DETECTION OF CERVICAL DISEASE WITH THE APTIMA HPV AND COBAS 4800 HPV ASSAYS USING SUREPATH LIQUID CYTOMETRY SPECIMENS

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Objectives: To compare the performance of Apta HPV screening and genotyping assays (AHPV and AHPV-GT) to the cobas 4800 HPV (cobas) test using SurePath liquid Pap specimens. Interim results from this study were reported previously and the final results reported here.

Methods: SurePath specimens were collected from consented women referred to colposcopy clinics. Colposcopy was performed and biopsies collected to ascertain disease status. SurePath specimens treated with Apta Transfer Solution (ATS) were tested with the AHPV assay and AHPV-positive specimens were tested with AHPV-GT. Untreated specimens were tested with the cobas test per the manufacturer’s instructions. The clinical sensitivity and specificity of each assay was calculated using a CIN 2+ endpoint. Absolute and relative risks were calculated for various HR-HPV and genotype interpretations.

Results/Conclusions: There were 136 cases of CIN 2+ from 1333 evaluable women (10.2% prevalence). The clinical sensitivity and specificity was 91.9% and 59.6% for AHPV and 94.9% and 54.9% for cobas. Specificity differences were significant (p-value < 0.001), while sensitivity was not (p-value 0.2188). Women with genotype-positive results (16 and/or 18/45) were at significantly higher risk of disease (31.7% for Apta assays and 30.2% for cobas) than women with other high-risk types (12.4% for Apta assays and 10.9% for cobas). Women negative for high-risk HPV had low risk of disease (1.5% for Apta assays and 1.1% for cobas). These results demonstrate sensitive detection of CIN 2+ disease by the AHPV and AHPV-GT assays using ATS-treated SurePath Specimens, similar to cobas HPV testing of SurePath samples.

DIRECT GENOTYPING FROM THE APTIMA® HPV ASSAY USING ANYPLEXTM II HPV28

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Objectives: In the APTIMA® HPV assay, 14 hr-genotypes (16,-18,-31,-33,-35,-39,-45,-51,-52,-56,-58,-59,-66 and 68) are detected, without type verification. During amplification of mRNA copies, double stranded DNA is produced as an intermediate step and the aim of this study was to evaluate if double stranded DNA could be used for genotyping, direct from the analyzed APTIMA tubes.

Methods: 108 samples were genotyped before and after APTIMA® HPV assay. Prior to analysis, 1 ml sample was used for DNA extraction. After mRNA analysis, APTIMA tubes were collected and 200 µl was DNA extracted for comparison. Pre- and post DNA was genotyped using the AnyplexTM II HPV28 (Seegene, Korea) detecting 28 different genotypes using real-time PCR and melting curve analysis at 30, 40 and 50 cycles.

Results: Sixty-three (58.3%) of the samples were positive for any of the genotypes included in the AnyplexTM II HPV28 prior to APTIMA® HPV assay and fifty-four (50.0%) samples were positive in the post analysis.

Sixty-five hr genotypes results were found prior to APTIMA® HPV assay, and fourteen hr genotyping results were lost at post analysis. Nine of the lost results were initially detected at 50 cycles indicating low viral load. When comparing detection levels before and after the APTIMA® HPV assay, thirty of all the genotyping results were detected at same cycle no and twenty of the results at a lower detection level. Interestingly, hr genotypes were detected in both pre and post analysis in 24 samples, where HPV-42 (n=9) and – 53 (n=6) were the most abundant genotypes.
COMPARISON OF HC2 AND COBAS REALTIME PCR IN ROUTINE HPV TESTING
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Background: Long-time the HC2 test was the gold-standard in routine HPV testing. Meanwhile several new assays have been validated and received an FDA approval. Cytomol, a major German lab, until 2013 used HC2 and shifted to cobas 8/2013.

Methods: We compared the results with cobas from 5.8.2013 to 4.8.2014 when only this test was employed with the same period in 2012/13 (exclusively HC2). The profile of the users remained unchanged. Around 40% of the tests were performed as adjunct to cytology, around 60% as triage or test of cure.

Results: 22.5% of 27,429 tests were positive with the HC2 high-risk (HR) probe (13 HPV types including HPV16/18) compared to 31.8% in 27,246 cases tested with cobas 12 types HR and HPV16/18 probes, an absolute increase of 9.3% and a relative rise of 41.3%. In cases tested out of the Thinprep vial (mainly used for private patients with a lower rate of abnormalities and in adjunct testing) 16.1% were positive with HC2 and 26.1% with cobas. From the proprietary tubes (mainly used for public healthcare patients and as triage) this was 23.8% vs 33.0%. 46.5% of the cases positive with cobas for HPV16 and/or 18 were also positive for the 12-types-HR-HPV set. Comparable data for HC2 are not existing because here only secondary testing for a combined probe set of HPV16/18/45 is available.

Conclusions: First data from routine use of cobas HPV testing point to a higher positivity rate and hence a potentially lower specificity compared to the HC2 assay.

DEFINITIVE SEPARATION OF HIGH GRADE FROM LOW GRADE DYSPLASIA USING THE COMBINED MORPHOLOGICAL AND MOLECULAR BIOMARKERS OF ONCOTECT 3DX
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Background: In prior studies, we have used 3Dx technology to demonstrate the ability to combine multiple measurable attributes with detection of E6/E7 mRNA to create a landscape capable of describing the cellular environment present in HPV infection. In an extension of this, we measured 13 cellular parameters to create a Logistical Regression Model taken from a compilation of 166 patients who were HPV DNA negative NILMs (negative for intraepithelial lesion or malignancy) and compared them to patients who were clinically defined as ASCUS (Atypical Squamous Cells of Undetermined Significance) n=37, or LSIL (Low-Grade Squamous Intraepithelial Lesion), n=29 taken from liquid based cytology specimens submitted for HPV DNA testing.

Materials and Methods: All submitted samples were processed using a semi-quantitative method designed to hybridize HPV mRNA and stain DNA using DAPI. The samples were collected on a Sony EC800 cytometer and the derived cellular parameters were tabulated with metadata in Excel and then ported into R, an open source language for statistics and graphics. Once in R, Random Forest analysis identified five parameters that differed from NILM and these parameters were used to derive the model designed to detect difference. The derived model was then tested by plotting true positives versus false positives to create an AUC (Area Under the Curve) and Boot Strapped 10 times to compare the initial accuracy of the model versus a cross validation of the model.

Results: The values of AUC (0.86-0.88) and Cross Validation Accuracy (0.851-0.861) reveal that the derived model is both highly specific and accurate in delineating ASCUS/LSIL from NILM in this small study. It is our goal to use similar concepts to objectively determine patients who should be referred to colposcopy from those who are merely infected. The use of highly specific cellular parameters using 3DX coupled with bioinformatic approaches appears to be a technique capable of highly accurate delineation beyond current standard of care.
A NOVEL, SIMPLE AND SPECIFIC TEST FOR HPV INDUCED NEOPLASIA VIA DIRECT DETECTION OF E6 ONCOPROTEIN

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The unacceptably high mortality caused by cervical cancers in developing countries calls for screening technology that readily allows widespread implementation in settings of need. The Onco\textit{E6}\textsuperscript{TM} Cervical Test (E6 Test) has been developed to meet requirements specific to many low and limited resource settings; the test features lateral flow format and is void of cold chain requirements for storage or sample collection. The procedure uses low complexity equipment which is virtually maintenance free, and comparatively inexpensive. The E6 Test is compatible with several specimen types (dry swab, PreservCyt media), and it can accommodate very low to medium specimen throughput per run. The E6 Test can be performed by personnel with little to no laboratory expertise, and operator training can occur via training video, or by in person training following a trainer-trainee model. The time from sample collection to result is about 2.5 hours, reducing the risk of patient loss on follow-up, and facilitating screen-and-treat. Directly detecting elevated quantities of the cancer causing viral oncoprotein E6, the test delivers high clinical specificity and positive predictive value (98.9\% and 40.8\% for CIN3+, respectively, as found in the Chinese START-UP study), thus greatly reducing over treatment upon a positive test result. We will present a clinical performance summary for the Onco\textit{E6}\textsuperscript{TM} Cervical Test, present features relevant to use low resource settings, and discuss implications of its use as a triage or primary screen in a variety of settings.

PERFORMANCE OF THE TROVAGENE URINE HPV ASSAY IN A REFERRAL POPULATION

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OBJECTIVE: Determine performance of the Trovagene Urine HPV test for the detection of high-grade cervical intraepithelial neoplasia (CIN) within a referral population.

METHODS: A total of 518 pairs of urine (in EDTA) and liquid based cytology samples in (ThinPrep PreservCyt medium) were collected. The Trovagene HR-HPV test was used for the detection of 13 high risk HPV types by consensus PCR amplification of the E1 region in both specimen types. Samples were anonymized, sent to Trovagene for testing with all urine specimens and currently 320 matching ThinPrep samples completed. This was part of the Predictors 4 study in which cytology samples were assayed by a range of other HPV tests in both ThinPrep and SurePath.

RESULTS: Sensitivity (SE) and specificity (SP) from cytology samples in ThinPrep medium was similar to other well established tests (SE= 98.0\% for CIN3+ and 95.7\% for CIN2+; SP= 22.8\% for < CIN2). SE from urine samples was only slightly lower (90.5\% for CIN3+ and 88.0\% for CIN2+) with similar SP (24.7\% for < CIN2). Positive/negative agreement between the 320 paired urine and ThinPrep samples currently analyzed was 84.4\% (95\% CI 79.8\%, 88.2\%) with 25 discordant pairs in each direction (kappa = 46.9\%). 90.5\% of the samples positive on cervical material were also positive in urine.

CONCLUSIONS: The Trovagene test had very good performance in ThinPrep samples - comparable to other established HPV tests, and good performance in urine that was slightly lower than for ThinPrep. Detailed performance from both urine and cervical samples will be presented.
AUTOMATED VERSUS MANUAL PRE-PROCESSING OF SUREPATH LIQUID-BASED CYTOLOGY SPECIMENS: ASSESSING IMPACT ON THE PERFORMANCE OF THE REALTIME HIGH RISK HPV ASSAY

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Cervical specimens collected in liquid-based cytology (LBC) media are the only sample types used for cervical cytology in the UK. The Abbott RealTime High Risk HPV assay, a highly automated qualitative multiplex real-time PCR for the detection of 14 high-risk HPV (hrHPV) types with simultaneous typing of HPV16 and HPV18, has been approved by the English NHS Cervical Screening Programme for HPV triage of low grade dyskaryosis and test-of-cure. As per manufacturer’s instructions, pre-analytic processing of LBC samples involves manual mixing and liquid transfer of the original sample into a secondary tube used directly on the Abbott m2000 System. Future implementation of hrHPV primary screening increases the need for automation of pre-analytics. We evaluated a custom-configured work-table-setup of the TECAN EVO Freedom instrument designed to automate pre-processing of SurePath samples and patient-identification matching between original SurePath vials and secondary tubes comparing results from 415 specimens (primary screening population: N = 260; triage and test-of-cure population: N = 155) pre-processed manually and automatically. hrHPV was detected in 91 samples after manual pre-processing and 94 positive results were obtained after automatic pre-processing. Excellent agreement of overall-hrHPV results (98.3% [95%CI:0.92-0.99]; kappa: 0.95) and individual results for HPV16, HPV18 and other-hrHPV (≥ 98.6% agreement; kappa ≥ 0.89) was found following manual and automated pre-processing. The TECAN EVO Freedom-configuration designed to automate pre-analytics required for preparing SurePath samples for hrHPV-testing resulted in assay performance comparable to that following the manufacturer-validated manual process, saves ~1.5mins hands-on-time per sample processed and allows for complete specimen identification tracking and documentation of process control.

INTRODUCTION OF CEPHEID® HPV TESTS INTO CERVICAL SCREENING IN MALAWI

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Objectives: Visual inspection with acetic acid (VIA) has a poor record in interpreting cervical abnormalities accurately and skills are readily lost. HPV tests could provide an objective primary screen with triage to VIA of positives. This project aims to test this in an unscreened population in rural Malawi

Methods: LBC samples were obtained from women attending VIA clinics within a new programme. Collection media included ThinPrep, saline and Cepheid®’s own collection medium. HPV tests were carried out on the same day as collection and later linked to clinic data.

Results: In the first 475 samples, HPV positivity was 20.2% overall and 31.8% in women known to be HIV positive. Of 90 positive samples, 17.8% harboured HPV 16, 19.6% HPV 18/45 and 56% HR-HPV other types. Comparison of collection media was available for 146 paired samples, with complete agreement in 133 (91%) and discordant or invalid results with one or more collection media in 13 samples. Results of HPV status linked with VIA results and clinical outcomes will be presented.

Conclusions: The Cepheid® HPV test proved easy to do with a turnaround time of 2 hours possible from clinic back to clinic. If the cost is acceptable, HPV testing may prove useful as primary screen in some settings in low resourced settings.
Objectives: High-risk human papillomavirus (hrHPV)-DNA testing is frequently performed parallel to cytology for the detection of high-grade dysplasia and cervical cancer (termed CIN 3+) particularly in women above 30 years of age. Although highly sensitive, hrHPV testing has only limited specificity. Furthermore it is well known that only a proportion of CIN lesions progress to cervical cancer. Therefore, markers with prognostic value would substantially improve cervical cancer diagnostics.

Methods: In a retrospectively, cross-sectional study with two gynecological hospitals cervical scrapes from more than 500 patients with histopathology-confirmed diagnosis a panel of 5 promising DNA methylation marker regions comprising a newly developed test termed GynTect® were analyzed using methylation specific PCR. In addition, for 29 patients who attended the colposcopy clinic at least twice (up to nine previous visits) before obtaining the endpoint diagnosis CIN3, the GynTect® markers were determined and the results correlated to histopathological and cytological findings.

Results: High sensitivity and specificity was observed for the detection of CIN3+ if at least 2 out of 5 GynTect markers were methylated. In the longitudinal study earlier detection (up to six years) of the severe disease compared to histopathology was obtained in 19 of 29 patients (66%), indicating that methylation of the GynTect® marker panel may show the likeliness of progression to severe lesions.

Conclusions: GynTect® may provide a promising diagnostic tool for identifying patients with CIN3+ among hrHPV-positively tested women. Furthermore there is strong evidence that these markers may be of prognostic value.

Objective: To assess the suitability of Novaprep HQ+ Orange medium for the detection of HR HPV DNA by HC2.

Methods: Specimens were prepared with HPV16+ cells (SiHa and CaSki), HPV18+ cells (HeLa) and HPV- cells (C33-A) diluted in Novaprep HQ+ Orange medium and in STM as reference medium. Repeatability was evaluated by coefficient of variation (CV%) calculated on 14 determinations (three levels of HPV16 and HPV18 DNA concentrations). Reproducibility was assessed between 3 lots of Novaprep HQ+ Orange medium by CV% calculated on 14 determinations. A method-comparison study was conducted on HPV16+ and HPV- specimens diluted in Novaprep HQ+ Orange medium and STM. A study is ongoing to assess the stability of specimens stored at +4°C, +21°C and +40°C in 3 lots of Novaprep HQ+ Orange medium. Samples were tested with HC2 according to the manufacturer’s instructions.

Results: Repeatability and reproducibility showed CV%<8% whatever the genotype (HPV16/18), or the lot was. The comparison study showed a 100% sensitivity and specificity for HR HPV detection from samples diluted in Novaprep HQ+ Orange medium. Furthermore, an excellent correlation was achieved (r²>99%) between RLU/CO measured from cell samples diluted in Novaprep HQ+ Orange medium compared to those diluted in STM. Specimens are stable for at least 10 weeks at +4°C, +21°C and +40°C. In conclusion, Novaprep HQ+ medium adequately preserves HPV DNA and is suitable for HR HPV DNA detection by HC2.
VALIDATION OF TWO METHODS OF COLLECTING INFORMATION ON HPV TEST: ELECTRONIC CLINICAL HISTORY AND MANUAL REGISTER ON CERVICAL CANCER SCREENING IN SPAIN.

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Objectives: In Catalonia (Spain) cervical cancer (CC) remain as the main screening tool the cytology and also introduced for the first time, the Human Papillomavirus (HPV) DNA test under some very specific situations (triage of ASC-US, underscreened women (women aged >40 years without screening history in the last 5 years) and post-surgery treatment). The aim of this study was to examine the percentage and the reasons of each HPV test performed in CC screening for the period 2007-2011 using two methods of collection information: clinical history and manual register.

Methods: Two sources of information were available: a) electronic health record system used by 77% of primary care doctors of the public system in Catalonia for period 2008-2011, b) A specific form for collecting information about HPV tests including reasons, age, center for period 2007-2009 used by 100% of primary care doctors.

Results: Preliminary descriptive results indicate that from 2008 to 2011, 55,460 HPV tests (positivity of 16.8%) were registered in the electronic history. 54.8% were performed in underscreened women and 15% for ASC-US triage. In contrast, during the period 2007 to 2009, 66,486 forms for HPV testing were filled (positivity of 21.5%), 61.4% of which were performed in underscreened women and 22% for ASC-US follow-up.

Conclusions: Although preliminary descriptive results suggest that there may be some differences between the two methods of data collection, more comprehensive validation analysis adjusted for period and percentage of covered population are underway and will be presented at the conference.

HIGH-RISK HPV mRNA IN RELATION TO FUTURE HIGH-GRAD E LESIONS AMONG HPV-DNA POSITIVE WOMEN WITH MINOR CYTLOGICAL ABNORMALITIES

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Objective: The aim was to determine the sensitivity and specificity of the APTIMA HPV mRNA assay (Hologic) in predicting future development of high-grade cervical intraepithelial neoplasia (CIN) among high-risk HPV-DNA-positive women with ASCUS or CIN1 cytology.

Methods: Archived SurePath cervical samples of women ≥ 35 years of age with high-risk HPV DNA-positive atypical squamous cells of ASCUS (n = 211) or CIN1, (n = 131) were tested by the APTIMA HPV assay, and the women were monitored for development of histopathologically verified CIN2+.

Results: Twenty-nine percent (61/211) of the women in the ASCUS group, and 34.3% (45/131) in the CIN1 group developed CIN2+ within 4.5 years of follow-up. The prevalence of HPV mRNA was 90.0% (190/211) among women with ASCUS and 95.4% (125/131) among women with CIN1. The presence of HPV E6/E7 mRNA was associated with future development of CIN2+ among women with ASCUS and CIN1 (p = 0.02).

The APTIMA assay demonstrated high sensitivity in predicting future CIN2+ and CIN3 in the ASCUS (96.7% and 100%) and CIN1 (97.8% and 100%) groups. The corresponding specificity was low (5.4-12.7%). The negative predictive value of the HPV assay for detecting future CIN3 was 100% since no mRNA-negative woman developed CIN3 (0/27) as compared to 13.6% (43/315) of the mRNA-positive women (p = 0.03).

Conclusion: The APTIMA mRNA assay demonstrated high sensitivity but low specificity in predicting future CIN2+ among women with minor cytological abnormalities. The assay had high negative predictive value for future CIN3, indicating that HPV-mRNA-negative women are at low risk of progression to high grade CIN.
MONITORING THE IMPACT OF THE HPV VACCINATION PROGRAM AND CERVICAL CANCER CONTROL IN BHUTAN

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Objectives: Bhutan has started a national cervical screening program in year 2000 and a vaccination programme against human papillomavirus (HPV) in 2010 with coverage of >90% in girls aged 12-18. The collaboration between the government of Bhutan and IARC to monitor HPV prevalence over 10 years and pilot HPV-DNA-based cervical screening, offers an excellent opportunity to evaluate short- to medium-term impact of cancer control in LMICs.

Methods: We conducted a set of descriptive studies since 2011 to assess the burden of HPV infection and local screening program performances. A cytology-based and urine-based HPV prevalence survey were conducted among adult women and young female students, respectively; a case series of CIN3+ biopsies was assessed to quantify the fraction of cervical disease attributable to each HPV type; and finally, an assessment of screening coverage of women aged 25 years or more was performed in the capital, Thimphu.

Results: For the cytology-based and urine-based survey 2505 women and 1051 students were enrolled, respectively; for the case-series 323 biopsies were collected; and finally for the cervical screening assessment 1620 women were investigated. Among the general population and students HPV prevalence was 26% and 11%, respectively; 70% of cancers were attributable to HPV16/18; the screening coverage in the capital was 34%.

Conclusions: We will present detailed results of the baseline picture of HPV epidemiology in Bhutan prior to vaccination and will describe a 10-year IARC protocol for repeat surveys with which to compare future vaccinated cohorts of women to demonstrate vaccine impact.

POSSIBLE HAND-GENITAL HPV INFECTION TRANSMISSION AMONG HETEROSEXUAL COUPLES: RESULTS FROM THE HITCH STUDY

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Objectives: HPV is transmitted by mucosal contact but other modes of transmission may exist. We analyzed the HITCH cohort study data to understand the role of the hand-genital route in transmission.

Methods: At enrolment and 4-month follow-up visits, we tested for HPV genotypes in genital and hand samples from 213 female university students (aged 18-24) and their male partners. We assessed genotype-specific concordance beyond chance between hand and genital sites within and between partners and genital-genital concordance between partners.

Results: Prevalence was higher in genitals (penile: 59.2%, vaginal: 57.8%) than in hands (male: 36.2%, female: 34.3%). Observed-to-expected ratios (95%CI) for hand-genital concordance within individuals were 8.7 (6.8-10.6) for men and 8.7 (6.7-10.7) for women, whereas between-partner ratios were 8.0 (6.0-10.0) for male hand-female genital and 6.7 (5.1-8.4) for female hand-male genital. The ratio for between-partner genital type concordance was 6.4 (5.3-7.5). Genital positivity was associated with the partner’s same-type hand positivity, after accounting for the partner’s genital HPV status. Mutually-adjusted odds ratios of vaginal infection were 7.40 (4.7-11.6) for male hand positivity and 55.2 (40.0-76.1) for penile positivity. The equivalent figures for penile infection given female hand and vaginal positivity were: 5.68 (3.5-9.2) and 55.80 (40.1-76.8).

Conclusions: In an individual, HPV types in the hand tend to reflect those present in the genitals, regardless of gender. Hand positivity is also correlated with a partner’s genital positivity, even after taking into account the individual’s genital types. Hand-genital transmission may be a significant influence in the epidemiology of HPV infection.
OBJECTIVE:

To assess the availability of type-specific data on HPV infection in European women and identify data gaps.

METHODS:

PubMed/Medline and EMBASE databases were systematically searched to retrieve full publications reporting type-specific prevalence and incidence of genital infections in general European populations of women without clinically-manifested disease, as well as type distribution in histologically confirmed cervical, vaginal and vulvar neoplasms and cancers. Original studies and meta-analyses reporting on types 16 and 18 and at least one other high-risk type were included. Studies published before 2000, non-English publications, studies focusing on special populations (e.g., HIV-infected, pregnant), and small studies (N < 50) were excluded. Key information, i.e., study type, country, population characteristics, sample type, HPV assay used, and HPV data, was extracted from each study.

RESULTS:

A total of 236 publications met the inclusion criteria: 126 for type-specific infection prevalence in general female populations, 107 for type distribution in cervical lesions or cancers, 23 for type distribution in vulvar or vaginal lesions and 5 for type-specific infection incidence. Five countries (Italy, UK, Spain, Sweden and Netherlands) contributed more than half of these studies. By UN sub-region, 107 studies originated from Southern Europe, 66 from Northern Europe (includes the UK), 51 from Western Europe and 17 from Eastern Europe (includes the Russian Federation).

CONCLUSIONS:

There is a significant scarcity of published HPV type-specific data for Eastern Europe, representing a population of approximately 147 million women. Gaps also exist for HPV type-specific incidence in European women and type distribution in vulvar/vaginal lesions.

CORRELATION OF VIRAL LOAD AND PERSISTENCE FOR HPV16 AND HPV18 IN A DUTCH COHORT OF YOUNG WOMEN

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OBJECTIVE:

Persistent human papillomavirus (HPV) infection with an oncogenic high-risk HPV (hrHPV) type is necessary for the development of cervical cancer. Here we evaluate the association of viral load of HPV16/18 in persistent and non-persistent HPV16/18 infection in young women.

METHODS:

3282 Women aged 16-29y supplied vaginal self-swabs in two rounds, one year apart. Samples were tested for HPV-DNA using the SPF10-PCR DEIA LiPA25 system. HPV16 and HPV18 load was quantified via an adapted RT-PCR protocol targeting the L1 gene. Values were normalized for cellular content by β-actin RT-PCR measurement. Only women that were HPV16/18-positive at baseline and participated in two rounds were included in the analysis.

RESULTS:

271 Persisting HPV16 infections and 112 persisting HPV18 infections were found. Viral load was significantly higher in persistent infections than in clearing infections for HPV16 and HPV18 (p=0.015, p=0.018 respectively). HPV16 load was significantly higher in multiple infections (HPV16 + any HPV) than in single infections (p=0.003), but did not differ significantly for HPV18 (p=0.136). HPV16/18 loads in infections with or without α9/α7 HPV co-infections did not differ significantly (p=0.926, p=0.092). Integration of variables into logistical regression analysis showed significant contribution of viral load levels to HPV16 and HPV18 persistency status (p=0.001, p=0.011). Contribution of multiple and α9/α7 co-infection status was not significant for HPV16 and HPV18 (combined variables: p=0.171, p=0.692).

CONCLUSIONS:

Viral load is a proxy for persistent HPV16 and HPV18 infections, but needs to be combined with other factors to predict persisting infections.
Objective: This retrospective study examined if HPV-specific changes in viral load measured in serial cervical smears are predictive for the natural evolution of HPV infections.

Methods: A cervical histology database was used to select consecutive women with biopsy-proven CIN in 2012 who had at least two liquid-based cytology samples before diagnosis of CIN. Before performing cytology, 18 different qPCRs allowed HPV type-specific 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 ±0.3 HPV copies/cell/day 2) basal cell transforming infections leading to CIN 3+ 0.003 HPV copies/cell/day.

Results: 210 women were included, 80 had single type infections (80 infections with 2 measurements, 53 infections with 3 measurements) and 130 had multiple HPV types (382 infections with 2 measurements, 281 infections with 3 measurements). For 2 and 3 consecutive HPV specific viral load measurements there was a decrease in productive infections from CIN 1 to CIN 3+ and an increase in the number of transforming infections. We could clearly demonstrate regressing lesions with a persistent linear decrease in viral load ($R^2>0.9$; -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 allows triaging HPV-driven processes in transient virion productive and basal cell transforming, enabling prediction to high-grade CIN.
WHAT CAN TWINS STUDIES TELL US ABOUT THE AETIOLOGY OF CERVICAL CANCER?

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Objectives: To investigate environmental and genetic influences on variation in susceptibility to cervical pre-cancer in a classical twin study.

Methods: Pap smear histories were obtained from monozygotic (MZ) and dizygotic (DZ) adult twin pairs. Casewise concordance was estimated for ‘history of atypical squamous cells that cannot exclude high-grade squamous intraepithelial lesions or higher’ (≥ ASC-H). Within-pair correlations were estimated for behavioural risk factors for ≥ ASC-H using a subset of participants for whom these data were collected.

Results: Pap smear histories were available for 2,095 pairs (1,324 MZ and 771 DZ). Mean age at most recent Pap smear was 42.9 years (SD 11.2), with no differences by zygosity. Overall, ≥ ASC-H was reported in 354 (7.8%) women. Casewise concordance for ≥ ASC-H was 0.24 (95% CI 0.16–0.32) for MZ pairs and 0.16 (95% CI 0.07–0.25) for DZ pairs, (p=0.2) for difference. In a subset (389 MZ and 238 DZ pairs), correlations were greater for MZ pairs for: age at first sex (p=0.001), lifetime number of sexual partners (p<0.001), years smoking (p<0.001), pack-years smoked (p<0.001) and Pap smear frequency (p=0.04)."}

Conclusions: Twin pairs were concordant for ≥ ASC-H suggesting a familial influence; although the estimate was higher for MZ than DZ pairs, the difference was not significant. Known cervical pre-cancer risk factors were more correlated for MZ than DZ pairs. Therefore, within the limitations of our sample size, we found no evidence that genetic factors explain variation in the development of cervical pre-cancer. The study concordance estimates guide the design of future pooled studies on this topic.

48 MONTH INCIDENT HPV INFECTION RATES FOR WOMEN HPV NEGATIVE AT BASELINE: THE HPV FOCAL TRIAL

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Objectives: HPV FOCAL is a population-based randomized trial evaluating primary high-risk HPV DNA (HPV) testing plus liquid-based cytology (LBC) triage for HPV positives, compared to LBC screening with HPV triage for ASC-US. Reported are 48-month incident HPV infection rates and factors associated with incident infection in women baseline HPV negative.

Methods: In HPV FOCAL, women 25-65yrs randomly assigned to primary LBC (LBC arm) or HPV testing (Intervention arm (IA)). IA baseline HPVneg women exit trial at 48 months with LBC and HPV testing. Logistic regression analysis was conducted to determine factors associated with incident HPV infection.

Results: 9540 of 9553(99.9%) women in the IA had baseline HPV results: 770/9,540 (8.1%) were HPVpos [<35yrs: 334/1,862 (17.9%); 35-49yrs: 318/4,457 (7.1%); 50+ yrs: 118/3,221 (3.7%)]; and 8,770/9,540 (91.9%) HPVneg. Of the 2709 baseline HPVneg women with a 48 month result, 111/2,709 (4.1%) were HPVpos [<35yrs: 33/334 (9.9%); 35-49yrs: 53/1,312 (4%); 50+ yrs: 25/1,063 (2.3%)]. Of the 111 HPVpos, histopathology results available for 55(49.6%): 36(65.5%) negative; 13(23.6%) CIN1; 5(9.1%) CIN2; no >CIN3 identified. Logistic regression analysis found incident HPV infections were associated with age <35yrs (AOR 4.8; 95%CI 2.6, 8.9) and lifetime sexual partners (<6 partners: AOR 0.5; 95%CI 0.3, 0.9).

Conclusions: 48 month incident HPV rate in women initially HPV negative is 4.1%, compared to a baseline HPV prevalence rate of 8.1%. Women with incident HPV infections were likely to be younger, and have more sexual partners. In programs planning HPV primary screening, awareness of the incident infection rate will help to better plan for health services demands.
TRENDS OF HOSPITAL DIAGNOSTIC DEMAND OF PAPILOMAVIRUS. 1996-2013.

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INTRODUCTION AND OBJECTIVES: HPV diagnostic is a technique of rising demand in clinical setting due to its value anticipating cervical pathology. The aim of this work is to analyze trends on diagnostic demand and positive results during last 18 years.

MATERIALS AND METHODS: Retrospective descriptive study of HPV diagnostic in samples from two different hospitals representing 500,000 habitants by means of molecular diagnostic based on hybridization, PCR and LIPA.

RESULTS: From 16,915 analyzed samples, 6,064 (35.8%) out of those were positive for any type of HPV. From these, 5,346 (32%) had high risk HPV type (HR-HPV), corresponding 736 (4.4%) to co-infections with low risk type HPV (LR-HPV). In 721 samples (4.3%) it was exclusively diagnosed LR-HPV, with minor differences in these percentages along the studied period. During 18 years, increasing trend in diagnostic demand fits a parabolic shape (R²=88.5%), while performance for HR-HPV slightly declined in a 0.9% yearly. Paradoxically, the sharpest slope in decreasing performance occurred in 2001-2004, when demand decreased too. In the last period (2009-2013) demand continues increasing in ~100 samples yearly, while HR-HPV diagnostic performance increases in a 0.4% yearly.

CONCLUSIONS: HPV detection is one of the most increasing techniques in the Microbiology laboratory. Despite that, percentage of positive and ARHPV/samples ratio remains being satisfactory, above 35 and 25% respectively during 18 years period. The outstanding performance could be related to milestone on scientific knowledge of HPV. Regarding registered trend in this study, it is expected a continuous increasing in the diagnostic demand of HPV.

EPIDEMIOLOGY OF HPV 6, 11, 16, 18 ANTIBODIES AMONG ADULTS IN THE UNITED STATES ACROSS SIX YEARS: NHANES 2005-2010

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Objective: To assess the prevalence, distribution and trends of human papillomavirus (HPV) antibodies (Ab) contained in the quadrivalent HPV vaccine (HPV 6, 11, 16 and 18) among US adults.

Methods: The sample population consisted of 11,186 men and women ages 18-59 years with HPV antibody available data included in the National Health and Nutrition Examination Surveys (NHANES) 2005-2006 (pre-HPV vaccine), 2007-2008 and 2009-2010 (post-vaccine) . HPV type-specific Ab serostatus was established using the competitive Luminex Immunoassay of Antibodies to Neutralizing Epitopes on HPV 6, 11, 16 and 18 L1 VLPs. Both cervical and oral HPV types were assessed using the Roche Linear Array Assay. Data analyses consisted of weighted prevalence with 95% confidence intervals, accounting for complex sampling with the SVY module of Stata V13. A p value <0.05 was set for statistical significance.

Results: Prevalences of HPV Ab 6, 11, 16 and 18 were 16.4%, 5.9%, 12.4% and 4.8%, respectively. Among women, Ab prevalence increased from 15.7% in 2005 to 23.4% in 2010 (HPV16) and from 5.6% to 9.5% (HPV18) (p<0.05) with a dose response by number of vaccine shots.

Conclusions: Significant Ab prevalence increases were seen across survey years among women but not among men, likely attributable to the effect of HPV vaccination1.

ABUNDANCE OF PUTATIVELY NOVEL HPV TYPES IDENTIFIED IN EYEBROWS USING NOVEL CODEHOP PRIMERS AND A SINGLE TUBE NESTED ‘HANGING DROPLET’ PCR

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Objectives: Several PCR-based methods have been developed for simultaneous detection of a broad range of human papillomaviruses (HPVs) infecting skin. These traditionally include HPV-genera-specific single-round PCR using a single or multiple pairs of degenerate primers or nested PCR using two pairs of degenerate primers. In this study, an improved version of the original panPV CODEHOP primers for the broad detection of PVs across different PV-genera was used in combination with a single-tube nested ‘hanging droplet’ PCR to survey cutaneous HPVs.

Methods: DNA was extracted from 149 eyebrow hair specimens and amplified in a single-tube nested PCR reaction using a total of 70 cycles. Obtained amplicons were either directly sequenced or cloned and sequenced if they contained multiple HPV types or a novel type. Sequences were phylogenetically evaluated by the Bayesian algorithm using the BEAST package.

Results: HPV DNA was detected in 59.7% (89/149) of the analyzed eyebrow hair specimens. Multiple HPV types were found in the majority of 52 cloned PCR-amplicons. In addition to 32 officially recognized HPVs, a total of 35 putatively novel HPV types or subtypes were identified. Phylogenetic analysis revealed that all novel HPVs reside within the Betapapillomavirus (five types/subtypes) and Gammapapillomavirus (30 types/subtypes) genera, some forming novel viral species.

Conclusions: The panPV CODEHOP primers used with single-tube nested PCR are powerful tools for discovering novel HPV types. Continuous efforts to improve identification of novel cutaneous HPVs leads to a better understanding of their phylogenetic diversity and may eventually clarify their role in the development of skin cancer.

SIGNIFICANT REDUCTION IN THE INCIDENCE OF GENITAL WARTS IN YOUNG WOMEN AND MEN 5 YEARS INTO THE DANISH NATIONAL HPV VACCINATION PROGRAM

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Objective: Denmark introduced the quadrivalent HPV vaccine into the vaccination program for 12–15-year-old girls in 2008/2009, which resulted in a prompt significant decrease in genital wart (GW) incidence in young women (1). In 2012, the program was supplemented with a catch-up program for women 19–27-years-old. We aimed to further extend our nationwide evaluation of the effectiveness of the vaccination program by increasing both the cohort size (with prescriptions of Podophyllotoxin) and follow-up time (until 2013) of the previous Danish cohort study.

Methods: Incident cases of GWs were identified from the Danish National Patient Register or through redemption of prescription for podophyllotoxin in the Danish National Prescription Registry in 2006–2013. Age-specific incidence rates (IR) were estimated and annual percentage change (APC) was calculated using Poisson regression.

Results: GW incidence was either stable or increased in both sexes aged 16–25 years in 2006–2009, with the greatest increase seen in 18–19-years-old men (APC = 25.3%; 95% confidence interval (CI): 18.4; 32.7). After introduction of the vaccination program, GW incidence decreased significantly in both women and men aged 16–25 years with nearly elimination among 16–17-year-olds (IRwomen: from 957 to 72 per 100.000 person-years (APC = –56.0%; 95% CI: –59.8; –51.4); IRmen: from 378 to 84 per 100.000 person-years (APC = –37.8%; 95% CI: –41.5; –33.9) in 2009–2013, respectively).

Conclusion: We find a near elimination of GW among women in age groups with high vaccination coverage. A similar pattern was observed for men indicating a substantial protection by herd immunity.

References
EFFECTIVENESS OF QUADRIVALENT HPV VACCINE ON GENITAL WARTS IN A REAL-LIFE SETTING IN BELGIUM

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Objective: To measure vaccine effectiveness (VE) of quadrivalent HPV (qHPV) against genital warts (GWs) in 16-23 year-old women in a real-life setting in Belgium.

Methods: We conducted a cohort study in women eligible for HPV vaccination in Belgium between 2007 and 2013 who were affiliated to a large Belgium sick-fund (MLOZ). Demographic and reimbursement data for insurees are recorded in their database. Women were considered as fully vaccinated 30 days after all three doses of qHPV vaccine had been reimbursed and as unvaccinated if no qHPV vaccine was reimbursed. Women vaccinated with bHPV vaccine were excluded from the primary analysis. A first agreement for reimbursement by the sick-fund medical advisor for imiquimod (the first line, most common GW treatment in Belgium) was used as a surrogate for a first GW episode. The incidence rate ratio (IRR) was used to compare GW incidence in unvaccinated and vaccinated women. VE was calculated as: $VE = 1 - IRR \times 100$. Poisson regression was used to adjust for confounding factors.

Results: Among the 106,579 women (369,381 person-years) included, 33.6% were fully vaccinated at the end of follow-up. Overall, 274 GW treatment episodes were recorded. The age-adjusted VE for fully vaccinated women was 88.0% (95% CI: 79.4; 93.0). VE was higher in those who were <18 years than in those aged 18 to 23 years at vaccination.

Conclusion: High qHPV VE was observed in a real-life setting in Belgium. These estimates are consistent with the results from clinical trials and other effectiveness studies in different settings.

EFFECT OF TIMING OF SECOND DOSE OF QUADRIVALENT HPV-VACCINE ON CONDYLOMA INCIDENCE

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Objectives: To assess the incidence of condyloma after 2 doses of qHPV-vaccine, by time since 1st vaccine dose, in women first vaccinated before age 20.

Methods: We established a national population-based cohort of 1.3 million women, aged 10-27, living in Sweden from 2006-2012. Women were identified through Swedish health care registers. The primary endpoint was condyloma, identified through the Patient Register (ICD10) or the Prescription Drug Register (prescriptions for Podophylotoxin or Imiquimod). Exposure status was time-varying qHPV-vaccine dose, identified through the Prescription Drug Register and SVEVAC, the national register for HPV vaccination. Incidence rates of condyloma by time between 1st and 2nd dose were calculated. Women were censored at dose 3, emigration, death, or December 31st 2012, whichever occurred first.

Results: In Sweden, a total of 252,800 women received 2 doses of qHPV-vaccine from 2006-2012. There were a total of 185 condyloma cases and 110,866 person-years during follow-up. Between 0-9 months there is a fluctuating downward trend in condyloma incidence with a crude IR/100,000 range of 204 (95% CI 110-379) to 62 (95% CI 9-440). After >9 months between the 2 doses, there is instead a steep increase in incidence IR/100,000 = 746 (95% CI 388-1434).

Conclusions: Incidence rates of condyloma appear to decline with a longer time interval between qHPV-vaccine dose 1 and 2 with a maximum protection with 9 months between the 2 doses. With >9 months between doses, the incidence rate increases instead. However, no firm conclusions can be drawn at this time as further analysis is on-going.
QUADRIVALENT HPV-VACCINE EFFECTIVENESS AGAINST CERVICAL LESIONS: POPULATION-BASED STUDY

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Objectives: This study aims to quantify the effect of quadrivalent HPV (qHPV) vaccination on incidence of CIN2 or worse (CIN2+) in the Swedish population of girls and young women.

Methods: An open cohort of all women ages 13-30 years living in Sweden between 2006-2012 (n=1,294,822) was linked to Swedish nationwide healthcare registers. These registers were used to obtain qHPV vaccination status of women vaccinated in the opportunistic HPV vaccination program (years 2006-2011) and cases with histologically confirmed CIN2+, respectively. The effect of qHPV vaccination on incidence of CIN2+ was analyzed using Poisson regression. Women previously diagnosed with genital warts or with an abnormal pap smear (ASCUS or worse) were excluded.

Results: Vaccination effectiveness against CIN2+ was 71% (95% CI 52-83%) among girls first vaccinated between ages 13-16. For girls first vaccinated between ages 17-19, vaccine effectiveness was 29% (95% CI 9-44%). No significantly decreased incidence of CIN2+ was found when comparing women first vaccinated between ages 20-29, with unvaccinated women (vaccine effectiveness= 7%, 95% CI -13-24%).

Conclusions: We show a significant reduction in CIN2+ following qHPV vaccination in women younger than 20 years at first vaccination. Vaccine effectiveness against CIN2+ increased with decreasing age at first vaccination.

HPV VACCINATION IN YOUNG WOMEN AT 25 YEARS IN ITALY. EFFICACY, IMMUNOGENITY AND IMPACT ON SCREENING FOR CERVICAL CANCER

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Objective: Although teenagers are the main population for HPV vaccines, adult women who remain at risk of cervical cancer can also be vaccinated. To offer vaccination to 25-year old women at their first access to cervical cancer screening represents a good opportunity to evaluate the efficacy of vaccination at this age and to understand its impact on screening activity.

Methods: Women aged 25-years called to the Cervical Cancer Screening program were invited to this study (Grant from Tumour Tuscany Institute). Women were randomized in two groups: Study Group (Pap test, HPV test, Blood sample and free HPV vaccination) and Control Group (Pap test). After 3y, women in both groups performed Pap test and HPV test, and women in the Study Group also a second blood sample.

Results: 4481 invitation letters were sent in 2010. The compliance at enrolment was 18.6% (832/4481). In the Study Group the prevalence at enrolment for any HR-HPV type, HPV16 and 18 was 20.8%, 8.1% and 1.5%, respectively. The prevalence for any HR-HPV, HPV16, HPV18 and HPV31,33,45 was statistically significant reduced in women HPV16/18 negative or HR-HPV negative at enrollment. A reduction in cytological abnormalities was observed in the Study Group compared to the Control Group.
After 3y, sero-conversion was 100% for HPV16 and 98.8% for HPV18. Moreover a strong increase of antibody response was observed also for HPV31,33,45.

Conclusions: The vaccination is effective in reducing the HR-HPV infections in 25-year old women, especially in HPV16/18 negative (12%) and HR-HPV negative (7.1%) before vaccine injection.
MONITORING THE IMPACT OF THE HPV VACCINATION PROGRAM IN RWANDA

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Objectives: Human papillomavirus (HPV) vaccination is expected to offer an effective solution to the high cervical cancer burden in low- and middle-income countries (LMIC). However, reliable evidence of real-life effectiveness of vaccine programs is crucial to encourage national planners to implement and sustain cervical cancer prevention services. The government of Rwanda’s introduction of a HPV vaccine programme in 2011 with an estimated 93% coverage among the target population offers an excellent opportunity to evaluate short- to medium-term impact of HPV vaccination in an early introducing LMIC.

Methods: In 2013/14, the baseline picture of HPV prevalence in Kigali, Rwanda was characterised among unvaccinated cohorts of women by collecting cervicovaginal samples from a population-based, age-stratified sample of 2,500 women aged 18–59 years and from urine samples from 1,000 18-20 year olds in high schools. HPV DNA detection and genotyping is underway using GP5+/6+ PCR.

Results: HPV genotyping results are available for the first 1,049 cervicovaginal samples. HIV prevalence was 23.1%, and high-risk HPV DNA prevalence 21.9%. The most common high-risk HPV type was HPV16 (6.2%), followed by HPV58 (2.9%). High-risk HPV prevalence decreased by age, from 30.7% among 16-24yrs, down to 14.3% for 55+yrs, and was higher in HIV-positive (31.2%) than HIV-negative (16.0%) women. Other significant risk factors for HPV positivity included lifetime number of sexual partners, marital status and receiving cash for sex. Complete HPV results for both the cervicovaginal and urine samples are expected by December 2014.

Conclusions: This survey confirms Rwanda to be a setting of cervical cancer risk with a background of high HIV prevalence. We will present the entire results on the baseline picture of HPV epidemiology in Rwanda prior to vaccination and will describe a 10-year IARC protocol for repeat surveys with which to compare future vaccinated cohorts of women to demonstrate vaccine impact.

EFFECT OF CATCH UP VACCINATION ON HPV PREVALENCE IN ROUTINE CERVICAL SCREENING

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Objectives: Since 2008, Scotland has offered a national HPV immunisation programme to 12-13 year old girls which included an initial 3 year “catch up” for girls up to age 18. The “catch up” cohort are now of age to attend for cervical screening. The aim of this study was to determine HPV positivity in younger women and the effect of vaccination.

Methods: We enrolled 613 women (20-24 years) attending for routine cervical screening. Samples were tested by the Cobas HPV test. HPV outcome was stratified by vaccination status and cytology. Follow up histology, where indicated, was also assessed.

Results: Fifty two percent (n = 321) were vaccinated. The Cobas HPV test was positive in 36.3% and 45.0% of vaccinated and non-vaccinated women respectively. Prevalence of HPV 16/18 in the vaccinated was 3.7% compared to 13.7% in non-vaccinated women. Of these 58.3% and 65.0% respectively were co-infections. One in 10 infections in vaccinated women were due to HPV 16/18 compared to less than 1 in 3 in non-vaccinated women. There were 38 (11.9%) abnormal smears in vaccinated and 48 (16.6%) in non-vaccinated women respectively. We diagnosed 1 CIN2 and 1 CIN3 in vaccinated and 6 CIN 2 and 3 CIN 3 in non-vaccinated women.

Conclusion: Catch up vaccination changed overall prevalence by 8.7%. Positivity associated with HPV16/18 infection has been significantly reduced. We have preliminary evidence to suggest that catch-up vaccination has decreased the number of high grade lesions in the first round of screening.
**OC 3-8**

**PROJECTING THE POTENTIAL PUBLIC HEALTH IMPACT OF A UNIVERSAL VACCINATION PROGRAMME WITH A NINE-VALENT HPV VACCINE IN THE UNITED KINGDOM**

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**Objectives:** To estimate the incremental public health impact of a universal vaccination program with a nine-valent human papillomavirus vaccine (6/1/16/31/33/45/52/58) in the United Kingdom as compared to the current girls-only, vaccination program with a quadrivalent HPV vaccine (6/11/16/18).

**Methods:** A dynamic transmission model of HPV infection and related diseases was calibrated to the UK epidemiological data. Up to 70% of cervical cancer cases were attributed to HPV 16/18 for the quadrivalent vaccine, and an additional 20% to the five additional types included in the nine-valent vaccine. For non-cervical disease (vulvar, vaginal, anal and genital warts), the disease attribution assumption remained constant across both vaccines, producing conservative outcomes. In the base case, a two dose vaccination program with lifelong vaccine protection and a coverage rate of 87.5% was assumed for the 12-year age cohorts. Sensitivity analyses were conducted.

**Results:** The findings of the analyses indicate that universal vaccination with the nine-valent vaccine has the potential to:

i) reduce the incidence of HPV 16/18/31/33/45/52/58-related cervical cancer by 99.9% after 100 years, relative to 76% for the quadrivalent vaccine and,

ii) prevent over 100 years, an additional 92,057 cases of CIN1, 227,344 cases of CIN2/3 and 33,988 cases of cervical cancer in females, 1,700 anal cases in total for females and males and, 365,393 and 969,469 genital warts cases in females and males, respectively.

**Conclusions:** The introduction of universal vaccination with a nine-valent vaccine in the UK is estimated to significantly reduce the public health impact of cervical and other HPV-related diseases.

**OC 3-9**

**CHARACTERISTICS OF A CLUSTER-RANDOMIZED, PHASE IV HPV VACCINATION EFFECTIVENESS TRIAL**

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**Objectives** Human papillomaviruses (HPV) causes anogenital and oropharyngeal cancers. While HPV-16/18 vaccine is efficacious against HPV-infections and associated precancers the herd effects of different vaccination scenarios are open. Our cluster randomized trial (NCT00534638) assesses the herd effects of vaccinating girls vs. girls and boys.

**Methods** In 2007-9 we invited 80,272 1992-95 born early adolescents to a CRT in 33 communities stratified by low, intermediate and high HPV-16/18 seroprevalence. In 11 Arm A communities 90% of participating girls and boys received HPV-16/18 vaccine, in 11 Arm B communities 90% of girls received HPV-16/18 vaccine - boys received hepatitis B-virus (HBV) vaccine, and in 11 Arm C communities all received HBV-vaccine. HPV prevalences were determined at age 18.5 years.

**Results** Equal enrolment of birth cohorts comprised 32,176 (40% response) vaccinees:: 20,515 girls and 11,661 boys. At age 15 years, 79.3% completed a questionnaire, 98% resided at study communities. Smoking and alcohol consumption were similar in the trial arms, also mean-age of menarche (12.4 years) or 1st ejaculation (12.6 years), and sexual behaviour (among <25%, who had had sexual debut) did not differ by arm. Mean-age at the sexual debut 14.3 and 14.4 in girls and boys, and proportions of those with ≥ 5 sexual partners (6.5% to 7.5%) were comparable. By the end of 2014 our CRT will verify predictions on herd effect (17 to 31% herd effect) of vaccinating both girls&boys with moderate vaccine coverage.

**Conclusions** Uniform residential, life-style and sexual behaviour characteristics indicate successful randomization/enrolment. Our CRT will guide HPV vaccination programs by verifying effectiveness of modelled scenarios.

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EVIDENCE OF CROSS PROTECTION FOLLOWING USE OF BIVALENT HPV VACCINE IN THE NATIONAL HPV IMMUNISATION PROGRAMME IN ENGLAND

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Objectives: To monitor the changes in type-specific HPV prevalence since the introduction of national immunisation programme using the bivalent HPV vaccine in England.

Methods: Residual vulva-vaginal swab specimens from sexually-active young women undergoing chlamydia screening in community health services in England between 2010 to 2013 were tested for type-specific HPV DNA using a multiplex PCR and Luminx-based genotyping system. Prevalence of type-specific HPV infection was compared to a similar survey conducted in 2008 (before the introduction of the HPV immunisation programme). Prevalence ratios comparing the pre-immunisation and post-immunisation surveys were calculated using a multivariable log-binomial model, adjusted for demographic and sexual behaviour data. Estimated vaccination coverage was calculated using published national data.

Results: In the post-immunisation period, estimated vaccination coverage among women aged 16-18 years old was 60.2% in 2010-2011 and 73.4% in 2012-2013 (with 87% vaccinated over 12 years old). In this age group, the prevalence of HPV31, HPV33 and/or HPV45 was 8.6% in the pre-immunisation survey (n=1,054), 7.3% in 2010-2011 (n=933) and 5.4% in 2012-2013 (n=1,063). The prevalence of HPV16 and/or HPV18 was 19.1%, 8.7% and 3.7% in the pre-immunisation survey, 2010-2011 and 2012-2013, respectively. The adjusted prevalence ratio was 0.6 (95% CI; 0.4-0.9) for HPV31/HPV33/HPV45 and 0.3 (95% CI; 0.2-0.5) for HPV16/HPV18. No similar reductions were seen for other high-risk HPV types.

Conclusion: These ecological analyses suggest some evidence of cross protection following the introduction of a national immunisation programme using the bivalent HPV vaccine. This surveillance will continue, with attention to the duration of this protection.

TWO OR THREE DOSES FOR PRIMARY HPV VACCINATION SCHEDULES? SYSTEMATIC REVIEW AND META-ANALYSIS

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Objective: The aim of this study is to estimate immunogenicity and efficacy of two-dose HPV vaccine schedules in adolescent girls.

Methods: We searched Medline, Cochrane Central and trials registers from their earliest dates to January 2014. We included controlled trials that randomised adolescent girls to either a two- or three-dose HPV vaccine schedule, or compared a two-dose schedule in girls with a three-dose schedule in women. We extracted immunological and clinical outcome data. We used meta-analysis to calculate weighted mean differences (with 95% confidence intervals, CI) for comparisons between geometric mean concentrations (GMCs).

Results: We screened 1,174 studies and identified eight eligible trials. GMCs were non-inferior (HPV16) or superior (HPV18) in girls receiving a two-dose schedule compared with women receiving the three-dose schedule but between study heterogeneity was severe (three trials in high income countries). In direct comparisons, GMCs in girls receiving the two-dose schedule were lower but non-inferior (HPV18) or inconclusive (HPV16) compared with girls receiving the three-dose schedule. All data showed non-inferior seroconversion and seropositivity for the two-dose compared with the three-dose schedule. GMCs were significantly higher for two-dose schedules with a longer (6 vs. 2 months or 12 vs. 6 months) interval between doses. We found no published clinical efficacy data.

Conclusions: Two-dose schedules in adolescent girls result in immunological outcomes that are as good as or better than three-dose schedules in women. The interval between doses is important. Two-dose HPV vaccine schedules for adolescent girls might increase vaccine effectiveness if they simplify administration and reduce costs.
EFFECTIVENESS OF 3 VERSUS 2 DOSES OF QUADRIVALENT HPV-VACCINE ON INCIDENCE OF CERVICAL LESIONS IN A 3-DOSE VACCINATION SCHEDULE: A POPULATION-BASED STUDY IN DENMARK AND SWEDEN

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Objectives: This study aims to quantify the effect of quadrivalent HPV vaccination with three versus two doses on incidence of histologically verified CIN2 or worse (CIN2+) in girls and young women in Denmark and Sweden.

Method: A cohort of all women aged 13–30 years and resident in Denmark or Sweden in 2006–2013 was followed for HPV vaccination and first occurrence of CIN2+. Information on vaccination dates, number of doses, and dates of diagnoses were obtained from nationwide healthcare registers. Incidence rate ratios (IRR) with 95% confidence intervals (CI) were estimated using a Poisson regression model stratified on age at first vaccination (< 20, ≥ 20) and adjusted for attained age with number of doses as a time-varying covariate.

Results: Preliminary results show that women vaccinated with three doses of the quadrivalent vaccine had significantly lower incidence of CIN2+ compared with two doses. For women vaccinated before age 20 IRR = 0.54, CI = 0.42; 0.71, while for women vaccinated at older ages (≥ 20) IRR = 0.57, 95% CI = 0.52; 0.62. Moreover, two doses of the vaccine did not result in decreased incidence of CIN2+ for women aged ≥ 20 at vaccination compared with unvaccinated women.

Conclusions: We observed a higher risk reduction of CIN2+ after three doses of quadrivalent HPV vaccine compared with two doses both in women vaccinated at younger and older ages. However, for women vaccinated at younger ages the result was based on few events.

Analysis of ASCUS+, the timing between first and second dose on disease incidence, and further adjustment for mother’s education level is ongoing.

MEMORY B CELL RESPONSES AGAINST HPV16, HPV18, HPV31 AND HPV45 IN 12-15 YEAR OLD GIRLS RECEIVING CERVARIX® OR GARDASIL® VACCINE

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Objective: To evaluate memory B cell responses against vaccine (HPV16/HPV18) and non-vaccine (HPV31/HPV45) types following immunization with Cervarix® or Gardasil® and compare with binding and neutralizing antibody responses.

Methods: Serum and peripheral blood mononuclear cell (PBMC) samples were from 12-15 year old girls following three doses of Cervarix® (n = 42) or Gardasil® (n = 46) vaccine. Binding titers were estimated by L1 virus-like particle (VLP) ELISA, neutralizing titers were estimated using L1L2 pseudoviruses and memory B cell responses were estimated by ELISpot following immune activation (R848/IL-2) of PBMC using L1 VLP as antigens.

Results: As expected, all individuals elicited high titer (ca. 104-105) vaccine-type binding and neutralizing antibody responses accompanied by a memory B cell response of around 1% of total IgG-bearing memory B cells. Responses against HPV16 were higher than HPV18 by all measures and Cervarix® vaccination elicited higher responses than Gardasil®. Neutralizing antibody responses against non-vaccine types were less frequent and orders of magnitude lower titer than for vaccine types, with Cervarix® eliciting higher titers than Gardasil®. However, this pattern was not reflected in VLP binding (ca. 105) or memory B cell responses (ca. 0.5% IgG-bearing cells) in which measurable responses against these types were common and similar between the vaccines.

Conclusion: The immunological effectors of cross-protection are unclear although the differential detection of neutralizing antibodies against non-vaccine types is at least coincident with the differential cross-protection bestowed by the current vaccines. These current data suggest that the magnitude of binding antibodies and memory B cells are poor surrogates of this functional neutralizing antibody response.
**OC 4-5**

**CEL-MEDIATED IMMUNE RESPONSE AFTER HPV VACCINATION**

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**Objective:** to evaluate the cell-mediated immune response to HPV after bivalent HPV vaccination, assessing cell proliferation and quantifying cytokines.

**Methods:** Cell Culture was performed with peripheral blood mononuclear cells (PBMC) from 30 patients one month after the total HPV immunization. Cell proliferation was assessed by MTT reduction assay. For the analysis of cytokine mRNA expression, cellular RNA was extracted from PBMC. Relative quantification of cytokines (IFN-β, IFN-γ, IL-12, TNF-α, IL-6, IL-17 or IL-10) in comparison to the transcripts of β-actin gene (endogenous control) was performed using “real time” PCR. Data were submitted to one-way ANOVA (nonparametric test) analysis followed by the Bonferroni’s Multiple Comparison Test.

**Results:** The cell viability of the culture stimulated with the vaccine compared to the negative control was different (118% X 100%, p<0.001). The cytokines were upregulated in stimulated PBMC cultures following vaccination compared to unstimulated cultures. The median n-fold increase theses cytokines were: IFN-β, 334.4-fold (95% CI, 37.49 to 706.3); IFN-γ, 12.64-fold (95% CI, 2.44 to 27.72); IL-12, 46.33-fold (95% CI, 4.29 to 96.96); TNF-α, 2.36-fold (95% CI, 0.69 to 4.03); IL-6, 9.07-fold (95% CI, 1.64 to 16.5); IL-17, 7.33-fold (95% CI, 0.73 to 15.40); IL-10, 6.47-fold (95% CI, 0.84 to 12.09). The cytokines expressions in stimulated cultures were different. IFN-β expression was significantly higher than IFN-γ, TNF-α, IL-6, IL-10 and IL-17 (p<0.05).

**Conclusion:** proliferative PBMC responses against HPV-16 and HPV-18 were detected in women who received the vaccine HPV immunization. HPV vaccine stimulates antiviral immune response, with robust induction of IFN-β production.

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**OC 4-6**

**THE EFFECT OF HERD IMMUNITY IN DIFFERENT HUMAN PAPILLOMAVIRUS VACCINATION STRATEGIES: AN ECONOMIC EVALUATION OF THE BEST II STUDY**

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**Objectives:** Italian recommendations for HPV immunization target females aged 9 to 26 years. However, males can be vectors in virus transmission and are at risk of infection. The BEST II study was designed to evaluate the cost-effectiveness (CE) of different interventions targeting females as well as males.

**Methods:** We developed a dynamic Bayesian Markov model. Both genders were considered in a universal vaccination programme which was compared to screening-only and female-only vaccination. A range of HPV-induced diseases caused by HPV genotypes included in the quadrivalent vaccine were considered (cervical, vaginal, vulvar and anal cancer, and anogenital warts), and cross-protection effects were accounted for. The process of sexual mixing was estimated based on age-, gender- and behavioural-specific factors to estimate the force of infection dynamically. We considered several scenarios; the baseline assumed universal vaccination to be implemented for 12-year-old females and males. The follow-up period was 55 years.

**Results:** According to our analysis, universal vaccination was a cost-effective alternative when compared to screening-only (with an incremental CE ratio, ICER, of €1,200) and to female-only vaccination (ICER = €6,900). We performed extensive

**Conclusions:** Universal HPV vaccination of male and female cohorts is potentially cost-effective compared to cervical screening and female-only vaccination, when accounting for a wide range of HPV-related diseases. This is mainly due to the fact that universal vaccination increases effects of herd immunity and provides protection against HPV in males as well as in females.
**OBJECTIVES**: Safety statements issued from outside of Japan are based on scientific evidence with transparency such as those from WHO, WMA, FIGO, Australia, the UK, the US, Korea and Canada, etc. In contrast, the Japanese Ministry of Health Labour and Welfare (MHLW) has decided not to resume proactive recommendation for the vaccine for more than 1 year. We investigated differences in scientific evidence and socio-political factors among other countries, governing bodies and Japan.

**METHODS**: A comparison of statements and recommendations.

**RESULTS**: In Japan, local governments, health professionals and parents were confused by the national government’s suspension of recommendations for the HPV vaccine and as such uptake dropped from around 70% to below 8%. Safety statements issued from outside of Japan were based on scientific evidence with epidemiological surveillance both pre- and post-vaccination for analysis of causality between the vaccines and adverse events.

**CONCLUSION**: The Japanese government must issue strong recommendations with clear and honest communication to the public and the health professionals. Public health authorities should explain the effectiveness and safety of the HPV vaccine based on scientific evidence via mass media. Despite the presence of scientific evidence, immunization of an important vaccine has been suspended and this will have serious implications for the future in Japan. In 10-20 years, only in Japan, will there be may many patients suffering from cervical cancer globally.

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**THE IMPACT OF NATURAL IMMUNITY ON THE EFFECTIVENESS OF HPV VACCINATION**

**OBJECTIVES**: Population-level effectiveness of HPV vaccination has been estimated in models that all differ in assumptions on estimates regarding natural acquired immunity. We investigated the impact of different model assumptions on estimates of HPV vaccine effectiveness.

**METHODS**: We used STD SIM, an established microsimulation model with which we have previously studied mechanisms for HPV immunity, based on observed epidemiological trends with age. We considered two plausible mechanisms for naturally acquired immunity after infection: full immunity with variable duration (A), or cumulatively decreasing susceptibility to reinfection (B). Vaccine effectiveness was estimated using HPV-16, HPV-18 and cervical cancer incidence. The impact of naturally acquired immunity mechanisms was determined by comparing the vaccine effectiveness estimates between the two different naturally acquired immunity mechanisms. We also determined the impact of increased vaccine coverage (to 100%), the inclusion of boys, and lower vaccine efficacy (70% and 80%).

**RESULTS**: Model assumptions on acquired immunity mechanisms have a large impact on estimates of population-level vaccine effectiveness. While cervical cancer incidence decreases with almost 40% under mechanism A, this reduction is over 45% under mechanism B. The impact of model assumptions on cervical cancer reduction is even larger with a 10%-point difference between mechanisms when boys are included, and when assuming lower vaccine efficacy; and larger for the incidence of HPV-18 incidence compared to HPV-16.

**CONCLUSIONS**: It is crucial to expand knowledge on naturally acquired immunity to accurately estimate population-level vaccine effectiveness, as well as to determine optimal vaccination strategies.
LONG-TERM EFFECTIVENESS OF GARDASIL™ AMONG ADULT WOMEN IN COLOMBIA

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Objectives: The GARDASIL™ long-term follow-up (LTFU) study is an ongoing extension of the safety of the quadrivalent HPV vaccine (qHPV vaccine) and its effectiveness in preventing HPV6/11/16/18-related cervical intraepithelial neoplasia (CIN) or condyloma in 26-45 year-old women. Here, we present our data 8 years after vaccination.

Methods: The early vaccination group (EVG) who received qHPV in the base study included 804 Colombian subjects. The catch-up vaccination group (CVG) included 703 of the 806 eligible Colombian subjects who were vaccinated 5 years after the base (ages 31-50). Subjects underwent history, pelvic exams with Pap tests, and wart/lesion biopsy. Cytology and biopsy specimens were evaluated in a central laboratory with HPV testing, and endpoint adjudication performed by an independent panel. The primary analysis was for safety and effectiveness in the EVG but effectiveness data is available from the CVG as well.

Results: There were no cases of HPV 6/11/16/18-related CIN or condyloma in the EVG over the 8 years of LTFU. Additionally, there were no cases of HPV16/18 related CIN2 or worse or HPV 6/11 related condyloma. As there is no placebo group in the LTFU study, comparison to the two year incidence rates in the base study (HPV6/11/16/18 CIN or condyloma [0.4/100 person-years]; HPV16/18 CIN 2 or worse [0.1/100 person-years] and HPV6/11 condyloma [0.2/100 person-years]) shows maintained vaccine efficacy over the follow-up period.

Conclusion: The qHPV vaccine continued to be effective in this population of adult women. There were no new safety issues encountered in either arm of the study.

A PHASE II STUDY OF GARDASIL IN HUMAN PAPILLOMAVIRUS RESEARCH
THE MID-ADULT MALE VACCINE STUDY–THE MAM STUDY

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Objectives: To establish immunogenicity and safety of the Gardasil vaccine among mid-adult men ages 27-45 years. Specifically, to assess the proportion of HPV antibody sero-conversion one month post-dose three of the Gardasil vaccine.

Methods: The study population included subjects from Tampa, FL, US, and Cuernavaca, Mexico who met eligibility criteria (male, 27-45 years, completed 4 years of follow-up in our HPV natural history study). Subjects completed four visits over seven months, with Gardasil administered at months 0, 2, and 6. Sera, oral, anal, and genital specimens were collected, with computerized questionnaires completed at Months 0 (pre-vaccination) and 7 (post-dose three). Anti-HPV16 and HPV-18 IgG levels were determined by ELISA from sera collected at Months 0 and 7.

Results: 147 of the 150 participants enrolled completed all 4 visits. No serious adverse events were reported. The most commonly reported adverse events were injection-site pain and swelling, headache, and flu-like symptoms. In an initial analysis of 51 U.S. participants, 11 (22%) and 15 (29%) men had detectable antibody levels for HPV16 and HPV18, respectively, at Month 0. At Month 7, all 51 (100%) had sero-converted for both HPV types. Median HPV16 and HPV 18 titers at Months 0 and 7 were 25.2 and 2296.57 EU/mL, and 13.6 and 705.84 EU/mL, respectively.

Conclusion: Vaccination of mid-adult men produced a robust antibody response against HPV16 and HPV18. As men are diagnosed at older ages with HPV-related cancers, vaccination may be an efficacious strategy these cancers in men beyond the currently recommended vaccination age group.
UNDERSTANDING CERVICAL SCREENING NON-ATTENDENCE AMONG ETHNIC MINORITY WOMEN

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Objectives: Women from ethnic minority backgrounds are less likely to attend cervical screening than white women in England. This study explored the socio-demographic and psychological correlates of cervical screening attendance across ethnic groups.

Methods: Women aged 30-60 years were recruited from Indian, Pakistani, Bangladeshi, Caribbean, African and white British backgrounds (n=720, response rate = 65%). Participants completed face-to-face interviews with a multi-lingual interviewer.

Results: Half of the women had not been screened within 5-years (53%,n=374). Women from ethnic minorities were more likely to be unscreened than white British women, (44%-71% vs. 11%, p<.001). Migrating to the UK, speaking a language other than English and low education level were also associated with being unscreened. These socio-demographics reduced, but did not fully explain the association between ethnicity and screening attendance. Ethnic minority women were more likely to be scared of what screening might find (41%-65% vs. 25% of white women), to have low perceived risk of cervical cancer (22%-53% vs. 10%) and to think screening was not needed in the absence of symptoms (55%-63% vs. 7%). Psychological factors reduced the effect of ethnicity further, explaining some but not all of its influence on screening.

Conclusions: There are ethnic inequalities in cervical screening attendance in England and these are not fully explained by socio-demographics. Interventions for ethnic minority women should ensure they understand the meaning of a screening result, minimizing anticipated fear. Addressing perceptions of risk and beliefs about the efficacy of screening in the absence of symptoms may also be beneficial.

BARRIERS TO AND FACILITATORS OF COMPLIANCE WITH CLINICAL BASED CERVICAL CANCER SCREENING

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Objective. This study aims to identify possible barriers to and facilitators of cervical cancer screening by (a) estimating time and travel costs and other direct non-medical costs incurred in clinic-based screening, (b) investigating compliance with screening and reasons for noncompliance, (c) determining women’s knowledge of human papillomavirus (HPV), and (d) investigating correlates of HPV knowledge and compliance with screening.

Methods. Via self-administered questionnaires, data on sociodemographic characteristics, time and travel costs and other direct non-medical costs, mode(s) of travel, time, distance, companion’s attendance, HPV knowledge and compliance with screening were obtained from 1 510 women attending the Swedish organized cervical cancer screening program.

Results. Mean total time and travel costs per attendance were €56. Over half (53%) of the respondents took time off work to attend screening (mean time 147 minutes). A large portion (44%) of the respondents were noncompliant, 51% of whom stated difficulties in taking time off work. 64% of all respondents knew that HPV vaccination was available; only 34% knew it was important to continue to attend screening following vaccination. Age, education, and income were the most important correlates of HPV knowledge and compliance. For compliance, these factors were additionally time off work, companion’s attendance and HPV knowledge.

Conclusions. Time and travel costs of clinic-based screening can be substantial, may influence overall cost effectiveness of screening programs and constitute barriers to screening. Women with knowledge of HPV and who did not take time off work to attend screening were more likely to comply with screening.
**OC 5-4**

**ACCEPTANCE AND SATISFACTION OF CERVICAL CANCER SCREENING PROGRAM USING VISUAL INSPECTION WITH ACETIC ACID AMONG WOMEN IN MOROCCO**

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**Objective:** The aim of the study is to explore the acceptance and satisfaction of cervical cancer screening via Visual Inspection with Acetic acid (VIA) at Meknes-Tafilalet Region. Method: A cross-sectional descriptive study was conducted using face-to-face interview of women attending health centers and meeting the inclusion criteria. A sample of 24 health centers, which represent 20% of total health centers that perform VIA screening in the region, were selected using a simple random sampling proportional to the number of health centers in urban and rural area in each of the 6 provinces of the region.

**Results:** A total of 324 women were included in the study. Result revealed low awareness about cervical cancer (19.6%) and a very high acceptability of VIA screening (94.5%). Of the 306 women who accepted to undergo VIA test, 99% stated going to recommend the VIA testing to their friends and relatives. All women screened negative intended to repeat the test every three years. Those that have a VIA positive test affirmed they will perform confirmatory explorations.96.3% of the women believed that screening using VIA test could save their live. Cervical cancer was the concern of 98.6% of the women. Only 11.6% of them say they feel anxious about repeating the VIA test. The majority of women (98.6%) were satisfied regarding the service received at the health center.

**Conclusion:** The study demonstrated that VIA screening program is acceptable by Moroccan women, despite their poor knowledge about cervical cancer. Awareness strategy is needed to increase their knowledge.
**AWARENESS OF HUMAN PAPILLOMAVIRUS BEFORE AND AFTER INTRODUCTION OF HPV VACCINATION: A LARGE POPULATION-BASED SURVEY OF SCANDINAVIAN WOMEN**

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**Objective:** Population awareness of human papillomavirus (HPV) is crucial to ensure high coverage of HPV-vaccination programs. In this population-based questionnaire study, we assessed the proportion of Scandinavian women without awareness of HPV before and after introduction of HPV-vaccination. Furthermore, we investigated risk factors for non-awareness.

**Methods:** In 2004/05, before HPV-vaccination licensure, a random sample of women aged 18–45 years in Denmark, Sweden and Norway received a questionnaire on education, lifestyle, and HPV awareness (n= 78,001; response rate 71.3%). In 2011/12, after HPV-vaccination licensure, the same questionnaire was administered to a new random sample in each country (n=83,720; response rate 60.6%). We calculated country- and age-specific proportions of women who had never heard of HPV. Country-specific risk factors for non-awareness were estimated by logistic regression.

**Results:** The overall proportion of women who had never heard of HPV decreased markedly from 2004/05 to 2011/12 in Denmark (from 75.8% to 25.1%), Sweden (from 74.8% to 33.5%) and Norway (from 62.4% to 33.0%). In 2011/12, the lowest awareness levels were observed in Norwegian women aged 18–19 and 20–24 years (60.4% and 48.9%, respectively, had never heard of HPV). In all countries, women with low educational level; virgins; and daily smokers were at increased risk of non-awareness, in unadjusted analyses and after adjustment for age and education.

**Conclusion:** HPV awareness has increased after introduction of vaccination, but 25%–33% of Scandinavian women have still never heard of HPV. Information campaigns targeting women with low educational level may be beneficial.

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**KNOWLEDGE AND BELIEFS ABOUT HPV IN A VACCINATED COHORT OF YOUNG MEN AND WOMEN**

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**Objectives:** Australia has provided free quadrivalent HPV vaccination to girls aged 12-13 years in a school-based program since 2007. Take-up rates are estimated to be around 80%, with significant variation between states. The program was extended in 2013 to include young men – a global first. This study examines knowledge and beliefs about HPV in this cohort of young men and women.

**Methods:** A national survey of Australian secondary students, aged 16 to 18, has been conducted regularly since 1992 and was most recently conducted in 2013 – data reported here. Students were recruited from all states and territories and from all school sectors. A total of 2,136 students (61% female) completed a paper based or online survey that examined their knowledge and practices concerning sexual practices and sexual health.

**Results:** Overall awareness of HPV remains low overall, with young women showing significantly greater awareness. Self-reported vaccination was lower than the known vaccination rates. There were significant gender differences on an HPV knowledge scale. Separate regression analyses for young men and women showed similar demographic and behavioural correlates of good HPV knowledge namely age, HPV vaccination status and sexuality significantly predicted greater knowledge of HPV.

**Conclusions:** A significant proportion of young men and women are under-informed about HPV and vaccination. For the vaccination program to be as successful for young men as it has been for young women, targeted health education and promotion is essential and must be based on current and relevant evidence of awareness and understanding.
WEB-BASED SURVEY ON KNOWLEDGE FOR CERVICAL CANCER PREVENTION AMONG YOUNG WOMEN: COMPARISON IN JAPAN AND AUSTRALIA

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Objectives: Cervical cancer (CC) incidence and mortality among young women have been increasing in Japan. To develop effective measures to combat this, we conducted a knowledge and attitudes study about CC prevention.

Methods: Advertising banners targeting women aged 16-35 years in Kanagawa Prefecture, Japan, were placed on a social networking site (SNS), Facebook (FB), in a similar manner to an Australian (AUS) study conducted on 16-25 in 2010, and on our CC advocacy website. Eligible participants were emailed instructions for accessing our secure website where they completed an online survey including demographics and knowledge of human papillomavirus (HPV) and CC. Data for this study were compared with the preceding AUS study.

Results: Among 394 women who expressed interest, 243 (62%) completed the survey. Participants had high awareness and knowledge of HPV and CC, comparable to the AUS study participants. However, the self-reported HPV vaccination rate (22% among participants 16-25 years) and the recognition rate of the link between smoking and CC (31%) were significantly lower than in the AUS study (58% and 43%, respectively) (P<0.05). Significant predictors of high knowledge scores about HPV included awareness of HPV vaccine (P<0.001) and self-reported HPV vaccination (P<0.05).

Conclusions: SNS and websites are an efficient method to recruit young women into health surveys. The influence of several factors including the current suspension of HPV vaccine approval in Japan due to adverse reactions should be investigated as a next step, considering the HPV vaccination status in other developed countries including Australia.

KNOWLEDGE AND PREVALENCE OF HPV DISEASES IN LATVIA

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Objective In last decade public health strategy in Latvia is focused on cervical cancer prophylaxis but not on prophylaxis of HPV diseases.

Aim Evaluate the knowledge and occurrence of clinical signs related to HPV diseases of Latvian inhabitants.

Methods In 2011 a population survey was carried out in Latvia sponsored by MSD company. The study sample consisted of 3050 randomly selected respondents aged 18 – 45 years. We compared knowledge and occurrence of clinical signs related to HPV diseases into three groups of patients: I - no sexual experience, II – having 0 – 1 partners during the last year, III – having two or more partners during the last year.

Results Among all respondents the frequency of incorrect answers to the knowledge questions was as follows: HPV infections is a rare disease - 52%, HPV infection is a sexually transmitted disease - 14%, can cause genital warts - 20%, may resolve without treatment - 72% and affects both man and women - 15%. Association between HPV infection and cervical cancer recognized 74% of the respondents of groups I and II but only 63% of group III (p<0.001). Genital warts more frequently have been noticed by respondents of the groups II (112/2347) and III (31/439) comparing to group I (2/224) (p<0.01). Precancerous lesions had no women of group I, 8.7% of group II and 16% of group III (p=0.03).

Conclusions Knowledge about HPV diseases in Latvia is insufficient. People with more risky sexual behavior are less aware about the consequences of HPV related diseases.
A POPULATION BASED SURVEY OF SCHOOL NURSES’ ATTITUDES TO THE IMPLEMENTED HPV VACCINATION PROGRAMME IN SWEDEN

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Objective: To investigate school nurses’ attitudes to, and experiences of the school-based HPV vaccination programme, one year after its implementation in Sweden.

Methods: Data were collected using a web-based questionnaire in spring 2013, and 83.1% (851/1024) of the nurses answered the questionnaire.

Results: The majority (88.9%, n=756) agreed that HPV vaccinations should be the school nurses’ responsibility, and most also agreed (81.5%, n=693) that boys also should be offered the vaccine. Two thirds, 66.9% (n=570), stated that they had experienced difficulties with the vaccination and of these 59.1% (n=337) considered the task time-consuming. Three out of four nurses, 76.1% (n=648), had been contacted by parents who raised questions regarding the vaccine. The most common questions were related to side effects. There were strong associations between the nurses’ received education about the HPV vaccine and perceived knowledge about the HPV vaccine and a favourable attitude towards vaccination (both p<0.001). A school nurse with a high level of received education was 9.8 times more likely to have a positive attitude to HPV vaccination compared to a nurse with a low level of received education (p<0.001). Nurses with high perceived knowledge were 2.5 times more likely to have a positive attitude compared to those with a low level of perceived knowledge (p=0.006).

Conclusions: HPV vaccination is a complex and time-consuming task and the school nurses need adequate knowledge, education, skills and time in order to address questions and concerns from parents, as well as informing about HPV.

HUNGARIAN HIGH SCHOOL STUDENTS’ ATTITUDE TOWARDS THE HPV VACCINE

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Objectives: The study aimed to explore the attitude of high school senior girls – probably being at the dawn of their sexual lives - in Budapest, the Hungarian capital toward the HPV vaccine and their knowledge of cervical cancer.

Methods: 492 girls were recruited from 12 randomly selected establishments. We conducted a cross-sectional analysis by administering an anonymous questionnaire consisting of 54 multiple-choice questions concerning basic socio-demographic and lifestyle factors and questions assessing their knowledge about cervical cancer and HPV. We also tested their attitude toward the HPV vaccine and vaccines in general. Our sample was distributed almost evenly among grammar (52.6%) and vocational schools (47.3%)

Results: Only 33.7% of the girls were aware of the fact that cervical cancer was caused by an infection, though 74.7% marked that HPV caused cervical cancer. 70.1% knew HPV was an STD. 59.9% would make the vaccine compulsory and 79.5% would vaccinate their future children. 63.2% would give it to boys as well, significantly more girls from vocational schools. More than a fifth of the girls has been vaccinated already (23%) and 19.6% had family members who had also received the vaccine. 73.8% expressed their doubts regarding the efficacy of vaccines in general. 98.4% believed that attending cervical cancer screening was important.

Conclusions: The knowledge of the girls from our sample regarding cervical cancer and the HPV vaccine proved to be unsatisfactory. Girls in grammar schools seemed to have more thorough knowledge which corresponded with higher vaccine acceptance.
The incidence of infection increases with the sexual onset in puberty and adolescence: careless lifestyle, bad dietary habits and frequent change of sexual partners. The psychic and physical maturity are not strictly correlated in adolescent population. Why are adolescent social groups at increased risk? The need for sexual experimentation, poor knowledge on sexually transmitted diseases, hedonism as a value orientation, strong peer pressure, illusion of invulnerability, poor communication skills. We made our educational programme for adolescents: “Knowledge is pleasure”. A week before the lectures/events, adolescents made: - web sites - theatrical performances (concerts, poems, plays... ) - posters - lectures... on sexually responsible behaviour, and the prevention of youth violence and addictions. Adolescents are actively involved in the project (making boxes, brochures, flyers, posters, organizing peers). We made research during 3 years of the project (2007 – 2010), 51 high schools, 2295 students. 68% uses condoms (of which 40% at their first sexual intercourse), 12% hormonal contraception. 59% had first sexual intercourse under the influence of alcohol, 13% under the influence of drugs. 39% have heard about HPV, 32% have heard that HPV presents a cancer risk factor. 70% estimate that their risk of contracting an STD is high. 85% know that condoms provide protection from both unwanted pregnancies as well as STD, 45% have no knowledge about the role of hormonal contraception. HPV vaccination in Central and Eastern Europe (Croatia is part of this): Current HPV vaccines registered in 25/28 countries. Only 6 countries (Bulgaria, Czech Republic, Macedonia, Latvia, Romania and Slovenia) have integrated HPV vaccination into their national immunization programme and currently provide routine vaccination free of charge to the primary target population. The key reasons for lack of implementation of HPV vaccination into the national immunization programme: High vaccine cost or inappropriate pricing policy, low level of awareness among public, negative public perception, low level of awareness among health care providers, not high on political agenda. Parents perceive the vaccine as risky. The belief that the vaccine represents an experiment that uses their daughters as guinea pigs (serving the commercial interest of pharmaceutical companies) The belief that the vaccine embodies a conspiracy theory that aims to reduce the world population. General mistrust in the ineffective health system. There are many reasons why people are not getting the HPV vaccine: one of the biggest reasons is that physicians are hesitant to talk to 11- and 13-year-olds about vaccines when they feel compelled at the same time to talk about the sexual nature of the virus’s transmission. I believe we should think of this vaccine as what it is: a cancer-preventing vaccine. Social media and text messaging can increase the amount of knowledge on the prevention of STDs. 80% of teens report using social networking sites.
HPV VACCINE CONCERNS IN JAPAN – PUBLIC HEALTH AND SCIENTIFIC BACKGROUND

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Objectives: The Japanese Ministry of Health, Labour and Welfare (MHLW) has suspended recommendations for the HPV vaccine program for more than 1 year. We investigated factors influencing the suspension from the point of view of PUBLIC HEALTH AND SCIENCE

Methods: Although WHO (GACVS) issued a safety statement for HPV vaccine on the June 13th 2014, the MHLW decided to suspend recommendations for the HPV vaccine program on the 14th. Because the vaccine adverse reaction committee (VARC) was not able to assess whether there were indeed safety issues or not based on insufficient data provided by the Ministry, the VARC decided to suspend proactive recommendation of the HPV vaccine. On December 25th 2013, January 20th and July 4th 2014, the VARC investigated the alleged adverse effects of the HPV vaccine and reached the conclusion there was little evidence to suggest a causal link between chronic pain and the HPV vaccine (1.5 cases per 100,000 vaccine doses). It concluded that patients were suffering from functional somatic symptoms caused by a psychosomatic reaction. Although collaborative movements of Academia and advocate groups issued several statements for resumption of proactive recommendation, the media ignored them and the MHLW has not issued any statement.

Results: A poor surveillance system for adverse events cannot precisely analyze background incidence during both the pre- and post- vaccination period. The VARC has failed to submit their conclusions in a written report to the MHLW.

Conclusion: Epidemiological surveillance during both the pre- and post- vaccination period should be performed. Furthermore, a rapid high level scientific response is the best ways to prevent erosion of public confidence in immunization.

CORRELATES OF UPTAKE IN SCHOOL-BASED ROUTINE HPV VACCINATION: A REGISTER-BASED STUDY OF 90,000 GIRLS AND THEIR PARENTS

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Objectives: To assess correlates of HPV vaccination of prepubescent girls in a free of charge, school-based childhood vaccination programme.

Methods: Individual demographic, socioeconomic and health data was merged from national registries for Norwegian girls born 1997-1999, eligible for HPV vaccination during the first three years of the programme (n=90,842), and for their registered mother (n=90,540) and father (n=88,565). Correlates of daughter initiation of HPV vaccination were analyzed by logistic regression.

Results: In total, 78.2% of the girls received the first dose of the HPV vaccine, 74.6% received three doses, and 94.8% received the MMR vaccine. Overall, the rate of initiation of HPV vaccination was similar across most levels of the potential correlates investigated. However, low parental education was associated with a relatively high likelihood of daughter initiation of HPV vaccination. In contrast, parental income level was positively associated with initiation. Parental age was negatively associated with initiation. The associations were similar for characteristics of mothers and fathers, although often less pronounced for fathers. The lowest likelihood for initiation of HPV vaccination was found among girls who did not receive the MMR vaccine. Daughters with relatively old mothers, or mothers in the lowest income category, also had relatively low likelihoods of initiation.

Conclusion: Many girls receiving the MMR vaccine did not get the HPV vaccine, thus indicating opportunities for improvements in HPV vaccine uptake. Routine school-based vaccination generally provides equitable delivery, yet some disparities still exist. These findings should be taken into account in attempts to improve coverage, and ensure equitable delivery of the HPV vaccine.
PREFERENCE FOR 2- COMPARED TO 3-DOSE HPV VACCINE IN A GLOBAL SAMPLE OF ADOLESCENT VACCINE PROVIDERS

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Introduction: Highly effective prophylactic vaccines are available to prevent cancers associated with vaccine-preventable human papillomavirus (HPV) types. WHO’s Strategic Advisory Group of Experts on Immunization recently revised its HPV vaccination recommendations to include a 2-dose schedule for girls who receive their first dose before 15 years of age. Understanding provider acceptance of a 2- versus 3-dose schedule will inform global implementation of HPV immunization programs. We investigated the acceptability of a 2- versus 3-dose schedule among adolescent vaccine providers in five geographical regions.

Methods: A total of 151 adolescent vaccine providers were recruited by non–probability convenience sampling in five countries: Argentina(n=30); Malaysia(n=30); South Africa(n=31); South Korea(n=30) and Spain(n=30). Univariate analyses were conducted using structured survey data from telephone or in-person provider interviews. NCT#:GSK117339

Results: Most providers said that they would recommend a 2-dose compared to a 3-dose schedule: Argentina:80%; Malaysia:76%; South Africa:45%; South Korea:90%; and Spain:86%. Most common reasons for preferring a 2-dose schedule were lower cost (Argentina:27%; Malaysia:53%; South Africa:16%; South Korea:60%; Spain:47%), fewer office visits (10%, 30%, 19%, 37%, 10%, 33%, 35%), higher series completion, and less pain (37%, 10%, 33%, 53%).

Discussion: Adolescent vaccine providers preferred acceptance of a 2-dose over a 3-dose HPV vaccine schedule for their female adolescent patients in Argentina, Malaysia, South Africa, South Korea and Spain. Benefits reported for a 2-dose schedule appeared to vary by country. Most providers (>50%) in 2/5 countries surveyed cited potential advantages of lower cost, higher series completion, and fewer office visits.

LONG-TERM EFFECTIVENESS AND SAFETY 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 study (Protocol 015) to investigate the safety, immunogenicity, and effectiveness of quadrivalent HPV vaccine (qHPV) on the incidence of HPV 16/18-related CIN2 or worse in 16-23-year old women. Here, we analyze the effectiveness and safety of the vaccine in this population of women up to 10 years after the start of vaccination.

Methods: All women in the trial are followed through different national registries (Denmark, Iceland, Norway and Sweden) for effectiveness and safety data. Effectiveness and safety analyses started approximately 2 years following completion of Protocol 015 and will occur approximately every 2 years thereafter. 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.

Results: In an analysis of effectiveness after the first 10 years, there were 1,281 subjects that contributed to the follow-up period out of a total of 1,984 eligible subjects in the per-protocol population in Cohort 1. No new cases of HPV 16/18-related CIN 2 or worse were observed. There were also no cases of HPV 6/11/16/18-related CIN, vulvar cancer, and vaginal cancer observed.

Conclusions: qHPV vaccine shows continued protection in women through 8 years, with a trend towards 10 years. The qHPV vaccine continues to be generally safe and well tolerated up to 10 years following vaccination.
PHASE II EVALUATION OF A MULTIVALENT HPV L1 VIRUS-LIKE PARTICLE (VLP) VACCINE

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Objective: To develop a multivalent prophylactic HPV vaccine that protects against infection and disease caused by HPV16/18 (oncogenic types in existing prophylactic vaccines) plus additional oncogenic types.

Methods: Three Phase II studies compared the immunogenicity and safety of several vaccine candidates with the licensed quadrivalent HPV6/11/16/18 vaccine (qHPV vaccine) in young women ages 16-26. Study 1 was conducted first. Studies 2 and 3 were conducted after analysis of results from Study 1. In Study 1, subjects received one of three dose formulations of an 8-valent HPV6/11/16/18/31/45/52/58 vaccine or qHPV vaccine (control). In Study 2, subjects received one of three dose formulations (termed low-, mid-, and high-dose formulations, respectively) of a 9-valent HPV6/11/16/18/31/33/45/52/58 vaccine (9vHPV vaccine) or qHPV vaccine (control). In Study 3, subjects concomitantly received qHPV vaccine plus 5-valent HPV31/33/45/52/58 or qHPV vaccine plus placebo (control). All vaccines were administered in at day 1, month 2, and month 6.

Results: In studies 1 and 3, anti-HPV6/11/16/18 geometric mean titers at month 7 were non-inferior in the experimental arms compared with the control arm; however, there was a trend for lower antibody responses for all four HPV types. In Study 2, this immune interference was overcome with the mid- and high-dose formulations of the 9vHPV vaccine by increasing antigen and adjuvant doses. In all 3 studies, all vaccine candidates were strongly immunogenic with respect to HPV31/33/45/52/58 and were well-tolerated.

Conclusions: Based on the totality of the results, the middle dose formulation of the 9vHPV vaccine was selected for Phase III evaluation.

IMMUNOGENICITY AND SAFETY OF A NOVEL 9-VALENT HPV L1 VIRUS-LIKE PARTICLE VACCINE IN BOYS AND GIRLS 9-15 YEARS OLD; COMPARISON TO WOMEN 16-26 YEARS OLD

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Objectives: An efficacy study of the investigational 9-valent HPV (6/11/16/18/31/33/45/52/58) VLP vaccine (9vHPV) is not possible in sexually naive adolescents. Therefore, a study was conducted to provide immunological bridging from young women 16-26 years of age (the population used to establish 9vHPV vaccine efficacy) to adolescents 9-15 years of age. This report investigates whether the 9vHPV vaccine induces non-inferior serum antibody responses in boys and girls 9-15 years of age compared to young women 16-26 years of age.

Methods: Subjects (n=3,066) received 9vHPV vaccine as a series of injections administered at day 1, month 2, and month 6. Blood samples for anti-HPV serologic assays were obtained at day 1 and month 7. Systemic and injection-site adverse experiences (AEs) and serious AEs were monitored.

Results: At 4 weeks post-dose 3, over 99% of girls, boys, and young women seroconverted for vaccine HPV types; marked elevations in cLIA GMTs to HPV types 6/11/16/18/31/33/45/52/58 were elicited in all vaccine groups at 4 weeks post-dose 3. Non-inferiority of the GMT responses for each of the HPV types in both girls and boys, 9-15 years of age relative to GMT responses in young women was established. Administration of the 9vHPV vaccine was generally well tolerated. Only 2 vaccine-related serious adverse experiences were reported over the duration of the study.

Conclusions: These results support bridging the efficacy findings with 9vHPV vaccine in young women 16 to 26 years of age to adolescent girls and boys 9 to 15 years of age.
**OC 6-4**

**IMMUNOGENICITY AND SAFETY OF A NOVEL 9-VALENT HPV VACCINE IN GIRLS 9-15 YEARS OF AGE COMPARED TO THE QUADRIVALENT VACCINE**

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**Objectives:** Approximately 20% of cervical cancers are related to HPV 31, 33, 45, 52, and 58, and a 9-valent virus-like particle vaccine (9vHPV) containing antigens against HPV 6/11/16/18/31/33/45/52/58 has been developed. A double-blind study was conducted to assess immunogenicity and safety of 9vHPV vaccine compared with qHPV vaccine in girls 9 to 15 years of age.

**Methods:** Subjects (n=600) were randomized 1:1 to receive 9vHPV vaccine or qHPV vaccine (day 1, month 2, month 6), in 2 age-strata (9-12 and 13-15 years of age). Immune response to the 9 HPV types was evaluated by competitive LumineX Immunoassay. The primary objective was to demonstrate non-inferiority of 9vHPV vaccine compared to qHPV vaccine for both HPV 16/18, based on the lower bound of the 95% confidence interval (CI) for post-dose 3 geometric mean titre (GMT).

**Results:** Non-inferiority was demonstrated: at month 7, GMT ratios (9vHPV/qHPV) were 0.97 (95% CI: 0.85-1.11) for HPV16 and 1.08 (95% CI: 0.91-1.29) for HPV18. In addition, the GMT ratios were 1.07 (95% CI: 0.93-1.23) for HPV6 and 0.93 (95% CI: 0.80-1.08) for HPV11. After qHPV vaccine, all subjects seroconverted to the 4 vaccine types (HPV 6, 11, 16, 18) and a number of subjects seroconverted to non-vaccine HPV types (HPV 31, 33, 45, 52, 58), mainly HPV31 (73.5%) and HPV58 (54.8%).

**Conclusions:** The 9vHPV vaccine immune response was comparable to that of qHPV vaccine for HPV 6/11/16/18 and robust for HPV 31/33/45/52/58. Adverse experiences were comparable for both vaccines.

**ABSTRACTS**

**OC 6-5**

**SAFETY AND TOLERABILITY OF A NOVEL 9-VALENT HPV VIRUS-LIKE PARTICLE VACCINE**

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**Objectives:** The investigational 9-valent HPV (6/11/16/18/31/33/45/52/58) (9vHPV) vaccine includes the 4 HPV types (6/11/16/18) in the quadrivalent HPV (qHPV) vaccine and 5 additional oncogenic types (31/33/45/52/58). Here we present the results of an integrated safety analysis from data gathered over multiple clinical trials of the 9vHPV vaccine.

**Methods:** The safety of the 9vHPV vaccine was assessed in 6 clinical trials (protocols 001, 002, 005, 006, 007, 009) conducted worldwide. These studies collectively were conducted in female subjects 9-26 years of age and male subjects 9-15 years of age. Temperatures were monitored for 5 days postvaccination. Systemic and injection-site adverse experiences (AEs) and serious AEs were monitored for 15 days post-vaccination using Vaccination Report Card. Non-prompted and serious adverse experiences (SAEs) were also collected and reported.

**Results:** Overall, 92.2% of subjects who received 9vHPV vaccine reported an AE. Most adverse experiences were injection-site AEs (88.3%) and the most common were pain (85.3%), swelling (37.6%), and erythema (31.8%). The most common vaccine-related systemic AEs were headache (13.9%), pyrexia (6.6%), nausea (3.4%), dizziness (2.4%), and fatigue (1.9%). Five SAEs were determined to be related to 9vHPV vaccine. Few subjects (0.1%) discontinued due to an AE. There were no deaths related to the 9vHPV vaccine.

**Conclusions:** These analyses demonstrate that the IM administration of 9vHPV vaccine is generally well tolerated. Discontinuations were rare and no safety signals of clinical concern were identified.
TOLERABILITY AND IMMUNOGENICITY OF A MULTIVALENT HPV L1 VIRUS-LIKE PARTICLE VACCINE IN 16- TO 26-YEAR-OLD MEN

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Objectives: This study is designed to evaluate the immunogenicity and tolerability of a prophylactic 9-valent HPV (types 6/11/16/18/31/33/45/52/58) VLP vaccine (9vHPV) in young men 16-26 years of age in comparison to young women 16-26 years of age. Safety and immunogenicity data from this study will be used to bridge 9-valent HPV vaccine efficacy findings in 16-26-year-old women to 16-26-year-old men.

Methods: This study enrolled 1103 heterosexual men (HM) and 1099 women who had not yet received HPV vaccination. In addition, 313 men having sex with men (MSM) were enrolled and were evaluated separately for immunogenicity (previous results showed that antibody responses to GARDASIL were lower in MSM than in HM). All subjects were administered a 3-dose regimen (Day 1/Month 2/Month 6) of 9vHPV vaccine. Serum samples were collected for anti-HPV assays. Safety information was collected for ~12 months.

Results: Administration of V503 to both 16-26-year old males and 16-26-year old females was generally well tolerated. The geometric mean titers (GMTs) for HPV types 6, 11, 16, 18, 31, 33, 45, 52, and 58 for 16-26-year old males (HM) were non-inferior to those of 16-26-year old females at Month 7. For all vaccine HPV types, Month 7 GMTs were numerically lower in MSM than in HM. Over 99.5% of subjects were seropositive at Month 7 for each vaccine HPV type.

Conclusions: These results support bridging the efficacy findings with 9vHPV vaccine in young women 16-26 years of age to men 16-26 years of age.

END OF STUDY EFFICACY AND IMMUNOGENICITY OF A NOVEL 9-VALENT HPV L1 VIRUS-LIKE PARTICLE VACCINE IN 16-26 YEAR OLD WOMEN

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Objectives: An efficacy and immunogenicity study of an investigational 9-valent HPV (6/11/16/18/31/33/45/52/58) (9vHPV) vaccine was conducted in women 16-26 years of age to demonstrate immunological non-inferiority of HPV 6/11/16/18 response and efficacy against HPV 31/33/45/52/58-related persistent infection and disease. The report presents results through end-of-study (i.e. up to month 54).

Methods: 14,204 healthy 16-26 year-old women were enrolled into an international, double-blind efficacy and immunogenicity study of the 9vHPV vaccine. Subjects received 9vHPV vaccine or quadrivalent HPV vaccine (qHPV) as a series of injections at day 1/month 2/month 6. Primary analyses included subjects who were seronegative at day 1 and PCR negative from day 1 through month 7 for the HPV type being analyzed. Gynecological swabs (for HPV DNA testing) and Pap test were performed every 6 months. Subjects with abnormal Pap tests were referred to colposcopy.

Results: Anti-HPV 6/11/16/18 responses generated by 9vHPV vaccine were non-inferior to those generated by qHPV vaccine. Efficacy of 9vHPV vaccine against a composite endpoint of HPV 31/33/45/52/58-related high-grade cervical/vulvar/vaginal disease was 97.4% (95% CI: 85.0-99.0) 1 case in the 9vHPV vaccine group and 38 cases in the qHPV vaccine group. Efficacy against HPV 31/33/45/52/58-related cervical/vulvar/vaginal disease (any grade) in the PPE was 97.7% (95% CI: 93.3, 99.4). Efficacy against HPV 31/33/45/52/58-related 6-month persistent infection in the PPE was 96.0% (95% CI: 94.6-97.1).

Conclusions: The 9vHPV vaccine was highly efficacious in preventing HPV 31/33/45/52/58-related persistent infection and disease up to month 54. HPV 6/11/16/18 immune responses were non-inferior to that of qHPV vaccine.
Objectives: To examine the distribution of HPV types contained in an investigational 9-valent HPV (9vHPV) vaccine (6/11/16/31/33/45/52/58) in CIN2/3/AIS (i.e., CIN2+) from younger women (aged 16-26) and older women (24-45) using placebo data from three clinical trials of the quadrivalent HPV (qHPV) vaccine.

Methods: Protocol-mandated testing was conducted at regular intervals and included Pap tests and follow-up for abnormalities. Tissue from biopsies and excisions were tested for HPV via PCR. Lesions were diagnosed by a Pathology Panel consensus diagnosis. The prevalence of HPV types and type combinations in these lesions was calculated using a proportional attribution method whereby a fractional allocation for each individual HPV type was used when evaluating multitype infected lesions.

Results: A total of 945 CIN2+ lesions were detected. There was minimal regional variation for the 9vHPV vaccine types in CIN2+, with an overall prevalence of 82%, 83%, 69% for Europe, Latin America, North America and Asia. The contribution of the 5 additional oncogenic HPV types over that of the qHPV vaccine was 38%, 31%, 22% and 36% for these regions, respectively. According to literature, the annual incidence rate of CIN 2+ per 100,000 women ages 18-39 is 265-533 in the US alone. Applying these rates and US female population estimates to our findings, we estimate approximately 120,000-241,100 of CIN 2+ cases diagnosed in the US each year are attributed to HPV16/18/31/33/45/52/58.

Conclusion: A 9vHPV vaccine has recently been shown to be highly safe and efficacious. If future 9vHPV vaccination programs are effectively implemented, the majority (70-85%) of pre-cancerous cervical lesions could be prevented worldwide, in addition to approximately 90% of invasive cervical cancers.

EVALUATION OF HPV CLEARANCE FOLLOWING PROCERVIX VACCINATION IN HPV 16 / 18 INFECTED WOMEN WITH NORMAL CYTOLOGY

Introduction: In a Phase I study, ProCervix, a first-in-class adjuvanted bivalent HPV16/18 therapeutic vaccine was reported to promote increased and sustained viral clearance in HPV16/18 infected women with normal cytology1. Changes in cervical HPV virology were further examined to understand the influence of patient HPV history and characteristics on clearance.

Methods: A ProCervix dose escalation (100, 600 mcg/dose) was followed by a randomized evaluation of ProCervix600 with imiquimod or with placebo cream; and placebo vaccine+ imiquimod. Changes in cervical HPV virology were evaluated using 2 clinically validated real time PCR genotyping tests for HPV16 and HPV18, including one allowing for the individual quantification of 18 different HPV types, using fixed amount of HPV DNA.

Results: Six months post-vaccination, HPV positivity with both tests showed no difference between treatment groups. At final analysis (average follow-up of 16.7 months), ProCervix600+imiquimod led to more cleared patients (19/28) with more sustained clearance than placebo+imiquimod (3/7). Clearance, and protection against incident infections, appear to be HPV16/18 specific. Patient characteristics (age, pre-existing immune response, HPV 16 or 18 type infection, viral load, and multi-infections) did not appear to impact clearance and time to clearance. Most importantly, serial measurements of type-specific viral loads, including up to 5 years before vaccination, showed HPV16/18 clearance for patients with long term pre-existing infection (up to 4.3 years) and in CIN2/3 previously treated patients (4/4).

Conclusion: These encouraging HPV clearance results further support ProCervix clinical evaluation in women infected with HPV16 and/or HPV18.

References
1 Abstract SS9-4, Florence, Eurogin 2013S
**OC 7-1**

**RISK OF HUMAN PAPILLOMAVIRUS-ASSOCIATED HEAD AND NECK CANCER FOLLOWING A DIAGNOSIS OF CERVICAL INTRAEPITHELIAL NEOPLASIA**

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**Objectives:** Human papillomavirus (HPV) represents a necessary cause for the development of cervical cancer and its severe precursors (CIN2/3). Furthermore, HPV is associated with the development of a subset of head and neck cancers (HNCs). The aim was to investigate whether women previously diagnosed with CIN2/3 had an increased risk of developing HPV-associated HNC. Degree of HPV association was based on the current literature.

**Methods:** This nationwide register-based cohort study included all women born in Denmark between 1918 and 1990 (~2,900,000). Of these, 133,740 women were registered with a diagnosis of CIN2/3 in The Danish Cancer Registry or The Danish Pathology Data Bank. All women were followed from the age of 18 until date of HNC diagnosis, date of migration, date of death or December 31, 2012. Using Cox regression analysis with age as the underlying time scale, Hazard Ratios (HR) for the risk of HNC in women with previously diagnosed CIN2/3 were estimated (adjusted for birth year).

**Results:** A significantly increased risk of overall HNC of 93% (HR 1.93, 95% CI 1.68–2.21) was found for women with a previous diagnosis of CIN2/3. Restricting the analysis to include only highly HPV-associated HNCs (oro-pharynx) increased the risk (HR 2.12, 95% CI 1.66–2.71). The risk of medium HPV-associated HNC was (HR 1.67, 95% CI 1.30–2.13).

**Conclusion:** Our study shows a highly significant risk of HPV-associated HNC among women previously diagnosed with CIN2/3. At the conference further analyses e.g. on time between CIN2/3 and HNC will be presented.

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**OC 7-2**

**HUMAN PAPILLOMAVIRUS PREVALENCE AND GENOTYPE PROFILING IN HEAD AND NECK REGION: AN ITALIAN STUDY**

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**Objectives.** Data on HPV prevalence in the head and neck region for the Italian population are scarce. The objectives of this study were to assess HPV prevalence and genotype distribution in oropharyngeal samples from individuals with: 1. squamous cell carcinoma (SCC); 2. benign lesions; 3. no clinically evident lesions. Additionally, we evaluated p16 overexpression in SCC.

**Methods.** Healthy mucosa samples were collected by cytobrushings in PreservCyt (Hologic); SCC and benign lesions were retrieved from the FFPE tissue archive of the Regina Elena National Cancer Institute. For DNA extraction we used: 1. DNeasy Blood and Tissue kit (Qiagen) for FFPE samples; 2. Ampliclone Liquid Media Extraction kit (Roche Diagnostics) for cytobrushings. HPV testing was performed by the INNO-LIPA Genotyping Extra (Fujirebio) on FFPE samples, and the Linear Array HPV Genotyping test (Roche Diagnostics) on cytologic samples. p16 immunohistochemistry was performed using the CINtec® Histology kit (Roche Diagnostics).

**Results.** 132 SCCs, 44 benign lesions and 50 healthy mucosa samples were analyzed, with an HPV prevalence of 40.1% (45.0% in tonsillar and 44.1% in base of tongue SCC), 15.9% and 8.0%, respectively. Among SCCs only high-risk types were found, with HPV16 being present in 88.7% of the HPV-positive cases. p16 overexpression was observed in 97.8% of the HPV-positive SCCs, while only 14.7% of the HPV-negative SCCs displayed p16 staining.

**Conclusions.** HPV prevalence in all the groups analyzed is in agreement with data from the literature. HPV16 was confirmed as the most prevalent genotype in oropharyngeal SCC. Moreover, p16 overexpression seemed to be associated with HPV-positive SCC cases.
Objectives: Indigenous people in four high-income countries, namely Australia (Aboriginal and Torres Strait Islanders), Canada (First Nations), New Zealand (Māori), and the United States (Native American/Alaska Native), have a higher burden from some cancers. We present here the incidence profiles of cervical and head and neck cancer, two cancers associated with HPV.

Methods: Incidence data were derived from population-based cancer registries in three Australian states, namely Queensland (QLD), Western Australia (WA) and the Northern Territory (NT), New Zealand (NZ), the province of Alberta, Canada, and the US Contract Health Service Delivery Area (CHSDA) regions, including US Alaska and the four other US-CHSDA regions combined. We computed age-standardized incidence rates (ASR) by sex and ethnicity for 2002-2006 using the SEGI world-standard population. Rates from Alberta were calculated directly by the registry; head and neck cancer rates were not available.

Results: Cervical cancer incidence was greater for indigenous women compared to all women in the population of each jurisdiction, including Alaska, but not for the rest of the US (Alaska Native 7.8/100,000 vs US Whites 6.3/100,000). Head and neck cancer incidence was greater for indigenous men and women in the NT, WA, QLD, NZ and Alaska Natives.

Conclusions: Disparities exist in the incidence of cervical head and neck cancers, the former largely preventable through screening and HPV vaccination. HPV vaccination might also reduce the incidence of some head and neck cancers. Investigation of the risk factors for these cancers in Indigenous populations are needed, as are culturally-appropriate public health interventions that address barriers to screening and vaccination by indigenous people.
PREVALENCE OF HPV & P16INK4A IN MULTIPLE PRIMARY SQUAMOUS CELL CARCINOMAS OF THE UPPER AERODIGESTIVE TRACT

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Objective: The prevalence of HPV and p16Ink4a was investigated in patients with two or more primary HNSCCs.

Methods: A retrospective study was conducted in Royal Victoria Eye and Ear Hospital and St. James’s Hospital, Ireland, between January 2000 and June 2012. p16 immunohistochemical staining was performed using purified mouse anti-human p16INK4A (BD Pharmingen™). The Qiagen DNA extraction kit was used to extract genomic DNA and it was tested for the presence of HPV using consensus SPF10 primers followed by gel electrophoresis. HPV genotyping was performed by TaqMan Real-Time PCR for HPV16, 18, 33 and 45.

Results: 72 (11.9%) out of 604 patients were identified with a second primary malignancy (SPM). Third primary malignancy prevalence rate was 3.0% (18/604) while a fourth primary malignancy rate of 0.5% (3/604) was established. Overall 57 males and 15 females between the ages of 36 to 78 (mean 58 +/- SD 9) were reviewed. Over 75% of these patients smoked and consumed alcohol. About one third of them (21pts) had HPV positive first primary tumour. Among them, nearly 50% (10pts) developed a HPV positive SPM. Majority of the patients were HPV16 positive (12pts). A low p16Ink4a positivity rate for both first primary (16.4%) and SPM (16.4%) was established.

Conclusion: HNSCC patients that were both HPV and p16Ink4a positive develop a SPM and these patients have a better survival compared to patients who did not harbour HPV infections and were p16Ink4a negative.

ADULT ONSET RECURRENT RESPIRATORY PAPILLOMATOSIS: A RETROSPECTIVE REVIEW

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Background: Recurrent Respiratory Papillomatosis (RRP) is a disease caused by the Human Papilloma virus (HPV). Current literature suggests that the prevalence of adult onset-RRP is 1.8-2.3 per 100,000, that patients are from low socioeconomic backgrounds and that the median age of adult onset is 34. The evidence for malignant transformation is generally based on small series and case reports, a recent study found the malignant transformation rate to laryngeal carcinoma to be 2.7%.

Objectives: This retrospective study aimed to determine the demographics, prevalence and risk of malignant transformation of adult patients managed by an institution covering a population area of 800,000, between 2003 and 2014.

Methods: Patients were identified using pathology numbers. RRP history, demographics and outcome data were analysed using electronic clinical database. Socioeconomic status was determined using the National Statistics Socio-economic classification three class system. Age, gender and RRP history were analysed using measures of central tendency.

Results: 67 patients were identified, median age at diagnosis was 38 (range 19-92, mean 42.3). Socioeconomic status was determined: 3 patients were students or unemployed; 6 were class I (managerial, professional); 18 class II (intermediate); 7 class III (manual workers) and status was unknown for 33 patients. Three patients developed laryngeal cancer.

Conclusions: This study observed a RRP prevalence of 8.9 per 100,000. The malignant transformation rate was 4.5%. There appears to be an evolving pattern of RRP with older economically active patients being affected and a higher risk of malignant transformation.
**HPV IN ORAL SQUAMOUS CELL CARCINOMA IN INDIA: PATHOGENESIS AND CLINICAL IMPLICATIONS**

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**Objectives:** Human Papilloma Virus (HPV) associated Oral Squamous Cell Carcinoma (OSCC) is increasingly being reported worldwide. The present study was done to assess HPV in OSCC along with associated risk factors, related biomarkers, histological features, clinical features and survival.

**Methodology:** HPV was detected in tumour biopsy by Real Time PCR in 250 histologically proven cases of OSCC and confirmed by Conventional PCR with L1 consensus PGMY09/PGMY11 primers and genotyped for High risk type 16 and 18. Immunohistochemical localization of p16 & P53 was done by standard protocol.

**Results:** HPV was present in 23/250 (9.2%) OSCC cases, of which 56.52% cases had HPV 16 infection, 43.47% had HPV 18 and 26.08% cases were negative for both 16 and 18. Six cases co-expressed DNA of both HPV 16/18 subtypes. p16 overexpression was observed in 40.90% while P53 in 65.2% of HPV positive cases. Of the 23 HPV positive cases association with Tobacco chewing was evident in 19 cases, smoking in 12 cases and alcohol in 7 cases. Histological evaluation showed basaloid morphology in 2/23 cases, with a keratinizing squamous cell carcinoma in others. No significant difference in median survival of positive (16.5) vs negative (12.9) cases was observed (p=0.62).

**Conclusions:** Prevalence of HPV associated OSCC is low in India. Etiopathogenesis of HPV associated OSCC in India may also be different due to association of multiple risk factors and activation of different biomarkers pathways. Basaloid histology and increased survival was not evident in all our cases.

**HUMAN PAPILLOMAVIRUS DETECTION IN ORAL MUCOSA CAN SUGGEST GENITAL INFECTION?**

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**Introduction:** HPV is the etiological agent of cervical and anal cancer. However, little is known concerning the etiology of the oral infection and oral cancer.

**Objective:** To investigate whether oral infection could point out genital infection, determining the presence of HPV in both sites of infection.

**Methods:** Oral scrapes from healthy mucosa and genital smears of condylomatus lesions were evaluated by molecular methods. A hundred and ten samples from oral and genital sites were collected from patients attending the STD Clinic from Universidade Federal Fluminense. To screen and type HPV DNA, generic MY09/11 PCR and type-specific PCR, followed by restriction fragment length polymorphism (RFLP).

**Results:** HPV was detected in 85.5% of genital lesions (n=55) and in 43.6% of oral mucosa samples. In 13 of the 55 (23.6%) studied cases, both sites were infected. The agreement among genital and oral types were high: 9 cases showed the same infecting types in both mucosa. HPV 11 were the most prevalent (n=7), followed by HPV6 (n=2) and HPV45 (n=1). Two cases showed mixed infections infected by and HPV6/11 and one HPV11/45. Oral infection, separated by male and female showed statistical significance (p=0.004), with markedly higher prevalence of oral infection on men.

**Conclusion:** The oral detection of HPV can suggest genital infection in half of the cases but it further studies are required to elucidate the natural history of HPV infection, mainly in relation to oral lesions.

**Keywords:** HPV, oral cavity, genital tract, PCR
Objective: To establish the relationship between HPV with the presence of premalignant and malignant lesions of the oral cavity.

Methods: An observational, case-control study was conducted. During the months of October and November of 2013, there were attended 19 cases of patients with premalignant and malignant oral lesions in an oncology service (all confirmed by the pathology report), they were matched with 19 controls of similar age and sex. There were obtained two samples for each case and control by brushing of oral cavity and subjected to PCR RFLP (Polymerase Chain Reaction Polymorphism with the size of the restriction fragments).

Results: Oral malignant and premalignant lesions represented 26.76% of all head and neck care outpatient. Malignant lesions were mainly carcinomas (73.68%) and premalignant were mainly papillomas with foci of dysplasia (10.53%). The mean age was 51.82 ± 9.88 years and a predominance of females (63.16%). Premalignant and malignant lesions were multiple. Premalignant lesions were elevated and without ulceration (15.79%), located in the cheek and tongue. Malignant lesions had ulcerations and located in the palate and lip. Patients with premalignant lesions showed HPV in oral cavity more frequently (66.66%).

Conclusions: The identification of HPV was linked with the presence of premalignant and malignant lesions of oral cavity (p<0.05). Cofactors such as oral sex (p<0.05) and consumption of tobacco (p 0.05) were also associated with the presentation of oral cavity lesions, both in the analysis of individual association and multivariate analysis.

KEYWORDS: HPV, premalignant lesions, cancer, oral cavity.

Objective: HPV+ compared to negative OPSCC have a better clinical prognosis. The aim was to study density of TILs present in the tumour microenvironment of OPSCC and any relationship with patient survival.

Methods: Formalin-fixed paraffin-embedded tumours from 139 patients diagnosed with OPSCC between 2002 and 2011 at the Christie NHS Foundation Trust were investigated. HPV detection was by polymerase chain reaction, in-situ hybridisation and corroborated with P16 immunohistochemistry (IHC). TILS (CD3, CD4, CD8 and FoxP3 T cell subsets) were identified using multiplex IHC. Stained tumour sections were scanned and multispectral deconvolution used to delineate immune cells labelled with different chromogens. Myofibroblast transdifferentiation in the stroma was identified using antibody to smooth muscle actin (SMA). TILS density was performed using automated image analysis. TILS density, site and stromal activation status were analysed in relation patient survival.

Results: The density of CD3 T cells in tumours irrespective of HPV status was significantly higher in the epithelial versus the stromal areas. However, CD4 and CD4FoxP3 T cells were present at higher levels in epithelial areas whereas CD8 T cells were more prevalent in the stroma. Interestingly, significant differences in infiltration of CD4 and CD8 but not FOXP3 T cells were seen in the stromal sites of HPV positive compared to negative tumours. The higher T cell infiltration in the stroma of HPV+ tumours correlated with overall patient survival. A relationship with the stromal T cell infiltration, SMA expression and outcome is being evaluated.

Conclusions: Stromal infiltration of effector T cells correlates with better outcome in HPV+ OPSCC.
GENOMIC CHARACTERIZATION OF A NOVEL HUMAN GAMMAPAPILLOMAVIRUS GENOTYPE HPV-199 ISOLATED FROM NAZOPHARYNX

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Objective: To characterize and phylogenetically evaluate the complete genome sequence of a novel Gammapapillomavirus (γ-PV) HPV-199, isolated from a nasopharyngeal swab sample.

Methods: A partial HPV L1 gene sequence of 331 bp was obtained using broad-range FAP6085F/FAP64 primers (GenBank Acc. No. HG515499). A complete viral genome was amplified using inverse long-range PCR using primer set: KC82LNG-F (5'-GGCAATAAGGTGATTGTCCT-3') and KC82LNG-R (5'-TTTTTTACAAGGTTCAGCAACATC-3'). The resulting amplicon was cloned into a plasmid vector and sequenced using a primer-walking strategy. The complete L1 nucleotide sequences from all currently completely sequenced γ-PVs were obtained from the PV genome database (PaVE), aligned and the maximum likelihood (RAxML) phylogenetic tree was constructed.

Results: The complete genome of HPV-199 has 7,122 bp and a G+C content of 36.5% (GenBank Acc. No. KJ913662). The genome contains five early (E1, E2, E4, E6 and E7) and two late (L1 and L2) open reading frames (ORFs), but no E5 ORF. The long control region (LCR) of 514 bp is positioned between the L1 and E6 ORFs. Phylogenetic analysis revealed that genotype HPV-199 clusters into the γ-PV genus, species γ12 and is most closely related to HPV-127 (nucleotide identity 77%). Systematic search in the GenBank database identified one more partial L1 sequence with 99% nucleotide identity (Acc. No. KC752084), isolated from the skin.

Conclusions: The cloning and full characterization of novel HPV genotype improves our knowledge of the diversity of γ-PV; however, the involvement of γ-PVs in clinical manifestations requires further investigation.

COMPARATIVE ANALYSIS OF MICRONARNAS IN HPV-POSITIVE AND NEGATIVE OROPHARYNGEAL CANCERS HIGHLIGHTS DIFFERENT ONCOGENIC MECHANISMS

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Objective: Human-papillomaviruses (HPV) type 16 is a causative agent in an increasing subset of oropharyngeal squamous cell carcinomas (OPSCCs). These tumors are different, at the clinical and molecular level, when compared to tumors caused by traditional risk factors. However, their specific oncogenic mechanisms are still poorly characterized. Analysis of their gene and microRNAs expression profile might provide valuable information.

Methods: mRNA and microRNA expression profiles were analyzed by micro-arrays in 38 OPSCCs (21 HPV-/ 17 HPV16+). A microRNA signature specific to HPV status solely was identified by analyzing a learning/training-set consisting of 16 OPSCCs (8 HPV-/ 8 HPV16+). Potentially confounding factors (stage, sex and tobacco) were equally distributed in both groups. The robustness of this signature was confirmed by blind case-by-case classification of a validation-set composed of 10 tumors.

Results: We have identified a 25 miRNA’s signature, which discriminates HPV16-induced OPSCC from their HPV-negative counterparts. After the blind classification of the validation-set, the viral status was revealed: 8/10 tumors were correctly classified according to tumor etiology and 2/10 were misclassified. In silico analysis indicates that these 25 microRNAs play a potential role in Wnt and PI3K-pathways, cell-adhesion/cell-polarity and the cytoskeleton regulation. Analysis of the gene expression profile of 30 samples confirmed that genes related to these pathways/functions are differentially expressed according to HPV status.

Conclusions: Our study contributes to a better understanding of pathogenic mechanisms involved in the development of HPV-positive OPSCCs and in the identification of potential therapeutic targets. Further studies are needed to confirm these results.
HPV CONCORDANCE BETWEEN NECK METASTASES AND PRIMARY TONSILLAR CANCER.

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Objective: To study possible HPV concordance between the primary tumor and the neck metastasis.

Background: Oncological treatment for HPV positive oropharyngeal cancer has proved to be successful worldwide and surgery can be avoided in most of these patients. Neck metastasis with unknown primary, CUP, was earlier treated primarily with neck dissection, but gradually, at many centers, neck dissection is if possible avoided. If the cytological diagnosis is certain and shows HPV positivity, the primary tumor can be assumed to be an undetectable tonsillar or base of tongue cancer and the patient can primarily be treated solely with oncological treatment.

Methods: Tumor biopsies from thirty-eight patients with tonsillar cancer and cytology from their corresponding neck metastasis were investigated. DNA was extracted using the Roche High Pure RNA Paraffin kit and HPV DNA detection was performed using Magpix instrument (Luminex Corporation, Austin, TX).

Results: In 33/38 patients there was a perfect concordance between the HPV status of the primary tonsillar cancer and the metastasis. In 23 patients both the primary tumor and the metastasis showed HPV 16 positivity and in 10 patients both were HPV negative. One patient presented HPV 56 in the primary tumor and HPV 16 in the metastasis. Among the remaining four cases, two patients showed HPV 33 and HPV 16 in the primary tumor, respectively, while the metastasis did not present any HPV DNA, which could possibly be explained by a secondary infection of the tumor. In two patients the metastasis showed HPV 16 and HPV 33, respectively, but no HPV was found in the primary tumor.

Conclusions: The finding of HPV 16 DNA positive cytology from a neck metastasis strongly indicates an HPV positive primary tumor.

EXPRESSION OF APM COMPONENTS IN OROPHARYNGEAL CARCINOMAS IN RELATION TO HUMAN PAPILLOMAVIRUS (HPV) AND CLINICAL OUTCOME


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Objectives: Patients with HPV-positive, as compared to, tonsillar and base of tongue squamous cell carcinoma (TSCC and BOTSCC), respond relatively well to treatment with ~80% 5-year disease free survival after conventional radiotherapy/surgery. Treatment has lately been intensified, often resulting in serious side effects, and to avoid overtreatment there is a need for biomarkers to predict treatment response. Earlier studies have demonstrated a HPV-dependent correlation between HLA class I expression and clinical outcome. To further examine relations between components of the antigen processing machinery (APM), HLA class I, HPV and clinical outcome, the expression of LMP2, LMP7, LMP10, TAP1 and TAP2 was analysed in TSCC and BOTSCC.

Methods: Between 135 and 278 TSCC and BOTSCC biopsies, earlier examined for HPV DNA, from patients diagnosed 2000-2007 at the Karolinska University Hospital, were examined for nuclear and cytoplasmic expression of LMP2, LMP7, LMP10, TAP1 and TAP2 and the expression of these was correlated to each other, HPV status and clinical outcome.

Results: The expression of LMP2, LMP7, LMP10 and TAP2 was found to be absent/low in 42-88% of TSCC and BOTSCC and several correlations in the expression of the different APM components were found. In addition, a low nuclear expression of LMP7 or LMP10 was, for HPV-positive tumours, correlated to a successful treatment response.

Conclusions: Reduced expression of APM components is common in TSCC and BOTSCC and, for HPV-positive tumours and nuclear expression of LMP7 and LMP10 may potentially be used together with knowledge of tumour HPV status for prediction of clinical outcome.
METHYLATION OF HPV16 URR SP1 AND E2 BINDING SITES 3 AND 4 IN MATCHED HPV16 POSITIVE PRIMARY/METASTASIS OPSCC PAIRS

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Objectives: Methylation of E2 binding sites (E2BSs) of the HPV 16 URR has been shown to play a crucial role in activating the expression of the HPV oncogenes E6 and E7 in cervical lesions that may progress to cervical carcinomas. During progression of transformed cell clones HPV 16 genomes often integrate into host cell chromosomes and become independent of the strict transcriptional control by the URR. Hence, integrated HPV genomes frequently lose methylation of E2BSs once they become integrated. Until now, this has not been investigated in detail for HPV genomes in OPSCCs. We assessed methylation patterns in pairs of OPSCC primary tumors and metastases to determine the extent of E2BS3 and 4 methylation in those distinct stages.

Methods: DNA extracted from FFPE tissue of 26 HPV16+/p16 INK4a + matched pairs of OPSCC with metastases was bisulfite-converted and analyzed for methylation in CpGs in positions 31-58 of the HPV16 URR by pyrosequencing.

Results: Methylation levels were lower in metastases than primaries at CpG 43 (E2BS3) and 52 (E2BS4) showing mean differences of 8.7 and 11.3 percentage points (p<0.05 for both) with similar trends for the remaining positions. Primary tumor samples’ mean methylation levels covered the range from 0-100% with a distribution hinting at two subgroups with low and medium/high levels.

Conclusions: In line with observed changes in URR methylation patterns in cervical carcinogenesis in different stages of disease and states of viral genome integration, lower methylation in OPSCC metastases could be indicative of integration and associated demethylation of previously episomal genome copies.

META-ANALYSIS ON P16INK4A ACCURACY TO IDENTIFY HPV TRANSFORMATION IN OROPHARYNGEAL SQUAMOUS CELL CARCINOMAS

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Objectives: p16INK4a immunohistochemistry (IHC) is applied as a surrogate marker of HPV-induced transformation in oropharyngeal squamous cell carcinomas (OPSCC), however, with considerable variation of accuracy among studies. We performed a meta-analysis on the accuracy of p16INK4a IHC to identify a transforming HPV infection in OPSCC and compared the results to HPV DNA detection by PCR, by in-situ hybridization (ISH) and combined p16INK4a/HPV DNA detection by PCR.

Methods: All available studies that assessed p16INK4a IHC and HPV oncogene transcription by an amplification-based method in OPSCC were included in the analysis. Pooled sensitivity and specificity of the four tests were calculated.

Results: Eleven studies assessing for p16INK4a IHC, four assessing for HPV DNA by PCR, five for HPV DNA by ISH and three assessing for combined p16INK4a IHC and HPV DNA by PCR fulfilled the inclusion criteria. The pooled sensitivities of p16INK4a IHC, HPV DNA by PCR, HPV DNA by ISH and combined p16INK4a/HPV DNA by PCR in the oropharynx were 0.93 (95% confidence interval (CI), 0.86-0.98), 0.99 (95% CI, 0.91-1.00), 0.84 (95% CI, 0.71-0.94) and 0.95 (95% CI, 0.84-1.00), respectively. The pooled specificities were 0.84 (95% CI, 0.77-0.90), 0.91 (95% CI, 0.79-0.98), 0.91 (95% CI, 0.78-0.99) and 1.00 (95% CI, 0.96-1.00).

Conclusion: Our meta-analysis data indicate that p16INK4a IHC alone is a very sensitive and moderately specific surrogate marker of HPV-induced transformation in OPSCC. Combined p16INK4a IHC and HPV DNA by PCR testing considerably enhances specificity while maintaining high sensitivity and might thus provide a reliable diagnosis of HPV-transformed OPSCC.
DETERMINATION OF HUMAN PAPILLOMAVIRUS SIGNIFICANCE IN ORAL PREMALIGNANT LESIONS IN ASSOCIATION WITH CLINICAL PARAMETERS AND P16\(^{INK4a}\) IMMUNOHISTOCHEMISTRY

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Objectives: Human papillomavirus (HPV) DNA and RNA is found in a proportion of oral cavity squamous cell carcinomas, indicating a transforming role of the virus in a subset of those cancers. HPV DNA has also been detected in some oral premalignant lesions (OPL), however, with unclear significance.

Methods: Formalin-fixed, paraffin-embedded biopsies of mild, moderate and severe OPL from the “Oral Cancer Prediction Longitudinal study” (OCPL), British Columbia, Canada, were analyzed for HPV DNA, genotype and semi-quantitative viral load applying Luminex technology. The results were correlated to various clinical parameters and p16\(^{INK4a}\) immunohistochemistry.

Results: Thirteen of 149 (8.7%) samples were HPV DNA-positive with a low mean “median fluorescence intensity (MFI)” as a semi-quantitative measure of viral load (high-risk(HR)-HPV16:204.6 and HR-HPV18:186.8 MFI). HPV DNA-positivity was associated with higher lesion progression risk (p= 0.038). Half of the 149 samples demonstrated focal p16\(^{INK4a}\) expression (52%), 13% showed a diffuse pattern and 35% were p16\(^{INK4a}\)-negative. No significant association between HPV DNA-positivity and p16\(^{INK4a}\) expression was observed. Progression occurred in 7 lesions: 0/78 focal, 6/52 negative and 1/19 diffuse p16\(^{INK4a}\) expression (P= 0.01).

Conclusion: A higher progression risk in HPV DNA-positive OPL patients was demonstrated. HPV DNA-positivity in those lesions might represent a non-transforming bystander infection, particularly considering the low MFI and the lack of association with p16\(^{INK4a}\) expression. Assessment of HR-HPV oncogene transcription in the HPV DNA-positive OPL will be performed to differentiate truly HPV-transformed lesions from potential bystander infections. OPL with focal p16\(^{INK4a}\) expression appear to have a low likelihood of progression independent of HPV DNA status.

E6/E7 mRNA IN SITU HYBRIDIZATION IS SENSITIVE AND SPECIFIC TO PREDICT PATIENT OUTCOMES IN OROPHARYNGEAL SQUAMOUS CELL CARCINOMA (SCC)

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Introduction: The incidence of HPV-associated oropharyngeal SCC has increased rapidly and has been documented to have a significantly better prognosis. Various methods have been tested. P16, currently accepted as a surrogate immunomarker for HPV infection, is sometimes discordant with HPV16 DNA in situ hybridization (ISH). HPV DNA ISH is well-established and has excellent histologic correlation; however, interpretation can be difficult and sensitivity is low. RT PCR for HPV DNA is very sensitive; however, it lacks the histologic correlation and the specificity is compromised.

Methods and Results: E6/E7 expression is a hallmark of HPV-driven tumors; E6 and E7 proteins abrogate the functions of two critical tumor suppressors, p53 and RB respectively, to both initiate and maintain tumorigenesis. E6/E7 mRNA detection by a novel ISH (RNAscope) developed by Advanced Cell Diagnostics offers a both specific and sensitive method to detect transcriptionally active high risk HPV in patients with oropharyngeal SCC. Its high sensitivity is achieved through a robust signal amplification system which enables single-molecule detection. Its high specificity is due to effective background suppression to ensure extremely low noise, and the ability to correlate mRNA detection and morphology. The detection process can be performed on formalin-fixed and paraffin-embedded tissues on automated platforms. Compared to p16 immunostain and HPV DNA ISH, E6/E7 status is strongly prognostic and superior to p16 or HPV DNA alone.

Conclusion: E6/E7 mRNA ISH should be considered the gold standard method for high risk HPV detection and integrated in the molecular staging of HPV-associated oropharyngeal squamous cell carcinoma.
Organized primary HPV screening in Sweden. Colposcopic and histopathologic evaluation of HPV++ women aged 56-60.

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Objectives: To evaluate the colposcopic and histopathologic findings in HPV++ women aged 56-60 years in an organized primary HPV screening program.

Methods: The organized screening program in Stockholm county randomized 50% of all resident women aged 56-60 years to either primary HPV screening with cytology triage or to primary cytology and HPV triaging for ASCUS/LSIL. In HPV screening, HPV+/Cyt-women had a repeat HPV test 1 year later. Attendance rates were similar with the 2 policies (HPV screen: 5979 women and cytology screen: 6087 women). HPV+/Cyt- women at baseline and HPV persistence at follow up were invited to colposcopy, performed by the same expert gynecologist.

Results: So far, 65/80 Cyt-/HPV++ women have been HPV typed. 63% (41/65) were persistent for the same type, 34% (22/65) were ++ with "other" type (persistent with type persistence unknown) and only 3% had a new type. 49% (32/65) had atrophic epithelium. 45% (29/65) a transformation zone (TZ) type 3, 25% (16/65) type 2 and only 30% (20/65) had a type 1 TZ. 43% (28/65) had a colposcopic lesion, 15% (10/65) abnormal cytology from the endocervix and 23% (15/65) CIN2+ in the cervical biopsy or conisation following the colposcopy.

Conclusions: Primary HPV screening was acceptable to the population and resulted in similar attendance rates. Colposcopic evaluation of cytologically negative HPV++ positive women resulted (so far) in a PPV for CIN2+ in histopathology of 23% (15/65). Atrophic vaginal epithelium and TZ type 3 are challenges in the colposcopic management in this group. Cytological endocervical evaluation, blind biopsies and diagnostic conizations as well as risk-assessment are options for this group of women.

A novel approach in cervical cancer screening

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Cervical cancer is the second most frequent cancer. Pap smear screening has led to a substantial decrease in the mortality of cervical cancer over the last 50 years. Colposcopy evaluation done for abnormal smear patients, but it is expensive and requires considerable skill. Fluorescence spectroscopy measure fluorophores which might change according to preneoplastic and neoplastic tissues.

Objective: To assess performance of fluorescence spectroscopy in the diagnosis SIL and to compare this with colposcopy and Papicolou smear screening.

Materials & Method: Sixty five patients attending the outpatient clinic between June 2013 to May 14 with discharge per vaginum, low backache, pain in the abdomen, irregular bleeding and post coital bleeding were studied. After gynecological workup Pap smear and colposcopy done and Cervical biopsy was taken from normal and abnormal areas of the cervix and fresh tissue was sent to Saveetha school of Engineering for Fluorescence spectroscopy

Results: At 380 nm excitation of the tissue, SILs can be differentiated from normal columnar epithelia and inflammation with sensitivity of 79% specificity 74%, PPV 81%, respectively. At 460 nm excitation, high grade SILs can be differentiated from low grade SILs with an average sensitivity and specificity of 82% and 78%, PPV 74% respectively similar to that of colposcopy.

Conclusion: Fluorescence spectroscopy, a noninvasive, can be used by inexperienced health care personnel. This in vitro work can be implement it for in vivo using an optical fiber probe.
WHEN SHOULD ENDOCERVICAL CURETTAGE BE DONE?

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Objectives: Determine the yield of cervical intraepithelial neoplasia (CIN) 2, CIN 3, or cancer (CIN 2+) attributed to endocervical curettage (ECC).

Methods: Review of electronic medical records from colposcopy clinics.

Results: Between 3/1/1996 and 4/23/2013, 18,537 cervical colposcopies with no missing results evaluated abnormal cervical cytology and/or positive human papillomavirus tests; median age was 32 years and median number of cervical biopsies was 3. CIN 2+ was diagnosed in 15.3% (2,840/18,537). Of 2,840 colposcopies with CIN 2+, cervical biopsy and ECC showed CIN 2+ in 19.9% (566/2,840), cervical biopsy had CIN 2+ with ECC not CIN 2+ in 67.0% (1,902/2,840), ECC showed CIN 2+ with cervical biopsy not CIN 2+ in 9.7% (274/2,840), and cervical biopsy and ECC had no CIN 2+ in 3.5% (98/2,840). Yield of CIN 2+ diagnosed by ECC of CIN 2+ with cervical biopsy not CIN 2+ was 1.5% (274/18,537); for women age < 25 years, yield was 0.5% (25/5,433) which was lower than for women age 25 years and older (1.9%, 249/13,104, p< .001). Of 274 women with ECC of CIN 2+ and cervical biopsy not CIN 2+; 90.9% (249/274) were age 25 years or greater; 28.8% (79/274) were age 50 years or greater; 24.8% (68/274) had cervical cytology of Cancer, High Grade Squamous Intraepithelial Lesion, Adenocarcinoma InSitu, Atypical Glandular Cells, or Atypical Squamous Cells r/o High; 8.8% (24/274) had colposcopic impressions of CIN 2+; and 20.7% (55/274) had inadequate colposcopy.

Conclusion: ECC should be performed at colposcopy in women age 25 and older.

EQE OF CERVICAL DYSPLASIA: CYTOLOGY-BIOPSY EVALUATION IS MANDATORY FOR GOOD PATIENT MANAGEMENT

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Objective: HSIL on cytology is followed by cervical biopsy. A comparison of the biopsy findings in relation to the cytology has been carried out.

Methods: a comparative retrospective study called CBC (CytoBioppticControl) has been conducted on 20 patients whom abnormal Pap smear was followed by cervical biopsy. The study was performed by a group of cytologists and pathologists. The following CBC results were possible: confirmative (biopsy confirmed the cytology), non-confirmative (biopsy findings did not confirm the cytology report). If findings were non-confirmative, cervical cytology and biopsy were re-evaluated. If necessary, additional sections from the available biopsy material were prepared and evaluated.

Results: Among the 31 cases (20 patients), conformity was reached in 11 cases (35%) and a non-conformity in 20 cases (65%). Re-evaluation of these 20 cases lead to conformity in an additional 11 cases (35%); 2 cases (18%) after re-evaluation of the cytology, 4 cases (37%) after re-evaluation of the biopsy, 1 case (9%) after re-evaluation of both cytology and biopsy, 2 cases (18%) when deeper sections from the biopsy material were analyzed and 2 cases (18%) required a new biopsy.

Conclusion: each HSIL cytology result should be followed by a cervical biopsy revealing and confirming the dysplasia. In cases for which non-conformity was present between cytological analysis and evaluation of the biopsy material several reasons were found. In 2 out of 31 cases (6%) the non-conformity was due to inaccurately performed biopsy or sampling error by the gynecologist and in 9 out of 31 cases (29%) due to evaluation errors by the pathologist. This quality control study demonstrates that the CBC is absolutely mandatory for exact patient management as 45% of the non-conformity results could be rectified in second time.
Background and Objectives: In Scotland, women who received immunisation as part of an initial “catch up” phase have been invited for cervical screening since 2011. The SHEVa studies are designed to garner evidence on the clinical performance of HPV tests in immunised women. Here, we present preliminary data on the effect of immunisation on the performance of three HPV assays as a triage of low-grade (LG) cytology.

Methods: LBC samples from 1000 immunised and 1000 unimmunised 20 year olds collected between 2010-12 and stored in the Scottish HPV Archive were tested using three clinically validated assays [the rT HPV Assay (Abbott), the APTIMA HPV test (Hologic) and Onclarity HPV Assay (BD)]. A total of 245 (unimmunised) and 185 (immunised) women harboured low grade abnormalities; at time abstract preparation follow-up information is available for 152 and 98 of these (average length- 460 days).

Results: All assays detected all 23 cases of CIN 2+ in the unimmunised women, 2/3 assays detected the 5 cases of CIN 2+ in immunised women with one assay detecting 4/5 cases – this translated into high sensitivity: 80-100%, for all assays, irrespective of immunisation status. However, the PPV for CIN 2+ for the rT HPV, APTIMA and Onclarity assay was 18.1, 17.8 & 17.7 in the unimmunised women compared to 6.5, 5.3 and 6.5 for immunised women.

Conclusions: Further data associated with outstanding follow up will be presented. However, these preliminary data indicate that the PPV of HPV testing for the triage of low grade abnormalities may reduce in immunised women.
**ECO-ROCS (EVALUATION OF CLINICAL OUTCOME AFTER REDUCTION OF CONIZATION SIZE), STUDY DESIGN**

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**Objectives:** Cervical intraepithelial neoplasia grade 3 (CIN III) is the direct precursor lesion of invasive cervical cancer. Besides prophylactic vaccination, the only curative treatment is represented by conization. In correspondence with the definition of the LLETZ operation (LLETZ = large loop excision of the transformation zone) the lesion needs to be resected including the transformation zone. It is well known from the literature that the cone size directly correlates with the risk of preterm delivery in a future pregnancy. Therefore it would be highly desirable to keep the cone dimension as small as possible by maintaining the same level of oncological safety.

**Methods:** Aim of this ongoing study is to analyze if the resection of the lesion only without additional excision of the transformation zone is equally effective regarding oncological outcome. Therefore we perform this prospective, patient-blinded trial randomizing women who need to undergo a LLETZ operation because of CIN III at a ratio of 1:1 either into the group with additional resection of the transformation zone or into the group with resection of the lesion only. To evaluate equal oncological outcome we perform HPV tests 6 and 12 months postoperatively. Study is conducted in thirteen German study centers including 1000 women with CIN III. The study is designed to consider the “lesion only” operation as oncologically not inferior if the rate of HPV high risk tests is not higher than 5 percent compared to the HPV high risk rate of women undergoing classical LLETZ operation.

**Results:** First results will be presented.

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**INFLUENCE OF GLACIAL ACETIC ACID ON HPV DETECTION - A STUDY OF 140 CASES OF CIN2+**

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**Objectives:** Lysis of bloody liquid based cytology samples with glacial acetic acid (GAA) can aid cytological interpretation. Evidence suggests that while GAA treatment influences the quantitative read-out of HPV assays, it does not affect qualitative detection and associated performance. However studies which have reported this have been under-represented in terms of high grade disease. Consequently, we determined the impact of GAA on HPV detection in a prospective series of LBC samples enriched for high-grade abnormalities.

**Methods:** 207 LBC samples with high-grade cytology and treated with GAA were collated prospectively across two Scottish laboratories. A total of 140 had underlying CIN2+ (including 88 CIN3 & 10 cancers). All samples were tested with the Hybrid Capture 2 assay (HC2, Qiagen) and the rT HPV assay (Abbott). Any sample associated with a CIN2+ that was negative by either or both assays was genotyped.

**Results:** Sensitivity of the rT HPV for CIN2+ and CIN3+ was 92.8% (87.2, 96.5) and 94.3 (87.2, 98.1) respectively. Sensitivity of the HC2 for CIN2+ and CIN3+ was 97.2 (92.8, 99.2) and 96.6 (90.3, 99.2) respectively. “Missed” cases of CIN2+ were largely attributable to types outside the explicit analytical range of either assay.

**Conclusions:** Although we do not have an untreated “comparator”, the sensitivities reported above are comparable to published sensitivities in similar disease-enriched populations. The above data indicate that, in relation to the above assays, GAA treatment may have little impact on the detection of CIN2+.
**DYNAMIC SPECTRAL IMAGING FOR POST-TREATMENT TRIAGE**

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**Background:** NHSCSP (National Health Service Cervical Screening Programme) recently implemented test-of-cure for patients treated for cervical intraepithelial neoplasia (CIN).

**Objective:** To assess the accuracy of colposcopy with and without the DySISmap in detecting CIN2+ for patients with post-treatment negative cytology and high-risk HPV positive.

**Methods:** This is an observational ongoing study (Mar-2013 onwards). Patients are examined using the DySIS digital colposcope. Initial colposcopic impression and biopsy sites are recorded before and after the DySISmap. Outcomes include sensitivity, specificity and negative predictive value (NPV) for CIN2+. The accuracy of the DySISmap in detecting CIN1 lesions that will progress will also be assessed once adequate numbers of patients with follow up cytology (1 year) is available.

**Results:** The study currently includes 97 women. Histology was available for 74, and these are analyzed. Overall, 5 (6.7%) women had high-grade biopsy results. Of those, 1 wouldn’t have been biopsied without the DySISmap, in 3 DySISmap indicated high-grade but the colposcopist would have biopsied the same area (regarded as low-grade lesion) and in one the DySISmap was normal. Colposcopy was normal/low-grade in all 5. Sensitivity of standard colposcopy for CIN2+ was 0% improving to 80% with the incorporation of the DySISmap. Using directed biopsy results NPV of colposcopy and DySISmap for CIN2+ was 98%. Sufficient numbers of patients with 1 year follow up, in order to assess outcomes for those with CIN1 biopsy results, are expected at time of presentation.

**Conclusions:** Incorporating the DySISmap as an adjunct to colposcopy might improve colposcopy accuracy for this population.

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**REAL TIME IN VIVO MICROSCOPIC IMAGING OF THE CERVIX USING CONFOCAL LASER ENDOMICROSCOPY: PRELIMINARY OBSERVATIONS AND FEASIBILITY STUDY.**

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**Background:** Confocal laser endomicroscopy enables in vivo, real time, imaging of living tissues with a micron scale resolution through a fiber optic probe.

**Objectives:** This study evaluates the technical feasibility and safety of CLE on exocervix and junctional area. The study also aims at creating a first cartographic atlas of normal and dysplastic squamous and columnar cervical epithelium.

**Methods:** In vivo CLE was performed on 9 patients scheduled for a cervical Loop Electric Excision Procedure (LEEP) for High Grade Cervical Intraepithelial Lesions (H-SIL). The CLE images were compared with standard hematoxylin and eosin analysis of LEEP specimens. Histopathological diagnosis on the surgical specimen was established as per standard of care. CLE images were then reviewed by pathologists to point out specific histopathological features.

**Results:** pCLE of exocervix and transformation zone was successfully performed on 7 out of 9 patients. Uninterpretable images were obtained the 2 other cases, one using the AlveoFlex and one using the GastroFlex UHD after the application of acetic acid 2%. 82.47% of the sequences recorded with the GastroFlex were suitable for interpretation. No adverse event nor complications occurred.

**Conclusions:** CLE enables proper in vivo imaging of healthy and dysplastic cervical tissue. Images correlate well with histopathological features established through traditional histology. CLE could be of interest for the assessment and the treatment of cervical lesions by enabling fine margin delineation and extensive follow-up examination.
OVERTREATMENT IN SEE-AND-TREAT MANAGEMENT OF CERVICAL INTRAEPITHELIAL NEOPLASIA; A SYSTEMATIC REVIEW AND META-ANALYSIS

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Objective: To determine overtreatment rates in see-and-treat management of women referred for colposcopic evaluation because of suspected cervical intraepithelial neoplasia (CIN) grade 1-3, in order to define circumstances that justify, or indicate to avoid see-and-treat management.

Data Sources: MEDLINE, EMBASE, and the Cochrane Library were searched from inception up to 12 May 2014. Additional sources included references of retrieved papers and concept proceedings.

Methods of Study Selection: Studies were included that performed see-and-treat management in women with suspected CIN with a registered cervical smear result, colposcopic impression and histology result. The methodological quality was assessed with the Ottawa scales. We used the inverse variance method for pooling incidences, and a random effects model was used to account for heterogeneity between studies. Overtreatment was defined as treatment in patients with no CIN or CIN1.

Tabulation, Integration, and Results: Thirteen studies (n= 4,611) were included. The overall overtreatment rate in women with a high-grade cervical smear and a high-grade colposcopic impression was 11.6% (range 0-29.4%). The overtreatment rate in women with a high-grade cervical smear and low-grade colposcopic impression was 29.3%, and in case of a low-grade smear and high-grade colposcopic impression it was 46.4%. In women with a low-grade smear and low-grade colposcopic impression, the overtreatment rate was 72.9%.

Conclusion: The pooled overtreatment rate in women with a high-grade smear and high-grade colposcopic impression is at least equivalent to the two-step procedure, which justifies see-and-treat management in this subgroup of women.

TECHNICAL EVALUATION OF THE XPERT® HPV ASSAY IN A GENERAL SCREENING POPULATION - AN ASSESSMENT OF THE CLINICAL PERFORMANCE

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Objectives: The Cepheid Xpert HPV Assay is a qualitative real-time PCR assay for the detection of 14 high-risk HPV types. A sample collected in PreservCyt is pipetted into a single use cartridge and processed by the GeneXpert® System. The result is available in 1.5 hours. The study evaluated the Xpert® HPV Assay in a screening population by comparing the results with cytology, histology and two established HPV DNA tests - Roche Cobas Test (primary comparator) and Qiagen hc2 Test (secondary comparator).

Methods: A prospective study using residual cytology samples for women aged 20-60 years attending routine screening in London, Bristol and Edinburgh. Residual specimens (minimum of 8mls after processing for cytology) were aliquoted in random order 1:1 for Roche Cobas and Xpert HPV assay. Qiagen hc2 was always third.

Results: Of the 3414 cases included in the analyses, 439 had referral cytology of borderline or worse. Xpert® Assay and hc2 were positive (to any high risk HPV type) in all cases where referral cytology was moderate dyskaryosis or higher (N=64) and Roche Cobas was negative in two cases. Histology is currently available for 107 participants (London only). 16 reported CIN2 or worse. Xpert® HPV Assay and hc2 were positive in all cases whereas Roche Cobas was negative for one case of CIN3.

Conclusions: The performance of the Xpert® HPV Assay in a general screening population is comparable to the established HPV tests and offers flexibility with non-batching and rapid turnaround time of individual samples.
Objective: High-risk HPV (HR-HPV) testing is commonly recommended as a triage strategy for ASC-US cytology. This study evaluated the potential impact of using extended genotyping as an alternate triage strategy.

Methods: The BD Oclarity™ HPV assay was used to genotype HC2 (Qiagen) positive ASC-US cytology samples from the PaP Cohort Study at Kaiser Permanente Northern California. Half of 640 CIN3+, 1/2 of 1,118 CIN2, and 1/10th of 12,020 <CIN2 cytology samples were genotyped to determine 3-year cumulative risks for CIN3+. Using genotype-specific risk and prevalence data, we estimated the number of colposcopies that could be avoided with extended genotyping, and quantified the cost savings using cost data from the Medicare 2014 Physician Reimbursement Fee Schedule. All assumptions and inputs will be detailed in the poster.

Results: Among HR-HPV genotypes, 3-year cumulative risk of CIN3+ ranged from 1.7% for HPV 39/68/35 to 21.3% for HPV 16, highlighting the risk variation among HPV genotypes. Rather than referring all ASC-US HPV+ women to colposcopy, we examined a strategy of referring women with HPV51, HPV59/56/66, HPV39/68/35 and those who were HPV- to 1-year follow-up. Preliminary analysis found this strategy could avoid approximately 250,000 colposcopies annually, saving nearly $48 million in healthcare spending. Results from alternate strategies will also be presented.

Conclusions: Because ASC-US is so common and so often benign, it’s worth exploring strategies that call for less invasive follow-up. Among certain HR-HPV types, the risk of CIN3+ might be low enough to avoid colposcopy, and help to reduce costs to the healthcare system.

Disclaimer: The views presented in this abstract do not represent those of the National Cancer Institute (NCI) or Kaiser Permanente Northern California (KPNC)

DETECTION OF HIGH-RISK HUMAN PAPILLOMAVIRUS E7 PROTEINS

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

Objective: Persistent infection with high-risk human papillomavirus (hrHPV) types is a major prerequisite for development of precancerous lesions and cervical cancer. Upon integration of the virus into the host genome, Deregulation and overexpression of viral proteins E6 and E7 lead to loss of cell cycle control and, ultimately, neoplastic transformation.

Current cervical cancer screening methods rely mainly on cytological analyses (Pap smear) or detection of HPV nucleic acids. However, these tools fall short of discriminating between transient infections which can spontaneously regress and persistent infections, which have a greater chance to progress to high-grade squamous intraepithelial lesions.

A more effective and reliable screening approach may involve exploitation of the oncoproteins E6 and E7 for specific detection of cervical cancer and precancerous lesions. Development and validation of a new diagnostic test for progressive infectious diseases that allows detection, by ELISA, of HPV E7 proteins in cervical smears, could be an easy and beneficial solution for better diagnosis of cervical cancer and precancerous stages.

Results: After accurate characterization of the RabMabs, specific combinations of the antibodies were chosen to enable the set-up of the hrE7 ELISA system. The assay allows detection of the three hrHPV types 16, 18 and 45 with highest carcinogenicity. For validation purposes, the hrE7 ELISA system was validated with (i) recombinant E7 proteins as well as with (ii) cell lysates of HPV positive cell lines stored in ThinPrep buffer. The proof of concept was shown with HPV DNA negative and HPV16 DNA positive, clinical abnormal samples.
CLINICAL VALIDATION OF E1-BASED “HPV EASY DNA ARRAY” GENOTYPING TEST DEVELOPED BY AID/GENID

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Objectives: Clinical validation of HPV Easy DNA Array, an E1-based genotyping test for identification of 18 high-risk (16,18,26,31,33,35,39,45,51,52,53,56,58,59,66,68,73,82) and 11 low-risk HPV types (6,11,40,42,44,54,67,69,70,85,97). It includes the GAP-DH control for verification of adequate DNA content.

Methods: Multiplex PCR is used to amplify E1 gene sequences. The PCR product is detected and genotyped by reverse hybridization to immobilized DNA probes spotted as tripletts together in single 96 well-plate wells and read by an AID ELISPOT reader. Clinical performance was assessed by using 563 cervical scrapings stored in PreserveCyte. Qiagen DNA extraction was used for DNA. Accuracy of the test was established by comparison with the reference test GP5+/GP6+ PCR with MPG-Luminex read out.

Results: We found complete match to the reference test in 30.6% (172/563), ≥ 1 HPV type match in 33.4% (188/563), and both tests HPV negative in 26.6% (150/563), with concordance of 90.6%. In 53/563 samples we found discrepant results: 5.7% (32/563) were MPG positive/HPV Easy negative, 2.7% (15/563) were MPG negative/HPV Easy positive and 1.1% (6/563) had discrepant HPV types. The sensitivity for HPV detection was 92% and specificity 88%, with positive predictive value of 95% and negative predictive value of 82%. The sensitivity for detection of high grade lesions (CIN2 or higher) was 97% and specificity was 67%.

Conclusion: HPV Easy DNA Array showed good concordance with MPG-Luminex Assay. It performs very well with regard to identifying high grade lesions. Due to simplicity and high throughput potential this method may be suitable for genotyping in HPV primary screening.

CROSS-REACTIVITY TO UNTARGETED, LOW-RISK HPV GENOTYPES BY COBAS, APTIMA AND HC2

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Objectives: Cervical cancer screening is increasingly changing from cytology-based to HPV-based testing. However, HPV screening displays lower specificity than cytology due to false-positive test results partly caused by cross-reactivity to low-risk genotypes. Cross-reactivity is costly as it may cause unnecessary follow up and anxiety in women. We studied cross-reactivity in three widely used clinical HPV assays with a full genotyping assay as a reference.

Methods: Within the Danish Horizon study, cross-reactivity to low-risk genotypes in consecutive routinely evaluated samples from 5,022 women was examined for HC2 (Qiagen), cobas (Roche), and APTIMA (Hologic-GenProbe) using CLART HPV2 (Genomica) as the reference assay. Complete histology follow-up was retrieved after 2.5 years.

Results: In total, 109 (2%), 62 (1%), and 35 (1%) of all included samples had a positive test result on HC2, cobas and APTIMA, respectively, where no high-risk genotype could be detected. When only samples with a positive test result on a particular assay were used as the denominator, cross-reactivity represented 11%, 5%, and 4%, respectively. Cross-reactivity was most common in young women, in women with abnormal cytology, and in follow-up samples. The most frequent cross-reacting genotypes were HPV70, HPV53, HPV82, HPV61, and HPV66 (the latter for HC2 only). Cross-reactivity was associated with low viral input. In total, 6 cross-reacting samples were associated with ≥ CIN2, but none from primary screening at age 30-65 years.

Conclusions: All three assays showed cross-reactivity to low-risk genotypes. Yet, cross-reactivity was not common and did not tend to be associated with ≥ CIN2 in primary screening.
Objective. The study of the prevalence and the role of different types of HPV in the development of CIN and cervical cancer, including HPV types 52 and 58.

Materials and Methods. Examined 548 women with abnormal cervical aged 18 to 60 years. Performed clinical-anamnesis, gynecology, extended colposcopy, molecular biological, morphological methods. Molecular biological techniques included a multiplex PCR detection results in real-time to determine the HPV type 21 (6,11,16,18,26,31,33,35,39,44 (55), 45, 51,52, 53,56,58,59,66,68,73,82) with the definition of viral load. Study group comprised 383 HPV-positive women.

Results. Frequent HPV types were 16, 58, 31, 52, 33, 44 (55). HPV types 52 and 58 were more prevalent in women under 35 years of age (88%). Cytology revealed: NILM - 169 (44.1%), ASCUS - 16 (4.2%), LSIL - 82 (21.4%), HSIL - 99 (25.8%), cervical cancer - 17 (4.4%). When NILM HPV types 52 and 58 occurred in 22 (13%) patients with ASCUS - in 6 (6.2%) with LSIL - in 20 (24.4%), and HSIL - in 25 (25.2%), and cervical cancer - the 2 (11.7%). Patients with HSIL, in the presence of the transformation zone with mild and severe changes, biopsy of the cervix. CIN was diagnosed in 145 of HPV-positive women: CIN I - 46 (31.7%) patients, of whom 11 (23.9%) identified HPV types 52 and 58; CIN II - 48 (33.1%), 12 (25%) identified HPV types 52 and 58; CIN III - in 51 (35.2%) 13 (25.5%) of them were found to HPV types 52 and 58.

Conclusion. HPV types 52 and 58 were more prevalent compared to previously submitted data, were the cause of the abnormal cytology in 22.4% of patients. Comprehensive analysis of the results of the extended colposcopy and histology of biopsy specimens of the cervix show a significant role of HPV types 52 and 58 in the development of CIN varying severity.
Objectives: After conization, the risk of cervical cancer remains relatively high. In Denmark, women are followed after 6 months with cytology and HPV testing. In these women, we compared cobas (Roche) to HC2 (Qiagen), the most validated HPV test up to date. Cobas has internal controls and separate HPV 16 and HPV 18 typing, which HC2 does not.

Methods: Consecutive routine samples were collected from two Danish laboratories (Copenhagen University Hospitals in Herlev (n=234) and Hvidovre (n=177)). Testing on HC2 and cobas was undertaken according to the manufacturers’ recommendations. Clinical outcomes were retrieved from the Danish Pathology Data Bank. We calculated positive agreement as a conditional probability that both assays returned a positive result if at least one did, κ-coefficients, proportions of ≥ CIN 2 with a positive result on an assay (sensitivity), and of < CIN 2 with a negative result (specificity).

Results: In Herlev, HC2 had a positive result in 58/234 (25%) samples, and cobas in 65/234 (28%). Positive agreement was 62% (95% CI: 0.50-0.73), and κ -coefficient was 0.68 (95% CI: 0.57-0.79). In Hvidovre, HC2 had a positive result in 56/177 (32%) samples, and cobas in 66/177 (37%). Positive agreement was a 69% (95% CI: 0.57-0.80), and κ -coefficient 0.73 (95% CI: 0.62-0.83). From 17 ≥ CIN 2, HC2 detected 16 (94%), and cobas 17 (100%). HC2 returned a negative result in 233/325 (72%) < CIN 2, and cobas in 223/325 (69%)

Conclusions: Our study using SurePath samples suggested that in follow-up after conization, HC2 and cobas can substitute each other with similar clinical outcomes.

Objectives: The objective of this work was to use "in silico" techniques, such as virtual screening assays and toxicity prediction, to search for inhibitors to human papillomavirus (HPV) E7 oncoprotein.

Methods: Four different types of HPV E7 oncoproteins (1A, 11, 16 and 18), previously modeled by Nicolau Junior & Giulatti 2013, were submitted to virtual screening assays by using GOLD software and Chembridge molecular database. The highest scoring results were then visually analyzed, and their toxicity was predicted by Derek software.

Results: The 100 best scoring molecules were visually analyzed due to their molecular interactions with the active site of the E7 proteins. The most prone molecules to strongly bind to the E7 site were analyzed due to their theoretical toxicity to human beings, leading to compounds with good potential to become leads.

Conclusion: In silico search for potential E7 inhibitors has been shown to be a promising strategy towards the treatment of HPV infections and prevention of human cervical cancer.

HPV GENOTYPIC DISTRIBUTION IN PATIENTS WITH CERVICAL CANCER IN THAILAND

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Background: As Thailand’s considering integrating HPV-16/18 vaccine into the National Immunization Program, this study aimed to determine the require data on HPV prevalence and genotypic distribution in Thai invasive cervical cancer patients.

Methods: The study was conducted during June 25, 2012 – September 11, 2014 at Chulabhorn Hospital, Bangkok, Thailand, 160 consecutively collected specimens of BD Surepath liquid-based cytology consecutively collected from pelvic examination for clinical staging and analyzed by linear array HPV genotyping tests (Roche, USA). Fourteen were excluded.

Results: Of 146 patients, mean age 55.0 years and range 26-78 years, HPV infection was detected in 140(95.9%), including 123(87.9%) single infections and 17(12.1%) multiple infections. HPV16 and HPV18 were the most common subtypes in 67(45.9%) and 25(17.1%), respectively, with HPV58, HPV52, HPV33, HPV45, HPV56, HPV59 and HPV31 found in 13(8.9%), 12(8.2%), 6(4.1%), 4(2.7%), 3(2.1%), 3(2.1%) and 2(1.4%), subsequently.

Conclusions: Differing slightly from the worldwide data, this study revealed lower prevalence of HPV16/18 and higher frequencies of HPV58 and HPV52. Currently available HPV vaccines against HPV16/18 could potentially prevent 63.0 % of cases. The next generation of 9-valent HPV vaccine (6/11/16/31/33/45/52/58) may be thus required for most Thai patients (88.4%).

HLA-G POLYMORPHISM AND HPV TRANSMISSION AMONG HETEROSEXUAL COUPLES IN THE HITCH STUDY

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Objectives: Human leukocyte antigen (HLA)-G polymorphism influences innate and adaptive immune responses. Among heterosexual couples in the HITCH cohort study we examined the association between HLA-G alleles and genital human papillomavirus (HPV) infection in women and their male partners independently. We also analyzed whether allele sharing in a couple was associated with the likelihood that the partners had infections with the same HPV type, i.e., if allele concordance predicted transmission of HPV infection.

Methods: We tested genital samples from 274 couples for 36 HPV genotypes by PCR. HLA-G alleles were typed using direct DNA sequencing.

Results: The most common alleles among couples were: G*01:01:01 (95.6%), G*01:01:02 (60.1%), G*01:04:01 (33.9%), G*01:06 (21.4%), G*01:01:03 (19.6%), and G*01:03 (19.2%). The G*14bp deletion occurred in 86.1% of the couples. In gender specific analyses, only G*01:03 was associated significantly with risk (negatively) (odds ratio [OR] = 0.36, 95%Ci: 0.17-0.76), among women exclusively. In the allele-sharing and HPV-concordance analysis G*01:03 was not a significant predictor of HPV positivity in either or both partners, nor was it a predictor that a couple shared a type-specific infection. The number of alleles exhibiting significant associations at the 5% level did not exceed what was expected under the null hypothesis taking into account the high number of associations examined. HPV-clade specific analyses also did not reveal any pattern of consistency concerning allele presence or sharing and risk, whether individually or in a couple.

Conclusion: We found no evidence for a role for HLA-G in acquisition and transmission of HPV infection.
DO HPV 16 AND 18 PREDICT A HIGHER RISK OF CIN2+ THAN OTHER HIGH-RISK HPV GENOTYPES IN TRIAGE?

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Objectives: To estimate the positive predictive value (PPV) for CIN2+ of HPV DNA 16 and 18 versus other high-risk (HR) types, in single and multiple infections, in delayed triage among women aged 25-69 with ASCUS and LSIL cytology.

Methods: We included 6058 women with primary screen-detected ASCUS or LSIL cytology with a follow-up HPV test; Hybrid Capture II (Qiagen), between July 2005 and June 2010. The HPV positive cases were genotyped with in-house nmPCR(1), differentiating between the 13 high-risk HPV types covered by HCII. All women were followed-up for histologically confirmed CIN2+ within three years of index HPV test by linkage to the screening databases at the Cancer Registry of Norway.

Results: HC2 was positive in 46% of the women (2756/6058). HPV genotyping revealed single infection in 57% (1578/2756) and multiple infections with two or more genotypes in 32% of the cases (884/2756). In 11% of the cases (294/2756) we were not able to detect any genotype (HPV X). CIN2+ was confirmed by histology in 1191/2756 HPV test positive women (43%) and in 71/3303 HPV test negative women (2.1%). For single infection by HPV 16, we found the highest the PPV (61%) for detecting CIN2+, followed by HPV33 (53%), HPV35 (46%), HPV58 (41%), HPV31 (40%) and HPV18 (35%). Minor differences were detected for PPV in multiple infections which will be presented.

Conclusion: In this observational study we have shown that HPV16 and 33 predicts much higher PPV for CIN2+ compared to HPV18, both in single and multiple infections.

Reference:

“SAFETY IN NUMBERS” – HPV GENOTYPE-SPECIFIC VS. POOLED HIGH-RISK APPROACHES TO CIN3+ RISK STRATIFICATION

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Background: The widespread adoption of the Hybrid Capture 2 test (QIAGEN), which uses a pooled cocktail of probes to detect all high-risk HPV types, has led other investigators to continue to leverage this pooled assay design approach. It is now well established that genotyping HPV 16 and 18 provides improved risk stratification and 16/18 triage is an integral part of US screening guidelines. Nevertheless, all subsequent FDA-cleared assay designs utilize an all high-risk or 12-other pool approach.

Results: We present evidence that pooling of the 12 other high-risk types masks the real risk for CIN3+ disease in the same way that the Hybrid Capture 2 design underestimates the individual risk posed by HPV 16 and 18. This is a direct consequence of the fact that the 14 high-risk HPV types have different abilities to infect, persist and cause disease. This is evident from several published longitudinal genotyping studies and has recently been confirmed using the extended genotyping BD Onclarity™ HPV Assay.

Conclusions: Pooling of 12 high-risk types runs counterurrent to the recent recommendation to implement “equal management of equal risks” in making colposcopy referral decisions (1). If a pooled approach is used, the underlying individual HPV genotype risk may be masked by the pooled type result. We discuss the implications for national screening programs and suggest that this be part of the ongoing debate on future primary screening guidelines. Failure to learn from the HPV 16/18 “history lesson” may have important implications for informed management of HPV infected women.

Objective: To determine the prevalence of 18 STIs among 100 HIV+ and 150 HPV high-risk women as part of the ACCESS-ING pilot study, taking place in the North Tongu District in Ghana.

Methods: Extracted DNA from vaginal lavage samples, taken with Delphi Screener, were tested with multiplex PCR followed by Luminex bead-based hybridization. This assay detects 18 sexually transmitted infections (STI) assay, Schmitt et al., 2014, J Infection 69:123). 250 Patients, aged 18 to 60 years, were selected for this pilot study with 100 HIV+ patients 150 patients with a history of high-risk HPV positivity or cervical dysplasia (HPV-hr).

Results: Prevalence found for Chlamydia trachomatis was 0% and 4.3% among the HIV+ and HPV-hr patients, respectively. 2.1% and 0.7% were tested positive for Neisseria gonorrhoeae and 0% in both groups for Treponema pallidum. Prevalence found for Trichomonas vaginalis was 2.1% and 2.9%, for Herpes simplex type-2 8.3% and 3.6%. For Candidosis prevalence was 29.2% and 25.9% and for strong/very strong bacterial vaginosis 43.8% and 30.2%..

Discussion: For most STI the prevalence found among HIV+ patients was higher compared to HPV-hr patients, except Chlamydia trachomatis. Overall, the prevalence found for Chlamydia trachomatis among HIV+ and Treponema pallidum and Trichomonas vaginalis in both groups was lower than stated in the 2005 WHO estimates for the African region among women 15 to 49 years old. Therefore, prevalence in our pilot study population seems lower compared to the WHO African region. Full epidemiological data of North Tongu District will be analyzed during the main ACCESS-ING study.

Background: About 99.7% of cervical cancers are caused by persistent infection with high risk Human Papillomavirus genotypes (HR-HPV). However, data on persistence of HR-HPV is lacking for Ghana and most Sub-Saharan African countries.

Objectives: To determine the persistence of HR-HPV infections among women in the North Tongu District, Ghana.

Methods: As part of a cervical cancer study in 2010/2011, 500 patients were genotyped for HPV using nested multiplex PCR (Sotlar et al. 2004) from swab samples at University of Ghana School of Allied Health Sciences, Accra. A total of 104 women who tested positive for HR-HPV and remained untreated, were followed up in the current ACCESS-ING pilot study and re-tested for HPV. HPV genotyping in 2014 was done on cytobrush samples by GP5+/6+ PCRs followed by Luminex-MPG readout at Charité Universitätsmedizin Berlin. Those who tested positive for HR-HPV were recalled for colposcopy and treatment.

Results: Out of 104 women who were identified as HR-HPV+ in 2010/2011, 71% had no high risk HPV infection after approximately 4 years, 7% had 1+ persistent HR-HPV type and 21% had cleared but acquired new HPV infections. Persistent HPV types were HPV 16, 18, 35, 39, 51, 52, 58, and 68. Complete clearance of HPV decreased with age, and older women acquired more new infections.

Discussion: This study represents rare longitudinal data on HPV infection in Ghana. The clearance rate observed does not vary greatly from other study populations. Further analysis by colposcopy will give insight into possible disease progression of the persistent HPV infections.
**OC 11-8**

**PREVALENCE OF RISK FACTORS ASSOCIATED WITH HPV AND HIV POSITIVITY AND CERVICAL CANCER AMONG CAMEROONIAN WOMEN.**

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**Objective:** To determine demographic and lifestyle factors associated with HPV and HIV prevalence and the presence of cervical precancerous lesions and cancer (CIN2+) in a community based HPV self-sampling.

**Method:** We report data from 838 women aged between 25-65 years, recruited in two sequential cervical cancer screening campaigns that took place in Cameroon. Demographic and historical information were obtained from all participants and specimens were self-collected for HPV testing (cobas®). HPV positive women were reexamed and underwent biopsy and endocervical curettage. Associations were determined using logistic regression.

**Results:** The overall HPV prevalence was 39.0% and the HIV self-reported prevalence was 9.2%. 18 CIN2+ lesions were found among the 194 women who underwent biopsy. Housewives had higher risk of being HPV positive (OR=1.60; 95%CI: 1.12-2.30; p=0.010). HIV co-infection (adjusted-OR (aOR)=3.44; 95%CI: 1.91-6.20; p<0.001) and hormonal contraception (aOR=1.97; 95%CI: 1.21-3.17; p=0.007) were associated with higher risk of HPV infection. Women with CIN2+ had higher probability to be infected with multiple HPV genotypes (aOR=7.23; 95%CI: 1.55-33.66; p=0.012). Housewives (aOR=4.72; 95%CI: 2.10-10.61; p<0.001) had higher HIV prevalence, as women having multiple sexual partners (aOR=10.29; 95%CI: 2.34-45.29; p=0.002). HPV positive women who wore condoms during sexual intercourse were at lower risk of CIN2+ (aOR=0.15; 95%CI: 0.03-0.82; p=0.029). HPV 16/18 positive women had 4.65-fold increased risk to develop CIN2+ (95%CI: 1.35-16.01; p=0.015).

**Conclusion:** Young single women and housewives are at higher risk of HPV and HIV infection and subsequently to develop cervical cancer. Condom seems to prevent cervical cancer in HPV positive women, although it doesn’t protect against HPV infection. Screening strategies should target women at higher risk.

**OC 11-9**

**THE EFFECT OF HIV INFECTION ON PENILE HIGH-RISK HPV INCIDENCE AND CLEARANCE AMONG MSM**

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**Objectives:** Around 30% of penile cancers are caused by high-risk HPV (hrHPV) infections. Understanding the natural history of penile hrHPV is relevant for pathogenesis of penile cancer and to better understand penile-anal hrHPV transmission. Therefore, we aimed to compare the incidence and clearance rates of penile hrHPV infection between HIV-infected and HIV-negative MSM.

**Methods:** MSM aged ≥ 18 years were recruited in Amsterdam, the Netherlands, and followed-up semi-annually for 24 months. At each visit, participants completed risk-factor questionnaires. Penile-shaft self-samples were tested for HPV DNA and genotyped using the SPF10-PCR DEIA/LiPA25 system; HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59 were classified as hrHPV. Effects on incidence and clearance rates were quantified via Poisson regression, using generalized estimating equations to account for multiple hrHPV types.

**Results:** 750 MSM with a median age of 40 years (IQR 35-48) were included in the analyses, of whom 302 (40%) were HIV-infected. The incidence rate of penile hrHPV was borderline significantly higher in HIV-infected compared to HIV-negative MSM (adjusted incidence rate ratio 1.45; 95%CI 1.00-2.11), whereas the clearance rate was non-significantly higher (adjusted clearance rate ratio 1.27; 95%CI 0.95-1.70). HR HPV incidence or clearance among HIV-infected MSM did not differ significantly by level of nadir CD4 cell count.

**Conclusions:** Increased penile hrHPV incidence rates and similar hrHPV clearance rates were found in HIV-infected compared to HIV-negative MSM, after adjusting for reported sexual behavior. Our findings suggest an independent effect of HIV infection on incidence of penile hrHPV infections.
Objectives: To assess high-risk types of the human papillomavirus (HPV) in women diagnosed with a precancerous lesion. To analyze high-risk types of HPV and their role in the development of cervical intraepithelial neoplasia (CIN).

Methods and Results: One hundred fifty seven patients were analyzed based on molecular tests and histopathological data. The frequency of HPV DNA type 16 and twelve high-risk oncogenic types, referred to collectively as human papillomavirus – high-risk (HPV-HR), in patients diagnosed with precancerous lesions suitable for treatment, was 54.8% and 53.5%, respectively. The difference between the HPV DNA type 16 and HPV-HR types is not statistically significant.

Conclusions: High-risk HPV infections are closely linked to the development of precancerous cervical lesions, and subsequently cervical cancer. This study shows that the incidence of type 16 of HPV is similar to the incidence of either one or more of the HPV-HR types: 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, in patients diagnosed with cervical intraepithelial neoplasia suitable for treatment. Future studies could include the identification of specific HPV types of HPV-HR.

Objectives: This work aims to obtain the second epidemiological data on the distribution of high-risk HPV in Midi-Pyrenees (France) after a first study in 2011.

Methods: Over a period of 12 months in 2013, 1476 patients were studied for high-risk HPV and positive samples were genotyped with Abbott High-Risk HPV Real-time PCR on an automated platform Abbott M2000.

Results: 492 samples were detected positive for HPV HR (33.3%) and 984 samples were negative (66.7%). The distribution of genotypes is as follows: HPV 16 in 25.2% and HPV 18 in 7.3% and others HPV HR (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68)in 73.6%. The distribution of the prevalence of HPV still depends of the matter of the prescription. In the 1104 samples of ASC-US women, 39.6% of the samples were positive. In the 304 women included for “screening” or “systematic”, only 9.5% of the samples were positive.

Conclusions: HPV 16 and HPV 18 are still detected in less than 40 % of the positive samples. The others HPV HR group are mainly detected on more than 70% of positive samples. The ASC-US patients have still a prevalence of HPV HR significantly higher with over 30% of samples detected positive while patients performing the test for “screening” have a prevalence of less than 10% of HPV HR. This study confirmed the 2011 data, the base point for following the evolution of HPV genotypes in Midi-Pyrenees with the advent of vaccination, which target genotypes 16 and 18.
HUMAN PAPILLOMAVIRUS AND CHLAMYDIA TRACHOMATIS CO-INFECTION AND THE SEVERITY OF CERVICAL NEOPLASIA
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Objective: To correlate positivity for Chlamydia trachomatis (C. trachomatis) according to serology and polymerase chain reaction (PCR) assay with positivity for human papillomavirus (HPV) and to evaluate the relationship of these findings with the severity of cervical neoplasia in women with cytological abnormalities.

Methods: Between February 2007 and March 2009, 136 women were referred to a colposcopy clinic in Goiania, Brazil for cervical abnormalities. HPV-DNA was detected by PCR using PGMY09/11 primers and genotyping was performed by reverse line-blot hybridization assay. C. trachomatis positivity was evaluated both by enzyme-linked immunosorbent assay (ELISA) to detect IgG antibodies and by PCR-DNA detection using H1/H2 primers.

Results: The overall prevalence of HPV infection was 85.2%. Seropositivity for C. trachomatis was 25% (34/136). Thirty-one samples were simultaneously PCR-positive for HPV and seropositive for C. trachomatis. Although C. trachomatis seropositivity was higher in HPV-positive than in HPV-negative women, this difference was not significant. According to PCR analysis, 12/136 samples (8.8%) were positive for C. trachomatis. Again, the prevalence of C. trachomatis infection was higher, but not significantly so, in the women infected by HPV. Positivity for HPV, particularly HPV16 and HPV18, and seropositivity for C. trachomatis were significantly associated with the severity of cervical neoplasia (CIN 2 or worse). A significant association was also found after controlling for HPV16 and HPV18.

Conclusion: In this study, no statistically significant association was found between co-infection by HPV and PCR-detected C. trachomatis and the severity of cervical neoplasia, even after controlling for HPV type.

LIQUID BASED CYTOLOGY (THIN PREP) AND COMPUTERASSISTANCE COMPARED TO CONVENTIONAL CYTOLOGY; SEVEN YEARS EXPERIENCE IN 310 203 CASES
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Objectives: Our goal is to compare the detection rate for pre neoplastic lesions in the cervical cancer screening with liquid based cytology versus screening with conventional cytology. We compared two similar groups of women who participate regularly to the cervical cancer screening program in Germany. One Group was screened computer assisted with Thin Prep-Imaging-System (TIS) and the other group with conventional cytology (CC). Here we report the performance of Thin Prep imaging System compared with conventional cytology under routine conditions over seven years.

Methods: Cytomol is a private commercial lab specialized in diagnostics for cervical cancer prevention. With Thin Prep since 2000 an experience with 340.000 cases has been achieved. Since 1.1.2007 all thin layer specimens have been processed by Thin Prep imaging system. Because in Germany liquid based cytology is reserved to privately insured and self-paying patients while public healthcare only reimburses conventional cytology. To avoid bias we limited this analysis privately insured patients. Finding rates of cytology abnormalities with TIS and CC were compared. Cytology diagnoses originally reported in the Munich nomenclature II (MN; with the use of the unofficial Pap IIK category. From 01.07.2014 we are using the new Munich III nomenclature ), were translated to The Bethesda System.

Results: From 2007 to 2012, 310 203 slides have been analyzed among them 217731by TIS. Except of some bloody and very cell-rich samples 97.25% of the smears were accepted for analysis by the system. TIS had a rate of LSIL (= MN PapIIId/CIN1) of 2.05% compared to 0.48% for CC (92 472), an increase of 427%. HSIL (= MN Pap IIId/CIN2 + Pap IVa/b) was found in 1.14% with TIS versus 0.39% with CC (+292%). The ASC-US rate (MN Pap IIK + III) was 2.05% with TIS and 0.98% with CC. This increase of 209% is 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.
NORMAL SMEARS AND SPECIMENS WITH BENIGN CELLULAR CHANGES: ARE THEY REALLY EQUIVALENT? 
DATA FROM 95 934 CASES 

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Context: In Alsace, the cervical cancer screening programme distinguishes entirely normal smears from specimens with benign cellular changes, unlike Bethesda 2001 classification. Aim of this study is to know if this is clinically relevant in terms of CIN2+ detected within 10 years.

Methods: All normal smears and smears with benign cellular changes registered in 2001 inside the EVE screening programme, are included and followed for 10 years. Numbers in terms of person time are calculated. Increased risk of CIN2+ is assessed by cumulated incidence risk and Relative Risk.

Results: 95 934 smears are included, 65 021 normal ones (67.8%) and 309 13 with benign cellular changes (32.2%). In all, 729 CIN2+ are observed including 57 cancers. After ten years the CIN2+ risk is increased by 30% (RR = 1.36; IC95 = 1.17-1.58) for women with benign cellular changes compared to those with normal smears. But cancer risk is not significantly increased (OR = 1.01; IC95 = 0.59-1.77)

Discussion: Management of the two types of smear was equivalent. For example, mean number of follow up smears is 4.0 in case of normal smears and 4.2 in the second group.

Conclusion: The increased risk observed for women with benign cellular changes justifies the cytologic distinction made by Alsatian pathologists. Absence of increased cancer incidence supports an identical management.

BENEFIT OF CERVICAL SCREENING IN YOUNG WOMEN – A MATTER OF ADHERENCE TO THE RECOMMENDED SCREENING INTERVAL

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Objectives: The benefit of cervical screening for women below age 30 is debated. We aimed to assess the benefit of cervical screening for women in this age group using a national Swedish case-control audit.

Methods: The first case-control audit of the Swedish screening program was performed in 2006 and included 1230 cases during years 1999-2001, with five population based controls per case. The number of cases below age 30 was 63 (309 eligible controls), and the number of cases with stage IB or worse (IB+) was 33 (158 controls). Cancers with a Pap smear within 6 months prior to diagnosis were considered screen detected. Conditional logistic regression models were utilized to calculate odds ratios (OR) with 95% confidence intervals (CI).

Results: The OR for cervical cancer was 0.45 (CI 0.26-0.78) for women below 30 participating according to the recommended screening interval (3 years). For stage IB+ cancers the OR was 0.64 (CI 0.30-1.34) and for women age 27-29 years the corresponding OR was 0.36 (CI 0.13-0.99). Allowing for an extended screening interval of 6 years gave corresponding ORs of 0.62 (CI 0.35-1.12), 0.96 (CI 0.40-2.28) 0.69 (CI 0.22-2.15). Women aged 23-26 years showed no benefit of screening within the last 3 years (OR 1.09, CI 0.32-3.68).

Conclusions: We found no evidence to support cervical screening below age 25, while women from age 27 and up have a benefit of screening, also against stage IB+ tumours. Six years seem to be a too long screening interval for ages below 30.
**OC 12-4**

**AGE AT FIRST SMEAR AND CERVICAL CANCER RISK: RECENT UK INCIDENCE AND MORTALITY TRENDS**

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**Objectives:** To assess the effects of the increase in age at first cervical smear in England.

**Methods:** The screening age increased from 20 to 25 year in England in 2004 but not in Scotland. Recent trends in incidence and mortality in England and Scotland were compared against the expected effects.

**Results:** Annual mortality rates for women aged 20-29 years increased in England from 0.37 per 100,000 in 2003-05 to 0.56 per 100,000 in 2010-2012 while rates remained stable in Scotland where the minimum screening age remained unchanged (0.21 and 0.19 per 100,000 respectively). Incidence rates of cervical cancer-in-situ (CIS) fell in women aged 20-24 but increased sharply in women aged 25-29, from 306 per 100,000 (2004) to 644 per 100,000 (2012). Annual CIS incidence per 100,000 women aged 20-29 increased by a third in England, from 263 (2004) to 349 (2012) compared to a 25% decrease in Scottish women from 472 to 356 per 100,000.

**Conclusions:** Interpretation is complicated by the introduction of Liquid Based Cytology and HPV triage and a transient increase in screening coverage in 2009 following the widely publicised death from cervical cancer of a 27 year old celebrity. Increases in CIS registrations in women aged under 30 could be due to lesser lesions remaining untreated and progressing to CIN3 before their first screen after age 25. Cervical cancer mortality has increased since 2004 in women aged under 30 but the causal link with raising the screening age remains uncertain.

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**OC 12-5**

**CERVICAL CANCER INCIDENCE AFTER A NEGATIVE SMEAR: COMPARING SUREPATH AND THINPREP WITH CONVENTIONAL CYTOTOLOGY**

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**Objectives.** During the last 10 to 15 years, liquid-based cytology tests SurePath and ThinPrep have replaced conventional cytology as primary test method in the Dutch cervical cancer screening program. By comparing interval cancer rates (i.e. cervical cancer detected within 6 years after a negative primary smear); we examined the difference in sensitivity to detect clinically relevant cervical intraepithelial neoplasia (CIN)2+ lesions.

**Methods.** All negative primary smears taken from January 2000 until March 2012 within the Dutch cervical cancer screening program were retrieved from the nationwide registry of histopathology (PALGA) with a follow-up until March 2013. The 6-year cumulative incidence per 100,000 negatively screened smears was calculated for each screen method. Cox regression analyses were performed to assess the hazard ratio (HR) adjusted for calendar time, age, screen region and socio-economic status.

**Results.** When comparing SurePath with conventional cytology, the 6-year interval cancer cumulative incidence was significantly lower (44.6 (95% confidence interval (CI): 37.8 to 52.6) versus 58.5 (95% CI: 54.6 to 62.7)), just as the hazard (HR of 0.83 (95% CI: 0.70, 0.98)). One interval cancer was prevented at the expense of finding 9 extra CIN lesions. When comparing ThinPrep with conventional cytology, the 6-year cumulative incidence (66.8 (95% CI: 56.7 to 78.7) versus 58.5 (95% CI: 54.6 to 62.7)) and the hazard (HR of 1.20 (95% CI: 1.01, 1.41)) were (non-significantly) higher.
HYSTERECTOMY AND ITS IMPACT ON THE CALCULATED INCIDENCE OF CERVICAL CANCER AND SCREENING COVERAGE RATE IN DENMARK

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Objectives: The incidence rates of cervical cancer and the coverage rate of women in cervical cancer screening programmes are usually reported by including all women from the whole population. These rates are calculated without elimination of hysterectomized women from the population. We aimed to describe the incidence rates of total hysterectomy and its impact on the calculated incidence rate of cervical cancer, and screening coverage rate in Denmark.

Methods: Data were retrieved from five Danish nation-wide, population-based, health care registers. All total and radical hysterectomies undertaken for reasons other than cervical cancer between 1977 and 2010 were included in the analysis. The incidence rates of cervical cancer and the screening coverage rates for women aged 23-64 years were calculated with and without adjustments for hysterectomies.

Results: For year 2010, the prevalence of hysterectomy was estimated at 6% over the entire age span. The unadjusted overall cervical cancer incidence rate of 12.8 per 100,000 woman-years corresponded with the hysterectomy-adjusted rate of 13.5 per 100,000 woman-years. The difference between the two incidence rates was about 19% in women around age 70 years and above. The coverage rate of the women targeted by the screening program (23-64 years) increased from 76% (without adjustment) to 79% (with adjustment for hysterectomies).

Conclusions: In Denmark, hysterectomies do not have a large overall impact on the calculated incidence of cervical cancer and coverage rates. Nevertheless, in older women, adjusted rates would increase by up to ~20% compared to unadjusted rates.

EVALUATION OF THREE STRATEGIES TO INCREASE CERVICAL CANCER SCREENING COVERAGE AMONG WOMEN AGE 30 TO 70 YEARS OLD. CRICERVA.

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(2) Unidad de Soporte a la Investigación. IDIAP Jordi Gol. Sabadell, (Barcelona), Spain.
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Aims: To evaluate three strategies to capture women to cervical cancer screening with a poor history of cervical cancer screening(CaCX).

Methods: The CRICERVA study is a randomized controlled trial. Out of 32858 women aged 30-70 years old enrolled in SAP Cerdanyola area (Barcelona), 15963 with no history of cervical cancer screening in the previous three years. The study included three interventions (personalized letter(G1,N=4197); informative leaflet added(G2,N=3601), a telephone added call(G3,N=6088)) and a control group. (GNI,N=2079) (spontaneous visit). The participants were offered cervical cancer screening with Cytology, HPV and personalized interview on the reasons fro not attending to screening.

Results: The intervention increased the cervical screening in a 17.6% in the G1, an 16.7% in the G2, a 21.7% in the G3 and a 9.11% in the GNI. This represented a final coverage of the screening of a 82.8% in the G1, 83.3% in the G2, 82.8% in the G3 and a 53.4% in the GNI. The lack of information about the importance of the screening was the most common reason of non-assistance (58.1%).

Conclusions: Organized screening through a personalized contact and an informative letter and a fixed appointment resulted in important gains in coverage. Our data support the need for further effort to communicate the relevance of cervical cancer screening.
IMPACT OF OVERSCREENING, DATA FROM A FRENCH ORGANIZED PROGRAMME.

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Objective: French Guidelines state that smears should only be repeated every three years after 2 normal tests. However physicians are afraid of interval lesions. Aim of this study is to assess the impact of overscreening in terms of detected lesions and costs.

Methods: A retrospective cohort is built based on data from the Alsatian cervical screening programme where all smears and further exams are registered. All women whose second normal smear occurred between 1998 and 2002 and are rescreened a third time are included and followed 9 years. Delay between the 2nd and the 3rd smear is considered to define 2 groups: one “overscreened” [6; 23] months and “correctly screened” [24; 43] months. The first criteria is CIN2+ lesions detected and the secondary ones cancers and CIN1. Costs of excess smears and CIN1 are assessed.

Results: 83346 women are followed. Incidence rate of CIN2+ is 12.7 per 10000 patients a year for the overscreened group (N = 50350) and 10.6 per 10 000 patients in the correctly screened group (N = 32996). The age-adjusted OR is 1.18 [1.01; 1.39]. Overscreening does not protect from invasive cancer but detects more CIN1 (OR = 1.72; IC 95% [1.48; 2.00]). Considering only excess smears and CIN1, the excess cost of overscreening is 1 83301 € (+31%) and there is also an unnecessary obstetrical risk for the conized women.

Conclusion: Overscreening provides very low benefits in terms of detected CIN2+ and does not protect more from cancer. It is expensive and can be dangerous for young women, due to unnecessary CIN1 treatments.

CERVICAL CANCER PREVENTED BY SCREENING: LONG-TERM INCIDENCE TRENDS BY MORPHOLOGY AND REGION IN NORWAY

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Objectives: We analysed 54 years of incidence trends of cervical SCC and adenocarcinoma in Norway in order to explain regional differences in cancer burden in terms of background risk and preventive effects of screening.

Methods: The Cancer Registry of Norway was used to identify and classify by morphology all 19,530 malignancies of the cervix diagnosed in the period 1956-2010. We used join-point analysis to describe trends in incidence rates by morphology and region, and to project annual SCC incidence in the absence of screening based on trends of adenocarcinoma.

Results: The the age-standardised (w) incidence rates (per 100,000 woman-years) of cervical SCC and adenocarcinoma were, respectively: 13.4 (95%CI:12.7,14.1) and 1.1 (95%CI:1.0,1.4) in 1961-1965; 14.9 (95%CI:14.2,15.6) and 1.0 (95%CI:0.9,1.2) in 1976-1980; 9.7 (95%CI:9.1,10.2) and 1.7 (95%CI:1.5,2.0) in 1991-1995; 7.1 (95%CI:6.6,7.5) and 1.9 (95%CI:1.7,2.1) in 2006-2010. The average annual percentage change was -1.3 (95%CI:-2.0,-0.5) for SCC, and 1.5 (95%CI:1.1,2.0) for adenocarcinoma. The projected age-standardised incidence rate of cervical SCC in Norway, assuming no screening, was 29 in 2009-2010, compared to an observed incidence rate of 6.7. The estimated proportion prevented by screening ranged from 67 to 79 % between regions.

Conclusions: Despite regional variation in the incidence rates of both types of cancer, the proportion prevented by screening was similar. Cytology screening has impacted cervical cancer burden in all regions of Norway to a higher degree than is evident by overall incidence trends. The simultaneous substantial increase in cervical adenocarcinoma is presumably associated with greater exposure to HPV over time.
**ESTIMATING THE CONTRIBUTION FROM UNSPECIFIED UTERINE CANCER TO CERVICAL CANCER MORTALITY**

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Objectives: Monitoring effectiveness of cervical cancer prevention is feasible using population based registers. Data must be validated in order to effectively monitor changes in prevention strategies. All cervical carcinomas, but no other tumors should be included. Death certificates are issued using simplified diagnoses, causing a large proportion of endometrial carcinomas and sarcomas to be classified as unspecified uterine cancer. Algorithms have been developed to account for deaths due to cervical cancer, erroneously coded as unspecified uterine cancer. GLOBOCAN 2012 reports a cervical cancer mortality which is 35% higher than in the Swedish Causes of Death Register. One possible explanation is the attribution of unspecified uterine cancer deaths (ICD 10 C55) to cervical cancer (ICD 10 C53).

Methods: All deaths in the Swedish Causes of Death Register due to unspecified uterine cancer were linked to the Swedish Cancer Register from 1997-2011.

Results: 84% of all women with a reported cause of death “unspecified uterine cancer” already had a specific diagnosis in the cancer register (7% cervical carcinoma, 55% endometrial carcinoma, 3% non-uterine carcinoma, 19% uterine sarcoma). Less than 16% had an undetermined or no previous cancer diagnosis, some of which might be cervical cancers.

Conclusions: The contribution of women with a death certificate of “unspecified uterine cancer” (C55) but who actually died from cervical carcinoma is less than 10%, rather than 35%. Where possible, the Causes of Death and Cancer registers should be linked before applying reallocation algorithms to obtain valid data suitable for the evaluation of cervical cancer prevention.

**THE IMPACT OF CERVICAL CANCER SCREENING ON PRETERM BIRTH: A DECISION ANALYSIS**

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4 The Robinson Research Institute, School of Paediatrics and Reproductive Health, University of Adelaide, Australia

Objectives: Although screening and early treatment has reduced cervical cancer incidence and mortality considerably, treatment of cervical intraepithelial neoplasia (CIN) might increase the risk of preterm birth (PTB). We assessed the impact of different screening strategies on the risk of PTB and subsequent neonatal outcome, relative to maternal life years gained (LYG).

Methods: We simulated six screening strategies, varying in start age (20, 25, and 30 years) and interval (3 and 5 years) using the MISCAN model. For every strategy, the model produced the age-specific number of CIN diagnoses and LYG. We assumed that 25% of CIN 1 and 100% of CIN 2 or worse was treated using loop electrosurgical excision procedures, which led to a relative increase of the age-specific PTB risk of 60%. Age-specific pregnancy data were used to estimate the increase in PTBs due to cervical cancer screening.

Results: Three-yearly screening from age 20 resulted in 10,448 LYG per 100,000 screened women, at the expense of 997 additional PTBs (i.e. 94 and 29 cases of neonatal morbidity and mortality, respectively). When the start age of screening was postponed to age 30, the number of LYG reduced with 0.3% to 10,419, while the number of PTBs reduced with 77% to 225 (i.e. 21 and 7 cases of morbidity and mortality, respectively).

Conclusions: Starting cervical cancer screening at age 30 instead of age 20 slightly reduces the number of LYG, for a substantial reduction in PTBs. Therefore, screening young women with a childwise may do more harm than good.
EFFECT OF HR-HPV IMMUNISATION ON THE PERFORMANCE OF CERVICAL CYTOLGY.

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Background. The ability of cytology to detect cervical precancer is fundamental to cervical screening programmes. Reduction of HR-HPV types and associated disease in immunised women is expected to adversely affect cytology. The first immunised women entered the Scottish Cervical Screening programme (SCSP) in 2010/11, at age 20. The SCSP uses Thinprep® with Image Assisted Screening® and can link immunisation status with cytology and histology results directly.

Methods. Data on first cytology test result, immunisation status and histology were obtained for women born between 1 January 1990 and 31 December 1993. The Positive Predictive Value (of HSIL+/– moderate and severe dyskaryosis or worse), the Abnormal Predictive Value (of ASCUS, ASCUS-H and LSIL/ borderline changes and mild dyskaryosis), and the Total Predictive Value (of all referrals for colposcopy), Referral Value (the number needed to refer to detect one case) and the Mean CIN Score (a weighted average of the severity of disease detected at each colposcopy) have been derived for CIN2+ and CIN3+…1 The PPV (CIN2+) is the primary analysis and statistical significance was assessed using Chi2 tests with a Bonferroni correction.

Results. Full immunisation is associated with significant reductions in PPV (CIN2+), and APV, TPV, RV and MCS for both CIN2+ and 3+, but not for the PPV (CIN3+). These findings suggest that high grade cytology carries the same significance with regard to CIN3 in both fully immunised and unvaccinated women but not for CIN2. Low grade abnormalities are probably less significant in fully immunised women, with implications for management.

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CHARACTERISTICS OF GPs WHO DO NOT ENGAGE IN CERVICAL CANCER SCREENING IN FRANCE

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Objectives: In France cervical cancer screening (CCS) by Pap smear can be performed by general practitioners (GPs), and medical gynecologists. Medical gynecologists manage contraception, cancer screening, menopause, while deliveries and surgery remain to obstetricians. Women have direct access to gynecologists as primary care practitioners. Our aim was to measure GPs’ involvement in CCS and investigate the characteristics of GPs associated with no practice of CCS.

Methods: Data came from 3 cross-sectional surveys conducted among representative samples of French GPs in 1998, 2002 and 2009 (n = 6213). We conducted univariate and multivariate logistic regressions stratified on GPs’ sex to investigate the characteristics (age, solo or group practice, professional network, area of practice…) of the GPs associated with no practice of CCS ever.

Results: The proportion of GPs not performing CCS increased from 24.1% to 34.8% over the period (χ², p<0.0001). Women performed CCS increasingly more than men in all three years, from 8.7% more than men up to 17.1%. In multivariate analyses, female GPs with unregulated fees were more likely not to perform CCS (2.31, 95% CI: 1.50-3.56) as were male GPs belonging to no professional network (1.38, 95% CI: 1.15-1.66). Male GPs from the Paris metropolitan area were less likely to perform CCS until 2002.

Conclusions: Less and less GPs engage in CCS when the growing scarcity of medical gynecologists calls for more participation. Female GPs remain significantly more active in CCS than male GPs. The participation in CCS is determined differently according to the practitioner’s gender.
**OC 13-2**

**COMPLETE AGE SPECIFIC ROUND 1 RESULTS FROM THE CANADIAN POPULATION-BASED SCREENING TRIAL: HPV FOCAL**

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**Objectives:** HPV FOCAL is a longitudinal population-based trial, evaluating the efficacy of high-risk HPV DNA testing, with Liquid Based Cytology (LBC) triage for HPV positives, compared to LBC testing with HPV triage testing for ASCUS, for detection of ≥ CIN3. Presented are the complete round 1 results comparing differences in CIN detection and colposcopy referral rates for women <35yrs and 35+yrs.

**Methods:** Recruitment occurred between January 2008 and May 2012 with 25,243 British Columbia women aged 25-65yrs consented. Participants randomized to either primary HPV (HPV arm) or LBC testing (LBC Arm) and followed for 2 or 4 years.

**Results:** Data presented for 25,151 participants with first round results (baseline and 12-month follow-up). Overall, CIN3+ detection rates for women all ages were not statistically different between HPV (6.6/1000) and LBC (4.4/1000) arms. In women ≥35yrs, overall detection rate for CIN2+ and CIN3+ were higher in HPV vs. LBC arm (CIN2+: 10.3/1000 vs. 5.4/1000. CIN3+: 3.7/1000 vs. 2.0/1000 respectively) and significantly higher for CIN2+ detection. In women <35yrs, CIN2+ rates were significantly greater for HPV vs. LBC arm (47.7/1000 vs. 31.7/1000 respectively) but as for women ≥35yrs, not for CIN3+. In all ages, HPV testing referred significantly more women to colposcopy (HPV: 59.0/1000 vs. LBC 30.9/1000).

**Conclusions:** For women all ages completing first round screening, primary HPV testing with cytology triage detects significantly more CIN2+ lesions and referred more women to colposcopy. Findings will inform for eventual paradigm shifts to primary HPV testing in organized settings (age to commence HPV testing and resource utilization).

**OC 13-3**

**HUMAN PAPILLOMAVIRUS ASSAYS AND CYTOMETRY IN PRIMARY CERVICAL SCREENING OF WOMEN BELOW AGE 30 YEARS**

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**Objectives:** Recent studies suggested that certain HPV assays, particularly those based on detection of HPV mRNA instead of DNA, might have a role in screening young women. Within the Danish Horizon study, we compared clinical outcomes of three DNA assays (HC2, cobas, CLART), one mRNA assay (APTIMA), and cytology in 1,278 women attending routine primary screening at age 23-29 years.

**Methods:** Testing protocols of SurePath samples were agreed upon with the manufacturers prior to the study. Women with abnormal cytology were managed according to routine recommendations, whereas HPV-positive/cytology-normal women were invited for repeated testing in 18 months as part of the study. They were followed through the Danish Pathology Data Bank for 2.5 years.

**Results:** In total, 406 (32%) of the women had a positive test result on HC2, 519 (41%) on cobas, 481 (38%) on CLART, 342 (27%) on APTIMA, and 95 (7%) on cytology. ≥ CIN3 was detected in 44 women. Sensitivity of HC2 for ≥ CIN3 was 95% (95% CI: 85-99), of cobas 98% (95% CI: 88-100), CLART 100% (95% CI: 92-100), APTIMA 82% (95% CI: 67-92), and cytology 59% (95% CI: 43-74). Specificities were 71% (95% CI: 68-73), 61% (95% CI: 59-67), 65% (95% CI: 62-67), 75% (95% CI: 73-78), and 94% (95% CI: 93-96), respectively.

**Conclusions:** In young women attending primary screening, HPV assays were highly sensitive but had low specificity. Although APTIMA was significantly more specific than the three DNA assays, its specificity was substantially lower than that of cytology, demonstrating that false-positive tests were frequent.
REFERRAL POPULATION STUDIES IN EVALUATION OF HPV ASSAYS FOR PRIMARY SCREENING

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Objectives: Comparisons between HPV assays are dominated by studies of women with cytological abnormalities (“referral populations”), mainly for ease of execution and the high prevalence of CIN. However, referral populations represent a selected group within primary screening, where most women have normal cytology. Within the Danish Horizon study, we compared detection of HPV and ≥CIN2 in women with normal and abnormal cytology.

Methods: Consecutive routine samples from 4,997 women had valid tests on HC2, cobas, APTIMA, CLART, and cytology. All women with positive screening tests were offered follow-up. The outcomes were retrieved from the Danish Pathology Data Bank. Differences in cut-off values, adjusted for women’s characteristics, were assessed using a lognormal distribution.

Results: In total, 367 (7%) women had abnormal, and 4,630 (93%) normal cytology. The median cut-off values for HPV infections were lower (all p ≤ 0.001) in women with normal than abnormal cytology: for HC2 (rlu/co), 11.0 (IQR: 3.3-52.6) vs. 124.2 (IQR: 22.9-506.9); cobas (CT), 33.6 (IQR: 29.6-37.5) vs. 26.9 (IQR: 23.7-31.3); and APTIMA (s/co), 10.2 (IQR: 5.8-11.3) vs. 11.1 (IQR: 9.4-15.5). The disagreement in detecting HPV infections (n=1,660) was more frequent with normal (67%) than with abnormal cytology (26%). Even in ≥CIN2 (n=175), the disagreement between the four assays was twice as frequent with normal (26%) than with abnormal (15%) cytology.

Conclusions: Referral populations show higher concordance between HPV assays in detecting HPV infections and CIN than would primary screening populations. In choosing an HPV assay for primary screening, data from referral population studies should be used with caution.

THE LONG-TERM PROGNOSTIC VALUE OF HPV TESTING AND PAP CYTOLGY TO DETECT CERVICAL NEOPLASIA IN THE CANADIAN CERVICAL CANCER SCREENING TRIAL (CCCAST)

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Objective: To assess the long-term prognostic value of HPV DNA testing and Pap cytology to detect CIN2+ among women undergoing routine cervical cancer screening in Canada.

Methods: CCCast was a randomized controlled trial designed to compare the performance of Pap cytology and HPV testing to detect CIN2+ among women attending routine screening in Montreal and St. John’s, Canada (n=10,154). In this analysis, extended follow-up data (total=494,177.40 women-months) were evaluated for St. John’s participants (n=5,754). Cervical screening-related procedures and outcomes occurring since study enrollment were retrieved from the provincial database for these women. HPV results with genotyping information and Pap cytology results determined at enrollment were correlated with detection of CIN2+ during the entire follow-up period using time-to-event analyses.

Results: The cumulative incidence rate of CIN2+ among women testing HPV(-)/Pap(-) at baseline was substantially lower during 120 months of follow-up [1.15%, 95%CI: 0.60-2.18] when compared with women testing HPV(+)/Pap(-) or HPV(+)/Pap(+) [12.17%, 95%CI: 6.71-21.52; and 26.05%, 95%CI: 15.34-42.13 (respectively)]. At approximately 60 months of follow-up, the cumulative incidence rate among HPV(-)/Pap(-) women began to notably increase. Of the four baseline HPV(+) participants developing CIN2+ following 60 months of follow-up, one was HPV16(+) and two were HPV18(+)..

Conclusions: The consistently low rate of CIN2+ among women testing HPV(-)/Pap(-) at baseline suggests that double negativity confers a long-lasting protective effect (up to five years in this analysis). Determining the extent and duration of this effect may allow for enhanced efficiency in screening, while maintaining favorable outcomes and reducing potential harms of over-screening.
O C 13-6

EVALUATING THE POTENTIAL INTRODUCTION OF PRIMARY HPV DNA SCREENING IN ENGLAND

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Objective: Primary HR-HPV testing has shown high negative predictive value for cervical intraepithelial neoplasia stage 2 or worse (CIN 2+) in trials but has not yet been widely implemented. The aim of this study was to assess the health-impact and cost-effectiveness of primary HR-HPV testing, compared with primary cytology testing followed by HR-HPV-based triage, which is currently offered by the national cervical screening programme, to inform policy making in England.

Method: A novel stochastic model was developed to describe the transmission and natural history of HR-HPV infection, in which the competing hazards of CIN, invasive cervical cancer and natural clearance of infection vary as functions of time post-infection. The incidence of undetected cervical cancers was inferred using data from the Cervical Cancer Audit and Trent Cancer Registry. HPV type-specific infection parameters were identified for strains 16, 18, 31, 33, 45, 52, 53, 58 and 61 using data from Public Health England and the National Health Service Cervical Screening Programme (NHSCSP). Lifetime sexual activity and screening attendance behavioural patterns were characterised using data from a national survey on sexual behaviour undertaken in 2010 (Natsal-3) and the NHSCSP, respectively.

Results: The model predicts that primary HR-HPV testing leads to a net increase in total number of screening tests carried out annually. The model identifies increased detection of neoplasias by colposcopy. The benefit of primary HPV testing outweighs the cost of increased colposcopy and treatment. In conclusion, primary HPV testing is found to be cost-effective compared to existing cytology-based screening with HPV triage.

O C 13-7

HPV TESTING WITH CYTOLICAL TRIAGE VERSUS CONVENTIONAL CYTOTOLOGY IN AN ORGANIZED CERVICAL CANCER SCREENING PROGRAM

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Objectives: In 2012, HPV screening of cervical cancer was implemented for women aged 35 through 60 in Tampere (pop. 220,000). Conventional screening was continued in the surroundings (pop. 370,000). Prospective population-based cohort study. All women aged 35, 40, 45, 50, 55, and 60 years in 2012 who were invited for screening in Tampere and its surroundings were included in the study.

Methods: Research arm (Tampere): Screening with Abbott RealTime HR HPV test. A PAP smear was taken at the same time but analyzed only if HR HPV test was positive.
Control arm (surroundings): Screening with conventional cytology only.

Results: 5,637 women were screened in HPV arm and 6,563 women in PAP arm. In HPV arm, 53 women (0.94 %) were referred to colposcopy, while the referral rate in PAP arm was 83 women or 1.26 %. Based on colposcopy-directed biopsies, the detection rate of CIN2+ and 3+ in HPV arm + was 2.8/1,000 and 1.8/1,000, respectively. The corresponding figures in PAP arm were 1.5/1,000 and 0.9/1,000.
A LEEP was performed to 13 women in HPV arm and 12 women in PAP arm. The final histopathological diagnoses in HPV arm were: 1 adenocarcinoma, 2 SCC, 7 CIN3, 1 CIN2, 1 CIN1, 1 normal. In PAP arm, one SCC, 8 CIN 3, 1 CIN2, 1 CIN1, 1 inflammation were found in the LEEP specimens.

Conclusions: Primary HPV screening seems to be more sensitive in detecting CIN2+ and CIN3+ than conventional cytological screening.
**PERFORMANCE OF HPV DNA TESTING WITH INDIVIDUAL HPV 16/18 GENOTYPING FOR PRIMARY CERVICAL CANCER SCREENING AND TRIAGE, COMPARED TO CYTOMETRY**


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**Objectives.** To assess the performance of the HPV DNA testing and individual HPV 16/18 genotyping using cobas® HPV test (Roche) as a method for primary cervical cancer screening compared with liquid-based cytology (LBC) in a population of Greek women.

**Methods.** Four thousand and nine women aged 25-55 years attending routine cervical cancer screening at nine Gynecology Departments in Greece were recruited between August 2011 and November 2013. Cytologic evaluation was performed using LBC (ThinPrep®). An aliquot of each sample was used in order to detect 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 found positive for either cytology or HPV were referred for colposcopy.

**Results.** Among 3,993 valid tests the overall prevalence of HR-HPV was 12.7%. Cervical Intraepithelial Neoplasia grade 2 or worse (CIN2+) was detected in 41 women (1.07%). Sensitivity of cytology [atypical squamous cells of undetermined significance (ASC-US) or worse] and HPV DNA testing for the detection of CIN2+ was 53.7% and 100% respectively (64.3% and 100% for CIN3+), and specificity was 96.8% and 90.3% respectively (96.5% and 89.7% for CIN3+). Genotyping for HPV16/18 only had similar accuracy to cytology for the detection of CIN2+ (sensitivity: 58.5%; specificity 97.5%) as well as for triage to colposcopy for HPV positive women (sensitivity: 58.5% vs 53.7% for cytology).

**Conclusion.** HPV testing with individual HPV-16/HPV-18 genotyping could represent a more accurate methodology for primary cervical cancer screening in comparison to liquid-based cytology.

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**CLINICAL CHARACTERISTICS OF SAMPLES WHERE COBAS, HC2, APTIMA, AND CLART HPV ASSAYS RETURN DIFFERENT TEST RESULTS**

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**Objectives:** From the Danish Horizon study comparing HC2, cobas, CLART, and APTIMA assays, and confirmed by other studies, we reported that the detection of HPV infections varies greatly by assay. In primary cervical screening at ≥ 30 years (n=2,859), only 29% of positive test results were in agreement on all four assays. Here, we studied the characteristics of the disagreeing samples.

**Methods:** Testing protocols of consecutive routine SurePath samples were agreed upon with the manufacturers prior to the study. Abnormal cytology was managed according to routine recommendations, whereas HPV-positive/cytology-normal women were invited for repeated testing in 18 months within the study. They were followed through the Danish Pathology Data Bank for 2.5 years.

**Results:** Women with a positive test result on only one HPV assay (n=258) were considerably less likely to have infections with HPV16 (6%), other high-risk genotypes (14%), and multiple genotypes (14%), than women with a positive test result on all four assays (n=188; 22%, 78%, 64%, respectively). Their samples were also more likely to contain only low-risk, or no genotype. The median cut-off values were substantially lower than for women with > 1 positive test result, without overlapping IQR, indicating low viral load. Among women with ≥ CIN2 (n=50), no woman tested positive on only one assay.

**Conclusions:** Screening samples from HPV-positive women for whom the HC2, cobas, CLART, and APTIMA assays gave conflicting test results were systematically different and less likely to contain clinically important lesions than samples in which all four assays returned a positive test result.
THE ADDED VALUE OF RESCREENING CYTOLOGY NORMAL SAMPLES WITH POSITIVE HPV mRNA TEST FOR THE DETECTION OF CIN2+ IN PRIMARY SCREENING

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Objectives. To estimate the increased detection rate of CIN2+ in women with normal Pap-smears by rescreening Pap-smears from HPV mRNA positive samples.

Methods. From April 4th, 2013, the Department of Pathology, Ålesund Hospital, introduced a study by rescreening all normal Pap-smears that had a positive HPV mRNA test (NorChip PreTect SEE) (types 16, 18 and 45) in women younger than 40 years. Within the SymPathy database, a study population of 3947 women aged 23–39 years with no prior history of CIN1+ was established.

Results. 30% of women with normal cytology were tested via HPV mRNA (1060/3496), and 18 samples were positive (1.7%). After re-evaluation of the index cytology and subsequent follow-up smears, 10 women had colposcopy, resulting in four diagnoses of normal biopsies, 2 CIN1 and 4 CIN2+. The detection rate of CIN2+ among normal Pap-smears was 0.38% (95% CI: 0.32–0.44). In the ASC-US+ arm (n=318) of women without HPV sampling, 57 CIN2+ were detected. If we apply the CIN2+ detection rate among cytology normal / HPV mRNA-positive women (0.38%) to the arm of women with normal cytology without HPV testing, an increase in CIN2+ detection from 13.5–18.4% was estimated.

Conclusions. By testing all women with normal cytology with a specific HPV mRNA test, a significant increase in screening program sensitivity can be achieved. The volume of rescreened smears (1.7%) is very low. In addition, the study adds quality to educating the screeners by rescreening presumably false negative Pap-smears.

EXPLORE THE VALUE OF A RAPID, ON DEMAND TEST FOR HPV AS PART OF CERVICAL CANCER SCREENING PATHWAYS

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Background: Rapid, on demand testing has been developed for HPV detection in cervical screening samples. This technology has the potential to change patient pathways. There has been no evidence presented yet around how such a test may impact upon a cervical screening programme.

Aim: To map patient pathways under the current NHS Cervical Cancer Screening Programme in England (HPV triage), and HPV primary screening, comparing standard currently available HPV testing platforms with a rapid, on demand test.

1. To compile experts’ views on the value, opportunities and challenges of implementing a rapid, on demand test.

Methods: Semi-structured interviews with laboratory, epidemiology, policy, and clinical experts across England. To inform the mapping of the current screening pathway under HPV triage and HPV primary screening, and explore the potential value of introducing a rapid, on demand HPV test.

Results: Experts reported that using a lab based rapid, on demand HPV test on cytology samples qualifying for HPV triage could reduce the time to results by up to a week. Under HPV primary screening, a rapid near patient test in primary care could radically change the testing paradigm. Most HPV negative women could be notified within 1 day by the practice, and the proportion of samples sent for cytology could dramatically reduce. This could improve patient experience and reduce anxiety for women. Questions remain as to quality assurance, IT integration, and reimbursement, as well as potential logistical challenges to implementing this model of care.
Background: START-HPV (Study of primary screening in the Ardennes ‘department’ by Testing for HPV infection) is the first French primary screening pilot study using solely HPV testing. This study is supported by The INCa (French National Institute of Cancer). The objective is to evaluate the setting conditions of an HPV-based primary screening in France for the non-attending population, at the scale of a French ‘department’.

Methods: The eligible population consisted of all women living in the Ardennes administrative area, aged 31-65, without any Pap-smear in the last 3 years. They received an invitation for having a cervical sampling to be analyzed by HPV testing (Hybrid Capture 2, Qiagen). Two recalls were scheduled. The second recall was randomized between cervical smear (1/3) or self-sampling (2/3).

Results: 30,365 women were eligible and gave their consent. Global participation rate was evaluate at 11.1%. The mean age of attendees was 51 years old. Samples were taken by gynecologists (43.0%), general practitioners (30.6%), self-sampling (24.2%) and other health care professionals (2.2%). Samples were tested HPV positive in 9.6% of cases. Among HPV positive women, triage cytology demonstrated to be abnormal (≥ ASC-US) in 39.2% of cases.

Conclusion: Participation rate is rather low in this population, despite a significant implication of general practitioners and self-sampling use. This probably reflects the difficulty to implement a new screening organization in France. A telephone survey is ongoing among 800 non-participating women, to identify the major reasons for non-attending. Results of the survey will be presented.

INTRODUCING CAREHPV INTO A PUBLIC SECTOR SCREENING PROGRAM IN EL SALVADOR

Objectives: CAPE (Cervical Cancer Prevention in El Salvador) introduced a low-cost HPV-DNA test into a public sector program. El Salvador has one of the lowest screening rates in Latin America (19%). Coverage rates are poor and follow-up for abnormal cytology is inadequate. The aim, since October 2012, has been to implement a 3-phased program to screen 30,000 women. The true impact will be seen when the program is handed over to the government, and becomes the national screening program. Results of phase 2 are presented.

Methods: 8,035 women, ages 30-49, were screened. 6,737 had self- and provider-collected samples and 1,298 had provider-testing. The agreement between sampling methods was 83.6% (kappa of 0.71). HPV-positive women were referred to treatment using the strategy their community followed. Cohort A was referred to colposcopy; Cohort B had immediate visual triage and cryotherapy.

Results: 341 (12.5%) women were HPV positive in Cohort A; 325 (11.3%) were positive in Cohort B. 2,736 women in Cohort A (70.1%), and 2,889 women in Cohort B (77.9%) have completed 6-month follow-up. In Cohort A, all were referred for colposcopy—313 attended their appointment, and 243 were treated. 29.9% have not completed follow-up and were not included in the analysis. In Cohort B, 257/325 received treatment.

Conclusions: A program introducing HPV testing was successfully implemented in a low-resource setting. Requiring women to return for a colposcopy made them less likely to complete treatment. Outreach to women who had not been screened recently helped find women at higher risk for HPV.
RANDOMIZED IMPLEMENTATION OF HPV-TEST IN PRIMARY SCREENING IN THE NORWEGIAN CERVICAL CANCER SCREENING PROGRAM

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Background: In 2015 Norway will implement hrHPV testing for primary screening in 4 counties as a randomized health service. Women will be allocated randomly to either HPV-test or Pap-smear based on their date of birth.

Methods: The Cancer Registry of Norway is responsible for the coordination of preparations prior to implementation. Two working groups were charged with defining and monitoring preparations. One group is focused on preparatory changes in laboratory infrastructure and routines, gynaecological wards, and GPs, information to women and screening providers, necessary changes in the reminder routines and data handling in the Cancer Registry. The other group is focused on the academic safeguard along with developing protocols for the evaluation of the implementation project.

Results: Necessary IT-solutions for randomization in the laboratories are prepared. Protocols for the whole process from sampling to registration and reporting to the Cancer Registry are completed. A public tender for the purchase of a common HPV test platform and biobanking solution was initiated. There is an ongoing contact with the authorities for regulatory support and maintenance of HPV testing reimbursements. Likewise, information- and media strategies to reach out with understandable information to women, doctors, laboratories and population, have been prepared.

Conclusions: It is important to prepare a national implementation in close collaboration with all stakeholders. This is to ensure that the introduction takes place as seamlessly as possible with high quality. The goal is a robust implementation that provides security for individual women at all stages in the best possible way.

ABSTRACTS

CERVICAL CANCER SCREENING PROGRAM BASED ON CYTOLOGY AND HPV TEST: POSITIVE PREDICTIVE VALUE.

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Objectives: The aim of this study was to know the probability of undergo a disease given a positive result on cervical cancer screening (CCS), based on HPV test and cytology; this is, the positive predictive value (PPV), which is crucial both for health management and clinical counseling.

Methods: Castile and Leon is a Spanish region where CCS was established on 1986. Its female population is ~1.3 million women. Since 2008, HPV genotyping (CLART® HPV2) is conducted jointly with conventional cytology on women 35-64 y. Women 25-34 y. are screened only by cytology. Positive predictive values were calculated for different diagnostic outcomes during 2013.

Results: According to algorithm, 1,092 women out of 55,532 were positive (2.0%) and were recommended to attend to their gynecologist. Follow-up and histology was available for 496 women. PPV for cervical pathology overall was 52.8% (IC95 48.3-57.3) given a positive CCS. The probability of undergo a low-grade dysplasia was 21.0%, 29.2% for high-grade and 2.6% for carcinoma. Cytology (≥ ASC-US, any HPV test result) has a PPV of 58.3% (IC95 52.0-64.6) for cervical pathology: 19.0% for low-grade dysplasia, 35.3% for high-grade and 4.0% for carcinoma. HPV-16/18 (any cytology result) has a PPV of 53.2% (IC95 47.2-59.3) for cervical pathology: 22.3% for low-grade dysplasia, 28.1% for high-grade and 2.9% for carcinoma.

Conclusions: There are no significant differences in PPV between cytology and HPV test, overall. HPV test has higher PPV for low-grade dysplasia, being an advantage for CCS in the earliest stages, consistently with natural history of infection.
**HPV PREVALENCE FINDINGS IN THE DANISH HPV SELF-SAMPLING IMPLEMENTATION COMPARED TO ROUTINE SCREENING**

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**Objectives:** In Denmark, 45% of cervical cancers are diagnosed in screening non-participants. The on-going pilot implementation study in the Capital Region aims to improve the screening coverage rate by offering an HPV-based self-sampling test for free to non-attenders. Here we report on the HPV prevalence in the returned self-sampling brushes, with an age-stratified comparison to routine physician-taken samples from screening participants.

**Methods:** Non-attenders from the Capital Region (N= 54,585) were identified from the invitational module of the nationwide Pathology Data Bank. Women were randomly invited in batches of 1,000, and the recruitment is still on-going. Reminders were sent after 8 weeks. HPV prevalence was determined by testing with the BD Onclarity assay. For comparison, we used data from a BD Onclarity study on unselected routine samples, including predominantly primary screening samples.

**Results:** In 1,174 analyzed self-samples, the overall HPV prevalence was 15.3%. Age-stratified data showed that 20.7% of women aged 30-39 years had a positive test result; at 40-49 years, this was 13.3%; at 50-59 12.4%; and at ≥ 60 years 10.7%. For comparison, in a regular screening population (N=811), HPV prevalence was 20.4%, 13.2%, 7.5%, and 4.7% respectively.

**Conclusions:** HPV prevalence in women aged 30-49 years was similar between non-participants who accepted self-sampling and regular screening participants. In women aged ≥ 50 years, the prevalence rates were higher in the self-sampling group. These preliminary data suggest that the older self-sampling group has a higher risk of CIN, and might particularly benefit from self-sampling.

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**COMPARATIVE EVALUATION OF TWO VAGINAL SELF-SAMPLING DEVICES FOR THE DETECTION OF HUMAN PAPILLOMAVIRUS INFECTIONS**

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**Objectives:** To compare the performance of two devices for vaginal self-sampling of dry cell material (Evalyn Brush, Rovers Medical Devices; Qvintip, Aprovix) using a clinically validated high risk HPV (hrHPV) DNA test and evaluate their acceptability.

**Methods:** Both self-sampling devices (change of order with every patient) including instructions for use and a questionnaire were handed to 150 patients for self-collection prior to scheduled colposcopies with collection of cervical specimens by gynaecologists in a colposcopy clinic and a general gynaecological outpatient clinic. Matched self-collected and physician-collected specimens were transferred to individual liquid-based-cytology vials (ThinPrep) and tested for the presence of hrHPV using the RealTime High Risk HPV assay (Abbott). Biopsies were taken if indicated by colposcopy.

**Results:** Data from 99 patients evaluated at date showed high agreement of overall hrHPV detection rates between self-collected (Evalyn:90.9%; Qvintip:88.9%) and clinician-collected specimens. In addition, high agreement of HPV16 (Evalyn:98.0%; Qvintip:97.0%), HPV18 (Evalyn:99.0%; Qvintip:98.0%) and non-16/18/hrHPV (Evalyn:91.9%; Qvintip:89.0%) was found between both self-sampled and physician-collected specimens. Colposcopy and histological evaluation revealed 39 women without cervical intra-epithelial neoplasia (CIN), 24 CIN1, 26 CIN2 and 10 CIN3. Nine CIN3 cases collected with both self-collection devices and the reference smear were positive for hrHPV. So far women seem to appreciate the ease of use of the Qvintip device (63% versus 48%), while the majority of women (60%) favor a physician-collected smear.

**Conclusions:** High agreement of hrHPV results was found between self-collected (Evalyn, Qvintip) and physician-sampled specimens collected in ThinPrep vials. hrHPV detection rates in women with and without cervical disease and evaluation of the questionnaires will be reported upon completion of the evaluation.
GOOD AGREEMENT OF HPV DETECTION BETWEEN SELF-COLLECTED DRY SAMPLES AND PHYSICIAN-COLLECTED SPECIMENS IN CHINA

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Objectives. To evaluate the effectiveness of self-collected dry specimens obtained with the Evalyn Brush (Rovers Medical Devices, KV Oss, The Netherlands) for the detection of high-risk HPV (hrHPV) using a clinically validated test.

Methods. 202 patients of the gynaecological outpatient clinic of a Chinese university hospital took vaginal self-samples prior to scheduled colposcopies. Self-sampled and physician-collected cervical specimens were tested for the presence of hrHPV using the Abbott RealTime High Risk HPV assay. HrHPV results were compared with cyto-and histopathology diagnoses.

Results. HrHPV was detected in 80/101 (79.2%) self-collected and in 82/101 (81.2%) physician-collected samples from women with cervical intra-epithelial neoplasia grade 2 and higher (CIN 2+). Among 46 (45.5%) women in this group with CIN 3+ hrHPV was found in 42/46 (91.3%) self-collected samples and in 43/46 (93.5%) physician-collected specimens. In women without cervical dysplasia, hrHPV was detected in 13/101 (12.9%) self-collected and in 15/101 (14.9%) physician-collected samples.

High overall agreement of hrHPV test results (96.5%; kappa=0.93) was observed between both sampling methods. The overall sensitivity and specificity for CIN 3+ by self-sampling was 91.3% (95%CI:79.2%-97.5%) and 87.1% (95%CI:79.0%-93.0%), respectively which was only marginally lower than by physician collection (93.5%;95%CI:82.1%-98.6% and 88.1%;95%CI:80.2%-93.7%). Similar detection rates for HPV16 (37.0% vs. 38.7%), HPV18 (5.4% vs. 3.2%) and non-HPV16/18 (72.8% vs. 72.0%) were observed in self-collected and physician collected samples.

Conclusion. Comparable hrHPV detection rates were observed with self-collected cervical specimens using the Rovers Evalyn Brush and physician-collected specimens from women with histology confirmed cervical status.

HPV-BASED SELF-SAMPLING IMPLEMENTATION WITH AN OPT-IN STRATEGY TO IMPROVE CERVICAL SCREENING COVERAGE

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Objectives: In Denmark, 45% of cervical cancers are diagnosed in screening non-participants. The ongoing pilot implementation study in the Capital Region aims to improve the screening coverage rate by offering an HPV-based self-sampling test for free to non-attenders. Unlike in previous research studies, we rely on an opt-in strategy where invited women have to order the self-sample brush actively upon invitation. “Opt-in” was chosen to reduce the cost, and will probably be the most sustainable strategy for countries that will routinely roll-out self sampling. However, a concern is that an “opt-In” strategy might be potentially discouraging for participation. Therefore, we studied the actual response rates.

Methods: Non-attenders from the Capital Region (N= 54,585) were identified from the invitational module of the nationwide Pathology Data Bank. Women were randomly selected into batches of 1,000. They could respond by mail, webpage, mobile application, E-mail or phone. Reminders were sent after 8 weeks. Received kits are being analyzed with HC2, CLART, and Onclarity HPV assays.

Results: So far, 14,000 invitations have been sent. The overall response rate for the first batch was 41% in120 days after the invitation. Of the invited women, 36% chose to receive the kit and 63% of the latter returned a self-sample (mean age: 47.9 years). This increases the overall coverage rate by about 5-6%. So far, the overall prevalence of HR-HPV was 15.3% by Onclarity.

Conclusions: Self-sampling with an opt-in strategy was well accepted among non-attenders, and might be a good supplement to the regular call-recall screening program.
CLINICAL VALIDATION OF SELF HPV TESTING IN ROUTINE SCREENING IN SCOTLAND.

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Background: Presence of HPV in genital epithelium is necessary for the development of cervical pre-cancer. Clinically validated HPV detection allows identification of women who will benefit from further screening or cervical assessment. Sampling has to be minimally invasive and universally accessible to women.

Objectives: We clinically validate self vaginal sample against previously validated cervical sample for HPV routine cervical screening (women from age20 years) using Cobas HPV detection.

Methods: 5330 women attending for routine smear between April 2013 and July 2014 consented to self-collect vaginal swab for Cobas HPV testing. The same Cobas HPV test was used for HPV detection in Thinprep cervical samples. Women with abnormal smear results were referred to colposcopy as per routine protocol in Scotland. HPV positive participants with normal cytology where offered HPV re-testing in 4-6months. Those with two positive cervical HPV tests with HPV 16/18 were invited to colposcopy.

Results: HPV was detected in 14.7% of cervical and 16.5% of vaginal samples. Prevalence of HPV16/18 was 4.9% and 5.5% in cervical and vaginal samples respectively. Sensitivity of cytology, cervical HPV testing and vaginal self testing for CIN2+ lesions was 79.7%, 96.5% and 94.3% respectively. Intra- and inter-laboratory agreement data will be presented.

Conclusion: It is reasonable to assume that the majority of women would prefer minimally invasive self HPV testing. Our results indicate that self vaginal HPV testing is as sensitive as cervical HPV testing in identifying CIN2+ lesions. This study is on-going with completion date in March 2015.

ACCESSING*: SCREENING BY SELF-SAMPLING AND LOWER COST ARBOR VITA E6 ONCOPROTEIN TEST CAN DETECT DYSPLASIA IN HIGH RISK WOMEN

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9) *ACCESSING (Adequate Cervical cancer Capacity building, Education and Screening by new Scientific Instruments in Ghana): GIZ/ESTHER funded trial

Objectives: To demonstrate feasibility of self-sampling combined with usage of the low-complexity Arbor Vita OncoE6™ test, detecting elevated levels of the E6 oncoprotein of HPV types 16 and 18. The ultimate aim of this feasibility study is to screen the most vulnerable women in remote areas for cervical cancer by taking advantage of resident community health nurses living in remote communities in Ghana.

Methods: 250 high risk women (100 HIV+, 150 HR-HPV detection or lesion) were recruited at Catholic Hospital Battor. Vaginal lavage (Delphi Screener) and swab samples were collected and tested with AVE6. Full HPV genotyping was done by GP5+/6+ PCR followed by Luminex-MPG readout at the Charité Universitätsmedizin. AVE6 positive and/or high risk HPV+ underwent colposcopy and treatment.

Results: 91% (228/250) self-sampled. 85% found this easy/very easy and comfortable/very comfortable. 63% of the HIV+ and 34% of formerly HR-HPV+ women were HR-HPV positive, respectively. 10% of HIV+ patients were CIN2+, compared to 2% of the HR patients. Of the 13 CIN2+, 5 were detected AVE6 positive by lavage sample of these 3 by the swab sample. Full MPG-Luminex genotyping revealed 9 CIN2+ patients had HPV 16/18. Of these 9, 4 were detected by lavage and 2 by swab.

Conclusions: Self-sampling was well accepted and not inferior in detecting dysplasia than swab sampling. Self-sampling in conjunction with AVE6 can be used in communities to detect and triage women with highest risk for severe dysplasia or cervical cancer and allow secondary cancer prevention ‘on the doorstep’ in remote locations.
FEASIBILITY OF SELF-COLLECTION AND DRY SPECIMEN STORAGE FOR HPV MRNA TESTING TO DETECT CERVICAL LESIONS IN HIGH RISK WOMEN

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Objectives: High-risk HPV mRNA detection hold potential to improve cervical cancer screening in low resource areas. Studies reported comparable performance in detecting HPV between self-collected specimens stored in liquid media (stored wet) and physician collected specimens. Performance of HPV mRNA with self-collected specimens stored dry, which could enhance specimen collection and storage, is unknown. We compared HR-HPV mRNA testing on two self-collected specimens (stored dry versus stored wet) to detect high-grade cervical neoplasia or greater (≥ CIN-2) among women in Kenya.

Methods: We enrolled 200 female sex workers (100 HIV-negative; 100 HIV-positive) in Mombasa. Participants provided two self-collected specimens: one stored dry using a Viba brush (Rovers) and one stored wet with Aptomia media (Hologic) using an Evalyn brush (Rovers); and physician-collected specimens using CSCT- for HPV RNA testing (APTIMA) and conventional cytology. Women positive on any screen underwent colposcopy with local biopsy reading.

Results: HPV RNA positivity was somewhat higher in physician (39%) and self-collected wet (39%) brushes than self-collected dry brushes (36%). Sensitivity estimates for ≥ CIN-2 detection (n=27) were similar in HIV-negative women: self-dry (82%), physician (82%), and self-wet (82%), yet differed in HIV-positive women: self-dry (69%), physician (94%), self-wet (94%). Overall specificity for ≥ CIN-2 were 61% for physician, 59% for self-wet, and 60% for self-dry sampling.

Conclusion: HR-HPV mRNA prevalence using dry self-collected specimens was somewhat lower than physician or wet self-collected specimens. Sensitivity of mRNA testing in self-dry samples for ≥ CIN-2 appeared comparable for the three sample types in HIV-negative women, yet appeared inferior in HIV-positive women.
Objectives: HRA is the gold standard for the detection AIN. Our aim was to compare different screening strategies, including anal cytology and HPV testing.

Methods: Data was prospectively collected from an AIN screening service for HIV-positive individuals over a 17-month period. HPV DNA testing was performed using Abbott real time PCR, detecting 14 high risk (HR) HPV types.

Results: There were 179 attendances in 148 patients. 96% were MSM. The median age, time since HIV diagnosis and time on antiretroviral therapy were 48, 13 and 11 years, respectively. Median CD4 nadir was 215x10^9 /L (range 0-828). 48% (85/178) had any HR HPV; 20% (36/178) had HPV 16 and 12% (22/178) had HPV 18. 35% (63/178) had other HR strains. There were no associations between presence of HR HPV and demographic factors. 41% (66/160) had abnormal cytology with 8% (12/160) identified with severe dyskaryosis. 23% (35/155) had abnormal HRA with changes suggestive of AIN. Of those biopsied, 8% (2/25) had AIN3, 20% (5/25) AIN2 and 8% (2/25) AIN1. There was not always good agreement between the three screening tests. 55% (45/82) of those with HR HPV had abnormal cytology, compared to 21% (16/77) of those without HR HPV. 53% (16/30) of those with abnormal anoscopy had abnormal cytology. Conversely, 29% (16/56) of those with abnormal cytology had abnormal anoscopy.

Conclusions: 48% were detected with high risk HPV, although the prevalence of type 16 and 18 was lower than expected. There was no predictive association between all 3 screening methods.

Objective: We assessed persistence, incidence and clearance of anal canal HPV among men having sex with men (MSM) and among men having sex with women and men (MSWM).

Methods: Genotyping for 37 HPV types was conducted for anal specimens from HIV-negative men, aged 18-70 years, from Brazil, Mexico, and the USA. A total of 406 men (125 MSM and 281 MSWM) provided evaluable specimens at ≥ 2 visits over 2 years. Persistence was defined as ≥ 12-month type-specific prevalent or incident infection at consecutive visits. Hazard ratios (HR) and 95% confidence intervals (CI) were calculated by Cox regression.

Results: Median follow-up time was 2.1 years. HPV16 persistence was observed in 8% and 3% of MSM and MSWM, respectively. Of 12 MSM and 15 MSWM with prevalent HPV-16, a total of 42% and 20%, respectively, retained HPV-16 at 24 months. After combining MSM and MSWM groups, men who had multiple recent sex partners and always used condoms during the study had a 12-month incidence of high-risk HPV of 6.7% (95% CI 0%-19.3%) vs 19.5% (95% CI 12.7%-26.4%) among men using condoms inconsistently. There was significantly increased clearance among men in relationships of < 1 year (HR 1.7, 95% CI 1.1-2.5) compared to single men and increased clearance among moderate alcohol drinkers (31-60 drinks/month, HR 1.6, 95% CI 1.1-2.4) compared to men who drank 0-30 drinks/month.

Conclusions: MSM with prevalent HPV16 infection should be considered at risk for HPV-associated anal disease. Condoms may help prevent anal HPV infection among men with multiple sex partners.
THE EFFECT OF HIV INFECTION ON ANAL HIGH-RISK HPV INCIDENCE AND CLEARANCE AMONG MSM

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Objectives: The majority of anal cancers are caused by high-risk HPV (hrHPV) infections, which are especially common in men who have sex with men (MSM). We aimed to compare the incidence and clearance rates of anal hrHPV infection between HIV-infected and HIV-negative MSM.

Methods: MSM aged ≥ 18 years were recruited in Amsterdam, the Netherlands, and followed-up semi-annually for 24 months. At each visit, participants completed risk-factor questionnaires. Anal self-samples were tested for HPV DNA and genotyped using the SPF10-PCR DEIA/LiPA25 system; HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59 were classified as hrHPV. Effects on incidence and clearance rates were quantified via Poisson regression, using generalized estimating equations to correct for multiple hrHPV types.

Results: 750 MSM with a median age of 40 years (IQR 35-48) were included in the analyses, of whom 302 (40%) were HIV-infected. The incidence rate of anal hrHPV was significantly higher in HIV-infected compared to HIV-negative MSM (adjusted incidence rate ratio 1.6; 95%CI 1.3-2.0), while the clearance rate was significantly lower (adjusted clearance rate ratio 0.7; 95%CI 0.6-0.9). HrHPV incidence or clearance among HIV-infected MSM did not differ significantly by level of nadir CD4 cell count.

Conclusions: Increased anal hrHPV incidence rates and decreased anal hrHPV clearance rates were found in HIV-infected compared to HIV-negative MSM, after adjusting for reported sexual behavior. Our findings suggest an independent effect of HIV infection on both incidence and clearance of anal hrHPV infections.

CORRELATES OF HIGHER BURDEN OF ANAL HIGH-GRADE SQUAMOUS INTRAEPITHELIAL LESIONS (HSIL) IN THE STUDY FOR THE PREVENTION OF ANAL CANCER (SPANC)

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Objectives: Anal cancer precursor lesions (HSIL) are highly prevalent among homosexual men. In the cervix, larger HSIL size is associated with a higher risk of progression to cancer. Little is known about the relationship between burden of anal HSIL and demographic, behavioural and virological factors.

Methods: The SPANC study is a three-year prospective study of anal HPV and cancer precursors. Homosexual men aged ≥ 35 years undergo behavioural questionnaires, anal swabs and high resolution anoscopy (HRA)-guided biopsy (when required). Number of biopsy-confirmed octants with HSIL was used as a proxy for burden of HSIL.

Results: 450 participants were enrolled by June 2014 (median age 49 years; 29.1% HIV-positive). Among 368 participants biopsied, 139 (35.1%) had HSIL at baseline. Of those, 88 (63.3%) involved only 1 octant. In univariate analyses comparing 1 with >1 octant, more HSIL was associated with higher numbers of recent receptive anal intercourse with condoms (RAIC) partners (p=0.015), HPV16 (p=0.005), both having any, and having higher number, of high-risk (HR)-HPV types detected (p=0.049 and p=0.005 respectively). Age, HIV status, smoking, duration since first anal sex, lifetime and recent numbers of male sexual partners, prevalent low risk HPV types and prevalent HPV18 were not associated with higher HSIL burden. In the multivariate model, HPV16 (p=0.039) and higher numbers of recent RAIC partners (p=0.015) remained associated with HSIL burden.

Conclusions: Larger burden of anal HSIL was associated with prevalent HR-HPV, in particular HPV16, in this cohort. This suggests that larger burden of HSIL may be associated with increased risk of progression to cancer.
**OC 15-5**

**STRAFYING RISK OF DEVELOPMENT OF ANALCANCER – A PROPOSED CLINICAL ALGORITHM**

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**Objectives:** Anal high grade squamous intra-epithelial lesions (HSIL) are highly prevalent among homosexual men and have a lower rate of progression to cancer than the cervical equivalent. There is currently no published proof of effectiveness of anal HSIL treatment, recurrence rates are typically 50% at one year and side effects are common. Targeting treatment towards those at highest risk of progression may be a preferable approach.

**Methods:** In the prospective Study of the Prevention of Anal Cancer (SPANC), homosexual men aged \( \geq 35 \) undergo five visits involving anal cytology, HPV DNA testing and high resolution anoscopy with biopsy of visualised lesions. A clinical algorithm was developed which utilised age, detection and persistence of HPV16 and other high risk (HR) HPV types, anal HSIL and lesion size. At study completion, participants were stratified into low, moderate, elevated and highest risk of subsequent anal cancer.

**Results:** 450 participants had been enrolled into the study by June 2014 (median age 49, 29% HIV positive). Using the clinical algorithm, it is estimated that 16% of SPANC participants will be in the low risk stratum (no HR HPV or HSIL over time), 42% in the moderate risk, 35% in the elevated risk and 7% in the highest risk stratum (persistent HPV16 and/or HSIL).

**Conclusions:** Repeated testing can identify a small subset of homosexual men with persistent anal HSIL and HPV16. This subset of men is likely to be at greatest risk of progression to anal cancer and thus may benefit most from closer monitoring and potential treatment of their HSIL.

**OC 15-6**

**ANAL CANCER SCREENING IN HIV POSITIVE CZECH MSM**

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**Objective:** HIV positive men who have sex with men (MSM) have high prevalence of anal human papillomavirus (HPV) infection and are at an elevated risk of developing anal cancer. Preventive programs for anal cancer are worthwhile, as early detected lesions could be effectively surgically treated. Despite the high incidence and morbidity of anal cancer in HIV positive MSM, there are no official guidelines for how to effectively screen for anal cancer. More screening approaches are used in different countries based on experience and availability of examination and laboratory methods. Here we are presenting data from the anal cancer screening of HIV positive MSM from the Czech Republic using anal cytology and HPV testing.

**Methods:** Anal swabs from 45 HIV positive MSM patients were collected into a liquid-based cytological medium. Clinical and cytological findings were compared to HPV genotyping results and to the presence of E6E7 mRNA HPV transcripts.

**Results:** Cytologically, the most common finding included low-grade lesions. HPV genotyping revealed mixed infection of high risk, as well as low risk HPV types with HPV types 11, 6, and 16 being the most prevalent types detected. The presence of HPV mRNA was predominantly revealed in cytologically abnormal samples (p < 0.05).

**Conclusion:** This is the first data about anal cancer screening in HIV positive Czech MSM. Laboratory finding are in concordance with clinical findings, however HPV mRNA testing seems to be more useful for anal cancer screening purposes because of an increased specificity for more advanced lesions, but follow-up data is needed.
SEXUAL ACTIVITY AND FUNCTION IN PATIENTS WITH PREINVASIVE AND INVASIVE VULVAR LESIONS AFTER COMPLETED TREATMENT

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Background: Sexual activity (SA) and function (SF) are central outcome measures in women affected by preinvasive (VIN) and invasive vulvar (VC) lesions. Data on SA and SF after completed treatment are scarce.

Methods: Validated questionnaires including the Female Sexual Function Index (FSFI-d) were provided to 166 women who were surveyed after completion of primary therapy for VIN and VC at the University Medical Center Hamburg-Eppendorf and Asklepios Medical Clinic Altona between March 2011 and June 2012. Furthermore patients could assess the questionnaires online via homepage of the Vulvar Cancer Support Group (n = 14).

Results: With an overall response rate of 34.9%, 24 patients with VIN and 34 with VC were evaluable. Median age was 51.5 years, with 34 (58.6%) patients being postmenopausal. Median time since completion of treatment was 17 months. All women underwent vulvar surgery (laser/cold knife/combination). Overall, 14 (25.4%) women reported no SA during the last 4 weeks. SA and SF was similar in patients with VIN and VC. Additional analyses contrasting surgical treatment methods yielded no significant differences. Time since surgery did not affect SA and SF. Age, however, was negatively associated to all dimensions of the FSFI-d [desire (p=0.002), arousal (p<0.001), lubrication (p<0.001), orgasm (p=0.001), satisfaction (p=0.027), pain (p<0.001)].

Conclusion: Women who regain sexual activity after treatment of vulvar lesions have a good overall sexual outcome; the results might however be biased by selection with a response rate of 32%. Age is one the most powerful factor influencing SA and SF.

ROLE OF VULVAR CYTOLOGY VS COLPOSCOPY IN WOMEN WITH CHRONIC VULVOVAGINAL COMPLAINTS AND FUTURE ROLE OF HPV PCR AS A PRIMARY SCREENING MODALITY

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Objective: To evaluate the role of cytology, colposcopy and HPV PCR test in patients with chronic vulvovaginal symptoms.

Materials And Method: It was a cross sectional study conducted on 100 sexually active female with chronic vulvovaginal complaints > 6 months. Pregnant females, known case of malignancy of lower genital tract, patients with active bleeding per vagina were excluded. A detailed relevant history was taken on preformed questionnaire. All patients were subjected to vulvar cytology ,HPV PCR test and vulvoscopy. Vulval biopsy was taken in patients who found to be positive on either on cytology or colposcopy.

Results: Mean age of patient was 39.1 yr. Most common complaint was pruritus vulva followed by discharge per vagina. On inspection vulva , 18 patients had abnormality but cytology detected abnormality was found in 10 patients, colposcopic abnormality was positive in 30 patients(12 more subclinical cases) and HPV PCR test was positive in 18 patients. 34 patients were subjected to biopsy and abnormality found in 28 patients. Out of these 28, cytology was positive in 6, colposcopy positive in 28 ,HPV PCR in 12 patients. Sensitivity, specificity, PPV & NPV taking histopathology as a gold standard i.e. 33.3%, 97.5%, 75%, 86.95% of cytology, 89%, 97.6%, 89%, 97.6% of colposcopy, 50%, 96.4%, 75%, 90% of HPV PCR respectively

Conclusion: Vulvovaginal cancers are on rise, thus increase the importance of early detection of premalignant and malignant lesions. Cytology vulva has a limited role but High sensitivity and specificity of colposcopy vulva and HPV PCR indicates its role in detecting cases which often missed on naked eye examination. Women with persistent vulvovaginal complaints should be screened and keep in scrutiny for follow up as they are the potential candidates for vulvovaginal malignancies.
**OC 16-3**

**RANDOMIZED TRIAL OF TREATMENT AND FOLLOW-UP OF VAGINAL INTRAEPITHELIAL NEOPLASIA**

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**Objectives:** The aim of the study was to evaluate the effectiveness, tolerability and success rates of different treatment options (laser or topical imiquimod) for vaginal intraepithelial neoplasia (VAIN).

**Methods:** The study was a randomized controlled three-arm trial enrolling patients (n=24) with histologically confirmed VAIN 2-3 or persisting VAIN 1. The study groups were A) expectant management (n=8), B) laser treatment (n=8) and C) topical imiquimod (n=8). Each group had follow-up colposcopy visits at 4, 8 and 16 weeks including high-risk HPV testing, cytology and punch biopsies.

**Results:** The preliminary results indicate a tendency for higher HPV clearance rates among patients treated with topical imiquimod (50%, 4 out of 8). In the other treatment groups HPV persisted. Treatment with laser or topical imiquimod was equally effective (normal histology at 16 weeks in 5 patients (63 %) in both groups). Nearly all patients experienced side effects such as pain or fever with topical imiquimod, but none of them discontinued the treatment.

**Conclusions:** Topical imiquimod appears to be as effective as laser treatment in VAIN, but in addition it may assist HPV clearance and thus promote permanent remission.

**OC 16-4**

**RISK FACTORS AND OUTCOMES OF WOMEN WITH VAGINAL INTRAEPITHELIAL NEOPLASIA GRADE 2 AND 3 (VAIN 2/3) – AN ELEVEN-YEAR EXPERIENCE**

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**Objective:** To evaluate the risk factors and outcomes of women with vaginal intraepithelial neoplasia grade 2 and 3 (VAIN 2/3).

**Methods:** The medical records of women with VAIN 2/3 in a tertiary hospital over an eleven-year period with a minimum follow up of 12 months were reviewed. Patients’ demographic and clinical information related to the diagnosis, treatment and outcome were obtained and analyzed in an excel spreadsheet.

**Results:** A total of 120 women were diagnosed with VAIN 2/3 over an eleven-year period. After exclusions, ninety-seven women with VAIN 2/3 were analyzed. Median age at diagnosis was 53 years. Of these women, 59% were post-menopausal and 48% had previously undergone hysterectomy. Moreover, 80% had an abnormal PAP smear prior to the diagnosis of VAIN 2/3. Out of 20 women who had high-risk human papilloma virus (hrHPV) DNA test done, 75% had positive result. The diagnosis of VAIN 2/3 was mostly made after a biopsy of a suspicious lesion (73%). The rest were diagnosed on vaginal vault smear (16%) and on vaginal cuffs of radical hysterectomy specimens (11%). Of all these women, 79% underwent treatment and 31% experienced recurrence of disease, of which four women developed cancer of the vagina.

**Conclusion:** This cohort of women further delineates the demographics of VAIN 2/3. With the high number of abnormal PAP smear related to VAIN 2/3, careful evaluation of the vagina should be performed during colposcopy, as progression to cancer of the vagina in this cohort seems rather significant.
Objectives: The impact on total mortality caused by HPV-related cancer in each country needs to be determined, because the prevalence of oncogenic HPV types varies between countries. We analyzed trends in age-standardized mortality rates (ASRs) of HPV-related cancer and age-specific mortality rates of cervical cancer and oropharyngeal cancer in a population-based study in a Japanese setting.

Methods: Using the Kanagawa Cancer Registry in Japan, we identified patients with cancers of the oropharynx, cervix, vulva, vagina, anus and penis between 1975 and 2012. We estimated the ASR of each cancer by sex- and age-specific mortality rates. In addition, we calculated average annual percentage changes (AAPCs) in ASRs using joinpoint regression.

Results: ASRs of oropharyngeal cancers increased among both males (AAPC 8.8% between 1980-2000, p<0.05; AAPC 1.3% between 2000-2012, p<0.05) and females (AAPC 5.9% between 1991-2012). For anal cancers, ASR increased among men (AAPC 1.8% between 1977-2012, p<0.05). Age-specific mortality rates increased for cervical cancer among females under 40 years old (p<0.05) and oropharyngeal cancer among males over 50 years old. ASRs of vulvar, vaginal and penile cancers decreased over the study period.

Conclusions: We observed increases in ASRs of cancers involving the oropharynx among males and females, and involving the anus among males. We also observed increases in age-specific mortality rates for cervical cancer among young females, and in oropharyngeal cancer among older males. Preventable deaths caused by HPV among both males and females have to be monitored carefully using population-based methods in Japan.

Objectives: Expression of HLA molecules on tumor cells is essential for the recognition of tumor antigens by the immune system. HLA class II antigens are expressed by professional antigen-presenting cells, but also by several tumors of non-lymphoid origin. Strong HLA class II antigen expression has been described for a subset of HPV-associated cervical cancers. To characterize HLA class II antigen expression during HPV-induced cervical tumor development, we examined HLA class II antigen expression in cervical precancers and cancers.

Methods: Biopsies of CIN2, CIN3 and invasive SCC were analyzed by immunohistochemical staining with a monoclonal antibody specific for HLA class II antigens (LGII-612.14).

Results: HLA class II antigen expression was absent in the normal cervical epithelium adjacent to lesions. In contrast, expression of HLA class II antigens was observed in all 9 CIN2 lesions (100%), in all 13 CIN3 lesions (100%) and all 19 cancers (100%). More than half of CIN2 lesions (5 out of 9, 55.6%) showed HLA class II antigen expression on all lesion cells while only 7 out of 19 (36.8%) invasive cancers had HLA class II antigen expression on all tumor cells.

Conclusions: Our results suggest that HLA class II antigen expression is commonly induced in precancerous stages of cervical tumorigenesis, with a potentially decreasing frequency in cancers. This observation supports the concept that HLA class II antigen-negative cell clones appear to emerge during tumor progression, possibly as a result of counterselection of HLA class II antigen-positive tumor cells.
Background: Transformation of HPV-infected cells is linked to a severe shift of HPV-gene expression patterns. It goes along with epigenetic activation of E6 and E7 oncogenes leading to p16 overexpression, whereas the late genes are usually silenced.

Objectives: We aimed to investigate the methylation status of all 19 CpGs in the L1-gene in productive p16 negative intermediate and superficial squamous cells versus non-productive but transforming p16-positive lesions and find out the impact of chromosomal integration of HPV genomes on the methylation levels of CpGs within the HPV 16 late genes.

Methods: 48 tissue sections HPV 16 positive samples were examined for p16^INK4a and L1 gene expression as well as the presence of koilocytes in the specimens. 5 were diagnosed as CIN1, 9 as CIN 2, 25 as CIN 3 and 9 as squamous carcinoma (SCCs). Furthermore, 24 HPV16 positive fresh-frozen carcinoma samples were selected; 12 carcinomas with integrated and 12 with episomal HPV genomes. Methylation levels were obtained by pyrosequencing.

Results: L1-methylation levels were substantially higher in p16-positive transforming HPV-infections in comparison to the productive infections. Carcinoma samples with integrated HPV16 genomes had significantly higher methylation levels in the 3’ region of L1, in particular at CpGs 9 to 15.

Conclusion: These data underline the hypothesis that the methylation of the L1 gene substantially increases during neoplastic progression. Methylation was lowest in p16-negative productive HPV-infections presumably reflecting the active expression in these lesions. Methylation of the CpGs 9-15 may indicate integration of HPV genomes into the host cell genome.

Objectives: To identify new methylation markers for high-grade cervical intraepithelial neoplasia (CIN2/3) using innovative genome-wide methylation analysis and to assess their diagnostic performance in cervical scrapings.

Methods: Methylated DNA was enriched from normal cervices and CIN2/3 lesions followed by next-generation sequencing (MethylCap-seq) to identify differential methylation regions (DMRs). The 15 highest ranking differentially methylated genes were validated by MSP. For diagnostic evaluation, QMSP was performed in cervical scrapings from 2 cohorts: 1) cervical carcinoma vs. healthy controls and 2) patients referred from population-based screening with abnormal cytology in whom HPV status was determined.

Results: MethylCap-seq identified 176 DMRs comprising 163 genes. Nine of the 15 genes showed in the validation step significantly more methylation in CIN2/3 lesions compared to normal cervices (p<0.05). Subsequently, methylation levels of 8/9 genes were significantly higher in carcinoma compared to normal scrapings. For all 8 genes methylation levels increased with severity of the underlying histological lesion in scrapings from patients with abnormal cytology. In addition to the 8 new genes, also our previous four-gene panel (C13orf18/JAM3/EPB41L3/TERT) was analyzed. The best combination of genes (C13orf18/JAM3/AL590705.4) revealed sensitivity (74%) for CIN2+ comparable to hrHPV testing (79%), while specificity was significantly higher (76% vs 46%, p≤0.05) in a triage setting after abnormal cytology in population-based screening.

Conclusion: We identified new CIN2/3 specific methylation markers using genome-wide DNA methylation analysis. The diagnostic performance of our new methylation panel shows comparable sensitivity to hrHPV testing for CIN2+, but with higher specificity to prevent referral for unnecessary colposcopy. The next step before implementation in primary screening programs will be validation in population-based cohorts.

**OC 17-1**
**METHYLATION OF HUMAN PAPILLOMAVIRUS 16 L1 GENE IN CERVICAL INTRAEPITHELIAL NEOPLASIA AND CANCER**

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**OC 17-2**
**DICOVERY OF NEW METHYLATION MARKERS TO IMPROVE SCREENING FOR CERVICAL INTROEPITHELIAL NEOPLASIA GRADE 2/3**


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Objectives: To identify new methylation markers for high-grade cervical intraepithelial neoplasia (CIN2/3) using innovative genome-wide methylation analysis and to assess their diagnostic performance in cervical scrapings.
HPV ONCOTECT QUANTITATIVE E6, E7 mRNA AS A SINGLE TEST ALTERNATIVE TO HPV 16, 18 AND PAP REFLEX FOLLOWING PRIMARY HPV DNA SCREENING

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Background: HPV DNA has been approved for primary cervical cancer screening. Samples positive for HPV DNA are reflexed to HPV 16, 18 and if negative, the sample is reflexed to PAP.

Methods: In this substudy of samples from Spathis et al PLoS ONE 7:1-9, 2012, 596 samples (including 196 CIN 2+) with HPV DNA, HPV 16/18, HPV Oncotect E6, E7 mRNA, liquid based cytology, and histology results were analyzed. Using the DNA alone screening algorithm, we compared the performance of HPV Oncotect Quantitative E6, E7 mRNA as a single reflex test with HPV 16/18 and PAP as utilized in the published algorithm.

Results: HPV DNA alone had a specificity of 42% for CIN 2+ while HPV reflex to HPV Oncotect had a specificity of 82% for CIN 2+. HPV DNA+/16, 18+ had a specificity of 87% for CIN 2+ but a sensitivity of 56% for CIN 2+ compared to a HPV Oncotect sensitivity of 86% for CIN 2+. The false negative rate of HPV Oncotect compared to PAP as a reflex to HPV DNA+ samples was the same at 3.3%.

Conclusions: HPV Oncotect is a viable reflex option in the HPV alone screening algorithm that could replace both HPV 16, 18 and PAP reflex using a single, automated, non slide-based test. The combination of HPV DNA and HPV Oncotect also provides a superior clinical laboratory workflow.

GENOME-WIDE METHYLOME ANALYSIS DISCOVERS NOVEL METHYLATION MARKERS FOR BOTH CERVICAL ADENOCARCINOMAS AND SQUAMOUS CELL CARCINOMAS

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Objectives: Cervical adenocarcinomas (ADC) are mainly diagnosed at advanced disease stage. In the last decade, the incidence of ADC increased in most developed countries and represents about 20% of cervical cancers. One explanation for the increase of ADC is the less effective cytomorphological detection of ADC and its precursors in population-based screening programs. Analysis of DNA methylation markers might improve the detection of ADC in earlier stages. The aim of this study was to discover novel methylation markers for cervical cancer detecting both ADC and SCC.

Methods: To generate a global methylation-profile, methylated DNA from 20 normal cervices, 6 ADC and 6 SCC was enriched followed by next-generation-sequencing (MethylCap-seq). Differential methylated markers (DMRs) were selected for verification and validation by bisulfite pyrosequencing or methylation specific PCR (MSP). Quantitative MSP (Q MSP) was performed for the clinical evaluation on cervical scrapings from an independent cohort of 89 women with a normal cervix and 89 cervical cancer patients comprising ADC and SCC.

Results: Validation of the highest ranking 15 DMRs resulted in 5 markers exhibiting different methylation between normal and cancer tissues (p<0.05). Using QMSP analysis on cervical scrapings, the sensitivity of these 5 markers varied from 76.5% to 88.8% to detect both ADC and SCC with almost all normal scrapings negative (specificity: 94%-100%).

Conclusion: Using MethylCap-seq analysis, we identified 5 new methylation markers with a high sensitivity for both ADC and SCC in cervical scrapings. A large series of scrapings with ADC and its precursors is needed to validate its clinical relevance.
**OC 17-5**

**COMPARISON OF MANUAL PROCESSING FOR P16/KI-67 DUAL STAIN**

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**Objectives:** Immunocytochemical detection of overexpression of p16 is valuable for the triage of HPV-positive cytologically normal cases and borderline/low-grade abnormalities. Simultaneous detection of the proliferation marker Ki-67 significantly enhances its specificity. Meanwhile this test is also available in an automated staining system (Ventana Benchmark XT [VB]). In a routine lab with high-throughput testing we compared automated (VB) with manual (MS) staining.

**Methods:** 495 Thinprep specimens routinely collected for triage were each splitted. First two slides were prepared for p16/Ki-67 Dual Stain and secondly two slides for Pap cytology and another marker. Staining was performed according to the instructions of the manufacturer (Roche Ventana, Mannheim, Germany) and read by two cytologists. Positivity was grouped <3, 3-10 and >10 cells.

**Results:** With MS 236 smears were positive vs 247 with VB. About one third of the positives contained <3, 3-10 and >10 positive cells, respectively. Only 2 slides were rated invalid (one MS, one VB). In 19 of 495 cases (3.8 %) the results were discrepant: 4 cases MS-pos/VB-neg vs 15 cases MS-neg/VB-pos. In 18 of the 19 discrepancies <3 cells were rated positive. Only minimal differences between the two procedures were observed in the number of cells rated positive: in 9 of 232 cases positive with both approaches a minor difference was observed, only in one case a clear difference (<3 vs >10). Association with HPV status will be reported.

**Conclusion:** Automated staining with the Ventana Benchmark is slightly more sensitive than manual staining for p16/Ki-67. Only minor discrepancies are observed.

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**OC 17-6**

**ESTABLISHED AND NOVEL PROTEIN BIOMARKERS FOR EARLY DETECTION OF CERVICAL CANCER CELLS**

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**Objectives:** Early detection of cervical intraepithelial neoplasia (CIN) in cervical smears is key to efficient control of the disease. Current protocols, relying respectively on cytological assessment of cervical smears and detection of high-risk HPV DNA in such smears, fall short of reliably detecting CIN. Protein markers, such as viral oncoproteins and the host protein p16INK4A were proposed as more efficient tools for the detection of cervical precancerous and cancerous lesions.

**Method:** To assess the role, if any, of p16INK4A for maintenance of the transformed phenotype in established cervical carcinoma cells, we used shRNA-mediated knockdown of p16INK4A and followed cell proliferation and survival of p16INK4A-depleted cells. We also developed a sandwich ELISA to detect E7 proteins in cervical smears in a routine screening setting.

**Results:** Depletion of p16INK4A from cervical cancer cells led to a significant reduction of E7 gene expression and otherwise yielded mixed results, ranging from induction of cellular senescence to no effect on cell proliferation. E7 proteins are detected by E7-ELISA in cervical smears derived from women with histology-confirmed CIN.

**Conclusions:** Our data suggest that p16INK4A is dispensable for the proliferation of several cervical carcinoma cell lines. Together with recent work showing that E7 drives p16INK4A expression in cervical cancer cells, our results provide a potential molecular basis for the observed high expression level of p16INK4A in most cervical cancers. The results reported here further warrant the use of HPV E7 proteins as biomarkers for cervical cancer detection.
**Background:** L1 and L2 expression is restricted to intermediate and superficial layers of HPV infected squamous epithelia. Differential methylation of the viral genome has been proposed to control its transcription during the normal viral life cycle. In CaSki cervical cancer cells several hundreds of HPV 16 genome copies are integrated. The vast majority of these genome copies are methylated and not transcriptionally active. Only the E6-E7 genes of the most 3’ located HPV genome are transcribed. This genomic and epigenomic structure of HPV 16 in CaSki cells resembles episomal HPV-genomes in infected basal squamous epithelial cells.

**Objective:** We were interested to test whether epigenetic inhibitors like 5-aza-2′-deoxycytidine (DAC) can activate the expression of the L1 gene in undifferentiated HPV-infected keratinocytes carrying episomal HPV genomes.

**Methods:** CaSki cells as model for the episomal genome configuration were treated with 5aza-dC in different dosages and over different time intervals. The expression of L1 gene was quantified by RT-qPCR and Western blotting. The methylation state of the L1 gene upon DAC treatment was determined by bisulfide conversion and subsequent pyrosequencing.

**Results:** DAC induced DNA hypomethylation within one week of treatment. After 2 weeks of DAC treatment expression of the L1 gene was substantially upregulated.

**Conclusion:** These data underline the hypothesis that treatment with DAC of HPV-induced lesions may activate the expression of L1 (and possibly L2) in HPV infected basal squamous epithelial cells. This may trigger a T-cell response and elimination of the HPV-induced lesions. The clinical validity of this therapeutic concept is currently under investigation.
THE EPGENETIC DRUG 5-aza-2’-DEOXYCYTIDINE DOWNREGULATES THE VIRAL ONCOGENES E6 & E7 AND REDUCES PROLIFERATION IN HPV ASSOCIATED CANCERS

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Background: Epigenetic therapeutics are steadily increasing in importance for the treatment of various cancers. Several studies have so far indicated epigenetic mechanisms in the regulation of viral oncogene expression in HPV-induced cervical lesions. However, the effect of epigenetic drugs on HPV16 oncogene expression and growth patterns of HPV16-induced cancers remains unclear.

Objective: We examined the applicability of the DNA methyltransferase inhibitor 5-aza-2’-deoxycytidine (DAC) on HPV-16 transformed cell lines.

Methods: HPV-16 positive cancer cell lines of the cervix and head and neck were treated with various concentrations of DAC for 72 hours. Methylation levels were assessed using bisulfite treatment and pyrosequencing of the early HPV16 promoter and the long interspersed nuclear element-1 (LINE-1) as a marker for global methylation levels. mRNA and protein levels of the viral oncogenes E6 and E7 and related genes were determined via qRT-PCR and Western Blot. Proliferation and the level of differentiation were monitored.

Results: DAC treatment induced global DNA demethylation, as well as demethylation of CpG-sites in the upstream regulatory region of the HPV genome. This was linked to a significant decrease of E6 and E7 oncogene transcripts and E7 protein levels while p53 protein levels were upregulated. These findings were associated with dose dependent reduced proliferation and an induction of differentiation in the treated cell lines.

Conclusions: 5-aza-2’-deoxycytidine is a promising candidate substance in order to epigenetically suppress viral oncogene expression and proliferation.

COFILIN-1 AND CATHEPSIN-D ARE PUTATIVE EARLY CERVICAL CANCER BIOMARKERS

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Objectives: Despite preventive measures, there is still aual clinical need for reliable early cervical cancer biomarkers. Malignant transformation of the cervical epithelium is accompanied by qualitative and quantitative changes in protein expression profiles of the infected cells. The aim of our study was to identify proteins associated with cervical cancer by proteomic analysis and evaluate the potential use of these proteins as putative biomarkers.

Methods: In the present study, we undertook a systematic analysis for intracellular and secretome profiles of four cervical cell lines, i.e. HCK1T, a normal cervical epithelium cell line, HeLa [HPV18+], SiHa, [HPV16+], and C-33A [HPV-], exhibiting unique complementary molecular and phenotypic features, by employing 2D electrophoresis and MALDI-TOF mass spectrometry. Western blots and immunohistochemistry were performed on total cell extract and cervical tissue sections, respectively.

Results: 168 proteins in the total cell extract and 125 in the secretome were differentially expressed between normal (HCK1T) and cancer (HeLa, SiHa, C-33A) cell lines. The most significant group of differentially expressed intracellular proteins was related to cytoskeletal remodeling. Among these, the cytoskeletal protein Cofilin-1 was further analyzed. In both 2D electrophoresis and WB analysis, Cofilin-1 was significantly upregulated in HeLa and C-33A cells, compared to HCK1T. The secretome proteomic comparison has as prominent feature Cathepsin-D, a protease involved in cancer invasiveness, which was upregulated in SiHa cells compared to control HCK1T. The proteomic result was further validated by immunohistochemistry.

Conclusion: These novel data provide the impetus for further exploitation and validation of Cofilin-1 and Cathepsin-D as putative cervical cancer biomarkers.
SECRETOME MAP STUDY OF NORMAL AND MALIGNANT CERVICAL CELL LINES

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Objectives: Cervical cancer is caused by sexually acquired infection with certain HPV types. Two HPV types (16 and 18) cause 70% of cervical cancers and precancerous cervical lesions. The aim of our study was to identify biological processes involved in malignant cell interactions with surrounding tissues by proteomic analysis of the secretome from control and cancerous cervical cell lines.

Methods: Secreted proteins were isolated from four cervical cell lines: HCK1T, a normal cervical epithelium cell line, HeLa [HPV18+], SiHa, [HPV16+], and C-33A [HPV-]. The secretome samples were analyzed by 2D electrophoresis and gels were stained with Coomassie Blue. Image comparison was performed by the PDQuest software. Differentially expressed proteins between normal and cancer cell lines (fold change > 2) were identified by MALDI-TOF MS. Bioinformatic analysis was performed by the PANTHER software.

Results: A total of 125 proteins in the secretome (68 upregulated, and 57 downregulated) were differentially expressed between normal (HCK1T) and cancer (HeLa, SiHa, C-33A) cell lines. The percentage of secreted proteins identified in this list is 34.5%, which is much higher than the corresponding percentage (7%) in the total cell extract. The majority of differentially expressed proteins have catalytic activity and a significant number of upregulated enzymes in cancer cell lines are proteases implicated in extracellular matrix remodeling.

Conclusion: The proteomic analysis of the secretome from cervical cell lines indicates that the protocol used is effective for isolating secreted proteins. The proteases secreted by cancer cells are likely to play a role in the invasion of the surrounding tissue.