+ specimens, confirmed by LA, that were negative by Cervista.

Conclusions: This analysis across multiple laboratories demonstrated good agreement between the cobas® HPV Test and hc2 hrHPV test and moderate agreement with Cervista™ HR HPV. Linear Array testing indicated that the majority of hc2-cobas discrepants were due to low risk HPV cross-reactivity by the hc2 assay whereas the Cervista-cobas discrepants were more commonly HPV-negative by LA (when cobas results were negative) and were high risk HPV-positive when cobas was positive.

OC 16-2

COMPARISON OF THE CAREHPV AND HYBRID CAPTURE-2 ASSAYS FOR THE DETECTION OF HIGH-RISK HPV GENOTYPES IN HIV-POSITIVE AFRICAN WOMEN

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Objectives: The careHPV assay (Qiagen) is a new HPV DNA test for low-resource settings detecting 14 high-risk (hr) HPV genotypes. The objective of this study was to conduct the first comparison study with the Digene Hybrid Capture 2 (HC2) assay (Qiagen) for the detection of hr-HPV genotypes in HIV-infected African women enrolled in the HARP (HPV in Africa Research Partnership) cohort study.

Methods: The two assays were directly compared on 149 paired cervical samples, 75 collected in Johannesburg (South Africa) and 74 in Ouagadougou (Burkina Faso). For each woman, two consecutive cervical samples were collected, the first with the careHPV cervical sampler brush and collection medium and the second with the Digene cervical sampler device. The two assays were performed following the manufacturer's instructions. HPV genotyping, using the INNO-LiPA HPV genotyping Extra assay, was performed on samples for which a discrepant result was observed.

Results: Prevalence of hr-HPV was 37.6% (56/149) by careHPV and 34.9% (52/149) by HC2. Agreement between tests was 94.6% (141/149): 96.0% (72/75) in South Africa and 93.2% (69/74) in Burkina Faso. The Kappa test value of 0.88 indicated 'excellent agreement' ($p<0.0001$). Six samples (4%) were positive by careHPV and negative by HC2, all of which were positive for hr-HPV by INNO-LiPA. Two samples (1.3%) were negative by careHPV and positive by HC2: one sample was found positive for the low-risk type HPV6 by INNO-LiPA, and the other was positive for hr-HPV 51 and low-risk types HPV69/71 and HPV70 by INNO-LiPA.

Conclusions: There was overall excellent agreement between the careHPV and HC2 assays. These results indicate that careHPV would be as suitable as HC2 for cervical cancer screening among HIV-positive women in resource constrained settings.

OC 16-3

POINT-OF-CARE DETERMINATION OF HIGH RISK HPV INFECTION USING ONCOTECT 3DX® POC PARAMETERS AND BIOINFORMATICS

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Using a combination of morphometric, molecular, and inflammatory classifiers on liquid based cervical cytology samples, we were able to determine the presence or absence of high risk HPV in approximately 5 minutes without the use of molecular methods to detect HPV DNA. We determined the analytic performance of our POC assay using 65 normal cytology, HPV DNA+ samples and 43 normal cytology, HPV DNA- samples.

Using four classifiers (ectocervical mean corpuscular volume (MCV), pyknotic/non-pyknotic ectocervical cell ratio, ectocervical Post G1% (cell-cycle), and PMN%), we developed a classification scheme that allows parameterization of SVM classifiers. By using cross validation and brute-force combinatorial sampling, we optimized combinations of parameters for two types of SVM classifiers in order to boost general classification accuracy, and particularly the Specificity (true negative predictive ability) of the classifier. This has resulted in the following three classifiers with noted properties:

1 - C_SVC Radial basis function SVM
2 - C_SVC Polynomial SVM
3 - Nu_SVC Radial basis function SVM

The classifier performance yielded the following results:
#1 Clinical Accuracy 88%, Sensitivity 79%, Specificity 95%
#2 Clinical Accuracy 89%, Sensitivity 84%, Specificity 93%
#3 Clinical Accuracy 90%, Sensitivity 87%, Specificity 93%

Current iterations of the algorithm are being developed to maximize sensitivity knowing that false positive will go on to routine screening in cytology laboratories.

OC 16-4

COMPARATIVE EVALUATION OF THE REAL-TIME PCR BASED LIPA ASSAY FOR THE DETECTION AND THE GENOTYPING OF HUMAN PAPILLOMAVIRUS IN CERVICAL SPECIMENS VERSUS CLART® HPV-2 MICRO ARRAY AND CONVENTIONNAL INNO-LIPA EXTRA GENOTYPING TESTS

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Objectives. The detection and genotyping of Human Papillomavirus (HPV) by molecular techniques is of high interest for the screening of cervical cancer. The analysis of these HPV-related markers is also valuable for epidemiological studies, vaccine trials and fundamental research purposes. A new two-step approach combining a real-time PCR based on SPF10 primers for broad-spectrum HPV detection and a reverse line probe assay (LiPA) used for the typing of 28 different HPV genotypes has been developed (Real Time LiPA Assay (Innogenetics)).

Methods. A prospective study was conducted on 110 cervical specimens with cytopathological findings of ASCUS or LSIL, in order to compare this new assay to the Clart® HPV2 test (Genomica) and the conventional INNO-LiPA HPV genotyping extra assay (Innogenetics). A positive result was asserted when a sample was detected positive for HPV by at least 2 out of 3 assays. The sensitivity of SPF10 real-time PCR, INNO-LiPA extra and Clart® HPV2 was 95.2, 100 and 93.6 %, respectively. Complete or partial agreement's rates for single or multiple genotypes detection were 98.3, 100 and 96.6% between SPF10 Real-Time and Clart® HPV2 assays, SPF10 Real-Time and INNO-LiPA extra assays, and between INNO-LiPA extra and Clart® HPV-2 tests, respectively. The 8 most frequent HPV types attributed to cervical cancer (HPV 16, 18, 31, 33, 35, 45, 52 and 58) were equally detected by the 3 assays.

Conclusion. Since only positive samples obtained by the SPF10 real-time PCR are further genotyped by the LiPA assay, this new approach offers reduced hands-on time, workload and cost for HPV diagnosis.

OC 16-5

CLINICAL AND ANALYTICAL EVALUATION OF THE REAL-TIME PCR HPV ASSAY ON SUREPATH SAMPLES FROM WOMEN WITH ABNORMAL CYTOLOGY

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Objectives: The novel BD Onclarity HPV assay on the BD VIPER LT system (BD Diagnostics, Baltimore, US), detects 14 high risk HPV genotypes, with individual results on five genotypes (16, 18, 31, 51, 52) andthree pooled genotype results (33/58, 56/59/66,35/39/68). We compared the performance of this assay to that of Hybrid Capture 2 (HC2; Qiagen, Gaithersburg, MD) and Linear Array (LA; Roche, Pleasanton, CA) in women with abnormal cytology.

Methods: 403 SurePath samples showing ≥ASCUS were collected in September-October 2012 from routine cytology screening at Copenhagen University Hospital, Hvidovre, Denmark. All samples were tested on the Onclarity, HC2, and Linear Arrayassays in concordance with the manufacturers' protocols. Women were routinely recommended for repeated cytology testing or colposcopy. Their histological outcomes were retrieved in May 2013 from the Danish Pathology DataBank. The study protocol was approved by the regional ethical committee.

Results: Of the 403 women, 104 (26%) had ASCUS, 161 (40%) LSIL, 30 (7%) ASC-H, 107 (27%) HSIL, and one (<1%) had cytological signs of carcinoma. In total, 82% tested positive on Onclarity, 88% on HC2, and 83% on LA. The positive agreement between Onclarity and HC2 was 88%, and between Onclarity and LA it was 93%. The histological outcomes are at present known for 233 (58%) women. Among these, 51 had ?CIN3 (including 4 cervical cancers), and 30 had CIN2. All three HPV assays detected 50 ≥CIN3 (sensitivity: 98%). Onclarity detected 80 out of 81 ≥CIN2 (sensitivity: 99%), whereas HC2 and LA each detected 79 (sensitivity: 98%; not significantly different compared to VIPER). Onclarity tested negative in 34 out of 149 women with less than CIN2 or with CIN not otherwise specified (NOS) (specificity: 23%). HC2 tested negative in 17 women (specificity: 11%, significantly lower), and LA in 23 women (specificity: 21%, not statistically different). In conclusion, the clinical and analytical performance of Onclarity HPV test was similar to that of LA, whereas the clinical specificity was somewhat higher than that of HC2

OC 16-6

COMPARISON OF A NEW PCR TECHNIQUE WITH GOLD STANDARD SIGNAL AMPLIFICATION HPV TESTING IN ROUTINE

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Objectives: Up to now the Digene HC2 test (Qiagen) has been the gold standard for routine HPV DNA testing. Meanwhile several new HPV test systems are available, among them the cobas HPV assay (Roche Diagnostics) with simultaneous genotyping for HPV 16 and 18. We compared the performance of this test with the HC2 retrospectively and prospectively in routine cervical samples.

Methods: In the retrospective approach 1781 anonymized routine specimens pretested with the HC2 test out of Preservcyt (Hologic) and STM (Qiagen) vials (≤3years at RT) were analyzed with the cobas HPV real time PCR assay. Cases with discrepant results were retested with the Linear Array HPV genotyping test (LA) (Roche). Partially, histologic diagnoses were available.

For the prospective analysis 1154 anonymized routine specimens pretested ≤10 days ago with the HC2 test out of Preservcyt and STM vials were analyzed with the cobas test. LA genotyping of cases with discrepant result is planned.

Results: In 1566 (87.9%) of the retrospectively analyzed 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 263 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. With increasing severity of the cytologic findings the rate of HPV-16 and -18-positivity increased proportionally.
In the prospective analysis in 1100 of 1154 cases (95,3%) the results of cobas HPV and HC2 test were concordant. There was no difference in the rate of concordance between specimens from Preservcyt and STM vials.

Conclusions: In retrospectively and prospectively analyzed 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.

OC 16-7

DEVELOPMENT OF A SINGLE USE GENEXPERT CATRIDGE ASSAY FOR HPV DIAGNOSTICS

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Objectives: The objective of this study was the clinical validation of a single use test cartridge (the Xpert® HPV Assay) for the detection of high-risk types of HPV on the Cepheid GeneXpert® instrument systems. The Xpert HPV Assay is a qualitative, rapid (~60 min), real-time PCR test for the detection of fourteen types of HPV (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) from a small aliquot of a liquid-based cytology specimen (ThinPrep). The test specifically identifies HPV16, HPV18 and 45 in a pooled result, and concurrently detects the remaining high risk types.

Methods: A two-stage, multicenter, prospective study was conducted to recruit women of all ages referred for colposcopy evaluation based on one or more prior abnormal Pap test results, an abnormal Pap test result in combination with a positive high-risk HPV test result, or other clinical suspicion of cervical cancer. Two ThinPrep specimens were collected from each subject at the time of colposcopy to support cytology review and comparator testing with the Xpert HPV Assay, the Qiagen hc2 High-risk HPV DNA Test, and the Roche cobas HPV Test. A minimum of two cervical punch biopsies were collected from each subject as well as an ECC for unsatisfactory colposcopy evaluations in which there was poor visualization of the squamocolumnar junction. Pathology review of the biopsy and ECC specimens was first conducted locally and then retrospectively, in blinded fashion, by a panel of three expert review pathologists to determine a consensus final cervical disease status. Stage I of recruitment was used to estimate a set of clinical cutoffs for the assay relative to ?CIN2 and ?CIN3 disease end points. Stage II two was used to validate these cutoffs.

Conclusions: An interim analysis of the Stage II data set (n = 371 subjects) demonstrated overall agreement between the Xpert HPV Assay and the Qiagen and Roche HPV tests of 88.2% (95% CI 84.6-91.2) and 91.7% (95% CI 88.6-94.2), respectively. Clinical sensitivity and specificity of the Xpert HPV Assay as assessed by local pathology for ?CIN2 were 92.3% (95% CI 84.8-96.9) and 46.1% (95% CI 40.1-52.1), respectively. The Xpert HPV Assay appears to be a promising new tool for the detection of high-risk HPV.

OC 16-8

CLINICAL VALIDATION OF THE PAPILLOCHECK ASSAY ACCORDING TO INTERNA-TIONAL GUIDELINES FOR HPV TEST REQUIREMENTS FOR CERVICAL SCREENING

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Objective: To compare the clinical performance of the PapilloCheck assay (Greiner Bio-One, Germany) with that of the clinically validated high-risk HPV GP5+/6+-PCR according to the international guidelines for HPV test requirements for cervical screening1.

Methods: 1,437 cervical scrapings of women without evidence of CIN2+ and 192 of women with CIN3+, originating from a population-based screening cohort (POBASCAM) were tested with both PapilloCheck and the GP5+/6+-PCR. In addition, intra-laboratory reproducibility over time and inter-laboratory agreement of the PapilloCheck assay were assessed using another set of 550 cervical samples. Clinical sensitivity and specificity values of the PapilloCheck assay were compared with those of GP5+/6+-PCR by non-inferiority score testing using pre-viously defined thresholds for non-inferiority, i.e. relative sensitivity for CIN2+ ≥90 % and rela-tive specificity for CIN2+ ≥98 %. For intra- and inter-laboratory agreement a lower confidence bound not less than 87 % was used as threshold.

Conclusions: When restricting PapilloCheck analysis to the 14 hrHPV types targeted by GP5+/6+-PCR the clinical sensitivity and specificity were 95.8 % (95 %CI 92.8-98.8) and 96.7 % (95 %CI 95.7-97.7), respectively. By comparison, these figures were 96.4 % (95 %CI: 93.9-98.9) and 97.7 % (95 %CI: 96.9-98.5), respectively, for GP5+6+-PCR. Both the clinical sensitivity and specificity of PapilloCheck were non-inferior to that of GP5+/6+-PCR (non-inferiority score test; $P<0.0001$ and $P=0.007$, respectively). High reproducibility values for HPV-positivity were obtained for both the intra-laboratory reproducibility overtime (97.6 %; 95 %CI 96.3 %-98.6 %; kappa value 0.941) as well as the inter-laboratory agreement (94.0 %; 95 %CI 92.1 %-95.7 %; kappa 0.842). Thus, when only the 14 hrHPV types targeted by the GP5+/6+-PCR are considered, PapilloCheck fulfils the cross-sectional clinical and repro-ducibility criteria of the international guidelines for HPV test requirements for cervical screen-ing and can be considered clinically validated.

OC 16-9

PERFORMANCE OF THE ONCO E6 CERVICAL TEST IN THE START-UP CLINICAL STUDY IN RURAL CHINA

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Objectives: Development and implementation of appropriate screening technologies for lower resource settings constitutes an important priority towards reduction of cervical cancer. The Onco***E6***™ Cervical Test (E6 test; developed by Arbor Vita Corp.) directly detects elevated levels of E6, a viral oncoprotein necessary for cervical epithelial cell transformation. The E6 test is lateral flow based ("strip test") and can be performed without the need for complex infrastructure or a cold chain. Clinical performance of the E6 test was studied in START-UP, a large population-based screening study in China.

Methods: 7,543 women aged 25-65 years in three different rural locations in China were enrolled and specimens were applied to HPV DNA tests (careHPV and HC2), to VIA and to the E6 test (E6 of HPV types 16, 18, 45). Women who screened positive for any test and a 10% random sample of those negative on all tests underwent colposcopic evaluation.
Results: 14-18% of the study population tested positive for HPV DNA tests, 7.3% for VIA, and 1.8% for the E6 test. Specificity for CIN3+ was 98.9% for the E6 test, 86.6% / 83.1% (clinician- / self collected) for HPV DNA test (HC2), and 93.4% for VIA. Sensitivity for CIN3+ was 97.0% / 90.0% for HPV DNA detection (clinician- / self collected), 57% for VIA and 53.5% for the E6 test; Notably, the E6 test had by far the greatest positive predictive value (PPV) at 40.8% for CIN3+, compared to the other tests, all of them had a PPV of <10%.

Conclusions: START-UP study data reveal the Onco***E6***™ Cervical Test's distinct clinical performance characteristics than compared to HPV testing or to VIA. The E6 test's high clinical specificity and PPV for high grade disease suggest substantial reduction of unnecessary follow-up procedures when applied both in a triage setting or in primary screening. Implications of the E6 test's clinical performance and its ease of use for implementation in lower resource settings will be discussed.

OC 17-1

EVALUATING HPV VACCINATION STRATEGIES IN CANADA USING CANCER RISK MANAGEMENT HPV MICROSIMULATION MODEL (CRM-HPVMM)

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Objectives: CRM-HPVMM is a Canadian web-based decision-support modeling platform that projects the population-based impact of HPV vaccination strategies on HPV infections. The presentation provides a model overview and its functionality using sample vaccination scenarios, focusing on the issue on vaccinating boys.

Methods: CRM-HPVMM is a continuous-time, interacting-agent, Monte-Carlo microsimulation model that simulates sexual behaviour and HPV transmission. Six HPV serotypes (6, 11, 16, 18, other non- and carcinogenic) and two vaccines (bivalent, quadrivalent) were modeled. Model structure is largely based on Van de Velde et al (2010). Input parameters include demography, sexual behaviour (sexual debut, partnership formation/separation) and biology (virus transmission, clearance, natural immunity). The simulation ran with 250,000 individuals with 50 years of projection. Results were scaled to reflect the Canadian population aged 10+ in 2011.
Multiple parameter sets were estimated through Latin Hypercube Sampling to incorporate parameter uncertainty. Parameter sets were selected by comparing to behavioural/ biological data from Canadian epidemiological literature and population health surveys. Parameter priors and targets were informed by Van de Velde et al, except for HPV prevalence targets, which are principally from the FOCAL trial.
Various vaccination scenarios can be compared by altering three parameters: vaccine program design (age, sex, program years, participation rate, vaccine type), vaccine duration of protection (lifetime versus declining proportion protected), and efficacy (per-act transmission protection probability). Results of the impact of girls-only versus adding boys strategies (with varying vaccination rates) on HPV prevalence projections will be provided. Potential impact of vaccine duration of protection and efficacy assumptions on the projections will be also explored.

Conclusions: CRM-HPVMM is a powerful tool, allowing researchers and policy makers to run a complex model in a simple and flexible manner via the internet. A variety of timely vaccination scenario comparisons, including the potential benefit of vaccinating boys to be shown, can be readily assessed.

OC 17-2

TOWARDS THE TRUE VALUE OF UNIVERSAL HPV VACCINATION: BROADENING THE SCOPE OF BENEFITS IN EVALUATING VACCINE.

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Objectives Health-economic evaluations of vaccines tend to focus on a narrow set of benefits — most notably avoided medical care costs — to assess cost effectiveness. However, these fail to account for all benefits of prevention to society, such as addressing health inequalities and unmet need, access to treatment.
HPV-related conditions are universal, while HPV vaccination is not. The International Agency for Research on Cancer acknowledges HPV as a human carcinogen affecting both sexes. Australia, the US and Canada are funding universal HPV vaccination, acknowledging the importance of direct protection for boys. Europe starts to follow this trend. However, the value of universal vaccination is still questioned.
Our objectives are to identify the types of value generated by HPV vaccination, and which of these values can be captured by conventional health-economic evaluations.

Methods A targeted literature review was performed to identify types of economic value generated by HPV vaccination, and which of these values are included or omitted in existing health-economic evaluations. Identified publications were screened to classify all costs and benefits of HPV-related conditions and HPV vaccination.

Conclusions The review identified a range of economic value types generated by HPV vaccination. These fall into three groups: 1) can be captured and are routinely incorporated in economic evaluations; 2) can be captured but are not routinely incorporated in economic evaluations; and 3) cannot be captured using conventional economic evaluation methods. Group 1 includes health costs and utilities of cervical cancer and genital warts. Group 2 includes herd immunity; health costs and utilities of non-cervical diseases; non-health costs, such as private practitioner costs, welfare payments; patient productivity loss; family/career productivity; cancer sequelae and fiscal consequences. Group 3 includes equality of access to prevention and anxiety about contracting/infecting others with HPV.
The review highlighted a number of benefits of universal HPV vaccination not captured in existing economic assessments. Some of these cannot be captured using conventional economic modeling approaches. Alternative methods must be developed to allow better understanding of the true economic value of universal HPV vaccination.

OC 17-3

HPV6/11/16/18 VACCINE EFFICACY IN WOMEN 24 TO 45: FOLLOW-UP THROUGH 6.3 YEARS POST-VACCINATION

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OBJECTIVES
We present the results of an interim analysis of a long-term follow-up study of qHPV in women aged 24-45 designed to determine the long-term immunogenicity, effectiveness, and safety of the qHPV vaccine. This report summarizes data collected as of year 6 post-vaccination (relative to day 1 of the base study). Future analyses are planned at years 8 and 10 (end-of-study analysis).

METHODS
This extension study was conducted in Colombia. Subjects who were vaccinated in the base study from Colombia are referred to as the "early vaccination group" (EVG) in this report. Subjects vaccinated after the completion of the base study (catch-up cohort) have not had sufficient follow-up as of this report.

CONCLUSIONS
Enrollment into the base study was 1,610 in total (804 randomized to qHPV, 806 randomized to with placebo). A total of 1,360 Colombian subjects participated in this extension (84% of the subjects enrolled in base study in that country). No new cases of HPV 6/11/16/18-related genital warts/cervical dysplasia have been reported in the EVG (N=684). Month 72 antibody titers against HPV 6/11/16/18 are comparable to those observed at Month 48 (end of base study), indicating no further diminution of titers between 4 years and 6 years post-vaccination. No serious adverse experiences have been reported in the EVG between years 4 and 6.

In summary, data through 6.3 years after the start of the 019 base study shows that administration of the qHPV vaccine among women aged 24-45 is generally well tolerated. Anti-HPV 6, 11, 16, and 18 responses have persisted over the long-term, and no additional cases of disease related to vaccine HPV types 6/11/16/18 were observed.

OC 17-4

THE PARTICIPATION OF HPV-VACCINATED ADULT WOMEN IN A NATIONAL CERVICAL SCREENING PROGRAMME IN SWEDEN

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Objectives: Concerns have been raised that HPV-vaccination might affect the cervical screening behavior of women vaccinated. Therefore, this study aims to investigate the potential consequences of opportunistic HPV-vaccination on cervical cancer screening uptake in Sweden.

Methods: A cohort of all women resident in Sweden born between 1977 and 1987 (n=611154) that were invited to cervical screening, was followed between October 2006 and December 2011. Data were collected using the Swedish national healthcare registers. The first invitation date to cervical screening within the study period defined the start of follow up. The primary outcome was a registered smear identified via the National Quality Register for Cervical Cancer Prevention. Data on vaccination status, including both the bivalent and quadrivalent vaccine, were obtained using the Swedish National Vaccination Register. Hazard ratios (HR) were estimated using Cox regression adjusted for attained age, education level. The exposure was vaccination status defined as a time-varying covariate. Analyses were carried out where women could contribute person time to the entire follow-up period (maximum of 5 years) and where women were censored after 2.5 years (around one average screening interval) of individual follow-up.

Conclusions: There were 3959 women vaccinated with at least one dose of the HPV-vaccine within the study period. The screening participation proportion was 77% in women vaccinated compared to 74% in those unvaccinated. Preliminary results show that the HR of screening participation in vaccinated women compared to unvaccinated women was 1.14 (95% CI=1.10-1.18). The results remained unchanged when follow-up time was restricted to 2.5 years. In conclusion, opportunistic HPV-vaccination is associated with a slightly increased likelihood of screening participation in Swedish women, when adjusting for age and education level.

OC 17-5

HPV VACCINATION UPTAKE IN SWEDEN IN OPPORTUNISTIC AND ORGANIZED VACCINATION PROGRAMS

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Objectives: HPV vaccination was introduced in Sweden in late 2006 in an opportunistic fashion and from early 2007 the vaccine was subsidized by around 50% for girls aged 13-17. An organized HPV vaccination program was not introduced until 2012, targeting girls aged 10-12, with catch-up of girls aged 13-18. The objective was to estimate vaccination rates and population coverage over time among girls aged 11-12 and 13-18 with special attention to changes in organization of HPV vaccination in Sweden.

Methods: The Swedish Vaccination Register (SVEVAC) was used to estimate rates and coverage of HPV vaccination from 2007 to 2012, with year-, sex- and age-specific population data retrieved from Statistics Sweden. Starting in January 2012 an organized and school-based HPV vaccination program, free of charge, was introduced for girls aged 10-12, with a catch-up program for girls aged 13-18. The catch-up program was mostly run in an opportunistic fashion, but without any charge. In some counties the catch-up program was school-based.

Conclusions: For girls aged 11-12 the vaccination rate was negligible before the start of the school-based program with less than 0.5% yearly. After one year of school-based vaccination the vaccination rate was almost 82% with a population coverage of the same magnitude. For girls aged 13-18 the vaccination rate went up from 4% in 2007 to 8% in 2008 and 10% in 2009. It then fell back heavily to 7% in 2011 and only 3% in 2011. With the start of the catch-up program the vaccination rate increased to 37% in 2012. The population coverage of HPV vaccination for girls age 13-18 was around 24% at the end of 2011 and had increased to 68% at the end of 2012. The introduction of organized HPV vaccination with no charge has increased the population coverage of HPV vaccination in Sweden drastically.

OC 17-6

SYSTEMATIC EVIDENCE ASSESSMENT FOR HPV VACCINATION IN MALES IN THE EUROPEAN HEALTH CARE CONTEXT BASED ON THE GRADE METHOD

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Objectives: The goal of this project was to develop and apply an extended methodological framework for evidence assessment based on GRADE using the example of human papilloma virus (HPV) vaccination in males in a European health care context.

Methods: A pan-European multidisciplinary expert group was established to conduct this project. We developed an extended GRADE framework that includes explicit assessment of cost-effectiveness, medical needs, as well as patient aspects, ethical and social issues. Using an expert panel process, we assessed the feasibility of using this framework by applying it to HPV vaccination in males in a European health care context. Studies were assessed using the specific framework tools, results and feasibility were discussed and consensus was retrieved through a modified Delphi method.

Results: We identified three advisory committees (ACIP, USA; NACI, Canada; STIKO, Germany) using GRADE for vaccines assessment and recommendations. Some institutions included health economic and other data, others focused on vaccine efficacy and safety. None of the institutions formally graded evidence on economic data. We adopted the grading methodology of ACIP for the key factor 'Benefits and Harms' and developed an extended framework for (1) grading the evidence type and quality for economic evaluations, (2) systematically assessing epidemiology, burden of disease and unmet medical needs, as well as (3) patient aspects, ethical and social issues. The feasibility test demonstrated that all developed framework components were feasible in the application to the case of HPV vaccination.

Conclusions: The GRADE approach is feasible in assessing vaccinations and has been successfully applied to HPV vaccination in males. The assessment of benefits and harms can be extended by a more explicit assessment of the evidence on cost-effectiveness and other key factors such as unmet medical needs, and ethical, social and patient aspects. This extended framework can help to better inform policy- and decision-makers for vaccines.

OC 17-7

POTENTIAL IMPACT OF A 9-VALENT HPV VACCINE IN INVASIVE CERVICAL CANCER IN 4 EMERGING ECONOMIES (BRAZIL, CHINA, INDIA AND MEXICO).

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Objective: We estimated the relative contribution (RC) to invasive cervical cancer (ICC) of nine HPV types (HPV 6/11/16/18/31/33/45/52/58) targeted by an HPV vaccine under development, in for 4 countries: Brazil, China, India and Mexico. These countries include 21.7% of the world's population, and contribute to almost half of new ICC diagnoses and deaths occurring annually worldwide.

Methods: The RC estimations were based on data from an international study providing information on 1,356 (86.7% of tested cases) HPV DNA positive ICC cases from these 4 countries [1]. HPV DNA was detected by SPF_{10}-DEIA-$LiPA_{25}$ system. RC was estimated among HPV positive samples, and multiple infections were added to single types using a proportional weighting attribution. Globocan 2008 and 2010 World Population Prospects were used to estimate current and future projections of new ICC cases.

Results: Overall RC of the nine HPV types in the four countries was 89.3% (95%CI: 87.6-91.0), with specific RCs of 85.7% (82.3-88.8) in Mexico, 88.6% (85.2-91.0) in Brazil, 92.2% (87.9-95.3) in India and 97.3% (93.9-99.1) in China. HPV16 and 18 were the most common types, with the highest contributions in China and India (RCs of 85.5% and 80.1%, respectively). HPV6 and 11 single types were not identified in any of the samples. Due to population growth alone, projected estimates of ICC cases attributable to the nine types in these 4 countries are expected to rise from 288,193 (55.0% of the world expected cases) new cases in 2013 to 315,501 (53.7% of the world expected cases) in 2025.

Conclusion: The addition of HPV 31/33/45/52/58 to HPV types included in current vaccines could consistently prevent almost 90% of ICC cases across the four countries. Assuming the same degree of efficacy of current vaccines, the implementation of the nine-valent vaccine in the evaluated countries would substantially impact in the reduction of the world cervical cancer burden.

OC 17-8

SEROLOGICAL SURVEILLANCE OF HPV IMMUNISATION IN ENGLAND

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Objectives: An HPV immunisation programme was introduced in England in September 2008 for all 12 year old girls with catch-up for girls up to 17 years old. Reported data on vaccine administration has shown coverage above 80% for all routine cohorts and ranging from 40-75% in the catch-up cohorts. Vaccination induces higher antibody titres than natural infection.
We used sera from the Public Health England (PHE) Sero-Epidemiology Unit (SEU) to estimate and monitor HPV 16/18 seroprevalence due to a) immunisation and b) natural infection, in a population-based sample of young females.

Methods: Serum samples from 15-19 year old females attending for routine microbiological and biochemical investigations in 2010 and 2011 were obtained by the SEU from 13 laboratories in England. Samples collected in January-March following the due date of first vaccine dose were excluded to restrict analysis to expected 3-dose coverage.
Serum samples were tested for antibodies to HPV types 16 and 18 using a type-specific ELISA at the PHE Vaccine Evaluation Unit, Manchester. Antibody levels are measured using ELISA units per millilitre (EU/mL). Seropositivity was determined using the technical cut-off of 8 EU/mL for HPV 16 and 7 EU/mL for HPV 18. Women were classified as having vaccine-induced seropositivity if they were seropositive for both types and either had a high titre for at least one type (above the upper 95% range of titres amongst those with discordant seropositivity results; $>$485 EU/mL for type 16 or $>$339 EU/mL for type 18) or a moderate titre for both types (above the lower 95% range of titres amongst those seropositive for both HPV types; $>$77 EU/mL for type 16 and $>$38 EU/mL for type 18).

Results: Of 2123 samples with a valid result for HPV 16 and 18 antibodies, 66% (1407) were seropositive for both types, and 62% (1314) had vaccine-induced seropositivity. Seropositivity for HPV 18 only and for HPV 16 only was found in 38 (1.8%) and 125 (5.9%) of samples, respectively. The proportion of women with vaccine-induced seropositivity tended to be the same or higher than the 3-dose coverage estimated from laboratory-area administration data (51% overall) with greater differences at older ages.

Conclusions: Assuming samples are representative, reported data on administration of 3-doses may have given conservative estimates of the levels of HPV immunisation in England.

OC 17-9

IMMUNE RESPONSE TO TIPAPKINOGEN SOVACIVEC IN CIN2/3 PATIENTS

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Objectives: To evaluate immune responses to tipapkinogen sovacivec, an MVA based therapeutic vaccine with modified HPV16 E6 and E7 genes as well as the gene for human IL2, in CIN2/3 patients and compare with efficacy and with immune responses in normal healthy volunteers

Methods: In the phase 2 study NV25025, patients with biopsy confirmed CIN2 or CIN3 were randomized 2:1 to receive tipapkinogen sovacivec or placebo subcutaneously on Days 1, 8, and 15. Study procedures included cervical conization or LEEP at Month 6. ELISPOT analysis was performed on purified cryopreserved PBMCs isolated from samples collected prior to the first injection, and then at Months 1, 3, and 6. The responses were compared to those of healthy volunteers without CIN in a phase 1 study of tipapkinogen sovacivec.

Results: Of the 113 patients who received tipapkinogen sovacivec, 49% had a detectable ELISPOT response to vaccinia. Overall, 16% of treated patients exhibited evidence of a new cell-mediated immune response to HPV16. Of the 29 patients who were treated and had complete histological resolution of CIN with interpretable ELISPOT, 6 (or 21%) had HPV16 cell-mediated immune responses. Of the 84 treated patients who did not have histological resolution, 12 (or 14%) had an HPV 16 cell-mediated immune response. None of the 6 patients with histological resolution who received placebo had a measurable HPV16 cell-mediated immune response.
In a previous Phase 1 study, it was observed that healthy volunteers receiving the same dose and schedule of tipapkinogen sovacivec had post-exposure response rates of approximately 50% to HPV16 antigens and 90% to vaccinia based on ELISPOT assays of their PBMCs.

Conclusions: While the immunological response rate was low in the phase 2 CIN2/3 study, a greater percentage of patients with histological resolution of CIN had an HPV16 ELISPOT response compared with those without resolution. However, the relatively small differences between these groups were not statistically significant. Further studies to identify cell-mediated immune correlates of clinical responses in CIN are needed.

OC 17-10

SURVEILLANCE OF EFFECTS OF HUMAN PAPILLOMAVIRUS IMMUNISATION IN BELGIUM (SEHIB): SECOND INTERIM ANALYSIS.

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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, enriched with 5x240 additional abnormal samples (80 ASC-US, 80 LSIL, 80 HSIL) from the 5 university laboratories as well as corresponding biopsies. Samples are tested for presence of DNA from individual HPV genotypes using a sensitive rtPCR targeting E6/E7 genes.

Conclusions: Currently, 5596 samples are recruited, comprising 5173/6000 consecutive routine samples and 423/1200 abnormal samples. This analysis included the first 4382 samples for which HPV genotyping results were available in December 2012.Vaccination status was recorded for 2027 women. The prevalence of cytological abnormalities in the consecutive screening series was: ASC-US (3.4%), LSIL (3.1%), ASC-H (0.7%), HSIL (0.6%). Substantial inter-laboratory differences were noted. The overall prevalence of high-risk HPV was 13.6%. The prevalence was highest among women younger than 29, decreasing progressively with higher age, and was strongly correlated with cytological findings. Overall, HPV16 was the most common infection (4.5%), followed by HPV31 (2.5%) and HPV52/HPV51 (both 1.8%). In HSIL, the most common types were: HPV16 (61.3%), HPV18 and HPV31 (both 16.1%), and HPV51 (12.9%). The prevalence of HPV16 in <30 years old was lower in vaccinated (3.5 %) than non-vaccinated women (6.7 %), albeit not significant. However, the reduction in prevalence (non-vaccinated vs vaccinated) of HPV18 (-2.0%) and HPV6/11 (-2.7%) was significant.
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 infections was observed suggesting an early effect of HPV vaccination. More results are awaited to assess the full 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. First significant protective results were found for HPV 18/6/11 in women < 30 years of age.

OC 17-11

GENERATION OF CHIMERIC P16^{INK4A}-HPV16 L1 PARTICLES FOR SECOND GENERATION HPV VACCINES

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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}a 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 p16^{INK4a} and HPV16 L1, the major capsid protein of HPV and antigen of the available prophylactic HPV vaccines, with the aim of using the adjuvant-like effects of L1 particles to improve p16^{INK4a} immune responses and at the same time generating a vaccine candidate with combined prophylactic (L1) and therapeutic (p16^{INK4a}) properties.
Methods: Two constructs were generated and compared to evaluate the antigenic effect of different structural isoforms. Therefore, the complete p16^{INK4a} encoding cDNA sequence was cloned a) upstream and b) downstream of a modified HPV16 L1 sequence into a pGEX4T2 expression vector. The GST-fusion proteins were expressed in E. coli and protein expression and purification was evaluated by Coomassie staining and Western blot analysis. Furthermore, particle assembly was verified by electron microscopy.
Conclusions: Chimeric constructs of HPV16-L1 and p16^{INK4a} can be expressed in and purified from E. coli as demonstrated by Western blot using p16^{INK4a} and L1 specific antibodies. The fusion proteins are capable of forming stable virus capsomeres and these particles are currently evaluated for their capacity to induce neutralizing antibodies against HPV L1 and p16^{INK4a}-specific cytotoxic T cells.

OC 17-12

PHASE I STUDY OF P16^{INK4A} PEPTIDE VACCINATION IN PATIENTS WITH ADVANCED HUMAN PAPILLOMAVIRUS-ASSOCIATED CANCER

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Objectives: The tumor suppressor p16^{INK4a} is strongly overexpressed in HPV-associated neoplasia, whereas in normal tissues barely any p16^{INK4a} expression is detectable. Targeting this HPV type-independent antigen by vaccination could represent an interesting complementary therapeutic approach to E6/E7-based vaccination. We performed a phase I peptide vaccination trial to monitor toxicity and immunogenicity of p16^{INK4a} vaccination in patients with advanced HPV-associated cancers.

Methods: Ten patients with p16^{INK4a}-overexpressing, HPV DNA-positive advanced cancer (8 cervical cancer, 2 head and neck cancer) were included. The protocol comprised 12 applications of a synthetic 27mer p16^{INK4a} peptide mixed with Montanide® ISA-51 VG over a six months period. Objectives of the study were clinical safety and changes of humoral and cellular immune responses against the p16^{INK4a} peptide. T cell responses were monitored by interferon-gamma ELISpot and antibodies by ELISA.

Conclusions: No vaccine-related toxicity was observed in any of the patients. One patient (head and neck cancer) completed the entire study protocol with stable disease for now 10 months after the last vaccination. The remaining 9 patients had progressing disease and were excluded from the study after 4 to 12 weeks. While at baseline only one patient had pre-existing T cell responses (CD4) against the p16^{INK4a} peptide, p16^{INK4a}-reactive T cells (CD4) were successfully induced in four patients after 4 to 12 vaccine doses. None of the patients had pre-existing p16^{INK4a} antibodies, but 3 patients developed increasing p16^{INK4a} peptide-specific antibody titers after the 5th dose.

This is the first study demonstrating that p16^{INK4a} peptide vaccination is safe and well tolerated. The results show that spontaneous immune responses against p16^{INK4a} are rare, but can be induced by p16^{INK4a} peptide vaccination. Further studies are needed to assess clinical efficacy of the approach.

OC 17-13

HR-HPV IMMUNISATION IS ASSOCIATED WITH LESS CIN – DATA FROM THE SCOTTISH CERVICAL SCREENING PROGRAMME.

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Objectives. The school-based national HPV immunisation programme with Cervarix® began in Scotland in September 2008 with over 90% coverage of 12 year olds. A 3 year catch-up programme included women born from 1990 onwards. Women born in 1990 entered the Scottish Cervical Screening Programme (SCSP) in 2010. The computer programme (Scottish Cervical Call Recall System - SCCRS) that supports the SCSP integrates cervical cytology results, immunisation status and biopsy results following colposcopy. Scotland therefore has a countrywide population based database with which to examine the effect of HR-HPV vaccination on cervical disease. In addition, a longitudinal HPV surveillance programme is in place to monitor the impact of vaccination. The aim of this study was to examine the effect of immunisation on cervical disease.

Methods. The SCCRS database was interrogated to provide data on uptake, cytological diagnosis, referral for colposcopy and biopsy diagnosis, stratified by vaccination status.

Results. Over 50,000 women born in 1990-1992 have smears recorded on SCCRS. Uptake of cervical screening has increased by 24 % in fully immunised women in the catch-up cohorts. The reporting rate of all abnormal smears fell from 20.2% in non-immunised women to 15.3% in fully immunised women, a reduction of 24%, and 18% fewer immunised women are being followed up for cervical abnormalities. The reduction in abnormal cytology, most striking in high grade dyskaryosis (HSIL) with a fall of 62%, from 1.9% to 0.72% of all smears, is associated with 54% fewer referrals for colposcopy. Low grade dyskaryosis (LSIL) and borderline squamous changes (ASCUS) show smaller reductions, 22% and 20% respectively. Women with incomplete immunisation (only one to two doses) have rates between fully immunised and non-immunised women.

Conclusion. HPV immunisation is associated with a reduced burden of significant cervical disease and in referral for colposcopy. The fears that immunised women would not see the need to attend for screening do not appear to be realised in this cohort. Sustained high coverage of HPV immunisation will provide major benefits for women and cervical screening programmes and will save health service resources.

OC 18-1

BIOBANKING OF LIQUID BASED CYTOLOGY – A VALIDATION STUDY

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Objectives: Liquid based cytology (LBC) has extended the possibility of biobankning into cytology. In Sweden, women between 23-59 years are invited to screening every third to fifth year. In collaboration with the biobanking initiative BBMRI.se we are in the process of establishing a biobanking facility with the goal of a complete population-based biobanking of about 100 000 cervical cytology samples each year.
The overall objectives are to offer a platform for studies of molecular pathology of cervical dysplasi and cancer and surveillance of the vaccination programme. However, in order to study the procedures of biobankning a validation study concerning HPV detection by DNA and mRNA based analysis as well as morphology is ongoing.

Methods: 100 samples previously analysed within the clinical setting were included in the study. The HPV-DNA method detects HPV 6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and betaglobulin duplexed in 8 reactions and were analysed on the 7900 HT Real-Time PCR System (Life Technologies, USA). A commercially available mRNA based analysis, APTIMA, (Genprobe, USA) on the PANTHER platform, detecting 14 hrHPV types was also used. Prior to biobanking of LBC samples 250ul were used for DNA and 1000ul and mRNA analysis. Biobanking was performed allowing the cellular component of the LBC liquid to concentrate by robotically transferring 4 ml of the remaining sample volume to an intermediate tube and thereafter transferring 300ul of the clotted material into a 96-weel platform format. After biobankning, 20 ul of material was used for each analysis.

Results: DNA and mRNA results were compared before and after biobanking. Concerning HPV DNA analysis 71/80 positive samples had identical results, 5 of the remaining 9 were not conclusive due to negative betaglobulin control and 4 were considered negative with our present Ct-cut off level. 67 samples were initially positive HPV mRNA. Using biobanked material 14/67 were negative while only 1/33 initially negative samples were positive. Morphological comparisons, methodological considerations and an analysis of HPV types will be reported.

Conclusions: Biobankning procedure of LBC is a valuable and reliable method for HPV studies, especially using DNA-based methods.

OC 18-3

HSIL IN WOMEN YOUNGER THAN 30 YEARS SHOWS PREVALENCE PATTERN RESEMBLING LSIL

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OBJECTIVE To evaluate whether a relationship exists between patient age and cytology findings of HSIL: cytological CIN 2 (HSIL-CIN 2) and cytological CIN 3 (HSIL-CIN 3).

METHODS The study was a cross-sectional assessment of the prevalence of cervical lesions in women undergoing cytology screening for screening purposes in Campinas region, São Paulo State, Brazil. The sample consisted of all Pap smears performed in the Cytopathology Laboratory of Campinas State University (Unicamp), from January 2000 to December 2009 (10 years). For HSIL reports categorization was maintained into HSIL-CIN 2 and HSIL-CIN 3. Two groups for analysis were considered: tests of women with at least one previous screening test (screened group) and tests of women without previous screening test (unscreened group). The total of LSIL and HSIL results was 15.279 on the screened group, and 3.928 tests on the unscreened group. For trend analysis of prevalence rates according to age, the chi-square test (x2) modified by Cochrane-Armitage was used. The impact of age on the prevalence of results was assessed by calculating the prevalence ratio (PR) with a 95% confidence interval.

CONCLUSIONS The prevalence of HSIL-CIN 2 was higher than HSIL-CIN 3 in women under 30 years old in both groups: PR 4.73, 3.90-5.75 on the unscreened group and PR 2.72, 2.49-2.97 on the screened group. The prevalence rates of LSIL and HSIL-CIN 2 tended to decrease with age in both groups ($p<0,0005$) whereas HSIL-CIN 3 rates tended to increase with age on the unscreened group ($p<0,0001$) and was uniformly distributed on the screened group ($p=0,83$). Cytology analysis is still relevant in young women because HPV-DNA testing is not indicated as a screening test in this population. Most HSIL results in young women do not correspond to a truly high-grade lesion and this could be weakness of two-tiered classification. Since the findings of HSIL may have different clinical significance in young woman, the cytomorphological criteria between low- and high-grade intraepithelial lesions need to be adjusted within possible limits.

OC 18-4

MISSING ENDOCERVICAL CELLS IN SCREENING DOES NOT IMPLY AN INCREASED RISK OF CERVICAL CANCER: A POPULATION BASED EVALUATION

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Objective: The presence of endocervical cells (EC) in cervical samples is used in process audits as a quality indicator to indicate whether tests were taken from the transformation zone (TZ). There is some uncertainty as to whether missing EC in screening means an increased risk for cervical cancer. Specifically, can a result of missing EC be disregarded or does it warrant recalling women for new tests within the next screening interval, or before exiting from screening at the upper age limit?

Method: A nested case-control study was conducted as a part of Swedish national audit project. Cases were all women diagnosed with invasive cervical cancer during 2002-2010, retrieved from the National Cancer Register. Controls were matched on birth-year and place of residence, retrieved from the Total Population Register. Screening information was retrieved from the National Quality Register for Cervical Cancer Prevention. Only women with normal Pap smears during the last screening interval were eligible. Cancer risks were compared between women whose last Pap smear had EC present and whose last Pap smear did not have EC, using conditional logistic regression models.

Conclusion: 1,174 invasive cervical cancer cases and 23,389 eligible matched controls were identified. Among women within the screening ages (age 23-60), having a Pap smear without EC present was not associated with an increased risk of invasive cervical cancer in the subsequent screening interval (OR=1.03, 95%CI=0.84, 1.26). Among women over the screening age, having a Pap smear without EC present between ages 50 and 60 was not associated with an increased risk of invasive cervical cancer after age 65 (OR=0.80, 95%CI=0.44, 1.43). When stratifying by histological type, the risks did not differ between squamous cell carcinoma and adenocarcinoma in either age group. Absence of EC is not a risk factor for cervical cancer in women with otherwise normal smears in a screening program where EC is a quality indicator for TZ sampling. This finding supports that re-testing or referral to colposcopy visits is not necessary as a result of missing endocervical cells, not even at the exit smear at age 60.+

OC 18-5

REGRESSION OF CERVICAL CYTOLOGICAL ABNORMALITIES AND CIN LESIONS IN THE CONTROL COHORT OF THE PATRICIA STUDY

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Objectives: To evaluate the rate of regression, naturally and after treatment of cervical cytological abnormalities and precancerous lesions.

Methods: All the abnormal cervical cytology and histopathological lesions (cervical intraepithelial neoplasia [CIN] and adenocarcinoma in situ [AIS]) detected during the 4-year follow-up period in the control cohort of the PATRICIA study (NCT00122681) were followed and classified as regressed or non-regressed.

Conclusions: Among the 9299 control women (aged 15–25 years) followed for approximately 4 years, 2489 (26.8%) and 673 (7.2%) had at least one abnormal cytology or one histopathological lesion detected. Among the 3005 cases of abnormal cytology detected, 2375 (79.0%) exhibited natural regression without treatment, 406 (13.5%) regressed after treatment, and 224 (7.5%) did not regress with or without treatment.

Among the 882 histopathological lesions (AIS:13; CIN3: 132; CIN2: 325; CIN1: 412), 352 (39.9%) exhibited natural regression to a lower grade lesion or to normal cytology, 339 (38.4%) regressed after treatment and 191 (21.65%) did not regress. In 108 (24.2%) of the 447 treated lesions, regression was not observed during the follow-up period. CIN regressed naturally in 39.8% of patients. Time to regression will be illustrated by Kaplan-Meier curves.

Natural regression of abnormal cervical cytology appears to be common, occurring in approximately 80% of subjects.

OC 18-6

ATYPICAL GLANDULAR CELLS IN PAP TEST INDICATE PRECANCEROUS OR CERVICAL CANCER IN WOMEN UNDER 50, AND ENDOMETRIAL CANCER IN WOMEN OVER 50 YEARS OF AGE.

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Objectives: To analyse cervical dysplasia and high risk HPV in patients with atypical glandular cells in liquid-based Pap–test.
Methods: In 2008 to August 2012 224 patients were found with atypical glandular cells in pap test. The results from HPV-test, colposcopy, endocervical curettage, endometrial biopsy, electrosurgical excision procedure (LEEP) and hysterectomy were registered.

Results: Final diagnose was established in 199 women; endometrial cancer n=77, cervical cancer n=30, adenocarcinoma in situ cervix n=19, HighSIL n=18, LowSIL n=5, glandular atypia n=8, endometrial hyperplasia n=3 and normal finding n=39. All women with endometrial cancer were over 50 years of age and only one did not have vaginal bleeding.
HPV-testing was performed in 103 cases. In the women with cervical cancer the HPV was analysed in 27 cases and the HPV types found were type 16 (n=11), 18 (n=11), 35 (n=1) and no HPV (n=3). In the endometrial cancer patients no high risk HPV was found. HPV 18 was found in 6 and HPV 16 in 5 of 14 patients with glandular atypia or adenocarcinoma in situ.. Women younger than 50 year or younger with atypial glandular cells (n=95) had adenocarcinoma *in situ* (n=16), cervical carcinoma (n=25) and highSIL (n=18), Those 59 patients needed surgical intervention or other further treatment (62 %; 95 CI 52-72 %).

Conclusion: In women 23-50 years of age with atypical glandular cells in pap test indicated precancerous or cervical cancer in more than 60 %. In women over 50 years of age atypical glandular cells indicate endometrial cancer.

OC 18-7

ATYPICAL GLANDULAR CELLS IN SCREENING IMPLIES A VERY HIGH RISK OF CERVICAL CARCINOMA

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Objective: Atypical glandular cells (AGC) in cervical cancer screening may signal a wide range of conditions from reactive changes to malignancies in the cervix, or even endometrial or ovarian carcinomas. The risk of malignancy after a report of AGC can vary between different settings. We need to know if AGC in our screening program is a high grade finding that should prompt a direct referral to colposcopy and endocervical evaluation and sampling.

Method: A nested case-control study was conducted as a part of the Swedish national audit project. Cases were all women diagnosed with invasive cervical cancer during 2002-2010, retrieved from the National Cancer Register. Controls were matched on birth-year and place of residence, retrieved from the Total Population Register. Screening information was obtained from the National Quality Register for Cervical Cancer Prevention. Cancer risks were compared among women who were diagnosed with AGC, high-grade lesions, low-grade lesions or normality during the last two screening intervals prior to cancer diagnoses, using conditional logistic regression models.

Conclusion: 2,113 invasive cervical cancer cases and 53,511 eligible matched controls were identified. Among women within the screening ages (age 23-60), having a Pap smear reported as AGC conferred an increased risk of invasive cervical cancer in the subsequent two screening intervals, compared to normal smears (OR=15.73, 95%CI=11.99, 14.88), low-grade lesions (OR=3.67, 95%CI=2.71, 4.97) and high-grade lesions (OR=1.80, 95%CI=1.33, 2.43. AGC was especially associated with a dramatically increased risk of cervical adenocarcinoma, compared to normal smears (OR=27.43, 95%CI=18.47, 40.74). Among women over the screening age, having a Pap smear reported as AGC at ages 50-60 also conferred an increased risk of invasive cervical cancer after age 65, compared to normal smears (OR=13.62, 95%CI=2.98, 62.23). AGC implies a very high risk of subsequent invasive cervical cancer, especially for adenocarcinoma, and warrants direct diagnostic measures to find precursors and early carcinomas in the endocervix or higher up in the genital tract.

OC 18-8

SIX YEARS EXPERIENCE IN 180.000 CASES WITH LBC AND COMPUTERASSISTANCE COMPARED TO CONVENTIONAL CYTOLOGY

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Objectives: Major high-quality studies showed inconsistent results for the comparison of liquid-based cytology (LBC) with conventional cytology (CC). However, several studies indicated a significant increase in sensitivity for HSIL with the computer-assisted ThinPrep-Imaging-System (TIS) (Hologic) compared to CC and even manually read ThinPrep LBC (TP). Here we report the performance of TIS compared with CC under routine conditions over six years.

Methods: Cytomol is a private commercial lab specialized in diagnostics for cervical cancer prevention. With TP since 2000 an experience with 340.000 cases has been achieved. Since 1.1.2007 all thinlayer specimens have been processed by TIS. Because in Germany LBC is reserved to privately insured and self-paying patients while public healthcare only reimburses CC, to avoid bias we limited this analysis to privately insured patients. Finding rates of cytologic abnormalities with TIS and CC were compared. Cytologic diagnoses originally reported in the Munich nomenclature II (=MN; with the use of the inofficial Pap IIK category), which is still the reporting standard in Germany, were translated to TBS.
Results: From 2007 to 2012 261.373 slides have been analyzed among them 184.001 by TIS. Except of some bloody and very cell-rich probes 97.25% of the smears were accepted for analysis by the system. TIS had a rate of LSIL (=MN PapIIID/CIN1) of 2.01% compared to 0.45% for CC (77.372), an increase of 347%. HSIL (=MN PapIIID/CIN2 + Pap IVa/b) was found in 1.02% with TIS vs 0.33% with CC (+209%). The ASC-US rate (MN Pap IIK + III) was 1.91% with TIS and 0.90% with CC. This increase of 112% is much lower than the rise in LSIL and HSIL cases. It is suggestive that the higher sensitivity of TIS was achieved without lowering specificity. All these results remained stable over the years analyzed. With TIS 21.9 slides/h were screened, compared to 13.4 for manually read TPs and 8.5 with CC. However, the technical expenditure for TIS was much higher than for CC and also for manually read LBC.

Conclusions: In routine use of a commercial lab TIS provided improved screening quality and higher productivity at the cost of higher technical expenditure.

OC 18-9

IMPACT OF SCREENING TECHNOLOGY ON CERVICAL CYTOLOGY RESULTS BY AGE

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Objectives. Little is known about age-dependent variation of results using modern cervical cytology technologies. An earlier study from a single Danish laboratory found an increased proportion of abnormal cytology in younger, and a reduced proportion in older women when SurePath liquid-based cytology (LBC) was implemented. Automation-assisted reading increased these proportions in all ages. We investigated whether this observation could be reproduced across other Danish laboratories and brands of cytological technology. We accounted for an underlying time trend by analyzing data from a laboratory using manually read conventional cytology continually.

Methods. Data for women screened in the three laboratories between 1998 and 2007 were collected from the Danish national health care registers. For each laboratory, we compared age-specific proportions of samples with abnormal cytology by cytological technology, starting with manually read conventional cytology.

Conclusions. The study included 489,960 primary cytological samples from women aged 23-59 years, the target age of the screening program during the evaluation period. Implementation of SurePath LBC in one laboratory was followed by an increase in the proportion of abnormal cytology at age 23-29 years from 4.6% to 6.1%, relative proportion (RP): 1.31 (95% CI: 1.08-1.61), and a decrease at age 45-59 years from 2.9% to 2.0%, RP: 0.71 (95% CI: 0.60-0.83). Implementation of ThinPrep LBC in another laboratory was followed by a decrease in the proportion of abnormal cytology both at age 23-29 years, from 7.7% to 6.8%, RP: 0.89 (95% CI: 0.78-1.02), and at age 45-59 years, from 3.4% to 1.0%, RP: 0.30 (95% CI: 0.24-0.37). With implementation of imaging-assisted reading, regardless of the brand, the proportion of cytological abnormality increased by around 30% in all age groups. In the laboratory with unchanged technology, no trends in abnormality proportions were observed. In summary, the impact of LBC implementation on abnormal cytological results varied considerably across age groups and brands of technology.

OC 18-10

USING SUREPATH IN DUTCH SCREENING PROGRAMME LEADS TO INCREASED CIN DETECTION RATES AS COMPARED TO THINPREP OR CONVENTIONAL CYTOLOGY

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Objectives: Liquid-based cytology (LBC) (i.e. SurePath and ThinPrep) has replaced conventional cytology (CC) in the Dutch cervical cancer screening programme within the last decade, although no consensus in literature has been reached whether LBC is more sensitive than CC. The aim of our study was to study the effect of the implementation of LBC on cytological outcomes, cervical intraepithelial neoplasia (CIN) and cervical cancer detection rates.
Methods: All cervix uteri examinations which were part of the Dutch national screening programme were analysed from 2000 to 2009 using the nationwide registry of histo- and cytopathology (PALGA).Cytological outcomes (borderline/mildly dyskaryotic smear (BMD) and >BMD) were compared between the cytology tests by using logistic regression analyses (adjusted for age, interval, number of former primary smears, region and calendar time). In addition, CIN and cervical cancer detection rates were compared between the cytology tests in all women who attended the screening programme, and in women with BMD or >BMD outcomes separately.
Conclusions: Using SurePath resulted in higher detection rates as compared to CC for CIN I (Odds Ratio (OR) 1.20 (95% CI 1.14, 1.27)), CIN II (OR 1.14 (95% CI 1.08, 1.20)) and CIN III (OR 1.07 (95% CI 1.03, 1.11)). This was mainly because more >BMD smears were found (OR 1.16 (95% CI 1.12, 1.20)). In addition, the finding of a BMD smear more often resulted in a CIN I (OR 1.30 (95% CI 1.22, 1.39)) and CIN II diagnosis (OR 1.11 (95% CI 1.03, 1.19)) as compared to CC, although it less often resulted in a CIN III diagnosis (OR 0.87 (95% CI 0.81, 0.94)). When CC was replaced by ThinPrep CIN detection rates did not increase. Cervical cancer detection rates did not differ between all cytology tests.
These analyses demonstrated that using SurePath in the Dutch national screening programme is associated with an increased detection of CIN I, II and III as compared to using CC, while ThinPrep is not. Whether the increased CIN detection rates lead to a decreased cervical cancer incidence need to be explored further.

OC 18-11

SPHEROID STRUCTURES WITH STEM CELL-LIKE PHENOTYPE IN PROGRESSION OF HPV-ASSOCIATED CERVIX UTERI PATHOLOGY

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Cellular spheroid structures (CSS) play an important role in repair processes and tumorogenesis.

Objective: to analyse CSS structural variants with characteristics of cell immunophenotypes and HPV DNA localization in HPV-associated cervical pathology.

Methods: Liquid-based cytology cervical samples from 35 women with chronic cervicitis (12 patients), CIN 1 (9), CIN 2-3 (14) were analyzed by immunocytochemical methods to detect expression of $p16^{INK4}$a, Ki-67 (CINtec PLUS Cytology Kit, Roche), Oct4 (Spring Bioscience,USA). Biopsy material (paraffin sections) from these same women have been investigated with immunohistochemical detection of OCT4, CD34, Vimentin, CD44, E-cadherin,p16, Ki67, CK7, SMA and by in situ hybridization analyses of HPV DNA (16, 18, 33, 54, 56 types).

Conclusions: Cell spheroids were detected in PAP tests with typical morphology and Oct4 smears due to positive staining of this marker. Positive $p16^{INK4a}$ and Ki-67 cells have been found in 20-25 % of epithelial cells in L-SIL spheroids and in 90-95 % - H-SIL spheroids. Obtained data proved neoplastic nature of spheroid structures in L-SIL and H-SIL.Oct-4, $p16^{INK4a}$ and Ki-67 immunocytochemical analysis of spheroid structure cells in liquid-based cytology may be used in diagnostics of cervix uteri lesions. It was found that CSS were formed in transitional zone basal-parabasal layers of squamous epithelium and adjacent myofibroblasts. HPV DNA have been detected in epithelial and endothelial cells of CSS.There are two structural and functional types of CSS – repair CSS and tumor CSS, that differ in capillary content and oncomarker expression. OCT4 and Vimentin positive cells were localized in colonies in CSS peripheral regions.

OC 18-12

DIAGNOSTIC IMAGING OF CERVICAL INTRAEPITHELIAL NEOPLASIA THROUGH HEMATOXYLIN AND EOSIN-DERIVED FLUORESCENCE

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Objective: Pathologic diagnosis of cervical intraepithelial neoplasia (CIN) has a low inter and intra-observer variability, using standard light microscopy. We investigate if the natural fluorescent intensity of the squamous epithelium; derived directly from standard hematoxylin and eosin (H&E) stained slides, could uncover distinct tissue fluorescent signature.

Methods: Archived cervical biopsy slides, stained with H&E, were identified containing the following diagnoses: normal cervical tissue, CIN I and CIN II/III cases. Two data sets where used, one from Community Hospital and the another from an Academic Medical Center. In the latter, slides classified by Gynecologic Pathologist, CIN I cases where confirmed for HPV by PCR. A total of 108 slides were reviewed: 46 normal, 33 CIN I and 29 CIN II/III. Images from H&E slides where captured first with bright field illumination and then with fluorescent illumination, using Zeiss Axio Observer Z1 microscope and an AxioVision 4.6.3-AP1 camera at of excitation of 450-490 nm with emission captured at 515-565 nm. The 32-bit grayscale fluorescence images were used for image analysis. Statistics performed.
Fluorescent intensity increased significantly in normal epithelial tissue as cells mature from the basal to superficial regions. In CIN I cases this change was less and statistically different compared to normals. In high grade CIN lesions there was slight or no increase in fluorescent intensity in the basal to superficial regions, also significantly different to normals

Conclusion: Our imaging process uses the spectral emission directly from a standard H&E slides. Fluorescence changes of the squamous epithelium from basal to superficial regions may provide distinct objective criteria to aid in the classification of CIN. We present a novel approach to evaluate cervical biopsies using ex vivo fluorescence spectroscopy.

OC 18-13

VULVAR INTRAEPITHELIAL NEOPLASIA: ARE WE USING THE MOST APPROPRIATE CLASSIFICATION?

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Objectives: VIN (Vulvar Intraepithelial Neoplasia) is a clinically heterogeneous condition with well defined histopathological characteristics. It has a well known potential of progression to invasive carcinoma. There are three classifications in current use: WHO (World Health Organization), ISSVD (International Society for the Study of Vulvovaginal Disease) and Bethesda.
This study aims to clinically characterize women with VIN and ascertain the adequacy of the WHO histological classification (VIN1/VIN2/VIN3) in comparison to the ISSVD's.

Methods: Retrospective analysis of clinical data of patients with histologically confirmed VIN2/VIN3 (March 1998-March 2013); VIN1 and cases of concomitant diagnosis of invasive lesion were excluded.

Conclusions: In the period analysed, there were 18 cases of VIN2/VIN3. Lichenoid dermatoses (LD) were identified in 27.8% of the cases (16.7% lichen planus and 11,1% lichen sclerosus). Women with LD had a mean age of 64 years and the remaining 45 years.
LD patients were more often symptomatic (80% vs. 60%), did not present with brownish lesions (30% in the other group) or with lesions in the labia majora or interlabial sulcus (31%/15% in the other group); raised lesions were less frequent (20% vs 58%); the area most frequently affected was the vestibule (80 vs 69%). In the group with LD there were no cases of immunossupression (0 vs 38%) and there were no smokers (0 vs 37%).
No cases of other intraepithelial lesion in the lower genital tract were found in the group with LD, while it was found in 62% of the patients of the other group.
This data confirms the existence of two distinct clinical groups among VIN patients, with different risk factors and forms of presentation. It favours the use of the ISSVD classification, which encompasses usual type and diferentiated VIN (related to HPV infection and LD, respectively).

OC 18-14

TWO DISTINCT MORPHOGENETIC UNITS OF THE CERVIX AND VAGINA – LIFELONG CLINICAL RELEVANCE

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Background: Morphogenetic units are differentiated compartments, which develop from distinct precursor tissues, the anlagen or primordia. An anlage is defined as the earliest recognizable tissue complex with a fixed morphogenetic determination in the embryo. Such anlagen are important for development of cancer and precancer of the uterine cervix (CIN) and the vagina (VAIN). CIN develops predominantly inside the transformation zone (90%), while the original squamous epithelium of the ectocervix is involved in only 10%. VAIN occurs predominantly in the upper vagina (90%), while the lower vagina is involved in only 10%. The reason for this distribution in both organs is unclear.

Methods: Epithelialisation of the cervix and vagina was studied in 24 female fetuses between 14 and 34 weeks of gestation as well as in 2 newborns from the archive of the Division of Clinical and Functional Anatomy, University of Innsbruck, Austria. We analysed different cytokeratins, vimentin, Pax2, p63, bcl-2, bmp4 and HOX A13 in the entire human anlagen of the cervix and vagina immunohistochemistry and compared the results with the cell biological studies in rodents.

Results: Different prenatal mechanisms are involved in epithelialisation of the vagina and cervix and evidenced by three morphogenetic units:

1. Vaginal squamous epithelium of the lower vagina that derived from the urogenital sinus,
2. the Müllerian squamous epithelium from the upper vagina, fornices and ectocervix up to the squamocolumnar junction and
3. the Müllerian columnar epithelium of the endocervix with the plasticity to transform into squamous epithelium (transformation zone).

Conclusions: Both the cervix and vagina develop from two distinct morphogenetic units. These observations may offer new viewpoints with respect to natural history of cervical and vaginal cancer and precancer.

OC 18-15

DIAGNOSTIC AND PROGNOSTIC APPLICATIONS OF P16 AND HPV IN OROPHARYNGEAL SQUAMOUS CELL CARCINOMA

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Introduction: The incidence of smoking associated squamous cell carcinoma (SCC) is reduced, while the incidence of HPV related SCC is increasing. The association of HPV infection with oropharyngeal SCC has been gaining more and more attention recently.

Design and Methods:

1) Histological variants of oropharyngeal SCC will be reviewed, which is of a wide histologic spectrum. Understanding the characteristic histologic features for HPV associated SCC is important, which can prompt us to perform the right tests. The subsequent prognostic value and potential therapeutic implication is of ultimate importance for a HPV positive SCC.
2) The diagnostic utility of p16 immunostain and HPV in situ hybridization in metastatic SCC will be discussed using the data from our own study and literature. We studied the p16 and HPV expression for its diagnostic applications using a cohort of 56 primary SCC cases from multiple sites, including lung, anogenital, and head and neck.
3) P16 immunostain has been used as a surrogate marker for HPV infection in oropharyngeal SCC. This application has been expanded to diagnostic utility of a metastatic SCC of unknown primary, particularly a metastatic carcinoma within cervical lymph node.
4) Prognostic value of p16 immunostain and HPV 16 in situ hybridization for oropharyngeal SCC will be discussed.

Conclusion:

1) HPV associated oropharyngeal SCC demonstrates characteristic histologic features.
2) P16 immunostain and HPV in situ hybridization are valuable in diagnosing metastatic SCC of unknown primary.
3) 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 in those of p16 positive ad HPV 16 negative cases. A positive HPV 16 case should be considered as a metastatic disease from anogenital or head and neck primaries.
4) P16 and/or HPV positive SCC is of a favorable prognosis compared to smoking related SCC. P16 positivity may be an independent favorable factor for oropharyngeal SCC regardless of HPV status.

IW 1-3

LA COMUNICAZIONE NELLO SCREENING

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La letteratura sottolinea la difficoltà di comunicare sull'HPV, difficoltà dovuta alla storia naturale dell'infezione, dal fatto che essa sia sessualmente trasmissibile e dalla sua connessione con il carcinoma cervicale.

Gli screening, in quanto programmi di sanità pubblica rivolti a grandi numeri di persone, hanno la necessità di cercare la qualità anche nella comunicazione sull'HPV. Quindi da una parte devono porre attenzione alla qualità del rapporto personale con le utenti, rapporto che nella maggior parte dei casi avviene con operatori telefonici o con le ostetriche, e che può essere reso più efficiente tramite corsi sulle competenze relazionali. Dall'altra, ricerche qualitative condotte anche in Italia hanno messo in luce la necessità di utilizzare materiali informativi orientati verso i riceventi e testati con essi. Materiali non testati sono risultati scarsamente comprensibili e capaci di provocare ansia e disagio. I materiali sull'HPV diventano invece comprensibili quando sono brevi e concentrati sugli aspetti essenziali della sequenza infezione-cancro. L'informazione, inoltre, deve essere mirata alle esigenze dei diversi momenti informativi (invito, risposta negativa, risposta positiva) e capace di indicare ulteriori fonti di informazione. A tale riguardo il Gisci e l'Osservatorio Nazionale Screening hanno prodotto un set di materiali di base ed altri più approfonditi rivolti sia alle utenti sia agli operatori e disponibili in rete.

HPV-16/18/31/45 antibodies measured by ELISA and HPV-16/18 neutralising antibodies measured by PBNA, for the 2D (M0,6) group versus 3D (M0,1,6) group at Month 7 (ATP cohort for immunogenicity, initially seronegative subjects)

		Enzyme Linked Immunosorbent Assay (ELISA)			Pseudovirion Based Neutralising Assay (PBNA)		
Antigen	Group	N	Seroconversion, % (95% CI)	GMT, EU/ml (95% CI)	N	Seroconversion, % (95% CI)	GMT, ED50 (95% CI)
HPV-16	2D	488	100 (99.2, 100)	9400.1 (8818.3, 10020.4)	102	100 (96.4, 100)	77625.4 (63204.3, 95336.8)
	3D	352	100 (99.0 , 100)	10234.5 (9258.3, 11313.6)	92	100 (96.1, 100)	31206.4 (23990.7,40592.5)
HPV-18	2D	493	100 (99.3 , 100)	5909.1 (5508.9, 6338.4)	102	100 (96.4, 100)	23005.7 (19326.8, 27385.0)
	3D	382	100 (99.0 , 100)	5002.6 (4572.6, 5473.1)	93	100 (96.1, 100)	13958.1 (10915.3,17849.0)
HPV-31	2D	97	100 (96.3 , 100)	1680.9 (1426.4,1980.7)	/	/ /	/ /
	3D	91	98.9 (94.0, 100)	1224.9 (955.8, 1569.9)	/	/ /	/ /
HPV-45	2D	98	100 (96.3 , 100)	1798 (1494.2, 2163.6)	/	/ /	/ /
	3D	92	100 (96.1 , 100)	1073 (879.7, 1308.8)	/	/ /	/ /

N= number of subjects with pre-vaccination results available. EU= ELISA unit. Positive ELISA result defined as a GMT ≥8 EU/ml for HPV-16, ≥7 EU/ml for HPV-18, and ≥59 EU/ml for HPV-31 and HPV-45. Positive PBNA result defined as an antibody titre ≥40 ED50 for HPV-16 and HPV-18.

Un'altra implicazione per i programmi di screening è quella di cercare la massima coerenza tra i materiali informativi e quanto detto dagli operatori, in quanto il rapporto personale rimane il canale comunicativo privilegiate per le utenti.

Infine, è essenziale che gli screening siano consapevoli che la comunicazione sull'HPV "avviene" comunque, e solo in parte è governata da chi lavora nei programmi. Il resto è quello che "passa" sui giornali, la televisione, internet , nei vissuti delle persone: e questo gli screening non lo governano affatto. Quello che gli screening governano, soprattutto in un periodo di scarsità di risorse, devono farlo meglio, aumentando il livello di consapevolezza con cui usano determinati strumenti e imparando a dialogare con altre discipline. Il dialogo con il "non screening" nella sanità (altri specialisti, medici di medicina generale, sanità privata) e nella società civile (decisori politici, associazionismo, ecc) è comunicazione anche questa, probabilmente la più cruciale.

IW 1-4

LA COMUNICAZIONE CON LA POPOLAZIONE

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La comunicazione rappresenta uno degli aspetti più salienti ma allo stesso tempo critici in medicina. Deve rispondere a requisiti fondamentali, quali: chiarezza, completezza, tempestività, correttezza ed efficacia. Ha un riconosciuto ruolo nel garantire l'empowerment degli utenti e dei pazienti, favorendo l'accesso ai servizi e alle prestazioni, promuovendo e valorizzando un'autonomia decisionale, responsabile e consapevole, nonché consentendo la partecipazione critica ai processi assistenziali. Cruciale dunque l'adozione di strategie di comunicazione, integrate ed efficaci, attraverso una rete di collaborazione sinergica ed alleanza tra i diversi settori della società e i soggetti "competenti", quali Istituzioni, Enti, media, associazioni di pazienti, organizzazioni rappresentative gli utenti, operatori sanitari.
Nel 2011, a due anni di distanza dal lancio della campagna vaccinale anti-HPV, O.N.Da ha realizzato un'indagine conoscitiva per esplorare il grado di informazione, i motivi di adesione o di resistenza alla vaccinazione nella popolazione di madri in target con figlie tra 11 e 18 anni. Circa il 70% delle donne intervistate ha dichiarato di aver ricevuto informazioni chiare e complete sul vaccino HPV, anche se è emerso un consistente bisogno di rassicurazione su pericolosità (40%) e reale efficacia del vaccino (39%). Paure, insicurezza e perplessità su questi specifici aspetti rappresentano il maggiore ostacolo all'adesione alla campagna vaccinale e si traducono in un livello di copertura a tutt'oggi ben lontano dagli obiettivi preposti ed estremamente disomogeneo sul territorio nazionale.
O.N.Da, come Osservatorio dedicato alla salute femminile è da tempo impegnato sul fronte HPV (già del 2007 è stata approvata una mozione con l'obiettivo di contenere il costo finale del farmaco per le donne che non appartengono alla coorte di riferimento della campagna vaccinale), svolgendo una capillare attività di comunicazione rivolta alla popolazione attraverso la produzione di materiale informativo, l'organizzazione di incontri aperti alla popolazione e di conferenze, la promozione di campagne di sensibilizzazione e la realizzazione di indagini conoscitive.

IW 1-5

IMMUNE RESPONSES TO A 2-DOSE SCHEDULE OF THE HPV-16/18 AS04-ADJUVANTED VACCINE IN GIRLS (9-14) VERSUS 3 DOSES IN WOMEN (15-25): A RANDOMISED TRIAL

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Objectives: This phase III, randomised, open-label trial (NCT01381575, Clinicaltrial.gov) evaluates the immunogenicity of a 2-dose (2D) schedule of the human papillomavirus (HPV)-16/18 AS04-adjuvanted vaccine (HPV-16/18 vaccine) in girls aged 9–14 years (y) versus the 3-dose (3D) schedule in women (15–25y).

Methods: 1447 subjects were randomised (1:1:1) to receive HPV-16/18 vaccine either at Month (M) 0,6 (2D, N=550, 9–14y) or at M0,12 (2D, N=415, 9–14y), or at M0,1,6 (3D, N=482, 15–25y). M7 analysis focused on 2D(M0,6) and 3D(M0,1,6) groups. Anti-HPV-16/18/31/45 geometric mean titres (GMT) by ELISA, anti-HPV-16/18 GMT by Pseudovirion Based Neutralising Assay (PBNA), and HPV-16/18/31/45 specific T-cell and B-cell mediated immune (CMI) responses were measured.

Conclusions: At M7, in the according-to-protocol (ATP) cohort for immunogenicity, non-inferiority of the 2D(M0,6) schedule in girls compared with the 3D(M0,1,6) in women was demonstrated for HPV-16/18 ELISA antibody response. At least 98.9% of initially seronegative subjects seroconverted for anti-HPV-16/18/31/45 antibodies (ELISA) (Table). Anti-HPV-16/18/31/45 GMT (ELISA) and CMI responses were similar (descriptive analysis) between 2D and 3D groups. HPV-16/18 neutralising antibody responses (PBNA) appeared higher in the 2D group than the 3D group.

The study was funded by GlaxoSmithKline Biologicals S.A.

IW 1-6

THE ASC-US IN THE CYTOLOGY TRIAGE: CATEGORY AT RISK?

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Objectives. The cytology triage (filter test) must be able to categorize HPV+ patients between low-risk disease patients, requiring annual inspections, and high-risk disease, to be sent to colposcopy exams.
Aim of this work is the revaluation of selected cases of ASC-US/HPV+, in order to establish dichotomous cytological reclassification "Negative/Abnormal", and to evaluate sensitivity and PPV.

Materials and Methods. From 2010-2011 ISPO archive 155 ASC-US/HPV+ cases were selected. These cases range from women aged between 34 and 65, of which immediate results of the investigations (colposcopy/biopsy) and every possible follow-up at 24 months are known. Three cytologists have reviewed in blinded the 155 Pap-tests, defining for each one a diagnosis of majority, with discussion at the multiple microscope of those discordant cases.

Conclusions. At the revision we obtained the following data: the Pap-tests interpreted as "negative" were 89 (57%), "abnormal" 66 (43%), including 17 ASC-H, 45 LSIL, HSIL 4. At the immediate assessment, 18 cases judged "abnormal" had a CIN2+ lesion histologically (true positives); false negatives were 3. The sensitivity was 86% (95% CI 64% -97%). The initial PPV of cases ASC-US/HPV+ was 14% (95% CI 9% -20%), while the PPV at the revision was 27% (95% CI 17% -40%): the difference between these two values is statistically significant ($p \leq 0.0143$).
The revision and dichotomous reclassification "Negative/Abnormal" of selected cases of ASC-US/HPV+, based on defined and shared morphological criteria, allowing us to hypothesize a possible elimination of the category ASC-US maintaining a good sensitivity and optimal PPV. In addition, the research highlights a potential saving of colposcopic examinations (57%) related to a down-grading of 89 ASC-US to negative. The applicability in the triage cytology of the results of this review, requires further confirmations in the real condition of routine.

IW 1-7

CERVICAL CANCER RISK IN FOREIGN WOMEN AND EQUITY OF ACCESS TO HEALTHCARE SERVICES AND SURGICAL TREATMENT IN THE REGGIO EMILIA PROVINCE

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Objectives Cervical cancer incidence shows wide geographical differences, due mainly to the prevalence of Human Papillomavirus (HPV) and Pap test uptake. The study analyses the incidence of cervical lesions in foreigners from High Migration Countries compared to Italians and investigates whether there are any differences between the two groups in access to healthcare services and surgical treatment in the province of Reggio Emilia in the period 2002-2009.

Methods Standardised incidence ratios (SIRs) and 95% Confidence Intervals (CI) for CIN3 and cancer were calculated for foreigner vs. Italian women; foreigners were also classified according to the HPV prevalence in their origin country: high (HHPVC) or low (LHPVC).
Two indicators of not appropriate surgery in CIN3 and micro invasive cancers are presented: proportion of hysterectomies and proportion of cold knife conisations among conisations.
The diagnostic pressure outside screening programme for the two groups was estimated through the ratio between expected and observed CIN3 in women not participating.

Conclusions A higher risk was observed in women from HHPVC both for invasive cancer and CIN3 (SIR=4.1, 95%CI 2.2-6.9; SIR=2.0, 95%CI 1.7-2.5 respectively), while in those from LHPVC no difference for invasive cancer and a lower risk for CIN3 were observed (SIR=1.0, 95%CI 0.2-2.2; SIR=0.6, 95%CI 0.4-0.8, respectively).
A higher percentage of lesions in foreigners was screen-detected (86.5% vs. 80.4% in Italians), especially at the first screening test, and a lower diagnostic pressure outside screening programme emerged (0.17 in foreigners vs. 0.33 in Italians).
No differences by stage were observed for cancers, but a lower ratio CIN3/cancer rates was found (2.6 in women from HHPVC and 3.6 in women from LHPVC vs. 7.5 in Italians).
A high appropriateness in surgical treatment for precancerous and micro invasive lesions was found both in foreigners and in Italians (the % of hysterectomies was 3.4 and 4.7, respectively and the % of cold knife conisations was 3.7 and 8.6, respectively).

IW 2-1

THE ROLE OF HIGH-RISK HUMAN PAPILLOMAVIRUSES IN HEAD AND NECK CANCER

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Head and Neck Cancer (HNC) is the sixth most common malignancy reported worldwide and one with a high case fatality rate, with an estimated global burden of approximately 550,000 incident cases and 300,000 deaths per year. Approximately 50% of 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 the mucosal high-risk (HR) human papillomavirus (HPV) types, the etiological agent of cervical cancer. Epidemiological and biological studies have shown that HPV16 and HPV18 are the most oncogenic types within the HR group, and are respectively responsible for approximately 55% and 16% of cervical cancers worldwide. In HNC, the majority of the HPV-associated cases (between 86-95%) are associated with HPV16, while the remaining HPV types appear to play only a marginal role. Among the different types of HNC, oropharyngeal carcinomas are the most frequently HPV-associated, while HPV prevalence in cancers of the oral cavity, larynx and hypopharynx appears to be considerably lower. Epidemiological studies have shown that the fraction of oropharyngeal cancers linked to viral infection can substantially vary in different countries and over time. This variation in the percentage of the HPV-positive cases could be explained by variation of incidence and prevalence of oral HPV infections between countries and/or by variation of risk factors other than viral infection. Although the overall incidence of HNC is decreasing in western countries due to the awareness of adverse effects of tobacco in human carcinogenesis, the percentage of oropharyngeal cancers is steadily increasing in the USA and Europe, but whether this phenomenon is linked to an increase in HPV infection and/or other factors remains to be proven. Additional studies are required to confirm the increasing importance of HPV infection in HNC development in European populations, as well as to precisely determine the fraction of HNC at different anatomical sites attributable to HPV infection at the present time and to provide data for predictions for the coming years.

IW 2-3

MALATTIE HPV CORRELATE EXTRA-CERVICALI: LA MALATTIA VULVARE.

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L'infezione da Human Papilloma Virus (HPV) rappresenta una delle più comuni forme infettive a livello genitale esterno, la più frequente tra quelle virali. Le lesioni della regione vulvo-perineale correlate all'infezione da HPV si possono classificare in:

- **Lesioni cliniche:** visibili all'ispezione ad occhio nudo, più o meno sintomatiche, quali: condilomi acuminati, papillomatosi, forme neoplastiche intraepiteliali (VIN) o invasive (carcinoma)
- **Lesioni subcliniche:** evidenziabili con strumentazione ottica specifica (lente d'ingrandimento o colposcopio), in genere poco o nulla sintomatiche, quali: condilomi piatti, più o meno associati a VIN, microcondilomatosi.
- **Infezione latente:** la presenza di HPV-DNA, in assenza di aspetti clinici o subclinici, può verificarsi a livello vulvare, analogamente a quanto può accadere a livello cervico-vaginale.

La **condilomatosi florida** vulvo-perineale e anale è correlata per lo più alla presenza di HPV-DNA dei tipi 6 e 11 (90%), mentre la **microcondilomatos**i deve essere ben distinta dalla micropapillomatosi vestibolare, che rappresenta un aspetto parafisiologico, nel quale la possibilità di riscontro di HPV-DNA è del tutto sovrapponibile alla mucosa sana.

Le forme neoplastiche intraepiteliali o invasive possono riconoscere una eziopatogenesi legata ad HPV ad alto rischio (prevalentemente il tipo 16). Si tratta delle forme di **VIN "usual type"** (a differenza della forma "differentiated type"), nonché del **carcinoma squamoso della vulva di tipo HPV-correlato**, indifferenziato, con incidenza in età più giovanile (età media 55 anni) più raro rispetto a quello cheratinizzante, legato a dermatosi vulvare, ad un'età più avanzata, non HPV-correlato. Il **carcinoma verrucoso**, invece, mostra più spesso la presenza di HPV a basso rischio.

Il trattamento delle lesioni HPV-correlate vulvari dipende dalla loro natura ed estensione. I condilomi genitali esterni sono trattati con successo con terapia medica locale (Imiquimod) o con trattamenti distruttivi o escissionali locali. Le forme preneoplastiche e neoplastiche hanno dei protocolli terapeutici oncologici rigorosi, basati prevalentemente sulla terapia chirurgica.

IW 2-4

LA VACCINAZIONE NEL MASCHIO

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L'infezione da HPV è la più diffusa delle MST e le conseguenze cliniche (sia come patologia condilomatosa, che come lesioni pretumorali o francamente invasive) gravano su entrambi i generi, seppure con diverse percentuali di attribuzione ai singoli genotipi. L'attuale programma organizzato di vaccinazione HPV (benchè sia l'unico ad avere un carattere monogenere) esprime alcune importanti differenze regionali in relazione al coinvolgimento del maschio. La disponibilità al sostegno economico, nonchè una sensiblità per le istanze universali alla prevenzione, hanno spinto alcune realtà regionali (Emilia-Romagna, Marche, Sicilia) ad articolare la vaccinazione nel maschio sino a 26 anni con un'offerta di co-payment. Oltre alla parità di diritto all'accesso alle pratiche di prevenzione, l'offerta alla popolazione maschile è considerata anche cost-effectiveness in condizioni di insoddisfacente adesione della popolazione femminile (come nel caso italiano), nonchè come primario veicolo di protezione nei confronti della patologia HPV-correlata maschile: cancro dell'ano, del pene, cancri oro-faringei, condilomatosi ano-genitale.

La necessità di un ampio coinvolgimento maschile alla vaccinazione HPV emerge anche dalla consapevolezza della mancanza di momenti istituzionali finalizzati alla prevenzione (come lo screening nelle donne), o l'esposizione ad un considerevole rischio da parte di chi pratica comportamenti sessuali a rischio (MSM) e che, peraltro, non potrebbero avvalersi di un'eventuale herd-immunity.

IW 2-5

HPV E DERMATOLOGIA

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Il papillomavirus umano o HPV è un virus appartenente al gruppo dei papillomavirus, dotato di DNA circolare a doppio filamento, del quale sono descritti più di 100 genotipi. Le infezioni da HPV sono estremamente diffuse e possono rendersi responsabili non solo di lesioni benigne cutanee e mucose, ma anche di un sottogruppo di carcinomi a cellule squamose prevalentemente, ma non esclusivamente, localizzati nell'area perianogenitale.

Gli HPV con tropismo cutaneo, molto comuni nella popolazione generale, sono soprattutto causa delle verruche (volgari, palmo-plantari, filiformi, piane,…), spesso caratterizzate dalla regressione spontanea come risultato di una progressiva ed efficace attivazione del sistema immunitario. Tuttavia, evidenze sperimentali sempre più convincenti dimostrano la presenza di alcuni HPV (es. 5,8) in patologie cutanee premaligne o carcinomatose (cheratosi attiniche, malattia di Bowen e papulosi bowenoide, carcinomi verrucosi…) nella cui patogenesi il virus svolgerebbe un ruolo attivo, talora sinergico all'azione dei raggi ultravioletti. Il modello di carcinogenesi cutanea da HPV è rappresentato dalla epidermodisplasia verruciforme (sindrome di Lewandowsky-Lutz), una rara genodermatosi ad ereditarietà autosomica recessiva, nella quale la suscettibilità alle infezioni da -HPV comporta la comparsa, nell'infanzia, di lesioni ipocromiche e papule lichenoidi acrali, gravate di un rischio del 50% circa di trasformazione maligna.

Gli HPV a tropismo mucoso sono classicamente distinguibili in genotipi "low-risk" (LR) and "high-risk" (HR). I genotipi LR (in particolare HPV 6 e 11), così definiti perché non associati a carcinomi invasivi, causano lesioni proliferative benigne del cavo orale e dei genitali (papillomi squamosi, iperplasia epiteliale focale, condilomi acuminati, tumore di Buschke-Lowenstein); i genotipi HR invece sono capaci di provocare la trasformazione neoplastica degli epiteli infettati, attraverso l'inibizione dell'apoptosi e la disregolazione del ciclo cellulare. Come risultato, gli HR- HPV possono indurre la comparsa di lesioni squamose intraepiteliali a livello vulvare, penieno e anale, potenzialmente destinate ad evolvere verso carcinomi invasivi.

In questa relazione verranno brevemente passate in rassegna le principali caratteristiche cliniche delle patologie cutanee HPV-correlate, con particolare riferimento alle dinamiche di interazione tra HPV e ospite nella patogenesi dei carcinomi cutanei non-melanoma.

IW 2-6

RECENT ADVANCES IN HPV-BASED IMMUNOTHERAPY OF HEAD AND NECK CANCERS WITH THE HELP OF GREEN WORLD.

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Objectives: Cervical, anal-penile and a large set of head and neck (H/N) tumours are caused by high-risk Human Papilloma Viruses. The available preventive HPV vaccines do not provide a therapy for already infected/tumour-affected subjects. Therapeutic vaccines represent an innovative approach in particular for HPV-associated cancer. Plants and microalgae represent safe and low-cost production platforms that can also provide immune-enhanced properties. Our goal was to produce immunotherapeutic antigens in plants and to test their effectiveness in pre-clinical model of H/N HPV cancers.

Methods: We generated DNA vaccines based on E7 gene fused to plant-derived genes and protein vaccines by high level production of non-oncogenic E7 protein variants in plant with immunological activity. However some drawbacks of in whole-plant vaccine production encouraged us to explore the production of the E7-based therapeutic vaccine in Chlamydomonas reinhardtii, a microalgae organism easy to grow and transform and fully amenable to GMP guidelines. An expression cassette encoding HPV 16 E7GGG was introduced into the C. reinhardtii chloroplast genome by homologous recombination. Production of the E7 antigens was achieved and reached 0.12% of total soluble proteins.

Results and conclusions: These innovative products derived from plants and microalgae, were used to treat efficaciously HPV-related tumours in animal models. In addition, as pre-clinical model of H/N HPV cancer are not available, we developed new orthotopic mouse models for HPV-associated H/N cancer, suitable for imaging analysis. In this new model only few preparations of those reported active against the conventional TC-1 model were able to affect the tumour growth, mostly in heterologous prime boost setting.
In conclusion we showed the possibility to produce HPV therapeutic vaccines also in microalgae and the development of a new tool for pre-clinical studies of H/N cancers that may allow to examine immunotherapies in combination with traditional treatments (surgery/radio/chemotherapy).

IW 2-7

PREVALENCE AND MOLECULAR CHARACTERIZATION OF HPV-ASSOCIATED HEAD AND NECK SQUAMOUS CELL CARCINOMAS FROM NORTHERN ITALY

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Objectives: Human papillomaviruses (HPV), most frequently HPV16, are causally associated with a subset of head and neck squamous cell carcinomas (HNSCC) especially from the oropharynx. The incidence of HPV-associated HNSCC increased in Western countries in the last decades. We want to evaluate the prevalence of HPV-driven HNSCC in Northern Italy by analysing viral DNA, transcriptional activity, cellular protein expression levels and HPV antibodies.

Methods: Fresh frozen biopsies and/or formalin-fixed paraffin-embedded (FFPE) tissues were collected from 256 patients with incident HNSCC from 2003-2012 and serum from 84 of these patients. We used HPV-genotyping by MY09/11 (229, 90%) and/or BSGP5+/6+-PCR/MPG (145, 57%). HPV DNA+ samples were further tested for viral load, presence of viral oncogene E6*I transcript and HPV16 transformation-specific RNA patterns. Antibodies to early and late HPV proteins were analysed by Multiplex Serology.
HPV DNA was present in 22 (9%) cases, 21 (95%) with HPV16 and 1 (5%) with HPV58. HPV DNA+ was highest in the oropharynx (15/57, 26%). HPV16 load was high (>0.5 copies/cell) in 8 of 13 cases. E6*I transcripts and HPV transformation-specific RNA patterns were present in 14/22 of the HPV DNA+ samples. All of the 5 HPV DNA+RNA+ tumors with serum available showed strong HPV type-specific antibody responses to E6 and other early proteins. The HPV58 DNA+ case showed both E6*I RNA and antibodies to E6 and E7 of HPV58.

Conclusions: Prevalence of HPV in HNSCC from Northern Italy was 9%, lower than in Western countries. Oropharynx was the preferential site for HPV-associated HNSCC. About 64% of HPV DNA+ cases were HPV-driven, they showed viral gene expression and if serum available also antibodies to type-concordant HPV oncoproteins.

IW 2-8

COMPARING BIOMARKERS FOR TISSUE DETECTION OF HPV-ASSOCIATED OROPHARYNGEAL CANCER

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Objectives Human papillomavirus (HPV)16 infection-associated oropharyngeal squamous cell carcinomas (OPSCC) have relevant prognostic and therapeutic implications. Several algorythms have been proposed for their identification, however only recently has the virus transcriptional activity been recognized as evidence of virus-induced oncogenesis and as the gold standard for test validation. In situ hybridization (ISH) for the detection of genotype-specific HPV E6/7 mRNA is a new technique that allows the assessment of HPV oncogene transcription on routine bioptic samples, avoiding the need of fresh tissue for mRNA extraction and amplification. This study was aimed at comparing the diagnostic accuracy of different tests currently used for the characterization of HPV-associated OPSCC against HPV16 E6/7 mRNA ISH.

Methods 40 consecutive paraffin-embedded OPSCC were tested with ISH for HPV16 E6/7 mRNA and high risk (HR) HPV DNA, p16 immunostain, HPV DNA amplification and genotyping with SPF10 primers, and HPV16 E6 gene amplification. Test sensitivity and specificity were assessed using mRNA ISH as the gold standard.

Conclusions mRNA ISH identified viral transcripts in 15 of 40 cases, all positive by p16 stain. DNA ISH and PCR with both primer sets were negative in two distinct mRNA ISH-positive cases. No differences in age, sex, stage, smoking and drinking habits were observed between mRNA ISH positive and negative groups.

Test	N. positive (%)	N. negative (%)	Sensitivity	95% CI	Specificity	95% CI
HPV16 mRNA ISH	15 (37,5)	25 (62,5)				
p16	25 (62,5)	15 (37,5)	1	0,74-1	0,6	0,38-0,78
SPF10 HPV DNA	29 (72,5)	11 (27,5)	0,81	0,53-0,95	0,33	0,16-0,55
SPF10 HPV 16	15 (37,5)	25 (62,5)	0,6	0,32-0,82	0,76	0,54-0,89
HPV16 E6 PCR	22 (55)	18 (45)	0,93	0,66-0,99	0,68	0,46-0,84
HR HPV DNA ISH	16 (40)	24 (60)	0,93	0,66-0,99	0,92	0,72-0,98

Our results further validate the current diagnostic algorithm for HPV-associated OPSCC identification, which includes p16 stain and DNA ISH in p16-positive cases. mRNA ISH can be considered to further investigate p16-positive, DNA ISH-negative cases, although only 1 of 11 tested cases was positive. The low specificity of PCR-based assays confirms the observation that HPV DNA amplification does not discriminate passenger (transcriptionally inactive) from oncogenic, transcriptionally active HPV infections. Moreover, integrated HPV16 can be missed when using primers amplifying the L1 region, which is lost upon viral integration.

IW 3-3

VACCINATION AND SCREENING: THE SCREENING PROGRAM POINT OF VIEW

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Advances in knowledge of the natural history of cervical cancer, and in particular the identification of human papillomavirus virus (HPV) as the necessary cause of the disease, have led to two new preventive tools: vaccine and HPV test.

As mass vaccination campaigns target young girls and cervical cancer occurs virtually only women over 25-30, all women now at risk are not targeted by vaccination campaigns. Furthermore, the vaccine currently contains only two of the 12 high-risk HPV types - HPV 16 and 18 - responsible for about 75% of cancers in Italy. Thus, even vaccinated girls should be screened in the near future. As regards screening, the 2013 Italian recommendations allow screening programs to adopt HPV test as primary test starting from age 30-35 years, with an interval of at least 5 years after a negative test.

Several strategies have been proposed to efficiently and cost-effectively combine the two preventive tools.

The health system should avoid introducing new technologies, which would double costs with small marginal benefits: only an integrated preventive policy can maximise the benefits offered by vaccine and HPV test and control costs and dedicated resources.

IW 3-4

VACCINAZIONE E SCREENING: IL PUNTO DI VISTA DEL GINECOLOGO

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Obiettivi: Ad oggi persistono rilevanti differenze geografiche di adesione ai programmi di screening e alla vaccinazione. La copertura del Pap test è alta ma ancora insufficiente in alcune Regioni. Nonostante la vaccinazione contro l'HPV, nel corso dei trial clinici di fase 3, abbia mostrato valori di efficacia del 98-100% verso lesioni HPV-correlate nella popolazione naive, la copertura vaccinale non ha raggiunto del tutto gli obiettivi prefissati dal Piano Nazionale, registrando in alcune Regioni addirittura un lieve decremento. Alla base dei motivi di resistenza alla campagna, più che aspetti meramente organizzativi, ci sono fattori culturali e la scarsa consapevolezza dei rischi legati all'HPV, in parte connessi alla non adeguata informazione. Nonostante che in ambito di vaccinazione il pediatra ed il medico di medicina generale siano i referenti, rispettivamente in età preadolescenziale e adulta, nel caso della vaccinazione per HPV tale figura deve essere rivestita dal Ginecologo. Tale ruolo è determinato dalla vicinanza alle giovani donne e alle madri e dalle sue competenze specialistiche che gli permettono di fornire una adeguata informazione sull'utilità della vaccinazione e dello screening. Nell'ambito dell'analisi di efficacia della campagna informativa, abbiamo voluto valutare le conoscenze di una popolazione di adolescenti in merito all'infezione da HPV e alla vaccinazione.

Metodi: nel 2009, è stato sottoposto un questionario anonimo a 1105 studenti di Scuola Superiore con quesiti riguardanti l'infezione da HPV e la vaccinazione. Sono stati confrontati i questionari compilati da adolescenti maschi vs femmine e da 629 ragazze in relazione al loro stato vaccinale.

Conclusioni: Dai risultati del nostro studio emerge che i teenagers hanno una scarsa conoscenza sulle modalità di trasmissione e relativa prevenzione dell'infezione da HPV, sulle patologie HPV correlate e sulle norme comportamentali post-vaccinazione. Informazioni errate possono pertanto aumentare i comportamenti sessuali a rischio. La campagna informativa ha pertanto un' importanza fondamentale e il Ginecologo in sinergia con le altre figure, ha il compito di fornire un adeguato counseling ed accertarsi che le informazioni siano state recepite anche dalle più giovani.

IW 3-5

SCREENING WITH PRIMARY HPV-DNA TEST: FIRST RESULTS IN WOMEN AT THE SECOND SCREENING ROUND

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Objectives: In April 2012 the organized screening programme based on primary hrHPV-DNA test of Este (north-east of Italy) started its second round. We present the first results observed in the women at their second screening episode with a hrHPV test.

Methods: Women 28-64 yrs-old underwent hrHPV test three years after a negative hrHPV test.

Between April and December 2012, 941 of 1,374 women complied to invitation (crude attendance rate 68.8%; adjusted attendance rate 75.3%).

35 cases tested hrHPV+ (3.7%); 20% of triage pap smears tested positive (referral rate to colposcopy 0.74%). Only one CIN2 was diagnosed at colposcopy (detection rate for CIN2+ 1.06‰).

The table compares the main results of the second round of the hrHPV-based programme with those at the first round (carried out in 2009-2011) and with the previous screening programme based on pap smear during 2006-2008.

	hrHPV programme second round	hrHPV programme first round	previous pap based programme
Adjusted attendance rate (%)	75.3	51.5	43.9
Positivity to HPV test (%)			
-25-64 years	3.7 (35/941)	7.0	
-25-34 years	8.6 (12/139)	14.5	
-35+ years	2.7 (23/802)	5.7	
Positivity to triage pap smear (%)	20.0 (7/35)	39.6	-
Referral rate to colposcopy (%)	0.74 (7/941)	2.7	2.6
Detection rate for CIN2+ (‰)			
-25-64 years	1.06	4.5	1.5
-25-34 years	0	8.2	1.8
-35+ years	1.25	3.6	1.5
Positive predictive value for CIN2+ at colposcopy (%)	20.0 (1/5)	11.7	7.7

Conclusions: More than two thirds of women who had been previously screened with hrHPV test attended the invitation to the second round. Positivity to hrHPV was almost halved compared to the prevalence round. The reduction was higher in women 35+ yrs. Also positivity to triage pap smear was halved compared to the first round. Overall, the workload for colposcopy (that represented a major drawback of the first round of the hrHPV-based programme) was much reduced.

IW 3-6

IS IT TIME TO SHIFT TO HPV AS PRIMARY SCREENING TEST? DEFINING PRIORITIES TO IMPROVE CERVICAL CANCER SCREENING.

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Background. Most of the invasive cervical cancers in industrialised countries are due to lack of Pap test coverage, only a marginal amount is due to screening failures. This study is aimed at quantifying the proportion of invasive cancers attributable to lack of screening and to screening failures during the first two screening rounds (1996-2002) and in following rounds (2003-2008) in Emilia-Romagna region.

Methods. All invasive cancers registered in the regional cancer registry in 1996-2008, were classified according to screening history through a record-linkage with screening programme registry.

Results and Conclusions. The incidence significantly decreased from 11.6/100,000 to 8.7/100,000: more rapidly for squamous cell cancers (APC -6.2*; IC: -7.8, -4.6) than adenocarcinomas (APC -0.4; IC: -3.6, 2.8), for advanced (APC -6.6*; IC: -8.8, -4.3) than micro-invasive cancers (APC -1.7; IC: -6.7, 3.6). The proportion of cancers in women not yet invited and non responders decreased over the two periods from 45.5% to 33.3%. Instead, the proportion of women with a previous negative pap test <5 years and $>=5$ years before cancer incidence increased from 5.7% to 13.3% and from 0.3% to 5.5%, respectively. Even if non attendance to screening programme is still the main barrier to cervical cancer control, the introduction of a more sensitive test, i.e. HPV DNA test, could significantly reduce the burden of disease.

IW 3-7

VACCINATION IN YOUNG ADULT WOMEN: PRELIMINARY RESULTS FROM ITT TUSCANY TRIAL

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Objectives: Prophylactic vaccines, designed to protect against the HPV16 and HPV18 types most commonly associated with cervical cancer, have the potential to reduce the associated morbidity, mortality, and incidence of precancerous lesions. Anyway screening needs to be continued also for vaccinated women in order to prevent cancers arising from the other oncogenic types. Vaccination is expected to reduce costs of screening, not only for a reduced referral to colposcopy linked to a lower prevalence of disease but also for a longer screening interval.
Data from efficacy studies provide little information about the impact of vaccination on routine screening, as study women have overall younger age that those called for screening and were screened much more intensively than in regular programmes.
In Tuscany Region a randomised trial 'Effective surveillance and impact of HPV vaccination on screening for cervical cancer' is ongoing.
The aims of the study are understanding the impact of vaccination on regular screening activity, evaluating the seroprevalence suveys before vaccination and the immunity status after vaccination, studying the dynamics of infection before and after vaccination and comparing HPV status prevalence in cervical samples and in urine to evaluate the possibility to monitor HPV status in younger girls.

Methods: Four thousand women aged 25 years resident in Florence have been invited for the study.
Historical data from Florence screening program suggests that screening adherence in this age group to screening is generally between 15-20% in the short term. Offering at this age screening test and vaccination slightly increase the compliance to screening call.

Conclusion: The prevalence of HPV-HR infection was 16%, nevertheless most young women have the potential to benefit from vaccination since $<1\%$ are infected with both oncogenic HPV types included in the prophylactic vaccines.
Certainly, like all organized prevention programs is important that management of HPV vaccination will be organized within the national health system. Vaccination centres makes it possible to reduce inequalities in vaccination offer, organize and manage the monitoring of vaccinated women and assess the future impact of vaccination.

IW 4-2

LA CITOLOGIA DI TRIAGE

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Il documento di Health Technology Assessment (HTA) italiano " Ricerca del DNA di Papilloma Virus umano come test primario di screening dei precursori del cancro del collo uterino", recentemente pubblicato, considera raccomandabile il test HPV per lo screening primario, a condizione che le donne positive al test HPV non siano inviate direttamente a colposcopia, ma si utilizzi un sistema di triage citologico. L'introduzione di un test filtro fra HPV e colposcopia ha lo scopo riportare su valori accettabili la specificità del processo

Nel triage citologico cambia la frequenza di anormalità (circa il 30-50% dei citologici di triage) rispetto alla citologia di screening, ma non cambiano ovviamente i quadri morfologici. Per questo motivo la lettura deve comunque basarsi su sistemi di refertazione riconosciuti, quali il Sistema Bethesda 2001 (TBS 2001).In questo contesto comunque l'utilizzazione della categoria ASC-US deve essere limitata al massimo, classificando nel modo più netto possibile i relativi quadri morfologici (Negativo vs LSIL+).Nella scelta delle procedure di Controllo di Qualità Interno dovrebbe essere superato il concetto di standard accettabile e standard desiderabile, riconducendo questo standard al più alto livello qualitativo. I nuovi indicatori di qualità della citologia di triage sono il numero percentuale di citologie anormali (ASC-US+) ed il valore predittivo positivo (VPP) del test per lesioni CIN2+ istologicamente confermate. La percentuale di Pap test positivi dopo un test HPV positivo è uno dei punti di iniziale criticità da monitorare in modo continuo nella fase di avvio del nuovo programma. Ad oggi non siamo in grado di definire uno standard al quale fare riferimento . I dati a disposizione sono quelli degli studi di fattibilità e dei primi programmi passati al test HPV. I risultati di questi progetti indicano una forte variabilità, fra il 20% ed il 50% .In prospettiva si dovrà comunque indicare un range di variabilità di questa percentuale che ad oggi potremmo provare ad ipotizzare fra il 25% ed il 35%. Il valore predittivo positivo di casi HPV positivo/citologia positiva rappresenta il punto essenziale per valutare le performance della citologia di triage. Anche in questo caso non esistono standard di riferimento. Inoltre il confronto non può avvenire con i dati di altri programmi dove ci sono ulteriori elementi di variabilità , ma con i VPP storici dello stesso programma basato sulla citologia di screening: i risultati di questo confronto saranno fortemente indicativi della qualità della citologia di triage.

IW 4-3

ORGANIZZAZIONE DI UN LABORATORIO HPV DI GRANDI DIMENSIONI

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Obiettivi. L'introduzione della ricerca del DNA dell'HPV nell'ambito di programmi di screening richiede che i test utilizzati siano standardizzati e validati, che abbiamo una sensibilità e specificità cliniche ottimali per la rilevazione di lesioni cervicali di alto grado, e che il processo sia effettuato garantendo il controllo di tutti gli step.

Metodi. Per valutare le performance analitiche e cliniche è stata fatta una ricerca bibliografica, e sono stati valutati i test a disposizione oggi. Inoltre sono stati presi in considerazione i risultati dell'Health Tecnology Assessment (HTA), relativa all'introduzione del test HPV come test di screening primario.

Conclusioni. **Numerosi sistemi sono oggi disponibili, relativi alla rilevazione dei genotipi al alto rischio dell'HPV, sia in pool** che individualmente. Tuttavia solo pochi sono stati sufficientemente testati e validati da un punto di vista analitico e clinico. Alcuni test sono stati validati mediante valutazione nell'ambito di studi di screening di popolazione, dove l'obiettivo primario era rappresentato dal triage dell'ASCUS, altri invece sono stati validati clinicamente e analiticamente seguendo le linee guida recentemente proposte (Mejier C 2009). Inoltre, l'analisi del report dell'HTA suggerisce come da un punto di vista di affidabilità dei dati e di adeguata produttività, sia necessaria la centralizzazione dei test per HPV in laboratori con dimostrato expertise, che possano gestire un elevato numero di richieste in automazione, in modo da potersi avvalere di un processo organizzato a partire dalla fase preanalitica fino alla emissione del referto, con monitoraggio continuo della qualità del test, attraverso sia procedure di controllo di qualità (interno ed esterno) sia tramite il controllo delle performance cliniche.

IW 4-4

IL TYPE REPLACEMENT: IL RUOLO DEL LABORATORIO

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Obiettivi: Recentemente, sono stati introdotti due vaccini contro il papillomavirus umano, il Cervarix® (GSK), vaccino bivalente che protegge dall'infezione contro due tipi di HPV ad alto rischio oncogeno, HPV16 e 18, e il Gardasil® (Sanofi Pasteur MSD), vaccino quadrivalente che in più protegge anche dall' infezione contro HPV6 e 11, questi ultimi responsabili del 90% dei condilomi.
A partire dal 2006 sono ormai più di 40 i paesi in tutto il mondo che hanno introdotto la vaccinazione HPV all'interno dei propri programmi di immunizzazione nazionali. In Italia la vaccinazione HPV è stata introdotta nel 2007 ed è rivolta gratuitamente a tutte le bambine di 12 anni.
HPV16 e 18 sono responsabili del 70% dei cancri cervicali, seguiti da HPV45 e 31 (responsabili di un ulteriore 10%) e HPV33 e 52 che contribuiscono per circa un altro 5-7%.
Sebbene ci aspettiamo che la vaccinazione riduca la prevalenza dei tipi di HPV contro cui il vaccino è attivo, vi è preoccupazione per l'effetto che questo potrebbe avere sulla distribuzione degli altri tipi oncogenici; infatti la vaccinazione potrebbe determinare una riduzione dei cancri correlati ad HPV16 e 18, ma contemporaneamente potrebbe portare ad un aumento dei cancri causati da HPV non 16 né 18 (type-replacement).

Metodi: Il fenomeno del type-replacement può essere studiato valutando la prevalenza tipo specifica di HPV sulla popolazione femminile generale, (meglio se confrontando popolazione vaccinata e popolazione non vaccinata), e successivamente valutando la prevalenza dei singoli tipi di HPV direttamente su lesioni cervicali, quindi sul cancro. Tale fenomeno deve essere valutato attraverso sistemi di genotipizzazione HPV che siano in grado di individuare tutti i tipi ad alto rischio oncogeno.

Conclusioni: Attualmente non possiamo dire con certezza se si verificherà il type replacement e la risposta definitiva a questo quesito sarà fornita in futuro da studi ampi e a lungo termine e da studi di sorveglianza post-vaccinale.

IW 4-5

COMPARISON OF THE HC2 –HR RESULTS USING QIASYMPHONY FULLY AUTOMATED SAMPLE PREPARATION AND MANUALY CONVERTION 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 HC2 High-Risk HPV DNA Test® (HC2 test) 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 RCS-HC2 HR-HPV test. 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 tested using the RCS Digene HC2 High-risk HPV test. 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 MC was 91.5%, 83% for positive samples and 97% for negative. RLU/co range of discordant samples: <1: 3.4%, 1–2.5: 100%, 2.5–10: 33,3%, >10: 1,4%. Evaluating intra-laboratory reproducibility the Meijer et al. guidelines indicated a k value of 0.5 on at least 500 specimens, 30% of which tested positive for a clinically validated test. QIAsymphony AXpH showed good reproducibility of results, with a k value of 0.81, much above the 0.5 indicated by the guidelines. 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 PreservCyt samples. A complete set of 96 samples can be processed in less than 4.5 hours. This significantly reduces the laboratory workload.

IW 4-6

THE ONCLARITY™ HPV ASSAY: EVALUATION OF A NEW HPV TEST

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Objectives. The BD Onclarity™ HPV Assay is an amplified DNA test for qualitative detection of high-risk types of human papillomavirus (HPV). This assay is performed with the BD ViperTM LT System: the assay is able to detect and genotype all high risk HPV types (16,18, 33, 45, 59, 31, 35, 39, 51, 52, 56, 58, 66, 68). Using PerservCyt® Solution, the aims of this study are: I) to compare the results of the BD Onclarity HPV Assay on the Viper to reference cytology results and histology results from biopsy; and II) to compare the results to the Qiagen HC2 HPV DNA Test (HC2; Qiagen, Gaithersburg, MD) and Roche Linear Array HPV Genotyping Test (LA; Roche, Pleasanton, CA).

Methods. The BD Viper LT System, is capable of automated extraction of nucleic acids from HPV specimens as well as amplification and detection of target nucleic acid sequences (qualitative results POS/NEG and/or genotyping) by Polymerase Chain Reaction (PCR) technology.
Samples from 572 prospective subjects were enrolled in the study. An interim analysis on 300 subjects (142 NILM, 19 ASCUS and 139 >ASCUS) was performed: of the 197 subjects with histology results, 96 were <CIN2 and 101≥CIN2. All testing was according to manufacturer's protocols.

Conclusions. Clinical Performance of the BD Onclarity™ HPV Assay compared to histology results (?CIN2 vs <CIN2) based on these preliminary data shows a sensitivity of 97% (95%CI: 91.5-99) and a specificity of 49% (95% CI: 39.2-58.8). The Qiagen HC2 shows a sensitivity of 96.7% (95% CI: 90.8-98.9%) and a specificity of 40.9% (95% CI: 31.4-51.0%). The concordance between Viper and HC2 by each cytology category (NILM, ASCUS and >ASCUS) and combined shows an Overall Percent Agreement of 94.7% (95% CI: 91.5-96.7) and a Positive and a Negative Percent Agreement of 92.8% (95% CI: 87.6-95.9) and 96.6% (95% CI: 92.3-98.5) respectively.
The concordance for HPV genotypes 16 between BD HPV Test and LA shows an Overall Percent Agreement of 98.3% (95% CI: 96.2-99.3).
These data show that the BD Onclarity™ HPV Assay is an effective system for the qualitative detection of HPV. The study is ongoing and more detailed conclusions will be presented.

IW 4-7

MUCOSAL AND CUTANEOUS HUMAN PAPILLOMAVIRUSES DETECTED IN RAW SEWAGES

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Objectives. Epitheliotropic viruses can find their way into sewage. The aim of the present study was to investigate the occurrence, distribution, and genetic diversity of Human Papillomaviruses (HPVs) in urban wastewaters.

Methods. Sewage samples were collected from treatment plants distributed throughout Italy. The DNA extracted from these samples was analyzed by PCR using five PV-specific sets of primers targeting the L1 (GP5/GP6, MY09/MY11, FAP59/64, SKF/SKR) and E1 regions (PM-A/PM-B), according to the protocols previously validated for the detection of mucosal and cutaneous HPV genotypes. PCR products underwent sequencing analysis and the sequences were aligned to reference genomes from the Papillomavirus Episteme database. Phylogenetic analysis was then performed to assess the genetic relationships among the different sequences and between the sequences of the samples and those of the prototype strains.

Conclusions. A broad spectrum of sequences related to mucosal and cutaneous HPV types was detected in 81% of the sewage samples analyzed. Surprisingly, sequences related to the anogenital HPV6 and 11 were detected in 19% of the samples, and sequences related to the "high risk" oncogenic HPV16 were identified in two samples. Sequences related to HPV9, HPV20, HPV25, HPV76, HPV80, HPV104, HPV110, HPV111, HPV120 and HPV145 beta Papillomaviruses were detected in 76% of the samples. In addition, similarity searches and phylogenetic analysis of some sequences suggest that they could belong to putative new genotypes of the beta genus. In this study, for the first time, the presence of HPV viruses strongly related to human cancer is reported in sewage samples. Our data increases the knowledge of HPV genomic diversity and suggests that virological analysis of urban sewage can provide key information useful in supporting epidemiological studies.
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IW 4-8

PREVALENCE OF GENITAL WARTS IN THE ITALIAN GENERAL FEMALE POPULATION

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Objectives: Worldwide, several million cases of genital warts occur each year. The objective of this study was to estimate: 1) the prevalence and the incidence of external genital warts (eGW) in a sample of women attending gynecological ambulatories and 2) the total number of women with eGW diagnosed in Italy.

Methods: In the years 2009-2013, 89 local gynecologists were included in this prospective study. They reported demographic data for every woman aged 15-64 years that they visited for any reason. For women diagnosed with eGW, behavioral and clinical data were recorded. Prevalence of eGW was calculated as the proportion between the number of women with eGW and that of women visited; incidence of eGW was calculated as the proportion between the number of women with a new diagnosis of eGW and that of women visited. Standardized prevalence by age was used to estimate the number of eGW cases in the Italian female population aged 15-64 years.
Results: Between October 2009 and May 2013, 25,284 women aged 15-64 years were included; 120 women were diagnosed with eGW (prevalence: 4.7‰, 95%CI:3.9-5.7). The highest overall prevalence was observed among 15-24 year-old women compared to women older than 25 years (10.5‰ vs 3.8‰), with a trend significantly decreasing by increasing age (p-value$<$0.001), and among women living in Southern Italy compared to those living in Central and Northern Italy (6.2‰ vs 3.4‰, p$<$0.001). The cumulative incidence was 3.6‰ (95%CI:2.9.-4.4). The estimated total number of women with eGW among women aged 15-64 years in Italy, was approximately 84,147.

Conclusions: These data confirm the prevalence of eGW reported in a retrospective Italian study conducted among gynecologists (Vittori et al. 2008), and stress the importance of clinical networks in investigating STI epidemiology, as well as promoting safe sex, implementing early diagnosis, treatment and prevention.

IW 4-9

CELLULARITY CONTROL: KEY TEST FOR THE EVALUATION OF SAMPLE ADEQUACY IN THE MOLECULAR DIAGNOSIS OF HPV AND OTHER SEXUALLY TRANSMITTED DISEASES

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Objectives. Increase of molecular tests for pathogens as high risk papilloma virus (HR-HPV), *Chlamidia trachomatis* (CT) and *Ureaplasma spp*, performed on DNA extracted from biological materials like urine and cervical swabs, should not be carried out without an adequate standardization of the pre-analytical phase. With the introduction of HPV test as a first-level test in cervical cancer screening, evaluation of sample adequacy (previously performed on Pap smear) becomes essential. Aim of this study was to evaluate the role of internal control (IC) to standardize pre-analytical phase and the role of the endogenous control (EC) in the suitability evaluation of biological matrices, and their influence on false negative results.

Methods. 120 cervical swabs were pre-treated and extracted following 3 different protocols. Extraction performance was evaluated by amplification of: IC, added in each mix extraction; human gene HPRT1 (EC) with RT-PCR to quantify sample cellularity; L1 region of HPV with SPF10 primers (InnoLipa). 135 female urine, 553 cervical E-swabs (CS) and 332 ThinPrep swabs (TP) were tested for *C. trachomatis* (CT) and *U. parvum* (UP) with RT-PCR and for HPV by endpoint-PCR. These samples were also tested for cellularity quantification.

Conclusions. Extraction protocol with higher average cellularity (Ac)/sample showed lowest number of samples with inhibitors; highest HPV positivity was achieved by protocol with greater Ac/PCR. CS and TP under 300.000 cells/sample showed a significant decrease of HPV (p$<$0.005) positivity. Female urine under 40.000 cells/ml were inadequate to efficiently detect CT (p$<$0.05) and UP (p$<$0.05). Our data show that IC and EC allow optimization of pre-analytical phase, with an increase of analytical quality. Cellularity/sample allows better sample adequacy evaluation, crucial to avoid false negative results, while cellularity/PCR allows better optimization of PCR amplification. Further data are required to define the optimal cut-off for adequacy evaluation: 300.000 cells/sample or more?

IW 4-10

HUMAN PAPILLOMAVIRUS (HPV) INFECTION IN DIFFERENT ANATOMICAL SITES OF HIV-INFECTED MEN

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Objectives: This study aimed at evaluating the prevalence of Human Papillomavirus infection (HPV DNA) and viral genotypes harbored in oro-pharyngeal, urine and anal canal samples of HIV-infected men.
Methods: In oro-pharyngeal and urine samples, HPV DNA was detected through a specific nested-PCR assay amplifying a 150 bp segment of ORF L1 and genotyping was carried out through sequencing. Anal canal swabs were analysed using INNO-LiPA® HPV Genotyping Extra (Innogenetics, Belgium). HPV prevalence was expressed as a crude proportion with corresponding 95% confidence intervals (95% CI) calculated with Wald test assuming a normal approximation. Statistical analysis was performed using OpenEpi, version 3.01.
Conclusions: HPV testing was performed on samples collected in the same day from oro-pharyngeal, urine and anal canal of 91 HIV-infected men (median age 41 years, IQR: 36-46 years) mostly MSM (98.9%). The overall prevalence (and 95%CI) of HPV infection was: 27.5% (19.4-37.4), 23.1% (14.4-31.7) and 98.9% (96.7-100) in oro-pharyngeal, urine and anal canal samples, respectively. A total of 13 different genotypes were identified in oro-pharingeal swabs, 9 (63.2%) belonging to the HR-clade and 4 (30.8%) to LR types. Sequencing of urine samples showed the presence of 5 different HPV types, 2 HR (40%) and 3 LR (60%). A multiple infection was found in 90% (83.8-96.2) of anal canal swabs and 92.2% (86.7-97.8) of HPV-positive sample showed an infection sustained by at least one HR type. Infection in all three sites examined was assessed in 7 subjects (7.7%; 95%CI: 2.2-13.2), whereas 26 men (28.6%; 95%CI: 19.3-37.9) were HPV-positive in 2/3 anatomical sites. HPV-16/-18 types were found in 44 anal swabs (44/90; 48.9%, 95%CI: 38.6-59.2) and in one oro-pharingeal sample. The present study detected HPV infection in a high proportion of HIV-infected MSM, with the highest prevalence in the anal canal. On the basis of data obtained, further investigations and long-term longitudinal studies are needed in order to developed new strategies for prevention of well known anal malignancies and to understand the meaning of oro-pharingeal colonization and the risk of head and neck cancer in HIV-infected subjects.

IW 5-3

FOLLOW-UP AFTER POSITIVE HPV TEST, NEGATIVE PAP TEST AND NEGATIVE COLPOSCOPY

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Prevalence of HR-HPV in female population depend on age (1). HPV test is unappropriated under age 30 because high prevalence of transient infections. Using HPV test as first level test (and triage with pap test –PT- if it is positive) or using cotest (HPVtest and PT) to screen women aged ?30, we identify women that are HPV+ but PT negative. These women account for about 3.6-3.9% of screened women (1,2), declining prevalence with age -from 7% under 35 years to 2.6% over 41 years(1)-. The prevalence of HPV+/PT- women depends also on the performance of cytology in that country. These women have a three years risk of CIN2+ up to 17%(3) or a five years risk of CIN3+ up to 4.5% (2), higher than women with only PT negative, and HPV+/PT- women repeat first level test in a year; the HPV test performed after one year persists positive in about 50% of them. Because in women with persistent positive HPV test the 5 years risk of CIN3+ lesions increase up to 7.4%, (2) women with repeated HPV+/PT- are sent to colposcopy.

If colposcopy is satisfactory and TZ is normal, usually the woman return in a year for a screening test, that may be PT or HPV test or PT and HPV test. There are not enough data on this population in medical literature. If women are checked with PT, those with positive PT are sent to colposcopy, or after two negative PT they return to usual screening. If women are checked with HPV test (or with HPV test and PT) and it is still positive (with negative PT) we don't know exactly in which way we have to manage them. We can presume a further increase of 5 years risk of CIN3+ lesions (three yearly consecutive HPV positive test), and so we have to send women to colposcopy; but we have to consider the negative predictive value of three yearly consecutive negative PT, associated with one negative colposcopy, so we could send women to three years cytologic follow-up: in this case, how will be the "interval cancer" in this population? There is a real risk of increasing colposcopy, unnecessary biopsies and treatments in women who have only the persistence of HPV infection, that may clear (5). There are no EBM data on the use of others viral tests, and there are no data to guide our choices in these population.

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IW 5-4

PROTOCOLLO DI GESTIONE NEL FOLLOW-UP POST-TRATTAMENTO E POST-COLPOSCOPIA NEGATIVA: IL PUNTO DI VISTA DEL GINECOLOGO

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Le pazienti trattate per displasia cervicale sono a rischio di recidiva e devono quindi essere seguite con un adeguato follow up. Il rischio di sviluppare un cancro cervicale in tali donne è da 2 a 5 volte maggiore rispetto alla popolazione generale. Gran parte di questo aumentato rischio dipende da una scarsa compliance delle pazienti; molti studi dimostrano che più del 50% dei carcinomi si sviluppa in donne che abbandonano il follow up. Perciò, l'attenta partecipazione ai programmi di follow up deve essere incoraggiata [1].
Dopo un trattamento distruttivo o escissionale sulla cervice uterina, le linee guida della SICPCV prevedono un controllo cito-colposcopico ogni 6 mesi per i primi 2 anni, dopo i quali si consiglia il ritorno allo screening (Pap test ogni 3 anni) [2].
Secondo il protocollo adottato presso il nostro Centro, il follow up delle pazienti post conizzazione varia in base alla positività/negatività dei margini di resezione del cono. Se i margini risultano negativi, c'è un minor rischio di recidiva; se, invece, sono positivi, il rischio di recidiva è maggiore e i controlli devono essere più frequenti.
Dopo una conizzazione, a margini negativi, si consiglia un controllo cito-colposcopico ogni 6 mesi per i primi 2 anni, seguito da un controllo annuale per 5 anni. Se, invece, i margini sono positivi, si consiglia un controllo dopo 3 mesi dall'intervento. La gestione della paziente poi varia a seconda dell'esito del controllo; quando indicato, viene effettuata una seconda conizzazione.
La gestione della donna con Pap test positivo e colposcopia negativa varia in base al risultato del Pap test.
Se la diagnosi citologica è ASCUS, ASCH o LSIL e la colposcopia è negativa, la donna viene inviata ad un controllo cito-colposcopico a 6 mesi: se questo risulta negativo, torna allo screening; se la citologia risulta positiva, a colposcopia negativa, viene effettuato un trattamento escissionale diagnostico sulla portio oppure programmato un nuovo controllo a 6 mesi.
Se la diagnosi è HSIL e la colposcopia è negativa, viene programmato un controllo a 3 mesi: se risulta negativo, la donna viene inviata ad un nuovo controllo a 6 mesi; se risulta positivo, si effettua un trattamento escissionale diagnostico.
Se la diagnosi è AGC e la colposcopia è negativa, si programma una valutazione endocervicale/endometriale: se questa risulta negativa, si effettua un controllo a 6 mesi, se positiva si procede con il trattamento [2].

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IW 5-6

QUALE TEST HPV NEL FOLLOW-UP

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Obiettivi Le donne dopo trattamento di lesione di alto grado (CIN2+) o con test di primo livello positivo e successivi approfondimenti di secondo livello negativi (colposcopia negativa e/o biopsia <CIN1) sono a maggior rischio di sviluppare lesioni, per cui vengono inserite in protocolli di follow-up con controlli ripetuti. Poter stratificare queste donne in base al rischio di sviluppare CIN2+ a medio-lungo termine è importante per personalizzare i protocolli di gestione. Diversi studi hanno mostrato che la ricerca di sequenze HPV dei tipi ad alto rischio (hrHPV) ha maggiore sensibilità rispetto alla citologia nell'identificare le donne a più alto rischio, ed un maggiore valore predittivo negativo.

Metodi In uno studio di coorte retrospettivo abbiamo valutato un gruppo di 760 donne precedentemente trattate per lesione CIN2+. Durante il follow-up (FU, minimo 2 anni, fino a 10+) sono state seguite mediante colposcopia, citologia e test HPV (ricerca e tipizzazione, disponibile per 469 casi). Complessivamente, 61 donne (8%) hanno avuto una lesione CIN2+ (35 CIN2 e 26 CIN3+) nel FU: 39 al primo controllo (3-6 mesi dopo l'escissione) e 22 dopo un primo FU negativo. Il rischio di recidiva CIN2+ è risultato più alto nelle donne con lesione iniziale CIN3+, giunzione non visibile (Odds Ratio, OR 4,84; Intervallo di Confidenza al 95%, IC95% 1,48-15,9), margini interessati (OR 9,32; IC95% 4,51-19,3) e test hrHPV+ al primo FU (OR per HPV16 36,3, IC95% 10,3-127,7; OR per altri hrHPV 26,9, IC95% 7,33-99,1; OR per HPV basso rischio 1,28, IC95% 0,14-11,7). Per gli stessi parametri sono stati osservati OR ancora più elevati per recidiva CIN3+.

Conclusioni La maggior parte delle donne (più del 90%) ha avuto un outcome favorevole, senza recidive per 2-10 anni. La visibilità della giunzione, lo status dei margini al momento dell'intervento e il risultato del test HPV al primo FU sono risultati essere i fattori più predittivi di rischio di recidiva. La ricerca di sequenze HPV di tipi ad alto rischio (con eventuale tipizzazione parziale per HPV 16) al primo FU (3-6 mesi dopo l'escissione) permette di stratificare i casi in base al rischio, e prevedere FU differenziati.

IW 5-7

FOLLOW-UP DEL CIN 1 NON TRATTATO

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Gli elementi essenziali nella gestione del follow-up del CIN 1 non trattato, sono l'età della donna e la sua anamnesi citologica.

Donne di età inferiore a 25 anni

Nelle donne al di sotto dei 25 anni di età con diagnosi istologica di CIN 1 preceduta da esito citologico ASC-US o LSIL si può semplicemente ripetere un Pap test a distanza di un anno, mentre in quelle con diagnosi istologica di CIN 1 preceduta da esito citologico ASC-H o HSIL è consigliabile ripetere colposcopia e Pap test ogni 6 mesi per due anni. Il trattamento non è raccomandato in questo gruppo di giovani donne, indipendentemente dall'esito citologico di invio. Nel caso che durante il follow-up sia diagnosticata una lesione CIN 2-3, sono accettabili sia il trattamento che l'osservazione nel tempo: se viene specificato CIN 2 sarà preferibile continuare l'osservazione con citologia e colposcopia, mentre se viene specificato CIN 3 o qualora la colposcopia non fosse soddisfacente è raccomandato il trattamento escissionale.

Il termine "giovani donne" non dovrebbe sottintendere una specifica età, ma essere esteso a tutte le donne che – dopo avere ricevuto un'adeguata consulenza dai propri medici curanti – considerano che i rischi su future gravidanze legati al trattamento delle loro anomalie cervicali siano superiori al rischio che si possa sviluppare un cancro durante il periodo di osservazione delle suddette anomalie.

Donne al di sopra dei 25 anni

Nelle donne al di sopra dei 25 anni, un CIN 1 preceduto da esito citologico ASC-US o LSIL può essere seguito con colposcopia e Pap test annuali per due anni, mentre un CIN 1 preceduto da ASC-H o HSIL può essere sottoposto a trattamento escissionale, oppure essere seguito con ripetizione di colposcopia e Pap test ogni 6 mesi per due anni. Dopo due anni è possibile optare sia per un trattamento escissionale diagnostico, che per una continuazione del follow-up annuale.

Ovviamente la diagnosi di una lesione cervicale di alto grado (CIN 2-3) o un esame colposcopico insoddisfacente, impongono il trattamento escissionale.

I trattamenti ablativi del CIN 1 (DTC, crioterapia, laser) – pur essendo contemplati in tutte le linee guida – non sono consigliati, per il rischio di perdere una possibile lesione CIN 3 o superiore. Se si decide per il trattamento, questo sia escissionale (modulabile a seconda della dimensione della lesione e penetrazione nel canale cervicale).

IW 5-9

AN ON-LINE QUALITY ASSURANCE PROGRAMME FOR COLPOSCOPY IN A POPULATION-BASED CERVICAL SCREENING SETTING IN ITALY: RESULTS ON COLPOSCOPIC IMPRESSION

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Objectives. To report the results of a web-based colposcopy quality assurance programme from a population-based cervical screening service in the Emilia-Romagna Region of northern Italy.

Methods. In 2011, a web application was made accessible on the website of the regional Administration. Fifty-nine colposcopists ,out of the registered 65, participated. They logged-in, viewed a posted set of 50 high-quality digital colpophotographs selected by an expert Committee, and classified them for colposcopic impression, graded into four classes, normal, minor changes, major changes, suspicious for invasion (IFCPC 2011). Kappa coefficients for intercolposcopist agreement and colposcopist-Committee agreement were calculated.

Colposcopist-Committee agreement was greater than intercolposcopist agreement (overall kappa 0.69 versus 0.60, $p = .000$). Kappa for colposcopist-Committee agreement was 0.83 on normal colposcopic findings (NCF), 0.53 on abnormal colposcopic findings (ACF)-minor changes, 0.66 on ACF-major changes, and 0.80 on cancer (all p values for pairwise comparisons $<.001$, except for NCF versus cancer [$p = .078$]). There was no systematic tendency for colposcopists to under- or overestimate the colposcopic findings (two-tailed sign test, $p = .13$). Overall colposcopist-Committee agreement was greater among women aged ≥35 years ($p = .000$) and for colposcopists with previous quality assurance experiences ($p = .008$). Only 0.2% impressions of NCF predicted a lesion worse than CIN1. The impression of cancer predicted a lesion less than CIN3/AIS in 0.5% cases. The histological substrates of ACF-minor changes were dispersed over a large spectrum.

Conclusions. The reproducibility of the colposcopic impression according to the IFCPC classification, when used by trained colposcopists examining high-quality images, is higher than is generally perceived. ACF-minor changes is the least consistent impression

IW 5-10

HR-HPV TESTING IN THE FOLLOW-UP OF WOMEN WITH CYTOLOGICAL ABNORMALITIES WITHOUT CIN2+ LESIONS AT THE FIRST COLPOSCOPY

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Objectives: The follow-up after abnormal Pap smear and negative colposcopy is not clearly defined. This study aimed to investigate the role of hr-HPV testing in the management of abnormal Pap test and negative colposcopy for Cervical Intraepithelial Neoplasia grade 2 or worse (CIN2+).

Methods: The study enrolled 1029 women with abnormal screening cytology (years 2006-2010) and negative colposcopy for CIN2+ which subsequently performed a hr-HPV test. Incident CIN2+ lesions were identified through linkage with cancer registry, hospital discharge records, neoplastic pathology reports and the archive of screening program (years 2006-2011).

Conclusion: During the follow-up, the cohort developed 133 CIN2+ lesions; only one among hr-HPV negative women.
The probability of developing CIN2+ on follow-up time as 0.44% (95% Confidence interval (CI) 0.1-3.1) and 41.8% (95% CI 31.8-53.5) for hr-HPV-negative women and hr-HPV-positive women.
A woman with a positive hr-HPV test had about 105 times higher probability of developing a CIN2+ lesion than a woman with a negative hr-HPV test (Hazard Ratio (HR)=104.5, 95% CI 14.5-755.1), adjusted for index Pap test result, age and status of cervix squamocolumnar junction.
Our results confirm that hr-HPV testing is able to select the real group of women at risk of developing CIN2+ lesions in the follow-up of abnormal cytology and first negative colposcopy.

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SINGLE AND MULTIPLE HPV INFECTION AS RISK FACTOR FOR CIN2+: EFFECT OF AGE AND HPV TYPE.

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Objectives: To evaluate the age distribution and risk of CIN2+ of single and multiple HPV infections, stratified by presence HPV 16, in a 1036 women evaluated for an abnormal pap smear

Methods: 3661 patients referred for an abnormal pap were sampled for HPV typing by Linear Array (Roche Diagnostics; 1036 underwent histological evaluation. The age specific risks (Odds Ratios and 95% CI) of CIN2+ by type of infection were estimated using the single low-risk infections as reference category. Age and type of infections also entered a multivariable logistic regression model. DNA-HPV positive samples were categorized into six mutually exclusive groups: no infections, single infections (HPV16 alone, other high and low risks HPV types) and multiple infections with or without HPV16.

Results: Forty-four (4.3%) patients had no infections, 558 (53.9%) single infections and the remaining 434 (41.9%) had a more than one HPV type. Mean age was significantly lower in women with multiple (36.8+/-9.5) and single (39.6+/-9.7) infections vs no infection (43.9+/-10.5, $p < 0.001$). Considering low-risk HPV as reference, high risk HPV DNA of any type in single or multiple infections was significantly associated with an increased CIN2+ risk at any age. The highest risk of CIN2+ was associated with single HPV16 infection, followed by HPV 16 in multiple infection; single non 16 high risk HPV infection and multiple infection without HPV 16 had comparable increased risk of CIN2+. Age was not clearly associated as independent factor with an increased risk of CIN2+ ($p = 0.049$). Risk for CIN2+ was highest for HPV 16 as single infection in women 18-30 (OR = 20.0, 95%CI: 4.3-103.2, $P < 0.001$) with a slightly lower risk for women 31-40 (OR = 14.9, 95% CI: 4.2-53.5, $P < 0.001$) and 41+ (OR = 19.1, 95%CI: 6.9-55.0, $P < 0.001$). CIN2+ risk was also higher in multiple infections including HPV16 at any age (HPV16+: OR = 16.6 95% CI: 3.9-80.5, OR = 9.2 95% CI: 2.6-32.8, OR = 7.9 95% CI: 2.7-24.2 vs HPV16-: OR = 4.1 95% CI: 1.1-19.0, OR = 1.9 95% CI:0.6,62, OR = 2.5 95% CI: 0.99,6.6 for age 18-30, 31-40 and 41+ respectively). High risk HPV single infections, excluding HPV 16, ha a risk comparable with multiple infections negative for HPV16 (18-30: OR = 5.3, 95% CI: 1.2,27.2, 31-40: OR = 3.9, 95%CI: 1.2-13.1, 41+: OR = 6.5 95%CI: 2.6-17.4).