ABSTRACTS

This publication contains the abstracts submitted for the EUROGIN 2010 Congress, held in Monte Carlo, from February 17 to February 20, 2010. More than 800 abstracts were received and reviewed. For uniformity, all abstracts have been formatted electronically. Occasionally, symbols in electronically submitted abstracts may have been lost or changed in the re-formatting process. Please advise the congress staff of errors that distort the data or change the meaning.

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

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

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

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

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Papillomaviruses disease is initiated through infection of a cell or cells in the epithelial basal layer, followed by their subsequent proliferation, which may involve the wound-healing response. Whether establishment of a productive infection requires the infection of a stem cell or a transiently amplifying cell, and whether this requirement differs between high and low-risk HPV’s, is currently unclear. In high-risk HPV infections, the so-called viral ‘oncogenes’ (E6 and E7) play a key role in driving cell proliferation in the lower epithelial layers of infected tissue, and in all HPV types, these genes are involved in the amplification of viral genomes in the intermediate cell layers in preparation for virus assembly in the upper epithelial layers. The different pathologies associated with HPV infection are thought to reflect differences in viral gene expression and viral protein function, and their subsequent effects on cell morphology. Whether an infection leads to neoplasia or to the formation of a productive lesion depends on the site of infection and the extent of aberrant viral gene expression. While the events that influence viral gene expression are still unclear, it is generally thought that different pathologies result from infection of columnar cells of the endocervix, cells of the transformation zone, or cervical cells outside the transformation zone and that the virus may be more or less successful in directing a productive infection at these different sites. The general model is that the overexpression and/or persistent expression of the viral oncogenes in such cells can predispose to chromosomal abnormalities and the accumulation of genetic changes, which contribute to high-grade neoplasia and eventually to cancer.

Worldwide, IARC Globocan estimates a total of 493,000 annual incident cervical cancer cases, nearly all of which are human papillomavirus (HPV)-associated. To estimate the burden of HPV-associated, non-cervical cancer cases globally, the number of estimated incident cancer cases were utilized from previous reviews of the global burden of cancer, while attributable fractions due to all HPV types or specifically due to HPV types 16/18 were taken from recent systematic reviews on the HPV-type distributions of different cancers. A total of 177,000 HPV-related, non-cervical cancers were estimated in 2002 (Table 1). Among HPV-related, non-cervical cancer cases, oral cancer was the most common (64,500 cases), followed by cancer of the larynx (38,200), anus (23,800), oropharynx (18,600 cases), penis (12,600 cases), vulva (10,800 cases), and vagina (8,700 cases). Esophageal cancer was not included due to insufficient evidence supporting a causal etiologic role of HPV in esophageal carcinogenesis.

The impact of current HPV vaccines against oncogenic HPV types 16 and 18 may extend beyond cervical cancer to the prevention of non-cervical cancers caused by these carcinogenic HPV-vaccine types. Assuming wide coverage rates are obtained on the population-level, the prevalence of non-cervical cancers attributable to HPV 16/18 can provide a crude estimate of the potential impact of the HPV 16/18 vaccines on these non-cervical cancers. The prevalence of HPV 16/18 for specific cancers are: vulva (30.6%), vagina (54.5%), penis (36.7%), anus (72.2%), mouth (22.3%), oropharynx (31.8%), and larynx (20.1%). Overall, HPV 16/18 account for ~27% of the non-cervical cancers, and ~88% of the HPV-positive non-cervical cancers. First-generation prophylactic HPV vaccines thus have the potential to prevent ~45% of all HPV-associated cancer cases, including ~70% of invasive cervical cancer and ~25% of non-cervical cancers. A worldwide increase in HPV vaccination for cervical cancer may have a notable influence on the incidence of HPV-associated cancers other than cervical cancer.
Meta-analyses of HPV type-distribution across a range of cervical lesion severity are helpful for estimating the potential impact of HPV type-specific vaccines and screening tests, and for understanding the oncogenic potential of different HPV types. Previous IARC meta-analyses of cervical lesions have clearly shown that the relative importance of HPV-16 and 18 increase, and geographical differences in HPV type-distribution decrease, with increasing severity of cervical lesions. HPV-16/18 is estimated to account for 16–32% of low-grade cervical lesions, 41–67% of high-grade cervical lesions, but 70% of all cervical cancers worldwide.

IARC meta-analyses are being continually updated and expanded both in terms of their representation of geographical regions and number of HPV types, but more importantly in terms of the detailed stratification of lesions by cytological/histological diagnosis, particularly at the lower end of lesion severity spectrum. To this end, the current update of the IARC dataset includes a systematic review of all relevant data published up to June 2009. Type-specific HPV prevalence data currently exist for 278,633 women with normal cytology; 10,014 and 5,943 women with low-grade and high grade cytological abnormalities respectively; 7,818, 3133, 6148 and 1,215 women with histologically confirmed CIN1, CIN2, CIN3 and carcinoma in situ respectively; 23,796 cases of squamous cell or unspecified carcinoma of the cervix, and 3,361 cases of adenocarcinoma of the cervix.

We will present data on HPV genotype distributions by histology and cytology across this broad disease spectrum to study the causal role and clinical importance of particular HPV types in cervical disease progression.

Objectives: Anogenital carcinomas other than cervical carcinoma are rare but: 1) their incidence has increased in some high-resource countries in the last decades, notably in women and men below age 50 years; and 2) the incidence of anogenital carcinomas is likely to be under-reported in developing countries.

Methods: Meta-analysis investigated human papillomavirus (HPV) prevalence in vulvar, vaginal and anal intraepithelial neoplasia (VIN, VAIN, AIN) grades 1-3 and carcinoma from 93 studies conducted in four continents and using PCR assays.

Results: Overall HPV prevalence was 67.8%, 85.3% and 40.4% among 90 VIN1, 1,061 VIN2/3 and 1,873 vulvar carcinomas; 100%, 90.1% and 69.9% among 107 VAIN1, 191 VAIN2/3 and 136 vaginal carcinomas; and 91.5%, 93.9% and 84.3% among 671 AIN1, 609 AIN2/3 and 955 anal carcinomas, respectively (1). HPV16 was found more frequently (>75%) and HPV18 less frequently (<10%) in HPV-positive vulvar, vaginal and anal carcinomas than in cervical carcinoma. HPV6 and 11 were common in VIN1 and AIN1 but not in VAIN1. HPV prevalence in vulvar carcinoma varied most by histological type (69.4% in warty-basaloid and 13.2% in keratinised type) and was also higher in women 60 years or younger and in studies carried out in North America. HPV prevalence in anal carcinoma was higher among women (90.8%) than men (74.9%), but no difference by gender emerged in North America. The majority of AIN2/3 derived from studies of HIV-positive individuals and/or men who have sex with men. Among AIN2/3, HIV infection was associated with higher HPV prevalence, more multiple-type infections, and a relative under-representation of HPV16.

Conclusions: Approximately 40% of vulvar, 76% of vaginal and 85% of anal carcinoma may be avoided by prophylactic vaccines against HPV16/18. This proportion would be similar for the corresponding high-grade lesions of the vagina and anus, but higher for VIN2/3 (75%) than for vulvar carcinoma.

Burden of HPV and relative importance of HPV types in carcinogenesis

HPV infections are the commonest of the sexually transmitted infections. Among women with normal cytology within the age ranges 25-65, typical included in screening programs, the average HPV DNA prevalence has been estimated at 10% with significant geographical variation. Among women with normal cytology and cervical HPV infections, one can often find multiple HPV types, a great variability in the HPV type distribution including a significant presence of low risk types. HPV 16 is the dominant type in most studies. In contrast as cervical lesions develop the global HPV DNA prevalence increases to 75-85% in LSIL and to 85-100% in HSIL and invasive carcinomas. In cervical cancer cases, the number of types is restricted to 12-15 types, the vast majority of cases harbour only one type and a clear type distribution pattern is found. On worldwide estimates, HPV 16 is consistently the most common type, accounting for some 50% of all cases followed by HPV 18, 45, 31 and 33. Some variability in the ranking thereafter has been described. For the subgroup of cervical adenocarcinomas, HPV 16 and 18 are found in similar proportions followed by HPV 45. The individual contributions of any other type are of minor quantitative importance. The three HPV types combined (16, 18 and 45) account for close to 75% of the squamous cell carcinomas and close to 90% of the adenocarcinomas. Cervical cancer in the Asia Pacific region shows some departure from world’s average. The five most frequent HPV types found as single infections in cervical cancer are 16, 18, 45, 58 and 52 and in cervical adenocarcinoma 16, 18, 45, 59 and 33.

Transmission of HPV infections

HPV infections occur early after sexual initiation and persist as long as women remain sexually active as a function of her number of partners and of the behaviour of her partners. Some studies have now provided some evidence that HPV prognosis in terms of risk of persistency and progression is independent of age at infection. In contrast to other sexually transmitted infections, few studies are available on the probability of HPV transmission from one infected partner to another by sex and on the risk factors for transmission.

LBC: THE REAL PICTURE

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Liquid-based cytology (LBC) is now extensively used throughout the developed world for cervical cytology screening. It was approved by the FDA largely as the result of a 5-centre trial in the USA,1 which demonstrated a higher detection rate for low-grade cervical intraepithelial lesion (LSIL) or worse. A number of early studies indicated greater sensitivity for high-grade intraepithelial lesions (HSIL) but this has not been supported by meta-analysis.2 Early enthusiasm for a new technique, extra training at the time of its introduction, an improved sampling device and lack of histological correlation in the early studies may have suggested that LBC had a greater advantage than was originally thought.3

In the UK, LBC was recommended by the National Institute of Clinical Excellence (NICE) for use in the NHS Cervical Screening Programme largely because pilot studies in Scotland, Wales and three laboratories in England demonstrated a significant fall in rates of inadequate tests.4,5 This, and the conclusion that LBC was at least as sensitive as conventional cytology, resulted in its implementation nationwide. LBC has undoubtedly reduced rates of inadequate tests in the UK but, as NICE said at the time, there were no UK criteria for judging adequacy of the sample although the Bethesda system addresses this issue.6 It was estimated that reduced inadequate rates would balance the costs but there is no evidence to suggest that has been achieved.

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Even if sensitivity is not significantly increased, LBC has major advantages over conventional cytology. First, it provides clean, well-fixed preparations that are easier and quicker to screen; second, it removes obscuring blood and exudate; second it provides residual material for ancillary studies such as testing for high-risk human papillomavirus; third, it provides a more consistent cell preparation for computer-assisted screening. There are two commercial LBC systems which are equally distributed across UK laboratories; and which each have advantages and disadvantages for cervical cytology and non-gynaecological cytology respectively.

LBC was introduced in the UK with a major programme of training for ‘sample-takers’, cytotecnologists and pathologists, which, taken with the advantages of the technology, has almost certainly improved cervical cytology laboratory performance.

References

TC 2-2

HPV/PAP TRIAGE IN ROUTINE PRIMARY CERVICAL SCREENING

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HPV testing has a higher sensitivity, a slightly lower specificity, but a significant higher negative predictive value (NPV) for high-grade CIN lesions (CIN2 or worse, CIN2+) compared to conventional or liquid based cytology. Randomised controlled trials comparing HPV testing alone or in combination with cytology versus sole cytology showed that 30-50% more CIN2+ lesions are detected by HPV testing. The higher NPV of HPV testing makes extension of screening interval possible, which makes the implementation of HPV testing as primary screening tool in cervical screening cost-effective. Nevertheless, HPV testing has a slightly lower specificity for CIN2+ compared to cytology. To increase the specificity additional triage of HPV positive women is therefore important. Based on two large trials with long term follow-up in The Netherlands (POBASCAM and VUSA-SCREEN) we evaluated more than 15 screening strategies including reflex cytology, repeat cytology at 12 and 24 months, repeat cytology with HPV at 6 months, and repeat cytology with genotyping for HPV16,18 at 6 months. To select the best strategy we calculated relative sensitivity for CIN2+, number of colposcopy referrals, and influence on cervical cancer incidence, taking into account that only one follow-up moment is preferable because of the loss of at least 20% of women in follow-up. We selected three strategies, which resulted in similar good results and these will be discussed.

TC 2-3

SQUAMOUS PRECANCER LESIONS: TWO- OR THREE-TIER TERMINOLOGY?

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Background: Squamous cell (SCC) carcinoma is the main histological type of cervical cancer (CC). During the past decades, three different morphological classifications have been introduced to classify cervical precancer lesions. These include a) the dysplasia-carcinoma in situ (CIS) classification, b) the CIN (cervical intraepithelial neoplasia) classification, and c) the Bethesda System (TBS). At present, the two most commonly used nomenclatures are the 3-tier CIN terminology and 2-tier TBS, the former being favoured by Europeans while the latter is a purely American innovation. The key difference between the two classifications is bound to the question, whether the intermediate category (CIN2) should be maintained or abandoned.

Results: As supporter of the European view, this author favours the concept of maintaining CIN2 in the histological classification, based on the following arguments: 1) Morphological criteria of CIN2 are well defined; 2) CIN2 has a natural history distinct from that of CIN1 and CIN3; 3) CIN2 has implications in management of women with abnormal PAP smears; 4) Novel biomarkers suggest linking of CIN2 and CIN1, not CIN2 and CIN3, and finally 5) HPV cofactors associated with progression to CIN1, CIN2 and CIN3 display unique profiles, implicating true biological differences between the three CIN grades. Because of the fact that there is a well established morphological and biological category of intermediate grade lesions (CIN2), the currently used 2-tier classification cannot 1) adequately describe the morphology, and even more importantly, 2) accurately predict the natural history of cervical precancer lesions. It is not justified to call any lesions as high-grade, if >40% of them undergo spontaneous regression.

Conclusions: Two possible solutions are: 1) to be satisfied with the CIN classification as it now stands, because it remains to be the most satisfactory descriptive terminology available despite its known shortcomings, or 2) to make an attempt to design a novel 2-tier classification, based on newly defined histological criteria. In the latter case, testing the biological validity of such a new classification would be of utmost importance, and as always, histological classification must be based exclusively on morphological criteria. Under current circumstances, to obtain the maximum clinically relevant information from cervical biopsy, maintaining CIN2 is justified, because it denotes for a distinct category of CC precursors, with progressive potential intermediate between the low-grade (CIN1) and high-grade (CIN3) lesions, and importantly, a risk profile distinct from that of CIN1 and CIN3.
NEW STRATEGIES FOR CERVICAL CANCER SCREENING

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Screening for cervical cancer precursors by cytology has been very successful in countries where adequate resources exist to ensure high quality and good coverage of the population at risk. Mortality reductions in excess of 50% have been achieved in many developed countries; however the procedure is generally inefficient and unworkable in many parts of the world where the appropriate infrastructure is not achievable.

A summary and update of recently published papers looking at the use of HPV in primary screening will be given. Primary screening with Hybrid Capture 2 (HC2) generally detects more than 90% of all CIN2, CIN3 or cancer cases, and is 25% (95% CI: 15–36%) relatively more sensitive than cytology at a cut-off of abnormal squamous cells of undetermined significance (ASCUS) (or low-grade squamous intraepithelial lesions (LSIL) if ASC-US unavailable), but is 6% (95% CI: 4–7%) relatively less specific. Several approaches are currently under evaluation to deal with the lower specificity of HPV DNA testing as associated with transient infection. These include HPV typing for HPV-16 and -18/45, markers of proliferative lesions such as p16 and mRNA coding for the viral E6 and/or E7 proteins, with a potential clinical use recommending more aggressive management in those who are positive.

In countries where cytology is of good quality, the most attractive option for primary screening is to use HPV DNA testing as the sole screening modality with cytology reserved for triage of HPV-positive women. Established cytology-based programmes should also be gradually moving towards a greater use of HPV DNA testing to improve their efficacy and safely lengthen the screening interval. The greater sensitivity of HPV DNA testing compared to cytology argues strongly for using HPV DNA testing as the primary screening test in newly implemented programmes, except where resources are extremely limited and only programmes based on visual inspection are affordable. In such countries, use of a simple HPV DNA test followed by immediate ‘screen and treat’ algorithms based on visual inspection in those who are HPV positive are needed to minimise the number of visits and make best use of limited resources. A review of studies for visual inspection methods is presented.

ACCURACY AND LIMITATIONS OF COLPOSCOPIC PERFORMANCE

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The paradigm of colposcopic examination after abnormal cervical cytology is still considered the standard for evaluation of cervical neoplasia. Despite colposcopy being a subjective test, its purpose is to identify disease, obtain representative specimens for histology, and direct patient management. Data will be presented to support the following key points:

- There is significant error in colposcopic assessment.
- Colposcopy with or without single “target” biopsy is an imperfect diagnostic gold standard because of its inherent pitfalls in disease ascertainment.
- Even with specialized training and experience, colposcopic biopsy misses 26% – 42% of prevalent CIN 2+.
- Grouping CIN2 with CIN3 improves performance as biopsied CIN2 often has CIN3 associated with it on definitive excision.
- The most significant factors influencing accuracy are the size of the lesion, the number of biopsies taken and the HPV type.
- Biopsy induced regression also seems to have an effect on natural history, particularly for small lesions.
- Endocervical curettage has a minimal impact on colposcopic sensitivity.
- These data should be taken into account in planning therapy especially for patients in whom the biopsy is less severe than the referral cytology.
- Taking more biopsies is the only compensating strategy.
The diagnosis of atypical glandular cells (AGC) is relatively uncommon with a prevalence of 0.4% in the U.S. A cytology report of AGC is much more concerning than a diagnosis of ASC-US because women with AGC are much more likely to have HSIL, AIS, or invasive cervical cancer which occurs in nearly 40% of women or endometrial carcinoma which occurs in nearly 20%. Therefore, initial work-up of women with AGC includes colposcopy and endocervical curettage to exclude HSIL, AIS, and cervical cancer and endometrial sampling to rule out endometrial hyperplasia and cancer. Endometrial sampling is not necessary in women under the age of 35 years unless they have risk factors such as abnormal bleeding or polycystic ovarian syndrome that places them at higher risk of having endometrial pathology. For women in whom the cytology was reported as “atypical endometrial cells”, colposcopy can be deferred until the results of endometrial and endocervical sampling are available since they are more likely to have endometrial as opposed to cervical disease. Subsequent management of women with AGC depends on the colposcopic and endometrial findings, the HPV DNA status and whether the referral AGC cytology was classified as atypical endocervical, endometrial, or glandular cells not otherwise specified (NOS). If significant disease is not identified during initial workup, the patient can be followed with repeat cytology and HPV DNA testing. If the referral AGC cytology was atypical glandular cells “favor neoplasia” or AIS, the patient should undergo either a cold-knife or loop excisional conization.

**Objectives:** To review the most often used methods to treat genital warts in routine and specialized practices.

**Methods:** Review of literature and personal experience.

**Conclusions:** External genital warts represent a major psychological, medical and financial burden, particularly in the 20- to 30-year sexually active population. Approximately 30% of EGW's may spontaneously regress; however, time to regression is variable and may take up to several months to years. In routine practice, therefore, waiting for regression is not an option as in most instances, treatments are requested by the patients. Wart therapies are delivered mainly based on the patient’s preference, extent of disease and treatment-related costs. They are carried out either by the healthcare providers or self-applied. In the former case, cryotherapy, topical BCA/TCA 85% to 90% solutions, podophyllin 8% to 12% in tincture of benzoin or scissors excision are the most frequent initial therapeutic methods. Home therapies include topical podofilox 0.5% solution/gel, imiquimod cream 5%, and more recently, the green tea extract sinecatechins ointment 15%. The latter is the first and only US FDA botanical product available for home therapy for EGW’s. The difference between sinecatechins (with powerful antioxidant activity) and podofilox and imiquimod resides in the significantly lower recurrence rates reported in randomized, placebo-controlled trials in complete responders. Recurrences with the former are less than 7% compared with 19% and 65% for the two latter products, respectively. Treatment of extensive EGW’s (> 20 cm² total wart area) includes CO² laser vaporization, electroexcision/fulguration and scissors excision. In the immunosuppressed, treatment is challenging. Combination therapies yield the best results on the order of 50% to 80% complete response with 20% to 30% recurrence rates, however. One of the most attractive approaches is surgical “debulking” followed by the topical, off-label home therapy using Cidofovir 1% gel, a nucleotide analogue with antiviral activity, on recurrent lesions. For vaginal and intraanal lesions of limited extent, cryotherapy, topical application of BCA/TCA solutions 85%, imiquimod cream 5% or excisional biopsy provide for about 80% complete response rates after an average of 4 treatment sessions. Recurrences on the order of 30 to 50% may be expected. For larger lesions, infrared coagulation, CO² laser vaporization or electrofulguration are the most appropriate modalities. For distal urethral lesions, CO² laser vaporization or 85% TCA application provide cure after 1 or 2 sessions. All treatment-resistant (> 8 weeks) and atypical warty lesions should be biopsied. Similarly, treatment modality should be switched after 2 months of unsuccessful results. The ultimate solution for EGW’s is primary prevention using the HPV-4 vaccine containing 6/11/16/18 L1 VLP’s in uninfected individuals.
HPV can infect vulva, vagina and cervix; at these sites the viral infection can lead to morphological recognizable lesions; the colposcope is an ideal instrument to characterize these lesions; however despite similar appearance, the clinical meaning of HPV related lesions is different at different sites and implies different management strategies. It is well known that the same HPV infection has an oncogenic potential which is deeply affected by the type of tissue infected; in other words the host plays a fundamental role in cancer development; as an example, vaginal cancer is very rare while cervical cancer is very frequent even if the vagina and the cervix are exposed to the same HPV types and are in the same individual. This observation stresses the role of host in the oncogenic outcome of HPV infection.

The morphological manifestations of HPV infection may be subclinical or clinical. Subclinical HPV infection has a different clinical meaning too on the vulva, vagina and cervix. On the cervix HPV infection with oncogenic viral types can lead to the formation of CIN that is recognized as cervical cancer precursor lesion; colposcopy is an ideal tool to detect these lesions and guide the treatment in order to maximize fertility preservation. Non oncogenic viral infection of the cervix can mimic CIN and, when the productive infection with benign HPV types generates epithelial overgrowths, non oncogenic HPV type infection can mimic even cervical cancer; however the regular organization of the tissue can be the clue for benign identification; a biopsy can confirm the absence of malignant changes. On the vulva HPV infection is frequent. Vulvar infection with oncogenic types is less relevant than on the cervix as HPV related vulvar cancer is a rare occurrence; oncogenic viral infection of the vulva can lead to the development of VIN, usual type; it can be observed in different configurations, as a white or pigmented lesion, or even as red area. Usual type VIN is easily recognized with the naked eye provided the clinician pays attention to the vulva. Application of acetic acid on the vulva should be discouraged as it reveals many acetoreactive areas which are not related to the oncogenic process and can only harm the patient. In conclusion, HPV infection of the female genital tract has distinctive morphological counterpart; besides a common clinical appearance the clinical meaning of these lesions is quite different and affect deeply the management plan.

VIN 1 versus VIN 2/3: A TWO-DISEASE CONCEPT

Historical Background
The previous terminology for squamous vulvar intraepithelial neoplasia (VIN) was introduced by the ISSVD in 1986. Squamous VIN was categorised as VIN 1, VIN 2 and VIN 3 according to the degree of abnormality.

Reasons for Modification
There is no evidence that the VIN 1-3 morphologic spectrum reflects a biologic continuum or that VIN behaves similarly to CIN. VIN 1 is an uncommon histologic finding and constitutes basally located cellular changes or minimal atypia. These findings are usually reactive or are an HPV effect. There is no evidence that VIN 1 is a cancer precursor. Studies have demonstrated considerable interobserver and intraobserver variations in the VIN 1 diagnosis and concluded that this diagnostic category is not reproducible. Finally, certain lesions, usually occurring in a background of lichen sclerosus, have basally located atypia, representing high grade Differentiated VIN. The majority of VIN lesions are categorised as VIN 2 or 3. Good histologic agreement is obtained when VIN 2 and 3 are combined as a single diagnostic category.

VIN, usual type, is HPV related in most cases and seen adjacent to approximately 40% of squamous cell carcinomas of the vulva. The less common VIN, Differentiated type, is seen primarily in older women, often in association with keratinising squamous cell carcinomas and, in some cases, is associated with lichen sclerosus.

SUMMARY
VIN Terminology 2004
- The term VIN 1 will no longer be used.
- The term VIN should apply only to histologically high grade squamous lesions.
- Two categories of VIN should be used to describe squamous VIN:
  - The more common type of VIN will be termed VIN, usual type. (VIN 2, VIN 3) These lesions are generally associated with high-risk HPV types, especially HPV 16.
  - The less common type of VIN lesion will be termed VIN, differentiated type. These lesions generally are not associated with HPV.
- The occasional example of VIN that cannot be classified into either of the above VIN categories may be classified as VIN, unclassified type (or VIN, NOS).
- Classification is performed on the basis of morphologic criteria only and not HPV type or clinical appearance.
The currently available HPV vaccines have only prophylactic features hence are of no benefit for HPV-infected women that are already on their way to develop a lesion. Whereas these vaccines present excellent efficiency in clinical trials and are expected to be of similar performance in vaccinated populations, the achievements towards therapeutic vaccines are lagging behind. There is agreement that the T cell arm of the immune system is responsible for controlling established infections although one might speculate that antibodies induced by a VLP-based prophylactic vaccine may prevent spreading of an HPV infection thus inducing a quasi therapeutic effect. The reason for the delayed progress in the field of T cell-dependent immune therapy is the complex interaction of the different players (immune cells, cytokines), the uncertainty about the viral target antigen and the appropriate stage of disease for application (cancer, precursor, persistent infection) as well as the so far limited interest of major pharmaceutical companies.

Yet very recent developments with peptide-based and vector-dependent vaccine candidates generate optimism for the advent of HPV-specific immune therapies.

**TC 4-1**

**HPV VACCINATION: LONG TERM VACCINE EFFICACY BY AGE GROUPS**

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Introduction and methods: Prophylactic vaccines, the bivalent (protects against 16 and 18, the cause of 70% of cervical cancers worldwide) and the quadrivalent (also protects against ~90% of genital warts as has 6 and 11 protection too) are both licensed in over 100 countries worldwide. Registration of these vaccines has been based on immunogenicity, safety and efficacy, as reported in phase 3 trials. The primary focus of these vaccines is prevention of infection and hence prevention of disease from the vaccine related genotypes to which the vaccines are targeted.

Results: The primary focus of these prophylactic vaccines is coverage of young girls prior to the becoming infected: consequently this will vary with culture, and first age of sexual debut. From phase 3 vaccine trials, efficacy for such populations is expected to be around 100% effective in reducing the risk of HPV16/18-related high-grade cervical, vulvar, and vaginal lesions (for those using either bivalent or quadrivalent vaccine), and HPV6/11-related genital warts (for those using the quadrivalent). The efficacy of quadrivalent HPV vaccine against high-grade cervical and external anogenital neoplasia remains high through 42 months post vaccination.

Although HPV infection occurs readily after initiation of sexual intercourse, it is still unusual for a woman to have been exposed to all types covered by the available prophylactic vaccines. Intention to treat (ITT) population data also has shown us that the vaccines are equally effective for those types for which an individual woman has not been previously infected. Data are available now out to eight years and show continued efficacy and immunogenicity.

In addition, both vaccines have shown some cross protection for phylogenetically related HPV genotypes of the A9 and A7 series, from infections, as well as related disease. How long and to what extent this cross protection will last, will require long-term follow-up.

Trials in women up to 45 years of age show equal efficacy for infection and disease for those not infected by a particular vaccine related HPV.
TC 4-2

EFFICACY OF GARDASIL® IN MEN AGED 16-26 YEARS NÄÏVE TO VACCINE HPV TYPES AT BASELINE:
THE LATEST DATA

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Background
Men are at risk for developing genital warts, penile, perineal, perianal, and anal neoplasia and cancer associated with HPV 6, 11, 16 or 18. In addition, male HPV infection contributes significantly to infection and subsequent cervical disease in women. This study examined the efficacy of the quadrivalent HPV (type 6/11/16/18) L1 virus-like particle vaccine against incidence of HPV6/11/16/18-related external genital lesions (EGL) (external genital warts, penile/perineal/perianal intraepithelial neoplasia, and penile/perineal/perianal cancer) as well as genital HPV6/11/16/18 infection in young men.

Methods
In this randomized, double-blind, placebo-controlled trial, 4,065 young men [3463 heterosexual men (HM) and 602 men having sex with men (MSM)] aged 16-26 years were administered quadrivalent HPV vaccine or placebo at enrollment, month 2, and month 6. Subjects underwent genital exams as well as swabbing of the penis, scrotum, intraanal (MSM only) and perineal/perianal region at enrollment, months 7, 12 and at 6-month intervals afterwards. After enrollment, all new genital lesions were biopsied for pathological diagnosis and PCR testing. Efficacy analyses were performed in a per-protocol population (PP) seronegative at day 1 and DNA-negative from day 1 through month 7 to the relevant vaccine HPV type. Median follow-up was 2.9 years after dose 1.

Results
Vaccine efficacy against HPV6/11/16/18-related EGL was 90.4% (95% CI: 69.2, 98.1) [92.4% (95% CI: 69.6, 99.1) among HM and 79.0% (95% CI: 87.9, 99.6) among MSM]. Vaccine efficacy against HPV6/11/16/18 persistent infection was 85.6% (95% CI: 73.4, 92.9) [83.7% (95% CI: 71.1, 91.5) among HM and 94.4% (95% CI: 64.4, 99.9) among MSM]. Quadrivalent HPV vaccine was well-tolerated in men. Compared with 64% of placebo recipients, 69% of vaccinated subjects reported at least 1 adverse experience (AE), the majority being injection-site AEs. No serious AEs were considered vaccine-related.

Conclusion
The quadrivalent HPV vaccine was efficacious in reducing the burden of HPV6/11/16/18-related EGL and persistent infection in HM and MSM aged 16-26 years naïve to the relevant HPV type at baseline. The vaccine was well-tolerated in men.

TC 4-3

IMPACT ON SCREENING, DIAGNOSIS AND TREATMENT

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

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Objectives: The recent introduction of vaccines against human papillomavirus (HPV) has brought to light the need for population-based surveillance. Although the primary impetus for development of HPV vaccines was indeed cervical cancer prevention, it will take decades to measure the impact of the vaccines on cervical cancer incidence. It is therefore important to consider monitoring of earlier endpoints to assess the positive as well as any unforeseen effects of HPV vaccination during the early phases of population-based HPV vaccine implementation. A variety of potential programs to estimate the near term impact of HPV vaccines have been formally established or are currently under consideration including surveillance of vaccine coverage, circulating HPV genotype prevalence, type-specific HPV serology, genital warts, cervical screening Pap practices and diagnoses, cervical intraepithelial neoplasia (CIN) of any grade and cervical cancer (CC) precursor lesions (CIN 2 and 3 and AIS). In addition, surveillance related to safety of HPV vaccination during early population implementation is continuing. Overall, surveillance information will facilitate necessary and appropriate integrations of vaccination and screening as complementary prevention strategies.

Methods: Current efforts as well as limitations of a variety of surveillance information to assessing HPV vaccine safety, population-based vaccine coverage and disease impact and as applied to facilitating necessary integrations with primary screening programs will be discussed.

Conclusions: A number of potential surveillance approaches to monitor HPV vaccine coverage, impact and safety are viable in the context of varying public health and financial resources. HPV vaccine implementation should not be delayed in settings where issues of feasibility or resources cannot support surveillance.

ISSUES RELATED TO BEST AGE TO VACCINATE

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Three important questions need to be taken into account when identifying an appropriate age to vaccinate—1) is there an age for optimal efficacy; 2) what is the duration of protection? and 3) what is feasible for distribution? Numerous studies have been published showing the high efficacy of the HPV vaccines in women who appear to be HPV naive at entry with naïve being defined by serology status. Unfortunately, serology remains an insensitive test to define naïve. Even with this limitation, the analysis showed that the efficacy was much lower in women with positive serology (i.e. past evidence of infection) or positive DNA test from the cervix. These studies make it clear that identifying naïve women is critical in finding an age for optimal efficacy. Natural history studies show that 40% to 70% of women acquire HPV within 2-5 years of onset of sexual activity. The rates of HPV 16/18 acquisition are somewhat lower but range from 10-30%. In the US, 24% of females are sexually active by age 15 years, 40% by 16 and 70% by 18 years. Consequently, from a public health perspective, vaccination should occur before sexual debut or shortly thereafter to achieve optimal efficacy. This short window swayed most policy groups to target an age where most girls in their population are non-sexually active. Clearly, the individual 19 year old who has not initiated sex would benefit. However, from a public health view, there is no evidence to recommend universal vaccination of a population where over 90% are sexually active and most have been for 3-5 years. One of the concerns of vaccinating too early is duration. Data from Gardasil trials show that there is excellent duration for HPV 16 antibodies but less so for HPV 18. On the other hand, booster studies suggest that there are adequate circulating memory cells years later to infer protection despite low antibody levels. Studies of Cervarix show excellent duration with similar findings associated with boosters. Clearly, only time will tell, but evidence suggests that targeting non-sexually active girls will confer protection 10 years later.

One of the greatest challenges to vaccination is access. If “best” age is set during middle or high school, the challenge is in delivery. These age groups are not easily targeted for a three series vaccine schedule. School-based vaccination strategies are the most successful since it is a captive audience but raise ethical and political concerns. Even school based strategies are likely to work better in the younger middle school child rather than high school since older students are more likely to be truant or drop out, missing the occasion to be vaccinated. In summary, the issues related to age to vaccinate remain complex and divided. Clearly, industry is a stakeholder and more vaccinations are perceived as better. Parents remain skeptical about the safety of new vaccinations. Physicians are overwhelmed with parent concerns and reimbursement issues.
CERVICAL CANCER AND HPV-RELATED DISEASE: PUTTING NEW PREVENTION TOOLS TO WORK IN LOW- AND MIDDLE-INCOME COUNTRIES
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Background: Cervical cancer is the leading cause of cancer mortality among women in many developing countries. 80% of the world’s cancer deaths occur in developing countries where access to screening, diagnosis, and treatment is limited. Cervical cancer is stigmatized in many communities and may cause women to be ostracized from family, friends and sources of care. Genital warts caused by HPV cause reproductive complications and shame; many recur despite treatment.

New Prevention Tools: Several new tools to prevent cervical cancer and genital warts can be put to work in low- and middle-income countries (LMIC). Two prophylactic HPV vaccines are highly efficacious in preventing precancerous cervical lesions and/or warts. Projects in several LMIC countries demonstrate that these vaccines are safe, acceptable, and readily delivered through schools or campaigns. Public sector programs in a few middle-income countries are now introducing vaccines as a result of more affordable prices. A rapid, HPV DNA screening test suitable for low-resource clinics in LMIC has been shown to be a highly sensitive. In an Indian trial in which a single round of this test was combined with diagnostic colposcopy and treatment, cancer incidence and mortality declined significantly. If scaled up, screening with rapid HPV tests could be used in LMIC where cytology is infeasible. Expanded HIV treatment in LMIC may reduce the immune suppression associated with rapid progression of HPV infection to precancers and severe genital warts. Recent inclusion of morphine on essential drug lists of many LMIC should increase access to palliative cancer care.

Next Steps: Collective advocacy of programs in cervical cancer control, vaccination, and HIV treatment is needed to increase awareness of and access to these new prevention tools, to develop implementation methods in LMIC, and to secure funding for sustained integration into health systems.

WHAT WOMEN WANT: RESULTS FROM RECENT SURVEYS
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In the UK, it has been estimated that the screening programme saves approximately 4,500 lives every year. Nevertheless, even in the UK, approximately 2800 women per year still develop cervical cancer (Peto et al, 2004) and the diagnosis and treatment of pre-cancerous cervical abnormalities results in significant anxiety. HPV testing has greater sensitivity than cytology, but gives more ‘false alarms’ and the psychosocial consequences of testing positive can be very damaging for women and their partners (Cuschieri et al, 2006). Primary prevention of HPV infection with a vaccine, is an obvious goal. However, despite the remarkable efficacy shown by the vaccines in trials, around 20% of cervical cancers will not be prevented by vaccination. It is clear that screening programmes, where they exist, will need to adapt when HPV vaccination becomes widespread. HPV testing of some kind is likely to be the way forward, but this raises many issues - not least the high transient positivity rates in women under 35. The introduction of HPV vaccination could reduce the number of colposcopy referrals by 26% and the number of excisional treatments by 69% (Paavonen et al, 2009). A modelling study from the Netherlands has suggested that adding vaccination to the screening programme will be cost effective. Meanwhile, a public health impact analysis in Germany has estimated that implementation of HPV vaccination could reduce the number of cervical cancer deaths from 1376 to 250 per year (Schnieder et al, 2007).

Surveys of the needs and wishes of women (eg Voice of Women survey, Needs of Network members (April 2009 audit), 1000 Femmes 1000 Vies) all show that women are anxious with regard to the results of cervical smear and HPV tests and that they very much want more information. The challenge is how to provide this in a format that is acceptable, accessible and understandable.

References
Peto J, Gilham C, Fletcher O & Matthews FE. The cervical cancer epidemic that screening has prevented in the UK. Lancet 2004; 364 (9430): 249-56
The Pap smear has been the most successful cancer prevention screening test to date. However, during its first 40 years of use it was not known to reflect the cellular changes of a sexually spread virus. As a result, most women did not know of this connection and many still do not. Two developments in the last decade have changed this knowledge-base significantly. The first was the introduction of HPV testing in the management of abnormal cervical cytology and later, in primary cervical screening, directly connecting through clinical practice the link between the virus and cervical screening. The second was the extensive media and educational campaign that accompanied the introduction of the HPV vaccine. Unfortunately, many clinicians continue to avoid discussing with their patients the origin of abnormal Paps and cervical cancer. Part of this avoidance is secondary to concern over the time that many perceive would be required to educate patients about such a “complicated” topic and part is secondary to concern that linking HPV to an abnormal Pap result will increase patient anxiety and result in questions clinicians often feel inadequately prepared to provide answers to. Fortunately there are a number of simple solutions to providing education to patients so that they may make the best use of science in informed decision-making. These include providing educational brochures and posters in the waiting or exam rooms, educating medical assistants regarding HPV in order so that they may help answer patient questions, listing informative websites that carry reliable expert-reviewed information on HPV, and utilizing clinician time efficiently during the exam to focus on short but information-rich preventive health messages that includes both options for primary prevention and preparation for the patient to better understand cervical screening and HPV. In this part of the WACC Forum we will discuss the responsibility of health professionals in educating their patients on both primary and secondary prevention of cervical cancer, and will provide an overview of the tools by which this may be accomplished within the framework of a routine well-woman's health exam.

Very important public health targets have been discussed and agreed upon by the international community that are directly linked with cervical cancer prevention and early diagnostics. In 2001, the Millennium Development Declaration set the goals and targets for the next 15 years and became the strategy for politicians, economists as well as health systems specialists. The World Health Organization (WHO) has assisted its Member States to reach the Millennium Development Goals by developing more detailed strategies and policies and assisting many countries in capacity building of human resources. In 2004 during the World Health Assembly, representatives of 191 countries approved WHO reproductive health strategy to accelerate progress towards the attainment of international development goals and targets. One of five core aspects is “combating sexually transmitted infections including HIV, reproductive tract infections, cervical cancer and other gynaecological morbidities”. Creating a dynamic environment of strong international, national and local support for rights-based sexual and reproductive health (SRH) initiatives is necessary to overcome inertia, galvanize investment and establish high standards and mechanisms for performance accountability. Political commitment and advocacy must be sufficiently strong to sustain good policies and programmes. Countries are urged to build strong support for investment, to mobilize crucial constituencies, to support a national SRH agenda, to create supportive legislative and regulatory framework and make concerted use of the mass media. The results of the work carried out in the area of cervical cancer prevention and management differs a lot between the countries of the WHO European Region. Many challenges still remain including surveillance, analysis of the data, priority setting and comprehensive health systems approach. WHO is assisting public health officials to find the most effective way in their countries to decrease morbidity and mortality from cervical cancer by helping to introduce organized cervical cancer screening, improving the quality of services or evaluating the effectiveness of actions taken. Examples from the countries of the WHO European Region will be shared during presentation.
Human Papillomaviruses infect many different epithelial sites, with a subset of Alpha types causing mucosal lesions. Amongst these are the high-risk papillomaviruses such as HPV16 and 18 that cause cervical cancer, as well as the low-risk types such as HPV11 that usually cause only benign papillomas. Comparative analysis has identified many similarities in the way that these different HPV types behave following infection, but has also revealed key differences in their biology. These differences reflect their diverse life cycle strategies and transmission routes, and underlie the significant differences in the diseases that they cause. Thus Beta HPV types cause widespread in-apparent infections that usually go unnoticed in immuno-competent individuals, whereas Alpha types have a plethora of immune-evasion strategies which can facilitate the persistence of visible papillomas. Of particular importance is the nature of viral gene expression in the epithelial basal cells, and the variation in expression patterns at different sites. For the high-risk HPV types, which infect a variety of different genital sites, aberrant viral expression at the transformation zone is thought to underlie neoplastic progression and facilitate the accumulation of secondary genetic changes in the host cell genome that eventually leads to cancer. The interaction with the immune system is important in determining whether lesions persist, regress or become latent, and remains an important area for future research.

**PS 2-1**

**CELLULAR NETWORKS TARGETED BY THE HPV EARLY PROTEINS E6 AND E7 AND THEIR LINK WITH ONCOGENESIS**

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**Objectives:** The pathogenesis of human papillomaviruses (HPVs) results from complex viral and host factor relationships mainly driven by the interplay between the host proteome and the early viral proteins E6 and E7, two key factors playing a major role in the oncogenic properties of HPVs. These two small proteins interact and often induce the degradation of numerous cellular proteins involved in immune response control and apoptosis, and also modify the proliferation behaviour of infected cells, thereby becoming competent for viral replication. Such proliferation can degenerate into immortalisation and transformation of the infected cells, leading to invasive cancer. Deciphering virus-host protein-protein interactions (PPI) is the easiest way to advance our understanding of the infectious process and conceptualise it in molecular terms. Our goal is to characterize the links between cellular networks targeted by E6 and E7 and HPV’s pathogenic traits.

**Methods:** To gain insight into their pathological relevance these interactomics studies must be able to discriminate between generic viral housekeeping PPI and pathogenic interactions. Taking in account the extreme diversity of HPVs, we have mapped E6 and E7 virus-host protein-protein interaction networks for a representative set of 11 HPVs corresponding to different tropisms and pathologies. Robustness of the resulting dataset is achieved by combining, two orthogonale approaches, yeast two-hybrid (Y2H) and a newly designed high-throughput Gaussia luciferase-based protein fragment complementation assay (PCA). The completeness of E6 and E7 interaction datasets for different genotypes allowed us to develop a new comparative interactomics approach based on the overlapping of E6 and E7 interactome profiles by using 2D hierarchical clustering and correspondance analyses.

**Conclusions:** Numerous interactions and common occurrences correlated with pathogenicity provide specific hypotheses on HPV strategies for replication, persistence and malignant transformation. Identified pathogenic markers will constitute prime targets for drug design.
GENETIC REARRANGEMENTS AND TRANSCRIPTIONAL PROFILING IN HPV ASSOCIATED CANCERS

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Genomic profiling by array CGH showed that gains at chromosome arms 1q, 3q, and 20q, as well as losses at 8q, 10q, 11q, and 13q are common in cervical carcinomas. For a better understanding of the consequences of these recurrent chromosomal alterations we integrated genomic and transcriptional profiles of the same cervical carcinomas. Chromosomal gains of 1q, 3q and 20q resulted in increased expression of genes located at 1q32.1-32.2, 3q13.32-23, 3q26.32-27.3, and 20q11.21-13.33, whereas a chromosomal loss of 11q22.3-25 was related to decreased expression of genes located in this region. Thus, this integrated approach identified chromosomal hotspots with altered gene expression within large commonly altered chromosomal regions in cervical cancer. Subsequent chromosomal profiling of CIN2/3 lesions identified two subsets following unsupervised hierarchical clustering, one of which with chromosomal profiles closely resembling cervical SCCs (with characteristic gains at 1q, 3q, and 20q). These findings suggest that gains at chromosomes 1q, 3q and 20q are potential hallmarks of advanced CIN2/3 lesions with a high short-term risk of progression. To determine whether HPV-associated SCCs arising from different organs have specific chromosomal alterations in common, we next compared chromosomal profiles of cervical SCCs with HPV-positive and negative HNSCCs. Potential organ-specific alterations and alterations shared by SCCs in general were investigated as well. Unsupervised hierarchical clustering resulted in one mainly HPV-positive and one mainly HPV-negative cluster. Interestingly, loss at 13q and gain at 20q were frequent in HPV-positive carcinomas of both origins but not in HPV-negative HNSCCs. Within the group of HPV-positive carcinomas, HNSCCs more frequently showed gains of multiple regions at 8q whereas cervical SCCs more often showed loss of 17p. Finally, gains of 3q24-29 and losses of 11q22.3-25 were frequent (>50%) in all sample groups. In this study hrHPV-specific, organ-specific, and SCC-specific chromosomal alterations were identified. The existence of hrHPV-specific alterations in SCCs of different anatomical origins, suggests that these alterations are crucial for HPV-mediated carcinogenesis.

HOST EPIGENETIC CHANGES IN CERVICAL PRECANCERS AND CANCERS

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Objectives: The detection of specific methylated genes could yield biomarkers for cervical carcinogenesis. Over the last decade, a large number of studies evaluating methylation of host genes in cervical disease have been published. Here, we summarize the results of published methylation studies analyzing cervical tissues and cells, including the specimen types, markers and assays evaluated and to assess the opportunities and challenges facing this line of research.

Methods: A systematic literature review was performed to identify the most promising methylation marker candidates for cervical carcinogenesis. Most markers have been analyzed based on previous studies in other tumor entities.

Conclusions: 15 markers, DAPK1, RASSF1, CDH1, CDKN2A, MGMT, RARB, APC, FHIT, MLH1, TIMP3, GSTP1, CADM1, CDH13, HIC1, and TERT have been analyzed in 5 or more studies. The published data on these markers is highly heterogeneous, with methylation frequencies from 0-100% reported for the some markers in different studies. Stratification for material analyzed and methods used did not resolve the heterogeneity. Only three markers, DAPK1, CADM1, and RARB, showed homogeneous results in most studies and had increased methylation frequencies in cervical cancers. Based on these data, there are currently no convincing methylation marker candidates to be used in cervical cancer screening. Methylation profiling studies of cervical carcinogenesis might yield new candidates that are more specific for HPV-related carcinogenesis. New candidate markers need to be thoroughly validated in highly standardized assays.
High risk human papillomaviruses (HR-HPVs) infect squamous epithelial cells of the anogenital and oral tract as well as of the skin. The rate of associated lesions depends on the anatomical location of the infected epithelium, endocrine, genetic and immunological parameters. Despite their wide distribution they usually cause lesions that may undergo neoplastic transformation only in very few particular epithelial sites. In this context we investigated the molecular signatures that regulate papillomavirus gene expression. Data that we collected suggest that there are three distinct modes of HPV gene expression:

i. the abortive infection during that no viral gene expression and no pathogenic effect of the virus can be observed;

ii.) the permissive infection during that viral gene expression occurs in a strict squamous differentiation dependent manner; and

iii.) the transforming infection during that the HPV oncogenes E6 and E7 are up-regulated in basal and parabasal cells, leading to chromosomal instability and subsequent neoplastic transformation. Epigenetic modification of the viral genome appears to be the underlying regulatory mechanism that governs these three phases of the HPV life cycle.

At the conference we will discuss distinct changes of the viral epigenetic signature that have a deep functional impact and explain many of the clinical features induced by HPV infections.
INTEGRATION OF CERVICAL CANCER SCREENING AND HPV VACCINATION PROGRAMS

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Objectives: Describe progress in integrating cervical cancer screening and efforts to deliver HPV vaccine effectively to priority populations since licensing of the quadrivalent HPV vaccine in 2006. Discuss efforts, successes, gaps and continuing barriers to achieving provider and community participation in delivery of HPV vaccine and effective and timely cervical cancer screening.

Methods: Analyze current data in both economically advantaged and disadvantaged communities about efforts to integrate HPV vaccine programs into existing cervical cancer screening programs. Study progress towards achieving a positive impact of community education on the value and importance of delivering the complete HPV vaccine series to pre-teenage and teenage girls before sexual debut, and the importance of beginning screening for cervical cancer precursors among women beyond age 21.

Results and Conclusions: Report will show that integration efforts in many areas are in the early stages of development, and while there is widespread acceptance and uptake of the vaccine among adolescents in developed and economically advantaged areas, integration with existing screening programs and bridging between vaccine for teenagers and regular cervical cancer screening among older cohorts of women is a work in progress. Because of a real concern for increasing incidence of cervical cancer among unscreened women, efforts must continue to educate providers and women about the importance of regular cervical cancer screening even among those receiving HPV vaccine. In middle-income and developing countries with high incidence of cervical cancer, and less opportunities for screening, efforts to make HPV vaccine a priority for children and adolescents, and the support of organizations like the United Nations and GAVI should be strongly encouraged.

CERVICAL CANCER RISK STRATIFICATION

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Advances in vaccination, screening, and diagnosis make it increasingly possible to prevent cervical cancer. However, if misused or poorly understood, these new tools will only increase costs and potentially harm patients without benefit. As a framework for standardized care that maximizes patient safety and well-being, a risk model should be adopted to guide clinical management now and in the future. The model would use thresholds of increasing risk for cervical precancer and treatable cancer to guide clinical decision-making for screening intensity, diagnostic evaluation, or treatment. For example, carcinogenic human papillomavirus (HPV)-negative women are at very low risk of cervical precancer and cancer (<1%) over the next 5-10 years and can be screened less frequently, i.e., longer screening intervals between screens. Extending screening intervals among carcinogenic HPV negative women is necessary for preventing over-diagnosis and treatment since newly-detected HPV infections convey much lower risks of concurrent and future cervical precancer and cancer than prevalently-detected carcinogenic HPV infections of unknown duration. Such a model would help integrate screening and HPV vaccination because HPV vaccination will variably reduce the risk of cervical precancer and cancer depending on the age of vaccination (relative to the age of sexual debut). More generally, the predictive values of a screening result will differ based on past history such as HPV vaccination and screening history. The NCI team is now working on developing a risk calculator to integrate past and current risk stratifiers and modifiers to provide risk estimation in a user-friendly manner. Importantly, experts would establish risk thresholds and stratum for clinical actions based on the patient risk to benefit ratio, independent of current (e.g., cytology, carcinogenic human papillomavirus testing, and colposcopy) and future methods of measuring risk (e.g., HPV genotyping and p16 immunostaining). Decisions regarding clinical management would be based on individualized risk measurement rather than increasingly complicated algorithms that are often difficult to understand and poorly adopted. A risk management model for cervical cancer prevention, based on appropriate clinical actions that correspond to risk stratum, can result in better allocation of resources to and increased safety for women at the greatest risk and increased well-being for women at the lowest risk.

Reference List

Mass, organised cytology-based screening programmes have been associated with a dramatic reduction in the incidence and mortality from cervical cancer in those countries that have implemented the programmes successfully. In countries south of the Sahara there are no mass screening programmes and the incidence of and mortality from cervical cancer in these countries remains unacceptably high. The greatest challenges to cervical cancer prevention in countries south of the Sahara include competing health needs, lack of human and financial resources, poor health care infrastructure, the failure of many countries to invest in their own people accompanied by poor governance and accountability, widespread poverty, war and civil strife. Add to these many complexities are the requirements of cytology based screening programmes. In the past 10 years, alternatives to cytology based programmes have been investigated.

The most studied alternatives to cytology have been VIA (visual inspection with acetic acid) with and without magnification, with and without VILI (visual inspection after application of Lugol’s Iodine). Data from a number a randomised trials have shown that ‘screen and treat’ using VIA as the primary screening modality is safe, acceptable to women but with limited efficacy in reducing cervical cancer precursors in treated women. ‘Screen and treat’ using HPV DNA testing with immediate treatment using cryotherapy has been shown to be twice as efficacious as VIA and treat in reducing cervical cancer precursors. In a recent large randomised trial in India, a significant reduction in cervical cancer was demonstrated using HPV DNA as the primary screen compared to screening with VIA or cytology.

Data from RCTs provide high quality evidence that alternative protocols to cytology based programmes have the ability to impact on cervical cancer incidence in developing countries in an affordable manner. However, widespread implementation remains a major challenge in most settings and will require political ‘buy-in’ from health ministries and treasuries of African countries. Primary prevention with vaccination is another very promising alternative, however the issues around implementation remain unresolved. These will be discussed in detail.

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Objective: To describe the cervical cancer control initiatives in the Asia- Pacific region.

Methods: Various cancer control initiatives in the Asia Pacific countries are reviewed and discussed.

Observations: More than half of global burden of cervical cancer is experienced in the Asia Pacific region. Although HPV vaccines have been licensed for public health use in several countries in the region, they are available through the national immunization program in Australia only. The cost of each HPV vaccine dose varies between 60-120 USD in the region and thus out of reach for most needy in the population. Where as large scale cytology screening is in place in countries such as Australia, New Zealand, Japan, South Korea, Hong Kong SAR of China and Singapore, population based cervical screening programs do not exist in other countries. For instance, there is no large screening available in countries such as India, Indonesia and China in the region; less than a million Pap smears are taken annually in India and China. On the other hand, in countries such as Thailand and Bangladesh, there are national efforts to improve coverage of population with Pap smear and visual screening. An encouraging aspect in the region is the vast amount of research going on in many countries in the region that evaluate the operational logistics of HPV vaccination, the comparative efficacy different HPV vaccination dose regimes such two doses, the cost effectiveness different alternative cervical screening approaches such as visual screening and HPV testing, the value of single life time screening, the clinical utility of rapid HPV and affordable HPV testing and the utility of nurse providers in delivering cervical cancer prevention services. The on going and completed research studies and findings have catalyzed an increasing awareness among policy makers and the general public that may hopefully lead to more widespread introduction of cervical cancer prevention services in this vast region.
GLOBAL PREVENTION OF HPV RELATED CANCERS: WHAT WILL IT TAKE?
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The development, testing and licensure of safe and effective vaccines to prevent Human Papilloma Virus infection is one of the great medical and public health achievements of this century. HPV vaccines are now licensed in more than 120 countries worldwide, but most of these countries have the vaccine available in the private market only. Most industrial countries have licensed one or both vaccines, and have recommended and funded the vaccine for use in pre-adolescent and adolescent girls, with varying recommendations for “catch up” immunization of women under the age of 26. Since the great majority of cervical cancer occurs in developing countries where cervical cancer screening is uncommon, the real impact of HPV vaccination on HPV related cancers will occur when developing countries start to add HPV vaccine as a routine vaccine in their National Immunization Programs (NIP) with vaccine funded by governments or donors. Inclusion of the vaccine into NIPs will be greatly aided by supportive recommendations and technical help from WHO’s Expanded Programme on Immunization (EPI), and financial support for HPV vaccine from GAVI. GAVI is an international organization that funds newer vaccines to most of the children in the 72 poorest countries. GAVI support was critical to uptake of Hepatitis B and Hib vaccines into the developing world. The GAVI Board has decided to fund HPV vaccine in the future if they can raise the money to do so, if manufacturers offer a workable price, and if countries wish to obtain the vaccine. Countries will need to pay a nominal copay (up to $0.50 per dose) and GAVI will pay the remaining cost up to the negotiated price from the manufacturer. One manufacturer has pledged to give GAVI a “no profit” price, and both manufacturers have stated they will tier prices for developing country markets for wealthier developing countries not eligible for GAVI aid. Financial issues are critical for uptake of these expensive new vaccines, but many other issues exist, such as cultural resistance to communicating about sexually transmitted diseases, the anti-vaccine movement, competition from other attractive new vaccines against pneumococcal pneumonia and rotavirus diarrhea, and delivering vaccines to adolescents, which most countries do poorly. Despite these hurdles, HPV vaccine has been highly successful to date and will hopefully be available in public sector programs without the decades delay seen with Hepatitis B and Hib vaccines.

REVIEWING THE STATUS OF HPV VACCINATION: CRITICAL ISSUES
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Critical issues of HPV VLP vaccine efficacy and immunogenicity remain. As often noted, duration of protection in adolescent girls and young women continues be the most critical efficacy issue. The absence of any suggestion of waning protection several years after VLP antibodies have reached a plateau provides a reason to be cautiously optimistic that protection will be long term, and perhaps life long, at least for the vaccine-targeted types. The degree to which the two commercial vaccines protect against infection/neoplasia by non-vaccine high risk types is receiving considerable attention. Some difference in cross-protection, particularly for HPV45, is likely. However it is unclear whether this difference is due to differences in adjuvants, the constellation of epitopes displayed by the VLPs in the two vaccines, or, less likely, in trial design. Protection against anal infection and AIN in men is currently being assessed. Similar studies of anal infection/AIN in women, and studies of HPV oropharyngeal infection/neoplasia in both sexes, would help to assess the potential public health impact of the vaccines. However, the latter studies may prove challenging unless a sensitive method for monitoring oropharyngeal HPV infection is validated. Immunogenicity studies suggest that two doses of vaccine in adolescent girls can induce VLP antibody titers comparable to those induced by three doses in young women. However, the durability of the antibody response after two doses has yet to be determined. Clearly a two-dose schedule would be preferable from a public health perspective. The question is whether such a program could be implemented in the absence of efficacy data, even if the antibody responses were durable. Trials of a nonavalent VLP vaccine have begun. It will be interesting to see if immune interference, i.e. lower antibody titers to the individual types, is observed. Several studies to assess safety and immunogenicity of the vaccines in HIV infected individuals are underway. The vaccines are likely to be safe in this group, since they are non-infectious subunit vaccines. The VLP’s highly ordered and repetitive epitope display may also make them relatively immunogenic in immuno-compromised individuals. Finally, and most importantly, the effectiveness of the vaccines in general use remains to be determined.
WHAT HAVE WE LEARNED ABOUT THE CROSS-TYPE PROTECTION OF HPV VACCINES?

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Objective: In populations generally naïve to vaccine type exposures, both bivalent (HPV16/18) and quadrivalent (HPV6/11/16/18) HPV vaccines have demonstrated high efficacy against infections and disease related to vaccine HPV types. Extended vaccine cross-protection to non-vaccine types has been demonstrated with the greatest reductions observed against HPV types most closely related to the vaccine types. Differences in the type-specific cross protection between the bivalent and quadrivalent vaccines have been reported. Broad cross-protection of any HPV vaccine would enhance reductions in overall disease burden but many questions remain. For example, it is unknown whether the cross protective immunity observed in relatively short-term clinical trials will be sustained. Presumably neutralizing epitopes between various individual HPV types even when closely related are only partially shared. Furthermore given that HPV co-infections are common and HPV 16 and 18 account for the vast majority of cervical cancer and its precursor lesion CIN3, it is unclear whether HPV vaccine cross-protection will contribute substantially to long-term population-based disease reductions. Available clinical trial data and the limitations of these data when considering real-world vaccine implementation will be presented.

Methods: Available cross-protection data from clinical trials of the bivalent (HPV16/18) and quadrivalent (HPV6/11/16/18) HPV vaccines are examined and compared. Potential contributions of vaccine cross-protection to clinically meaningful disease reductions are considered. Simple models of expected versus unexpected outcomes of disease reductions and replacements during implementation of HPV vaccines are used to project population scenarios.

Conclusions: Extension of HPV vaccine prevention to non-vaccine HPV types if substantial has the potential to increase overall reductions in HPV-related disease burden. Ultimately the impact of cross-protection from current HPV vaccines will be considered in the context of eminently available next generation HPV vaccines.

IMMUNOGENICITY COMPARISON OF TWO PROPHYLACTIC HUMAN PAPILLOMAVIRUS CERVICAL CANCER VACCINES AT MONTH 18

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Objectives: Vaccine-induced protection against HPV-16/18 has been demonstrated for human papillomavirus (HPV)-16/18 AS04-adjuvanted vaccine (Cervarix®; GlaxoSmithKline Biologicals) and HPV-6/11/16/18 vaccine (Gardasil®; Merck). The immunogenicity of these vaccines was compared 12 months after a third dose (Month 18).

Methods: In this blinded study (NCT00423046), women (N=1106) were stratified by age (18–26, 27–35, 36–45 years) and randomised (1:1) to receive Cervarix® (Months 0-1-6) or Gardasil® (Months 0-2-6). In the ATP cohort for immunogenicity, neutralising antibody (nAb) responses in serum and cervicovaginal secretions (CVS) were measured by pseudovirion-based neutralisation assay, and memory B-cell and T-cell responses by ELISPOT and cytokine flow cytometry, respectively.

Conclusions: Across all age strata in women sero/DNA-negative before vaccination for the HPV type analysed, serum nAb geometric mean titres for both HPV-16 and HPV-18 were higher with Cervarix® than Gardasil® (2.4–5.1-fold higher \([p<0.0001]\) for HPV-16 and 7.9–9.8-fold higher \([p<0.0001]\) for HPV-18). Percentage of memory B-cell responders (subjects with specific B cells) was higher with Cervarix® than Gardasil® for HPV-16 (86.7% vs 58.6%; \(p=0.0112\)) and HPV-18 (74.5% vs 45.2%; \(p=0.0087\)). Corresponding geometric mean ratio (GMR) for the frequency of circulating specific memory B-cells (calculated in responders) of 1.10 \((p=0.7649)\) for HPV-16 and 2.97 \((p=0.0015)\) for HPV-18. Percentage of CD4+ T-cell responders (subjects with \(\geq 500\) cells/million cells) was higher with Cervarix® than Gardasil® for HPV-16 (92.5% vs 40.0%; \(p<0.0001\)) and HPV-18 (78.6% vs 42.4%; \(p=0.0018\)) with corresponding GMR of 2.89 \((p<0.0001)\) for HPV-16 and 2.68 \((p=0.0012)\) for HPV-18. In CVS, nAb positivity rates with Cervarix® and Gardasil® were 20.9% vs 14.0% for HPV-16 and 7.0% vs 0.0% for HPV-18. Hence, 12 months after full vaccination (Month 18), immune responses remained higher with Cervarix® than Gardasil®. Although an immunological correlate of protection is not currently defined, differences in the magnitude of the immune response between these vaccines may represent determinants of duration of protection. Long-term follow-up of immune profile endpoints for both vaccines is ongoing for this study population.
COST-EFFECTIVENESS OF HPV VACCINES

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Objectives: The objectives are to review published models that have been used to evaluate the cost-effectiveness of HPV vaccination, to highlight key differences in the methods used and explain their potential impact on findings.

Methods: The review consists of cost-effectiveness studies published in the peer-reviewed literature before September 2009. The potential impact of key methodological choices is illustrated using a flexible model that can represent different structural assumptions.

Conclusions: Model components that have an important impact on the cost-effectiveness of HPV vaccines are: herd immunity, natural immunity, grouping of HPV-types, HPV diseases (cervical cancer, other HPV-related cancers, genital warts), waning vaccine efficacy and discount rates. Despite variations in methods and results, modeling studies are producing consistent conclusions: vaccinating young girls against HPV is likely to be cost-effective, and vaccinating boys will most likely not be cost-effective in countries that can reach high coverage rates in girls. However, results from analyses examining the effectiveness and cost-effectiveness of vaccinating boys when coverage rates in girls are low (≤ 80%) and catch-up strategies have reached conflicting conclusions.

OVERVIEW OF TRIALS OF HPV TESTING IN PRIMARY SCREENING

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The evidence emerging in the last 10 years for the efficacy of HPV testing in primary cervical cancer screening has evolved in sequential steps. Many cross-sectional investigations and a few randomized controlled trials (RCT) formed the first generation of studies that provided the initial level of evidence that HPV DNA testing is more accurate than Pap cytology in detecting high-grade cervical lesions. The much greater sensitivity of HPV testing relative to cytology comes at the expense of larger referral rates for colposcopy. The second level of evidence is the verification that the extra detection and treatment of high-grade lesions will reduce the rate of these abnormalities in subsequent screening rounds, thus demonstrating that many HPV-positive lesions may persist or progress and removing them at the outset translates into more safety for the patient. The third level of evidence comes from the claim that, being more accurate, HPV testing permits extending screening intervals safely for patients that are initially HPV negative. It is only after the above three steps can be completed that a persuasive enough case can be made for HPV testing as a cervical cancer screening strategy in resource-rich countries. A fourth level of evidence should come after the incorporation of HPV testing in screening programmes, i.e., the demonstration of reduced cervical cancer incidence and mortality. Many RCTs are currently ongoing but have produced valuable preliminary findings that bolster the above 4 steps in the burden of scientific proof for HPV testing in screening. Of note are the UK’s ARTISTIC, the Dutch POBASCAM, the Swedish SWEDESCREEN, the Italian NTCC, the Indian Osmanabad, and the Canadian CCCaST studies. Two other RCTs are addressing the value of HPV testing followed by Pap triage (of HPV positives) compared with the traditional paradigm of Pap cytology with or without ASCUS triage. These are the Finnish trial and the Canadian HPV-FOCAL RCT in British Columbia.
IMPLEMENTATION OF HPV TESTING IN THE NETHERLANDS
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Several trials with long term follow-up evaluating the effect of HPV testing alone or in combination with cytology have shown the higher sensitivity and higher negative predictive value (NPV) for CIN2+ at the cost of a slightly lower specificity. About 30-50% more CIN2+ lesions are detected by HPV testing compared to cytology. Based on data from the POBASCAM and VUSA-SCREEN trials cost-effectiveness analysis has shown that implementing HPV testing is effective in terms of earlier detection of CIN2+, life years gained, incremental net costs per Qaly gained and that the screening interval can be extended with 2 years. Guidelines for HPV testing have been developed and organisational efforts are in progress. Presently, the question whether the successful cytology-based screening program should be changed in a primary HPV test based program is under review by the Heath council, which advises the Minister of Health about implementation. The advice is expected in the Q1, 2 of 2010. Criteria used for making decision about the screening program will be discussed.

IS COTESTING A Viable SCREENING STRATEGY FOR THE US?
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SCIENTIFICALLY? UNEQUIVOCALLY YES:
1) Cotesting is significantly more sensitive for CIN2+ than cytology alone. This has now been demonstrated in randomized prospective studies published in NEJM and elsewhere. The barrier to realizing these benefits in clinical practice is the current inability to effectively triage the Pap negative HPV positive woman.
2) HPV positivity in the general population in the US is low enough to make cotesting a reasonable approach. Reports of HPV prevalence in small series of young women from STD clinics or urban charity clinics should not be confused with general population prevalence.
3) The negative predictive value of a negative cotest is high enough that annual testing and the attendant morbidity of unnecessary treatment of transient lesions with subsequent increases in prematurity can reasonably be avoided.
4) Additional benefits include cancer detection in women with negative Paps; avoidance of colposcopy in the ~15% of LSIL that will be HPV negative; ability to determine the primary site of adenocarcinoma (cervical versus endometrial) diagnosed following an AGC smear.
5) Patients and providers are accepting of cotesting and of extended intervals for screening in the absence of financial disincentives. Cotesting avoids “taking away” the cherished Pap smear, which in turn reassures patients and providers and permits implementation.

POLITICALLY? NOT WITHOUT REFORM OF PERVERSE FINANCIAL INCENTIVES WHICH DRIVE ANNUAL SCREENING:
1) Provider adherence to Guidelines including interval extension is poor when nonadherence is perceived as potentially improving provider compensation.
2) 7 Years following FDA approval of cotesting <=50% of women undergoing Pap screening have an associated HPV test, including ASC triage, despite nearly universal insurance coverage.

CONCLUSION:
Until reimbursement policies are aligned with practice Guidelines, compliance with Guidelines (including interval extension with cotesting) will be problematic.
Study design: The present cross-sectional multi-center trial was designed to compare the performance of oncogenic APTIMA HPV® RNA assay (Gen-Probe Incorporated) with oncogenic Digene HPV® DNA test (Qiagen Inc.) and LBC technique, in detection of CIN2+ and CIN3+ endpoints in 5006 women undergoing routine screening for 20 to 65-year-old women in France.

Results: LBC cytology is the least sensitive 69.3% (95% CI 59.3-78.1) and the second most specific (92.5%; 95%CI 91.6-93.2) test in detecting CIN2+ endpoint. HPV DNA test is slightly more sensitive (96.5%; 95%CI 91.2-99.0) than HPV RNA test (92.9%; 95%CI 86.5-96.9) at CIN2+, but the latter (92.6%; 95%CI 91.8-93.3) is more specific than HPV DNA assay (87.8%; 95%CI 86.8-88.7). At CIN3+ endpoint, both tests are equally sensitive (96.6%) but HPV RNA test is more specific, 91.1% (95%CI 90.3-91.9) and 86.3% (95%CI 85.3-87.2), respectively. Using (verification bias) corrected and uncorrected indicators, HPV DNA test is 5% and 20% less specific than HPV RNA test, respectively, at both CIN2+ and CIN3+ endpoints. All three tests perform better among women over age of 30, but the difference in specificity in favor of HPV RNA test remains unchanged in both age cohorts. In young (<30-year-old) women, HPV RNA test has the same specificity as LBC, being 8% higher than that of HPV DNA test. Combining LBC and the HPV test leads to 3-4% gain in sensitivity but compromises the specificity, similarly in both combinations, and most severely affecting younger women, with >10% difference in test specificity between the younger and older women.

Conclusions: Given the very high sensitivity, specificity equal to LBC and the substantially higher specificity (both uncorrected and corrected) as compared with HPV DNA test, APTIMA HPV Assay seems to be an attractive candidate screening test also for younger women, among whom the performance of cervical cytology and HPV DNA tests are limited.

ROLE OF P16 AND OTHER CELL CYCLE MARKERS TRIAGING HPV POSITIVE WOMEN IN A SCREENING SETTING.
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Objectives. Methods for triaging HPV positive women are warranted.
Methods. In the NTCC trial women on DNA samples from women who were HC2 positive we performed PCR by GP5+/GP6+ and typing by RLB, viral load physical status of HPV 16 and 18 infections by real time and p16 by ICC. We computed the sensitivity and specificity for CIN2 or more severe histology and we estimated the relative sensitivity and relative referral rate vs. conventional cytology.
Results. We studied 2921 samples. If only women with infection by HPV 16 or 18 were considered as positive (group A) sensitivity was 59.5% (95%CI 52.4-66.4) and specificity 64.9% (63.1-66.7). When also including types 33, 35 and 52 (group B) sensitivity increased to 74.5% (67.9-80.4) and specificity decreased to 58.4% (56.5-60.2). When adding HPV 31 (group C) sensitivity was 85.5% (79.8-90.1) and specificity was 47.9% (46.1-49.9). PPVs for groups A, B and C, was 11.1% (9.3-13.1), 11.6% (9.9-13.5) and 10.8% (9.3-12.4) respectively. Among women aged 35-60 yrs the relative sensitivity and relative referral rate vs. conventional cytology was 0.96 and 8.83 respectively for group A, 1.18 and 0.99 respectively for group B, 1.33 and 1.21 respectively for group C. By comparison these values were 1.53 and 1.08 respectively with triage by p16INK4A immunostaining. When excluding women with infection from HPV16 or 18 the positive predictive value (PPV) of HC2 positivity decreased from 6.9% (5.9-7.8) to 4.4% (3.5-5.3) and the PPV of LBC cytology ASCUS+ decreased from 8.8% (7.4-10.1) to 4.9% (3.7-6.0). Preliminary results on viral load and physical status of HPV 16 and 18 will be also presented.
Conclusions. Genotyping is less efficient than p16INK4A immunostaining for triaging HPV positive women. Both the PPV of cytology and that of HPV testing can be expected to be strongly reduced among vaccinated women.
In the US, primary cervical screening guidelines from both the American Cancer Society (ACS, 2002) and the American College of Obstetricians and Gynecologists (ACOG, 2003 and 2009) provided the option to screen women age 30 and over with a Pap and a HPV test (cotesting). This “revolutionary” option for primary cervical screening in the US was the result of review of the extensive literature available at the time, and subsequent discussion, debate and deliberation by expert panels appointed by each professional group (The ACS Pap Guidelines Group and the ACOG Gynecologic Practice Committee, respectively), and represents one approach to the introduction of HPV testing, or any new technology, into national guidelines. Another model, exemplified by the American Society for Colposcopy and Cervical Pathology (ASCCP) led 2001 and 2006 Consensus Conferences for the Management of Women with Abnormal Cervical Cytology and Cervical Cancer Precursors provided a broad base of initial support for recommendations incorporating HPV testing these management situations by inviting 29 major national and international organizations involved in women's health care to send voting delegates to the Consensus Conferences. Each conference was preceded by extensive literature review, drafting of potential recommendations derived from this review, posting of these recommendations for public comment and revising them accordingly. The draft recommendations were then presented with supporting evidence to the delegates at the Consensus Conference where each was subsequently voted on by the delegates. Proposed guidelines not approved by a two-thirds majority were then discussed and debated by expert work-groups, revised as deemed appropriate and taken back to the conference delegates for further discussion and vote. All guidelines were developed in this manner and only accepted when a super-majority voted affirmatively. Whether it be the introduction of HPV testing or other markers for risk for development of cervical cancer into screening and management algorithms, or decisions to continue screening with cervical cytology with, or without computer assisted screening, each of these models carries a dominant requisite: guidelines must be evidence based, and where evidence is lacking, must be based on consensus expert option. Additionally, acceptance of the recommendations is greatest when the organizations issuing the recommendations are widely respected and enhanced by a “consensus” process involving the majority of organizations with interest in the guidelines developed. These strategies will be discussed as well as present and potential future screening strategies using HPV testing as a primary screen in developed as well as developing countries, with or without, cytology.

In several countries with a cervical cancer screening programme, economic evaluations have been used to support a decision on the adoption of HPV16/18 vaccination. Economic evaluations are particularly important for HPV vaccination as vaccines are expensive and the incidence of cervix cancer is already low because of screening.

Health-economic models can be categorized into static and dynamic models. Evaluation of screening scenarios have been based on static models which calculate costs and effects for a single cohort. Vaccination scenarios, however, are more and more often evaluated by dynamic models that take into account that vaccination not only reduces the disease risk in vaccinated women but also in non-vaccinated women due to reduced exposure through herd immunity. When evaluating the cost-effectiveness of combined screening and vaccination interventions, the static screening model and the dynamic vaccination model need to be integrated to assess new optimal screening scenario for both vaccinated and non-vaccinated women. Although screening is to remain cost-effective in addition to vaccination as long as vaccines only protect against HPV 16 and 18, the length of the screening interval and the age of first screening need to be re-optimized. Furthermore, the use of HPV DNA testing instead of cytology that has been advocated for non-vaccinated populations may also lead to health-economic gains in (partly) vaccinated populations.

The aim of this presentation is to review the health-economic effects of vaccination and screening as predicted by economic models. Parameters will be discussed that influence the outcomes of health-economic studies and that are still controversial: vaccine and screening uptake rates, anticipated trends in the price of commercially produced vaccines and screening tests, and the adopted discounting rate for future costs and benefits.
MONITORING THE IMPACT OF HPV VACCINATION

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Internationally standardised HPV laboratory services will become essential for assisting in vaccine development, vaccination implementation and surveillance of HPV as evaluation/monitoring of HPV vaccination programs. First, laboratory services will be essential in continuing clinical trials globally. Optimal modes of implementation and strategies to achieve high coverage and population (“herd”) immunity will be the subject of phase IV/V trials. There is also a need for quality monitoring of vaccinations (e.g. when changing administration strategies) and for evaluation of new vaccines. Definition of susceptible populations in clinical trials requires testing for both HPV DNA and HPV antibodies. The current use of non-standardised assays is impairing progress, as trials are difficult to compare. The endpoints for trials will in the future most likely be based on early endpoints such as protection against infection, which will require standardised HPV DNA testing. Some simple trials (e.g. new vaccine batch, new method for transportation/storage et c) may use immunogenicity as endpoint, which will require standardised HPV serology. A basic ambition level for monitoring is to monitor population coverage and vaccine safety. A medium ambition level is to include monitoring of the population effectiveness of the vaccination program, which should be done using internationally comparable methodology to enable evaluation of which HPV vaccination strategies that are the most effective. As results should be available within reasonable timeframes, early evaluation possibilities are essential. Incidence of condylomas is of interest as a clinically evident disease that occurs with a short incubation time after the HPV infections. Monitoring of the spread of oncogenic HPV types will require HPV DNA testing strategies, e.g. in random population-based samples or selected sentinel clinics providing health services for teenagers. Medium and long-term evaluation of HPV vaccination programs would typically involve HPV DNA typing of HPV-associated diseases, such as high grade CIN, cervical cancer and other HPV-associated cancers. Such monitoring will also be providing increasingly solid data on the current burden of disease that would be preventable by vaccinations, which will be important in the continued prioritisation, optimisation and extension of HPV vaccination programs.

OVERCOMING BARRIERS TO ROUTINE HPV VACCINATION

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Objectives: Understand current barriers to delivery of routine HPV vaccination among priority populations and describe methods of reducing or eliminating those barriers to achieve high uptake of the vaccine and reduce the number of women needing follow up for abnormal cervical cancer screening results, and the incidence of serious pre-cancerous cervical lesions.

Methods: Identify knowledge and behavior gaps that result in non-acceptance of primary prevention tools and impact an inappropriate response to knowing that a sexually transmitted infection with HPV could ultimately result in invasive cervical cancer; review factors that contribute to hesitation or refusal to consider use of HPV vaccine; review results of surveys of physician and community about HPV related knowledge, attitudes and practices, review Community Guide recommendations for interventions that successfully increase uptake of vaccines and other preventive measures, and are more likely to increase knowledge and result in attitude and behavior change by the public. Also, review and describe adverse events reported following immunization with the HPV vaccine in the United States since June, 2006.

Results and Conclusions: Review will show that barriers to successful implementation of immunization programs exist in many settings and for a variety of reasons, including community attitudes and priorities, personal perception of vaccine safety, political will, and funding limitations due to competing health priorities. Safety is a very important issue however rates of serious complications associated with this vaccine were not greater than background rates of these conditions during 2006-2008. To achieve improved delivery of vaccine worldwide, particularly to those populations with the greatest need, we will need to provide appropriate education to clinicians and the public about the effectiveness and safety of the vaccine and propose means of dealing with the skepticism about vaccine use in general, and the stigma and emotional effects of having a sexually transmitted infection now which may result in a cancer diagnosis many years later.
ADVOCACY AND EDUCATION TO MOVE THE CERVICAL CANCER PREVENTION AGENDA FORWARD IN LOW-RESOURCE SETTINGS

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PATH

Exciting new tools for prevention of cervical cancer include HPV vaccines, HPV DNA screening tests, and simple, visual inspection methods for screening. Once broadly implemented, new strategies for screening and treatment, coupled with vaccination of those not yet infected with HPV, could reduce cervical cancer mortality in low-resource settings to the low rates common in wealthy countries.

The most rapid and extensive health benefits will result when national programs are able to offer vaccine to girls and affordable and effective screening and treatment to adult women. However, because the age groups needed to be targeted for prevention fall under different departments within health ministries, policy makers lack experience to guide the design of comprehensive cervical cancer prevention programs. Guidance also is needed for evaluating cost-effectiveness, assessing affordability and planning for sustainable programs.

Recognizing this gap, PATH and its partners prioritized providing decision-making assistance related to comprehensive programs for 2010 and 2011. The strategy includes 1) outreach to top level policy makers (such as Parliamentarians, Ministers of Health and their advisors, and donor agencies) and 2) technical assistance for mid-level program planners. The first strategy is being implemented through regional meetings, along with direct contact with key individuals in select countries. The second strategy involves in-depth work with multi-disciplinary teams, the focus being those responsible for immunization and those working on women's health.

PATH developed a new web-based interactive tool to help with planning comprehensive programs called the “Cervical Cancer Prevention Action Planner”. The planner will stimulate discussion among participants about how the tool could benefit cancer prevention planning now and how it might be improved in future.

SCREENING IN HIGH-RESOURCE COUNTRIES

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Objectives - There are multiple tests that can be used for primary cervical screening and multiple strategies for triage of women with abnormal primary screening results. Questions regarding how best to combine these various technologies, at what ages screening should be offered and what is an appropriate screening interval need to be addressed taking into account, test performance, the epidemiology of the disease in a given country, quality assurance requirements and the resources available for health care. Here we discuss the questions with regards to a high-resource country in which HPV infection is common.

Methods - We will review the literature with regards to the efficacy of conventional and liquid based cytology, HPV testing in primary cervical screening. The issue of quality assurance will be discussed. Recent studies provide results not only in terms of sensitivity and specificity to high-grade cervical epithelial neoplasia (CIN), but also in terms of duration of low risk following a negative result, and even invasive cancer incidence and mortality. We will briefly discuss the role of visual inspection, digital colposcopy and self-sampling in high-resource countries. We will consider a variety of algorithms for managing test results with low positive predictive value (such as low-grade cytological abnormalities, or HPV positive in the absence of cytological abnormality). Results on the use of various biomarkers in triage will be reviewed. Simple modelling will be used to compare the likely impact of various screening algorithms.

Conclusions - A high resource country without an established cytology based screening programme should consider introducing a programme offering 5-yearly HPV testing in women aged 30 to 50. HPV positive women should be triaged initially by cytology with repeat testing at 12 months for those who with normal cytology. A country with an established high-quality cytology-based programme should consider a staged introduction of HPV testing in such a way that it can be evaluated and abandoned if it proves to be no more effective than cytology.
Cytology based programmes have not been implemented in resource poor or many intermediate resource settings. Alternative methods to cytology have been actively investigated over the past years, with promising results in a research setting. Prophylactic HPV vaccines have introduced another potentially powerful tool in the armamentarium of prevention methods.

While Visual inspection with acetic acid evaluated in a number of cross-sectional studies, showed test characteristics similar to cytology in sensitivity with lower specificity and positive predictive value, randomised controlled trials comparing this strategy with HPV DNA testing and treat and even, cytology and treat, have not supported the efficacy of VIA and treat. VIA has many potential advantages mainly in terms of cost and providing an immediate result, but it has many drawbacks which include the subjective nature of the test, the need for ongoing training, in itself labour intensive, the lack of reliable quality control, the very low positive predictive value leading to significant overtreatment of women, coupled with the relatively low specificity. However, establishing VIA and treat programmes is currently the only protocol available to many very low resourced countries and while the impact of these programmes on cervical cancer incidence and mortality is likely to be modest to poor, it does allow for the creation of the infrastructure to provide screening and health care to older women. HPV DNA testing coupled with treatment has been shown to be much more efficacious in reducing both the incidence of cervical cancer precursors and cervical cancer in randomised controlled trials in South African and India respectively. The advent of the rapid HPV test (careHPV) which will provide a result within 2.5 hours of screening thus facilitating screen and treat approaches, offers many potential advantages over VIA.

While ongoing screening is important both in terms of cervical cancer prevention and providing health care to older women, primary prevention of cervical cancer using vaccination may be the most important tool for prevention. Vaccination is relatively well accepted and implemented in low resource settings reaching coverage of over 90% in some countries. While there are many challenges to the widespread implementation of HPV vaccination in poor countries, these are not insurmountable and with meaningful political will, should be implementable in the next few years.

**ROADMAP EUROGIN 2010: VACCINATION IN INTERMEDIATE- AND LOW-RESOURCE COUNTRIES**

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**Objectives:** To review factors that might influence opinions about vaccination in low- and medium-resource countries in Eurogin’s 2010 Roadmap.

**Findings:** The 2010 Roadmap may be influenced by several factors in low- and medium-income countries that have evolved since the 2008 Roadmap was written. These include new data on vaccine safety, immunogenicity, efficacy, and acceptability; evidence that high coverage of the three-dose series can be quickly achieved in schools and campaigns; funding constraints in organizations that subsidize vaccines related to the global financial crisis; WHO prequalification of both HPV vaccines, lower public sector HPV vaccine prices observed in middle-income countries; increasing awareness and demand for HPV vaccines among women; and innovative cervical cancer screening methods that might complement vaccination programs in low-resource settings.

**Conclusions:** This evidence suggests that acceptability, demand, and programmatic experience delivering HPV vaccines in low- and middle-income countries are growing. The challenges of affordable access, sustainable financing, scaling up vaccine delivery infrastructure, and monitoring program impact must be met to ensure widespread and equitable vaccine access.
Colposcopy and treatment costs are an important aspect of the cost-benefit evaluation of vaccination in countries with effective cervical screening programmes. The costs and benefits of vaccination are very different in countries where regular screening by cytology or HPV testing with reliable recall and treatment is impractical. There will be little short-term reduction in costs, but the eventual benefit in reduced cancer incidence and mortality should be much greater. The vaccine has no short-term effect in women who have already been infected, but a single HPV test a few years after vaccination may identify a substantial proportion of those who will eventually develop cancer, as only persistent or recurrent infections will be seen for HPV types included in the vaccine. The effect of vaccinating older women, followed a year or two later by a single HPV test and immediate treatment of infected women, should therefore be evaluated in long-term studies. Vaccination restricted to teenagers and young women cannot substantially reduce cervical cancer rates for many years, and in the absence of vaccination most lesions and infections are transient and do not need to be treated.

Universal HPV vaccination will not be achieved in intermediate and low resource countries until a cheap polyvalent vaccine is available. Such a vaccine will be produced eventually, but a reduction of a year in its development would reduce screening and vaccination costs by at least a billion euros in developed countries and prevent almost half a million cancers worldwide. The delay in producing such a vaccine is thus a crucial parameter that should be included in formal cost-benefit analyses. Developed countries might then be persuaded to devote considerable resources to a concerted collaborative effort to accelerate its development.

Objective: The purpose of the EUROGIN Roadmap is to offer updated state-of-the-art information on cervical cancer prevention to clinicians and researchers. The EUROGIN ROADMAP is intended to present emerging expert opinions and guidance in the absence of consensus.

Methods: Critical and new published information related to selected areas of primary (vaccination) and secondary (screening) prevention of cervical cancer are considered since the 2009 EUROGIN ROADMAP. Each year, the EUROGIN ROADMAP is initially developed from a selected set of topics. Speakers are invited to offering their individual expert opinions on the topics through public presentation. The ROADMAP topics are subsequently developed as a written document that is integrated and expanded on by a review group representing numerous areas of expertise.

Conclusions: Conclusions of the EUROGIN 2010 ROADMAP will be determined through an iterative process. The conclusions will be completed on-site through collaborations of the EUROGIN organizers, the ROADMAP chairpersons, the speakers and the larger group of expert reviewers.
OBJECTIVES: A subset of human papillomavirus (HPV) genotypes is responsible for ~5% of all cancer deaths globally, and uterine cervical carcinoma accounts for the majority of these cases. Their impact is greatest for women who do not have access to effective secondary preventive measures, and consequently over 80% of cervical cancer deaths worldwide occur in Developing nations. The understanding that persistent infection by this ‘oncogenic’ subset of HPV genotypes is necessary for the development of cervical carcinoma has driven the development of preventive vaccines. Two preventive vaccines comprising recombinant HPV L1 virus-like particles (VLPs) have been licensed. However, the current cost of these vaccines precludes global delivery, and they target only two of the ~15 known oncogenic HPV types, although ~70% of cervical cancer cases are attributed to these two types and there is evidence for some degree of cross-protection against other closely related types. A possible approach to broader immunity at lower cost is to consider vaccination against L2. This study shows that in women with VIN3, objective clinical responses can be achieved by therapeutic vaccination with synthetic long peptides (SLP®) derived from known cross-protective epitopes of HPV-induced high grade vulvar intraepithelial neoplasia (VIN3), a premalignant epithelial disorder, spontaneous regression of which occurs in less than 2% of patients and in which recurrence after standard treatment is high.

METHODS: Antibody responses of mice and rabbits to vaccination with HPV16 L2 polypeptides comprising residues 1-88 or 11-200 were compared with multi-type L2 fusion proteins, namely 11-200x3 types (HPV6, HPV16, HPV18), 11-88x5 types (HPV1, HPV5, HPV6, HPV16, HPV18), or 17-36x22 types (5 cutaneous, 2 mucosal low risk and 15 oncogenic types), and with a licensed quadrivalent HPV L1 virus-like particle (VLP) vaccine, Gardasil®. Vaccinated mice were challenged with HPV16 pseudovirions. The HPV16 L2 polypeptides generated robust neutralizing titers against HPV16, but lower titers against other types. In contrast, the antisera to the multi-type L2 fusion proteins, 11-200x3 and 11-88x5, induced high neutralizing titers against all heterologous HPVs tested, albeit lower than those to L1 VLPs. 11-200x3 formulated in GPI-0100 adjuvant or alum and 1018 ISS protected mice against HPV16 challenge at four months after immunization in the same manner as HPV16 L1 VLPs, whereas 11-200x3 alone or formulated with either alum or 1018 ISS was significantly less effective.

CONCLUSION: Adjuvanted multi-type L2 proteins can potentially be produced inexpensively in bacteria and they also have the promise of conferring much broader cross-type protective immunity than observed with L1 VLP immunization. However, L2 vaccine development lags behind L1 VLP vaccines and several technical hurdles remain.

Therapeutic vaccination with a synthetic long peptide (SLP®) vaccine mediated the eradication of established human papilloma virus type 16 (HPV16)-positive tumors in mice and controlled wart growth and latent virus infection in rabbits persistently infected with cottontail rabbit papillomavirus. Subsequent phase I/II studies with an HPV16 SLP® vaccine, consisting of 13 long peptides covering the HPV16 E6 and E7 anti-carcinoma accounts for the majority of these cases. Their impact is greatest for women who do not have access to effective secondary preventive vaccines. Two preventive vaccines comprising recombinant HPV L1 virus-like particles (VLPs) have been licensed. However, the current cost of these vaccines precludes global delivery, and they target only two of the ~15 known oncogenic HPV types, although ~70% of cervical cancer cases are attributed to these two types and there is evidence for some degree of cross-protection against other closely related types. A possible approach to broader immunity at lower cost is to consider vaccination against L2. This study shows that in women with VIN3, objective clinical responses can be achieved by therapeutic vaccination with synthetic long peptides (SLP®) derived from known cross-protective epitopes of HPV-induced high grade vulvar intraepithelial neoplasia (VIN3), a premalignant epithelial disorder, spontaneous regression of which occurs in less than 2% of patients and in which recurrence after standard treatment is high.

In a phase 2 trial, 20 women with VIN3 were vaccinated three times sc in the limbs with a mix of the HPV16 E6 and E7 synthetic long peptides formulated in Montanide ISA-51. The endpoints were objective clinical responses, defined as reduction of at least 50% in lesion size (partial response) or complete regressions, and HPV16-specific T-cell responses, determined before and after vaccination. The vaccine was safe, as no side effects exceeding CTC grade 2 were observed. At 3 and 12 months after the last vaccination an objective response was observed in 12/20 (60%) and 15/19 (79%) patients respectively. Nine of them showed a complete and durable regression of the lesions at 12 months and at 24 months. The strength of the vaccine-induced HPV16-specific T-cell response was significantly higher in the group of patients with a complete regression of their lesions as compared to non-responders.

This study shows that in women with VIN3 objective clinical responses can be achieved by therapeutic vaccination with synthetic long peptides that is able to induce effective HPV16-specific T-cell responses.

Literature
1. Vaccination against Human Papillomavirus 16 oncoproteins for vulvar intraepithelial neoplasia
ADENO-ASSOCIATED VIRUS (AAV) AS PROPHYLACTIC AND THERAPEUTIC HPV16 VACCINE

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Objective: Intranasally applied HPV 16 L1 recombinant adeno-associated virus (rAAV) vectors showed great promise as prophylactic HPV-specific vaccine. Here we explored the potential of vectors from different AAV serotypes for combined prophylactic and therapeutic vaccination.

Methods: Recombinant AAV5, 8 and 9 vectors expressing an HPV16 L1/E7 fusion gene were generated and used for intranasal immunization of mice. Antibodies to HPV 16 L1 in sera and vaginal washes were measured by ELISA and pseudovirion assay; HPV-specific CTLs were determined by ELISPOT.

Results: rAAV5 and rAAV9 but not rAAV8 showed efficient induction of humoral and cellular immune responses. The L1-specific antibody response evoked by the L1/E7 fusion gene was significantly lower than the one evoked by expression of the L1 antigen alone. In case of rAAV5 this deficiency was compensated by E.coli heat-labile enterotoxin (HLT) or monophosphoryl lipid (MPL) as adjuvant. Co-immunization of rAAV9-L1/E7 with rAAV5-L1 or boosting of rAAV9-L1/E7 with rAAV5-L1 dramatically increased L1 specific antibody titers without diminishing the CTL response to L1 or E7. Nasal immunization with rAAV5 or rAAV9 outperformed by far vaccination with HPV16-L1 VLPs or HPV16-L1/E7 CVLPs (with respect to humoral and cellular immune responses). Vaccination with these vectors led to a significant protection of animals against a challenge with different HPV tumour cell lines.

Conclusion: rAAV5 and rAAV9 vectors are promising vaccine candidates for a non-invasive nasal vaccination strategy.

INTRAVAGINAL APPLICATION OF IMMUNE RESPONSE MODIFIERS AFTER SYSTEMIC VACCINATION

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Objectives: Immunotherapies designed to eliminate lesions in HPV-infected women showed limited clinical results with poor correlation to the vaccine-specific immune responses measured in the blood. This strongly contrasts with the complete regression of subcutaneous (sc) TC-1 tumors observed in mice and suggests that an efficient targeting of protective immune responses to the genital mucosa (GM) as well as testing vaccine efficacy in more adequate models are necessary. Here we investigated strategies to overcome the basic immunosuppressive environment of the GM and effectively enhance the vaccine-specific immune responses in this mucosa. Finally we examined how such strategies may induce regression of genital tumors in mice.

Methods: Mice were sc immunized with an adjuvantized synthetic HPV16 E71-98 polypeptide vaccine. E7-specific CD8 T cell responses were determined by tetramer stainings, in vivo cytotoxic assays and ex-vivo IFN-γ ELISPOT. Intravaginal (ivag) immunostimulants (Toll-like receptors (TLR) agonists) were applied after vaccination. Tumor regression was evaluated in the sc TC-1 tumor model and in a new orthotopic model where luciferase-expressing TC-1 tumors developing in the mouse GM can be visualized by an in vivo imaging system.

Conclusions: The E71-98 vaccine induced 100% regression of sc tumors in both prophylactic and therapeutic settings and tumor regression was mainly provided by CD8 T cells. E7-specific CD8 effector cells were detected in the blood, different lymphoid organs and more importantly in the GM itself. There was no correlation between the responses measured in the periphery with those measured in the GM of individual mice, emphasizing the necessity to determine the immune responses in this mucosa. The additive ivag application of different TLR agonists enhanced 5 to 20 fold the E7-specific responses locally in the GM. This increased response correlated with a local recruitment of total CD8 T cells. Finally, preliminary data show that combining vaccination with ivag immune response modifiers can more efficiently regress large genital TC-1 tumors, suggesting that such strategy could be an efficient immunotherapy against HPV-16 and cervical cancer.
Objective: Numerous studies have demonstrated the relationship between persistent high-risk human papillomavirus (HRHPV) infection and development of high-grade cervical intraepithelial neoplasia (CIN) and cervical cancer. Therefore, HRHPV testing is recommended in combination with cervical cytology for evaluation of equivocal results, clinically relevant cervical lesions and as a screening adjunct for women ≥30 yr. Identification of HPV types, such as 16, 18 may help to identify women at highest risk for CIN3 and permit less aggressive management of women with other HRHPV infections, thereby potentially altering management guidelines. HPV typing will be valuable for assessment of prophylactic vaccine efficacy. A technology overview will be presented.

Methods: Various technologies have been developed for detection and typing of HPV DNA. There are 3 FDA approved assays, Digene (Qiagen) Hybrid Capture II, Hologic Cervista HPV HR Test (Invader technology), and Cervista HPV 16/18 Test. Digene HPV Genotyping RH Test is a reverse hybridization assay for identification of 18 HRHPV types using GP5+/6+-PCR and type-specific probes. Digene LQ Test uses multiplex, bead-based xMAP technology. The automated Abbott RealTime HR HPV Test detects 14 HRHPV types and differentiates HPV16, 18 and non-HPV16/18. Roche AMPLICOR HPV Test detects 13 HRHPV types and Linear Array HPV Genotyping Test detects and differentiates 37 LR/HRHPV types. Innogenetics INNO-LiPA HPV Genotyping Extra is a reverse hybridization line probe assay that identifies L1 sequences of 28 LR/HRHPV types. Other detection and typing assays include Genomica CLART HPV2 low density array (35 types) and Greinerbio-one PapilloCheck HPV-Screening (24 types). Three assays target HRHPV mRNA with the intent to improve specificity of detection of clinically relevant disease. GenProbe APTIMA HPV Assay utilizes transcription mediated amplification to detect oncogenic E6/E7 mRNA from 14 HRHPV types. NorchipPreTect HPV-Proofer Test and the bioMérieux NucliSENS EasyQ HPV use nucleic acid sequenced based amplification to detect E6/E7 mRNA of 5 HRHPV types.

Conclusions: A variety of methods are available for detection and genotyping of HPV. Selection of an HPV test must be based on analytically and clinically validated data that demonstrate reproducible and accurate performance with acceptable sensitivity and specificity for the detection of clinically relevant disease.
Cervical cancer arises through a continuum of dysplasia of the cervical epithelium extending from slight to severe cytologic atypia to cancer. The role of HPV infection is now well established in inducing cervical cancer but the molecular events underlying carcinogenic progression are poorly understood. In vitro systems have allowed to accumulate knowledge on the roles of the viral proteins in carcinogenic progression although mostly related to the viral oncogenes E6 and E7. As for the other viral proteins, their role in regulating the HPV-associated cellular transformation in vivo has been extremely elusive. For instance, the best in vitro differentiated keratinocytes systems have not yet allowed to fully understand the roles of the viral regulators such as E4, E5 or E2. In this respect, the viral E2 protein has been shown to exhibit a number of key functions in vitro that were not confirmed in vivo and remained controversial issues. We could demonstrate, for the first time, using clinical samples, that precursor lesions of HPV16-associated cervical cancers express high levels of E2. E2 staining of clinical samples followed the rules expected for detection of an immediate early viral protein, expressed before high levels of the viral oncogene E7 and onset of oncogenic transformation and correlated with our previous assumption that E2 is a transcriptional repressor of E7. This finding open up new directions in diagnosis and prognosis of cervical neoplasia as well as in new therapeutic approaches that have been so far mainly devoted to the oncogenic E6 and E7 proteins.

Objectives: The series of International Agency for Research on Cancer (IARC) HPV Prevalence Surveys, performed in over 25 regions around the world, has revealed a >15-fold variation in HPV prevalence as well as a considerable difference in age-specific curves of HPV prevalence (1). In some low-resource countries, the age-specific curve does not resemble that in high-resource countries, which show a peak in young women followed by a steep decrease with increasing age. Rather, in many populations in Asia and Africa (e.g. India, Nepal, China, Algeria, Nigeria and Guinea), HPV prevalence is similar in different age groups and, in some areas, very high also among middle-aged women. Therefore, it seems important to undertake follow-up surveys of women from areas of high-prevalence of HPV infection in middle-aged women. Such studies would help clarify the extent to which the high prevalence at middle-age represents persistence or reactivation of old infections or acquisition of new infections.

Methods: In at least three areas from Asia, sub-Saharan Africa and South America, follow-up visits will be carried out, at approximately five-year intervals. All women included in the original IARC HPV Prevalence Surveys will be recalled, interviewed again and re-tested for individual HPV types. Furthermore, two additional groups will be recruited in each area: 1) women aged 18-24 years, who were too young to participate in the original surveys, for comparison with the youngest women recruited earlier; and, 2) approximately 2,000 women aged 25-59 years to whom a simpler HPV-based screening approach (Care HPV) will be offered. Type-specific incidence, persistence and clearance between the baseline and the two follow-up surveys will be compared to the original surveys. Age-specific HPV prevalence and reporting of sexual behaviour will also be compared.

Conclusions: The follow-up data from the IARC HPV Prevalence Surveys are expected to clarify to what extent high HPV prevalence in middle- and older-aged women in these communities is related to the persistence or reactivation of old infections or acquisition of new infections, or cohort effects.

HPV testing has been clearly shown to be more sensitive than cytology, but also somewhat less specific. The lack of specificity may be in part due to the cross-reaction of the Hybrid Capture assay with low risk HPV types. New tests have been designed to avoid this cross-reaction and we need to determine how much of an improvement this will lead to. Tests based on mRNA and p16 also are aimed at only identifying persistent infections destined to progress to high grade CIN and eventually cancer, and they also require full validation in a screening context. Efficient algorithms using HPV as the primary screening modality need to be validated, and issues including the best age to start and stop screening, the interval between screens the use of cytology as a triage test and other approaches such as HPV typing, cell proliferation and cell cycle markers, need to be further elucidated. Ideally we need large studies that can demonstrate an impact of HPV testing on invasive cancer incidence.

This presentation will focus on a one critical gap in our knowledge of VLP prophylactic vaccines, establishing an immune correlate of protection, and one critical gap in our understanding of HPV therapeutic vaccines, effective trafficking of vaccine-induced T effector cells to CIN lesions. There are several reasons to believe that virion-neutralizing antibodies are the primary, if not the only, mediators of protection for HPV VLP vaccines. However, the remarkably high efficacy of VLPs in preventing persistent infection and CIN lesions in clinical trials has made it difficult to formally establish an immune correlate of protection. Most "breakthrough" infections and CINs in vaccinees occurred relatively early after vaccination, suggesting that many were manifestations of infections that were present at the time of vaccination. Hence, analyses based on these vaccine "failures" might suffer greatly from misclassification. The observation of partial cross-protection against several non-vaccine types might provide a better opportunity to establishing the level of antibodies, presumably neutralizing ones, that correlate with protection. The antibody titers of vaccinees who become infected with these types could be compared with vaccinees who do not. However, the fact many of the latter group will not have been exposed to the relevant types has to be taken into account. An alternative, or complementary, approach might be to use the murine cervicovaginal challenge model to determine the levels of vaccine-induced antibodies needed to prevent cervicovaginal infection of HPV pseudovirions after passive transfer of human sera.

Vaccine trials targeting HPV-induced CIN have for the most part failed to induce a significant clinical response. Measurements of T cell immunity have generally not correlated with responses in the cases in which regression was observed. These findings have raised questions of the quality and quantity of the cell mediated immune responses, particularly T cells, induced by the vaccines. However, an equally important question that has received relatively little attention is whether the vaccine-induced T cells reach the lesions to an appreciable extent. They may have limited trafficking to the lesions for two reasons. First, the vaccines were administered parenterally and so generate T cells that are licensed to traffic systemically. Second, CIN lesions are intraepithelial and generally are not pro-inflammatory. Therefore, there is no reason to think that the systemic T cell responses will preferentially traffic to the site of the lesion. Two strategies might serve to focus the immune responses to the lesions. First, immune response modifiers might be applied to the lesions to attract circulating immunocytes. Second, vaccination strategies that preferentially induce intraepithelial T cells in the female genital tract might be employed.
COMPARISON OF HPV AND CYTOLOGY TRIAGE ALGORITHMS FOR WOMEN WITH BORDERLINE OR MILD DYSKARYOSIS IN POPULATION-BASED CERVICAL SCREENING (VUSA-SCREEN STUDY)

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Objectives We studied the effectiveness of high-risk human papillomavirus (hrHPV) triage for immediate colposcopy in women with borderline or mild dyskaryosis (BMD).

Methods In the Utrecht province of the Netherlands, women aged 30-60 years who participated in the regular cervical screening programme were offered hrHPV testing and cytology (intervention group) or cytology only (control group). In the intervention group (n = 337), women with BMD were immediately referred for colposcopy only if the sample was hrHPV positive. Women with a hrHPV negative test were advised to repeat cytology at 6 and 18 months and were referred for colposcopy if and when the repeat test result was positive (BMD or worse). In the control group (n=329), referral of women with BMD was delayed until cytology was repeatedly positive at 6 or 18 months. The CIN3 detection rates were 10.7% (36/337) in the intervention group and 6.4% (21/329) in the control group (p=0.047). Moreover, hrHPV triaging resulted in shorter time to diagnosis (154 vs. 381 days). Although the number of colposcopy referrals was 51.5% higher in the intervention group than in the control group, the medical costs per detected CIN3 were slightly lower (4781 vs. 6235). If, in addition, hrHPV negative women had been referred back to routine screening at baseline, the CIN3 rate would have been 10.1% (34/337) and colposcopy rate would only have been 30.4% higher than in the control group.

Conclusions This study shows that hrHPV triaging of women with BMD is at least as effective for detecting CIN3 as repeat cytology, also when hrHPV negative women are referred back to routine screening.

REGARDLESS OF COLPOSCOPIC SKILL, PERFORMING MORE BIOPSIES INCREASES THE YIELD OF CIN 3 OR CANCER

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1. Though texts suggest that “Colposcopy can easily determine the location and extent of 90% of CIN lesions”, recent studies report lower sensitivities of colposcopic directed biopsy for CIN 3 or Cancer of 44%, 56.9%, and 83.7%.
2. The correlation coefficient for correlation of initial colposcopic impressions and review impressions is 0.24.
3. The mean average epithelial thickness of CIN 2/3 increases from 184 for lesions associated with colposcopic impressions of normal to 407 for impressions of Cancer suggesting that some false negative colposcopic impressions are secondary to thin CIN 2/3 which may not appear acetowhite.
4. For individual physicians, the sensitivity of colposcopic directed biopsy for CIN 3 or Cancer is between 28.6% and 81.5% (p<.001).
5. The sensitivity of colposcopic directed biopsy for CIN 3 or Cancer is higher for Cancer (90.3%) than for CIN 3 (59.2%, p=.002)
6. The sensitivity of colposcopic directed biopsy for CIN 3 or Cancer involving 3-4 quadrants of the cervix (92.3%) is higher than that for CIN 3 or Cancer involving 2 quadrants (81.1%) or 1 quadrant (58.5%, p<.001).
7. For individual physicians, the area under the quadrant-specific receiver operating curves correlating colposcopic impression with CIN 3 or Cancer is between 0.646 and 0.891 suggesting that some of the physicians are on differing ROC curves.
8. Physicians with higher area under the quadrant-specific receiver operating curves correlating colposcopic impression with CIN 3 or Cancer have higher proportions of Cancer and higher proportions of CIN 3 or Cancer involving 3-4 quadrants of the cervix suggesting that some of their greater accuracy is secondary to the population studied rather than the skill of the colposcopist.
9. For 6 of the 7 physicians studied, the yield of CIN 3 or Cancer per colposcopy increased as the number of biopsies of cervical quadrants without visible lesions increased from 0 to 4.
MANAGEMENT OF ABNORMAL PAP REVISITED: BY RISK FACTORS?
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Background: According to the time-honoured practice, abnormalities of certain degree of severity in the PAP smear should alert the referral for colposcopic examination. This practice should be completely independent of the terminology used to classify PAP smears, to take place almost as an automation, and be to equal to all women. Accordingly, colposcopic examination should be mandatory following any PAP smear with: 1) a suggestive invasive cancer; 2) the presence of L-SIL (TBS), mild dyskaryosis (BSCC) or Class III (Papanicolaou) (practice in the Nordic Countries); 3) the presence of moderate to severe dyskaryosis (BSCC); H-SIL (Bethesda); Class III-IV (Papanicolaou); 4) persistent lower grade abnormality (ASCUS, Class II, borderline) in 3 subsequent smears over a 12-month period; 5) the presence of any abnormality of the glandular cells (AGC and more). Recently, there has been an idea that whether the management of abnormal PAP smear (MAPS) could be based on the assessment of the risk profile of the patient, i.e., the risk of individual lesions to progress to different grades of CIN. In addition to oncogenic Human papillomaviruses (HPV) that are involved in practically all progressive lesions, several cofactors are needed in cervical carcinogenesis, and any such risk assessment essentially involves the question, whether different HPV cofactors are needed at different stages of CIN progression.

Objectives: To test the validity of this concept of using a risk profile as the basis of MAPS, we recently assessed whether HPV cofactors associated with disease progression to i) CIN1 are different from those required for progression to ii) CIN2 and iii) to CIN3, which would reflect genuine biological differences between CIN1, CIN2 and CIN3.

Study Design and Methods: Data of the NIS Cohort (n=3,187) and the LAMS Study (n=12,114) were combined, and cofactors increasing the risk of progression to CIN1, CIN2 and CIN3 were analysed using multinomial logistic regression models with all covariates recorded at baseline HR-HPV-positive women (n=1,105), representing a sub-cohort of all 1,865 women prospectively followed-up for two years in both studies.

Results: Altogether, 90 (4.8%), 39 (2.1%) and 14 (1.4%) cases progressed to CIN1, CIN2, and CIN3 respectively, progression times being equal in the two cohorts. Baseline HR-HPV was the single most powerful predictor of incident CIN1, CIN2 and CIN3. When controlled for residual confounding by analyzing HR-HPV+ women only, the risk profile of incident CIN1, CIN2 and CIN3 was unique, as shown by the completely different HPV cofactors associated with these three outcomes in univariate and multivariate analysis. The same holds true irrespective whether non-progression was used as the reference outcome, or if different CIN outcomes were compared with each other in multinomial regression models.

Conclusions: The present data suggest that the HPV cofactors associated with the risk for progression to CIN1, CIN2 and CIN3 are different, implicating genuine biological differences between the three CIN grades, which revisits the combination of CIN2 with CIN3 or (as suggested) with CIN1. Potentially, these data could pave the way to a new clinical practice of using risk profile assessment as the guideline of MAPS, but substantial amount of work is still needed to translate these data from a research setting to the level of individual women. The situation has its closest analogy in the use of markers as predictors of CIN; potential predictive markers shown to perform well in a research setting do not necessarily predict the clinical outcome of individual lesions, which still remains to be unpredictable.

MANAGEMENT OF ABNORMAL PAP REVISITED: BY LESIONS: GLANDULAR LESIONS VS SIL
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Management recommendations for abnormal cervical cytology and for treatment of CIN and AIS in the US are established and updated under the auspices of the American Society for Colposcopy and Cervical Pathology (ASCCP) and the American College of Obstetricians and Gynecologists (ACOG). The most recent updates were in 2006 by the ASCCP and 2007 by ACOG. The recommendations from this conference for the management of women with squamous abnormalities on cytology (atypical squamous cells-of-undetermined significance [ASC-US], atypical squamous cells-cannot rule out high grade [ASC-H], low-grade squamous intraepithelial lesion [LSIL] and high-grade intraepithelial lesion [HSIL]) are quite different from the management of women with cytologic glandular cell abnormalities (atypical glandular cells, not otherwise specified [AGC-NOS] and atypical glandular cells, favor neoplasia [AGC-“favor neoplasia”]) and adenocarcinoma in situ [AIS]. The primary reasons for these differences are secondary to differences in location of the typical squamous and typical glandular lesion and to human papillomavirus (HPV) association (or lack of it) with some glandular lesions. However, there are also significant similarities. For example, all women with ASC-H, LSIL and HSIL, as well as HPV-positive ASC-US, require colposcopy, as do all women with AGC, whether “not otherwise specified” [AGC-NOS] or “favor neoplasia”. However, only LSIL derived from postmenopausal women and ASC-US are “reflex HPV tested” to determine whether immediate colposcopy is warranted. Even though AGC includes the same word “atypical” as ASC, women with AGC are not “reflex HPV” tested initially as a determinant for need for colposcopic evaluation because some glandular cancers found after an AGC Pap are endometrial, tubal, or even ovarian, and therefore not of HPV origin. Only after colposcopy and other indicated procedures have ruled out CIN 2,3, AIS and these HPV negative non-cervical cancers in women referred for AGC-NOS is HPV testing valuable in determining further follow-up of women with glandular cell changes on cytology. Differences and similarities in the management of glandular and of squamous cytology and histology will be discussed in this session.
CS 2-1

THE DIFFICULTY OF ASSESSING EARLY INVASIVE CANCER

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The diagnosis of early invasive cancer (micro-invasion, Stage 1a) from a high grade premalignant lesion (CIN 2/3) can be difficult and will usually be determined by the pathology obtained by an excisional procedure. There are however features that will indicate the probability of an early invasive lesion. These are.

1. Irregular surface with ulceration or erosion. This feature may present as obvious stripping of the epithelium.
2. Dense aceto white changes with extremely rapid uptake of the acetic solution.
3. A large area of abnormal (atypical epithelium) usually involving at least 3/4 quadrants of the transformation zone (TZ). Previously we have shown that early invasion usually develops from large volume high grade lesions. The borders of the TZ are usually straight with rolled peeling edges.
4. Atypical and abnormal vessels are prominent. In high grade lesions coarse punctation and mosaic patterns predominate with wide distances between vessels. However these patterns of abnormal vessels give way to atypical vessels with their loops, corkscrews and pollarded vessels (defined like a tree whose top branch have been cut back to the trunk).
5. The final arbitor of exact staging will be the pathology which will define the depth of any invasion through the basement membrane, the extent of linear progression along the surface by the invasive tissue and the permeation of the capillary lymphatic spaces. These features can only be assessed by a large excisional procedure and can never be derived from a punch biopsy.

In summary colposcopy in association with pathology will define the presence of early invasive cervical cancer. It is not an easy process.

CS 2-3

THE DIFFERENTIAL DIAGNOSIS OF CERVICAL PRE-CANCER

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Objectives: The goal of colposcopy is to correctly identify cancer precursor lesions so that timely intervention may be instituted to prevent their progression to invasive cancer. However, there is a wide spectrum of lesions that very closely mimic high-grade squamous dysplasia, pre-invasive glandular disease of the cervix, and even invasive cancer.

Methods: Colposcopically-directed biopsies of cervical lesions have become widely accepted as the gold standard for the diagnosis of invasive cervical cancer and its precursor lesions. Nevertheless, this strategy is inherently subjective and dependent upon the training and experience of clinicians. \(^1,2\) Recent studies have confirmed that colposcopy, even in expert hands, has a much lower sensitivity than was previously recognized. \(^3,4\) In order to minimize diagnostic errors, colposcopists should be aware of benign lesions that may be confused with cancer precursors as well as cancer within the differential diagnosis of pre-cancer. The most common of these mimics will be presented.

Conclusion: Benign conditions such as immature squamous metaplasia, condyloma acuminatum, endocervical microglandular hyperplasia, post-radiation vascular changes, and decidual reaction, among others, must be included in the differential diagnosis of cervical pre-cancer and cancer. This presentation reviews the colposcopic features of some of the most commonly encountered cancer and pre-cancer mimics, and proposes a systematic approach to colposcopy in order to minimize diagnostic errors.

Screening for cervical cancer implies the search in the cervix and the vagina for cytological abnormalities or for oncogenic HPV types, depending on the type of primary screening test used, in asymptomatic women. In this way cervical screening includes also vaginal screening as the vagina is observed with the colposcope when an abnormal screening test triggers colposcopic examination. However it is well known that the same HPV infection or the same intraepithelial lesion has an oncogenic potential which is deeply affected by the type of tissue affected; vaginal lesions, even if similar to cervical lesions, do not behave in a similar way; vaginal cancer is very rare while cervical cancer is very frequent even if the vagina and the cervix are exposed to the same HPV types and are in the same individual; interestingly the vagina is constituted by more epithelial cells than the ectocervix, still cervical cancer is far more frequent. So colposcopy of the vagina should not induce overdiagnosis and overtreatment of clinically irrelevant lesions. On the vagina HPV infection can be revealed by the colposcope as a fine punctuation; micro-spikes can also be present; HPV infection can lead to the formation of aceto-white plaques; these plaques show the morphologic pattern of VAIN, but are often the expression of a benign and transient infection; the clinical meaning of these VAIN lesions is different from the analogous cervical counterpart as vaginal cancer development is a rare occurrence. On the contrary, VAIN can also appear as a thick tissue with vascular prominent pattern: these epithelial alterations are suggestive of progressing or early invasive lesions and should be watched and managed with great caution. Vagina can be also involved in disease originating in the vulva; vaginal extension of VIN or vulvar cancer is not uncommon. Vagina can also be involved by benign diseases originating from the vulva; while vaginal extension of vulvar lichen sclerosus is never observed, lichen planus, in particular erosive lichen planus, can involve the vagina. One disease that deserves attention is vaginal melanoma, even if this is a rare occurrence, since the amelanotic subtype is a frequent manifestation of the vaginal localization of this malignancy.

Public awareness of HPV is generally low, particularly around which HPV types cause warts and which can cause cancer. The sexually transmissible nature of the infection is of major concern and confusion to women. Qualitative studies have highlighted important features about HPV to be communicated. Reassuring information is that high risk HPV is an extremely common infection and can potentially clear up on its own, as is the knowledge that it will not cause genital warts. The fact that there is little impact on men’s health and that condoms are not necessarily protective reduced anxiety. However, women receive conflicting information, which leaves them confused and distressed (Szarewski, 2009). There is considerable variation worldwide in the type of health professional involved in interacting with the public regarding HPV and cervical cancer. With the onset of vaccination, paediatricians will be dealing with cervical cancer and HPV, probably for the first time. Meanwhile, gynaecologists and midwives are unlikely to have dealt with issues around vaccination before. In many ways, family doctors or general practitioners (GPs) and practice nurses are well placed to provide information, as they have usually been involved with both cervical screening and childhood immunisation. However, their knowledge about HPV is often poor and, in such a fast-moving research field, may be significantly out of date. Healthcare workers and the media constitute the two most preferred sources of information. Educational initiatives for health professionals are urgently needed. These must take into account attitudes, cultural issues and communication skills, as well as providing facts. Advocacy groups such as Jo’s Trust and the European Cervical Cancer Association (ECCA) are playing an increasingly important role in the education of both patients and health professionals. (Giordano et al 2008)

References


External genital warts can have 3 presentations: acuminated, papillary or flat. The more frequent warts are condyloma acuminata. Lesions are elevated and papillary. The clinical diagnosis is usually easy. But when the lesion is not typical, clinicians must be careful with look-alike lesions.

Differential diagnosis of external warts:

1. Physiologic changes:
   - Vestibular papillae
   - Fordyce glands

2. Benign tumors:
   - Syringomas
   - Acrochordon
   - Naevi

3. Sexually transmitted infections:
   - Molluscum contagiosum
   - Condyloma lata

4. Neoplasia:
   - VIN
   - Papillary cancer
   - Melanoma
   - Basel cell carcinoma

When the vulvar lesion is atypical, biopsies should be done.

Key Points

1. Candidiasis vulvovaginitis represents a frequent infection during reproductive years. Candida albicans is the most common infectious agent. Recurrent vulvovaginal candidiasis (RVVC) is defined as more than 4 episodes per year. RVVC may be caused by other species of Candida. Identification and fungigram may be useful before treatment. RVVC requires a long term preventive therapy.

2. Bacterial vaginosis (BV) is the most frequent type of infection during reproductive years. BV is frequently asymptomatic. Debate is still ongoing as being sexually transmitted or not. Recurrent BV is very difficult to treat. Treatment of partner does not seem to be useful.

3. Prevalence of Group A - B-hemolytic Streptococcus vulvovaginal infection is more important in prepubertal girls. Adults may be infected. In case of recurrence, treatment of partner may be useful.

4. Diagnosis of desquamative inflammatory vaginitis (DIV) is difficult and the etiology is unknown. Clindamycin cream represents the optimal treatment of DIV. Recurrence is frequent and should be treated with a mixture of clindamycin cream and hydrocortisone cream.
CS 3-3

VULVAL INTRAEPITHELIAL NEOPLASIA

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The importance of VIN relates principally to the symptoms it causes and its potential to progress to invasive vulval cancer. The terminology includes both squamous and non-squamous varieties. The non-squamous varieties include extramammary Paget’s disease and melanoma in-situ.

Squamous VIN

The incidence particularly in younger women has increased by 400% in the past 30 years in the USA. Most women with VIN present with symptoms - in particular pruritus, soreness, dyspareunia or lumps. Approximately 20% of women are asymptomatic. VIN lesions demonstrate a striking diversity of clinical features. Most lesions are visible to the naked eye. Approximately one third of women with VIN will have antecedent or concomitant lower genital tract neoplasia. Biopsies should be performed of the most significant lesions under local anaesthetic with a 4mm disposable punch biopsy. Occult invasive vulval carcinoma has been reported in 18-22% of excised specimens in women in whom a pre-treatment biopsy reported VIN alone.

Terminology

(see TC3)

Natural History

The natural history of VIN remains one of the contentious issues in gynaecological oncology. Evidence for the progression of untreated VIN to cancer includes morphological, molecular and clinical observations.

Management

Strategies need to be carefully balanced between the adverse sequelae (vulval mutilation, psychosexual trauma, etc.) associated with a radical approach to treatment and the potential risks of vulval cancer associated with an excessively conservative approach.

Treatment

needs to individualised.

1. Excisional techniques
2. CO₂ laser
3. Medical therapy (Imiquimod)
4. Photodynamic therapy

Recurrences occur in up to 30-50% of cases.

The introduction of prophylactic HPV vaccines has the potential to prevent half of vulval cancers and two-thirds of intraepithelial lesions in the lower genital tract.

Follow-up

Life-long surveillance of all women who have had a previous diagnosis of VIN 2-3 is essential. Approximately 4% of women treated for VIN develop vulval carcinoma during follow-up.

CS 3-4

VULVAR VESTIBULITIS

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Vestibulitis, recently renamed Vestibulodynia, or localized provoked vulvodynia, is a common cause of dyspareunia. Until 1981, dyspareunia was considered a result of vaginismus. Since then it was discovered that dyspareunia can be caused by Vestibulodynia, a physical condition characterized by hyperesthesia of the vestibule. In a population based study, 16% of women aged 18-64 reported histories of chronic vestibular burning, knifelike pain, or pain on contact that lasted three months or longer. This highly prevalent condition causes many women to abstain from intercourse. Using immunostaining by C-kit, Mast cell Tryptase and Heparanase, S-100 and PGP-5,9, we detected increased local heparanse expression, as well as subepithelial and intraepithelial hyperinnervation, and a significant increase in sub-epithelial inflammatory infiltrate, number of mast cells and degranulated mast cells, in cases of Vestibulodynia, compared to normal controls. A search for the local presence of H. Pylori proved negative.

As a result of our finding, anti-heparanse therapy is under clinical study. So far, the most effective therapy for Vestibulodynia is surgical excision of the vulvar vestibule (Vestibulectomy, Perineoplasty). Other treatments are rehabilitation of pelvic musculature using biofeedback techniques, topical oils or anesthetic creams, behavioral therapy, low dose tri-cyclic antidepressants, certain anti-convulsants, such as Gabapentin and Pregabaline, low oxalate diet, and local interferon injections.
Vulvar dermatosis are inflammatory usually chronic and relapsing conditions. They respond to topical corticosteroids. Two of them (lichen clerosus and lichen planus) may, very rarely, turn into squamous cell carcinoma.

1. **Lichen Simplex**

Lichen simplex is the most common itchy vulvar condition. It represents a reaction to chronic scratching usually associated with atopy. The lesions are mainly located on the cutaneous aspect of the vulva. Anal lichen simplex is frequently associated. The skin is thickened, pink, white or pigmented.

2. **Psoriasis**

Vulvar psoriasis is frequently itchy. The lesions mainly involve the the hairy portion of the vulva and the vulvar folds. They typically consist in red, thickened, more or less scaly and sharply demarcated plaques. Topical corticosteroids and moisturizing creams are the first line treatments.

3. **Lichen Sclerosus**

Lichen sclerosus (LS) is an inflammatory auto-immune disorder, Pruritus vulvae is the most common symptom. Vulvar LS mainly involves the mucosal aspect of the vulva (labia minora, vestibule, interlabial folds). Its main clinical features are palor, atrophy and architectural modifications (burying of the clitoris, resorption of the labia minora' contours, posterior synechiae of the labia minora.). Approximately 60 % of squamous carcinoma are associated with lichen sclerosus. However the risk of progression of VLS to cancer is low (< 5%). Precursors of squamous cell carcinoma associated with VLS are differentiated VIN, epithelial hyperplasia and classical VIN. Topical corticosteroids are the mainstay of the treatment. Surgery is needed in case of leucoplakia or focal resistance to corticosteroids or in case symptomatic interlabial or clitoral synechiae.

4. **Lichen planus**

Vulvar LP may be either erosive or not. Architectural modifications are frequently associated with erosive LP. Erosive lichen planus may involve the vagina and other mucosa. Response of erosive vulvar LP to topical corticosteroids is usually satisfactory Long term follow up is mandatory to detect complications such as VIN or squamous cell carcinoma which both are very rare.

5. **Contact dermatitis**, either irritant or allergic, is a very “popular” but rarely demonstrated cause of vulvitis.

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**CS 4-1**

**PATIENTS WITH CURRENT OR PAST HISTORY OF CIN**

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**Background:** Women with cervical intraepithelial neoplasia (CIN) and women having undergone treatment for disease such as conisation often actively ask for HPV vaccine. Since the vaccines were designed for prophylactic use the optimal time of vaccination is considered before the onset of sexual activity. Therefore vaccination of these women is often denied by physicians.

**Methods:** The current data are discussed. They clearly show that there is no therapeutic effect but that new infections and disease can be prevented after a relevant history.

**Conclusion:** The lecture should help the physician to counsel their patients on the basis of data and give them the correct information in cases of present or past CIN
Objectives: Low grade squamous intraepithelial lesions are frequently found during cervical cancer screening. They are most of the time associated with an Human Papillomavirus (HPV) infection. One can question whether the high transmission rate of HPV infection to the male partner represent a clinical risk for him and if preventive measures must be taken to prevent the occurrence of male diseases.

Discussion: More than 80 % of all LSILs are associated with HPV infections. The prevalence of HPV infection in male can range up to 40% with 60% of the male partner of LSIL female patients presenting with penile flat lesions. Nevertheless, the spontaneous cure rate for these infections is very high (90% at 5 years). HPV infections in male as in female are so frequent that it can be seen as a marker of sexual activity more than sexually transmitted diseases. Nevertheless, their consequences in female (cervical high grade lesions and cancer) are frequent. Their male counterparts are far rarer but in some patients can request deleterious treatment. The transmission prevention by the use of condoms can therefore be recommended in some selected cases even if its efficacy is not optimal.

Conclusion: Some medical situations can be seen as formal indication of condoms use in case of female cervical intraepithelial lesions.

Background: In males, anogenital infection with human papillomavirus (HPV) can lead to genital warts, penile, perineal, perianal, and anal neoplasia and cancer. Recent results have demonstrated the efficacy of the quadrivalent HPV (types 6/11/16/18) L1 virus-like particle vaccine (GARDASIL®) against HPV6/11/16/18-related external genital lesions in men (90.4% [95% CI: 69.2, 98.1]). Here we evaluate the safety and efficacy of the vaccine in a population of men who have sex with men (MSM).

Methods: 602 MSM aged 16-26 years were randomized in an international double-blind, placebo-controlled efficacy trial. Subjects received GARDASIL® or placebo at day 1, months 2 and 6 and had genital exams and HPV sampling from the penis, scrotum, perineal/perianal and intraanal area at day 1, months 7, 12 and every 6 months afterwards for up to 36 months. Persistent infection was defined as HPV6/11/16/18 DNA detected by PCR assay in 2 consecutive anogenital samples collected 6 months apart. Efficacy was calculated in a per-protocol population naïve to the relevant HPV type from day 1 through month 7. Endpoints were counted after month 7; median follow up was 2.9 years post-dose 1.

Results: Vaccine efficacy against persistent infection and any-time DNA detection related to vaccine HPV types in the per-protocol population was 94.4% (95% CI: 64.4, 99.9) and 48.8% (95% CI: 11.6, 71.2), respectively. Vaccine efficacy against external genital lesions was 79.0% (95% CI: -87.9, 99.6) (5 placebo cases versus 1 vaccine case). 70% of vaccinated MSM compared with 71% of placebo recipient MSM reported at least 1 adverse experience, with the majority being injection-site adverse experiences (58% in vaccine vs. 59% in placebo groups). There were no vaccine-related serious adverse experiences reported.

Conclusions: The quadrivalent HPV vaccine is well-tolerated and efficacious in preventing vaccine-type persistent HPV infection in MSM aged 16-26 years. More disease cases diagnosed during longer follow-up will provide more power for disease efficacy endpoints.
**CS 4-5**

**MANAGING ADVERSE EVENTS IN THE TEENS**

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Key points:
1. Adverse events in the clinical trials were common and were primarily associated with local site reactions.
2. Post licensure safety surveillance is important for all vaccine products and it remains important that health care providers have access to this data to educate their patients.
3. In the US, Vaccine Adverse event reporting system (VAERS) is a voluntary, national passive surveillance system. Manufacturer reporting to VAERS is required, but most information comes from physician, patients or other primary reports. Comparison to reported events in VAERS are made from known background rates of events associated with other vaccines as well as known rates of expected disease among the population vaccinated.
4. VAERS received 12, 424 reports of adverse events; 53.9 reports per 100,000 doses given. Of these 6.2% were considered serious including 32 deaths.
5. The majority of events include syncope, dizziness, local site reaction, nausea, headache, hypersensitivity and urticaria. Other events reported include venous thromboembolic events, autoimmune disorders, and Guillain-Barre syndrome, anaphylaxis, transverse myelitis and pancreatitis.
6. Of all reported events, only syncope and venous thromboembolic events (VTE) were elevated.
7. Manufacture guidelines now include recommendations that patients sit for 15 minutes post vaccination because of the risk of syncope and dizziness.
8. Since many of the vaccinated individuals were sexually active and on oral contraceptives, it remains to be determined if the risk of VTE is related to the vaccine or the group vaccinated. This risk was not seen in the vaccine trials.
9. Autoimmune disease remains a significant worry for providers and parents. Paper by Calleus et al suggest that the autoimmune rate, in 12-15 year olds, per 100,000 fully vaccinated girls should be between 2.8 (ulcerative colitis) to 8.4 (type I diabetes). In VAERS, the rate of autoimmune disorders was 0.2 per 100,000 doses.
10. To date, the HPV vaccine does not appear to have excessive risk compared to other vaccines. No vaccine is without risk, but the benefit of the vaccine must be considered. This information is important to relay to parents.

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**CS 5-1**

**HPV ASSOCIATED DISEASES IN PREGNANCY**

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The diagnosis and management of HPV associated diseases during pregnancy is challenging, and in many cases we don't have sufficient information to allow a definite evidence-based approach. Approximately 5% of pregnant woman will have an abnormal Pap smear and most of these lesions are LSIL. However — 1% of cervical cancers are detected during pregnancy and there is an increasing number of HSIL. Diagnosis and evaluation of these cases is not easy.

Guidelines published by the American Society for Colposcopy and Cervical Pathology (ASCCP) give general directions for the evaluation of abnormal Pap smear and management of CIN during pregnancy. Colposcopy in pregnancy is no different from that performed in non-pregnant state, but as the pregnancy advances, colposcopy becomes progressively more difficult. The primary aim of colposcopy for pregnant woman is to exclude invasive disease and defer biopsy and treatment until the woman has delivered. The safety of delaying treatment of pregnant woman has been shown in a number of cohort and retrospective studies. However if the colposcopic examination suggests that the woman may have invasive disease there is no point in taking a punch biopsy, because invasion cannot be adequately excluded by a small biopsy. What is required is a wedge or a cone biopsy at the most suspicious area taken under general anesthesia. Excision biopsies cannot be considered therapeutic and are indicated only in order to rule our invasion. As a general principle cone wedge and diathermy loop biopsies should not be undertaken if results are not going to change management.

Conization during pregnancy in the presence of suspected micro-invasive or invasive disease by cytology or colposcopy is frequently associated by significant morbidity. If early invasive cancer is diagnosed then treatment could be postponed until after delivery. Once the diagnosis of microinvasive carcinoma during pregnancy is made the patient may be safety followed up to the point of fetal viability. Depth of invasion and other prognostic features relating to microinvasive disease may influence the timing and the route of delivery.

**Conclusions**

There is no convincing evidence that the route of delivery influences the outcome of microinvasive cervical cancer. However, deep stromal invasion associated with high risk features (lymphatic or vascular space involvement) may require more radical management and delivery by cesarean section and radical hysterectomy. If invasive disease is diagnosed then timing of intervention will depend on the woman's wishes, the stage of the disease and the gestation age. Although cervical cancer in pregnancy remains uncommon, it is a clinical dilemma when it does occur. Progression of early stage cancer in pregnancy is rare and to postpone therapy until fetal lung maturity does not seem to reduce survival in women receiving standard treatment.
Many studies have shown that HIV infection is associated with increased prevalence, incidence and persistence of cervical human papillomavirus (HPV), the causal agent of cervical cancer, infection. Furthermore, HIV-positive women have been shown to carry a wide diversity of HPV genotypes with multiple concurrent types in the cervical area. As a consequence of high burden of persistent HPV infection, HIV-infected women are at high risk for cervical intraepithelial neoplasia. Few studies have evaluated the natural history of cervical disease among HIV-infected women since the beginning of the AIDS epidemics. In this population, the prevalence of abnormal cytologic findings was as high as 40% in 1999 ten years ago and remains above 25%. On the opposite, high-grade abnormalities were and are still infrequent. Women living with HIV-AIDS clearly have an increased risk of cervical cancer. Unlike the other HIV-associated cancers such as Kaposi's sarcoma and non-Hodgkin's lymphoma, the incidence of invasive cervical cancer did not decrease with the arrival of antiretroviral therapy. The field of cervical cancer may dramatically change in the future with the arrival of two highly efficacious human papillomavirus-16 and 18 vaccines supposed to prevent 70% of cervical cancers. New strategies for screening precancerous cervical lesions and cancers using HPV DNA tests in association or replacement of cytology are currently under evaluation, with promising results. Will HIV-positive women, who are now 17 million, benefit from these advances in prevention and screening?

Recent studies have shown that anal HPV infection was also in HIV-positive women and HIV-positive women were estimated to exhibit a significant increased risk for developing anal cancer as compared with the general population.

Key points:
1. HPV is extremely common after the onset of sexual activity with over 50% having cervical HPV within 3 years. Repeated infections are also common.
2. The development of LSIL is also common shortly after the acquisition of HPV
3. Over 90% of HPV and LSIL will regress in adolescents within 3 years. Only a minority continue to progress to development of CIN 2/3
4. CIN 2/3 can also develop within months after HPV acquisition; these CIN 2/3 are likely regress similar to LSIL. CIN 2/3 that develops after years of persistence are likely to persist or progress to cancer eventually.
5. Cervical epithelium in adolescents has different immune profiles based on topography (columnar vs. mature squamous epithelium). Although the adolescent cervix is likely naïve to HPV, it has overall a constitutive immune profile that will be protective and results in the high rates of clearance that is observed.
6. The high rates of HPV underscore the lack of clinical utility of HPV testing in adolescent populations
7. The high rates of regression of both LSIL and CIN 2/3 suggest that if identified, treatment is not warranted. Rather observation should be emphasized, specifically in that new infections are likely and treatment is associated with adverse events such as premature delivery.
8. High rates of regression of HPV, LSIL and CIN 2/3 also suggest that adolescents should not be screened for cervical cancer. Screening leads to confusion about treatment and triage.
9. Data from several groups have now shown that screening young women does not change cancer rates in these women or the age above (20-24 year olds). Otherwise, cancer development in young women is not predictable and likely not changed by the current methods of cancer screening.
HUMAN PAPILLOMAVIRUS INFECTION INCIDENCE IN A MULTI-NATIONAL STUDY OF MEN (THE HIM STUDY)

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Infection with the sexually transmitted human papillomavirus (HPV) is the cause of several different diseases in men and women. In the United States (U.S.) approximately 25,000 cases of cancer in men and women in 2007 were estimated to be attributable to infection with HPV. In addition to the diseases HPV causes directly in men, HPV is readily transmitted from males to females and significantly influences female disease risk, yet the natural history of HPV in men is largely unknown. Infection natural history data are an important component of decision models developed to inform HPV vaccination public policies and consensus statements. The objective of this study was to define the overall and age-specific incidence of type-specific HPV infection among men ages 18-70 yr residing in three countries: Brazil, Mexico, and the US, and to assess factors independently associated with acquisition of genital infections in men. A prospective cohort study of 1159 men evaluated every 6 months for a median follow-up of 27.5 months was conducted. Men were recruited from the general communities of Tampa, FL, US, the state of Morelos, Mexico, and Sao Paolo, Brazil.

The 12 month incidence of a new HPV infection was 39%. Overall, no significant differences in HPV incidence were observed by country or age group. Acquisition of grouped oncogenic HPV infections was significantly and independently associated with having higher number of lifetime sexual partners (HR=2.3 CI: 1.5-3.3 for 10-49 partners and HR=2.5 CI: 1.4-4.6 for >50 partners), and same sex behavior (HR=1.8 CI: 1.2-2.7 among men reporting sex only with men). In addition, incidence of oncogenic HPV statistically significantly decreased with age among circumcised men and increased with age among men who were uncircumcised. Study results support those of others indicating that HPV infection in males is independent of age, and male circumcision has health benefits in the prevention of infections that cause significant disease in men. The results from this study provide much needed data on the natural history of HPV infection in men, data essential to developing realistic cost-effectiveness models of male HPV vaccination internationally.

INTRODUCTION: HPV ASSOCIATED IN HEAD AND NECK CANCERS, IS THE VACCINATION A QUESTION OF DEBATES?

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1. Epidemiology of HPV positive head and neck cancers
2. Burden of head and neck cancers due to HPV infection
3. Risk factors of HPV positive and negative tumors
   a. Sexual behavior
   b. Alcohol and tobacco
   c. Viral aspects
4. Oral precancers and HPV
5. Epidemiology of oral HPV infection
6. Annual disease burden to HPV infection in head and neck region in Finland
7. HPV genotypes in oral mucosa
8. Risk factors of oral HPV infection
9. Sampling and HPV testing of oral HPV infections
10. Prevention of HPV infection in head and neck region
DE 1-2a

VACCINATION: ALTERNATIVE SCHEDULES: YES OR NO?

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Objectives: To review advantages and disadvantages of administering currently licensed prophylactic HPV vaccines according to the three-dose series advised by manufacturers.

Methods: Review of scientific literature and policies about HPV vaccine schedules.

Results: Several clinical practice guidelines, including those of the World Health Organization and nearly all national and regional vaccination committees, advise both vaccines be given as three doses over 6 months. These policies are based on trials showing the efficacy, immunogenicity, and safety of a three-dose series, even with substantial variation in dosing intervals; antibody titer peaks after dose three; and immunologic and modeling data suggesting that three doses optimize long-term immunity. Results of studies of the long-term immunogenicity of a two-dose and extended three-dose series and the efficacy of a two-dose series are pending. Research and evaluations in high-, middle-, and low-income countries show that schools, campaigns, and clinics can achieve high coverage of three doses when vaccinees and providers understand the value of the complete series. Providing all doses within 6 months instead of a longer interval precludes recalling vaccinees for a delayed third dose in late adolescence and may simplify measuring coverage and monitoring vaccination program impact. Three immunization encounters also maximize opportunities to offer other vaccines, health interventions, and education. Financing a three-dose series has become more feasible as vaccine prices have declined, especially in public sector markets in middle income countries.

Conclusions: While a two-dose series would be initially less expensive, more cost-effective, and less complex to deliver, adopting this approach now in the absence of evidence has disadvantages. It may be less effective at the individual or population level, it is inconsistent with the evidence base for current policy, it may confuse providers and vaccination candidates, and it may reduce opportunities for other health interventions. Ongoing studies of the immunogenicity, efficacy, and uptake of a two-dose series or extended three-dose series (i.e., over 12 or 24 months or third dose delayed to late adolescence) will determine if variations on the currently recommended three-dose series are effective, less expensive, easier to deliver, and more acceptable.

DE 1-2b

ALTERNATIVE VACCINE SCHEDULES: YES OR NO?

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Both available prophylactic vaccines have been shown safe and effective in extensive and expensive studies. Only one schedule is recommended for 9-26 years of age even if levels of antibodies are quite higher in younger population compared to the older population. Manufacturer’s recommended schedule were made on one schedule that showed the better phase II trials then phase III trials. But are the manufacturer’s recommended schedules the best economic decisions for a public health system with limited resources? Alternative trials about hepatitis A, hepatitis B and pneumococcal vaccines have shown that excellent results could be obtained with a lighter schedule than the manufacturers’ schedules.

A Canadian trial is taking place in 4 provinces (BC, On, Qc and NS) to measure the efficacy of a 2 dose calendar compared to a 3 dose program in terms of antibody production with the both available prophylactic vaccines. Also an elongated schedule 0-6-60 months schedule is being compared to the standard recommended schedule of 0-2-6 months. At this moment, Québec province as a free HPV prophylactic vaccine school based program. Females in their 4th year of primary school cycle will be offered the elongated schedule given at 0-6-60 months and females in their 3rd year of secondary school cycle given the recommended 0-2-6 month schedule. Only Québec province has decided to go forward with this elongated schedule.

Many reasons have helped made this decision: heightened immunogenicity in younger age population, spreading the doses usually helps build higher level of antibody, uncertainty about long term efficacy of the manufacturer’s recommended schedule, the possibility to boost the level of protection prior to the onset of sexual activity and operational aspect of Québec’s school based program have helped make this decision.
DE 1-3a

CATCH-UP VACCINATION: YES OR NO?

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Background: The two available VLP HPV vaccines were designed for prophylaxis of HPV infections and disease. Therefore the optimal use is considered before the sexual debut. This is the most cost effective approach, but cost effectivity is influenced by both, the medical effects and the price. The following points demonstrate that from the medical point of view it makes absolutely sense to vaccinate catch up cohorts and prevent disease:
- In the phase III trials of both VLP HPV vaccines >94% of the young women (up to 26 years) were sexually active and a high prophylactic efficacy (up to 100%) was demonstrated.
- Only a small proportion of the HPV positive women was infected with more than one type
- In the intention-to-treat populations including HPV positive women the vaccine was effective in mid term, a high efficacy has also been demonstrated in seropositive women.
- Women remain at risk for infections as long as they are sexually active and vaccinating them will prevent disease. The absolute number of reduction is the same in HPV naive or exposed populations.
- An efficacy in the order of 90% has been demonstrated in women aged 24-45 years.
- Even in women having undergone conization or treatment for genital warts, a significant prophylaxis against further disease could be demonstrated.
- Modelling shows that vaccinating a broad age range is more effective compared with vaccinating single cohorts of young girls.

Conclusion: Young and midadult women actively seek HPV vaccination and there are good medical reasons for vaccinating them. In countries without catch up programs Health economic decisions will probably be revised or extended in long term.

DE 1-4a

VACCINATION OF MALES: YES

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While the incidence of any particular HPV related cancer in men is low, the total cancer burden related to HPV in men is quite high, and similar to that experienced by women in countries that routinely screen for cervical cancer. Unlike the majority of HPV related cancers in women, there are no proven screening measures to prevent HPV related cancers in men. Of particular concern is the fact that two cancers caused by HPV in men are significantly increasing each year - HPV related oropharyngeal and anal cancers. Both cancers result in tremendous loss in quality of life both during and after treatment. Both cancers are costly to treat, and both cancers ultimately result in mortality. Prevention of the HPV infections that cause cancers in men is the only viable option to reduce HPV related cancers in men.

In addition to a direct benefit males can receive from HPV vaccination, there is the indirect benefit females will receive if males are vaccinated. Reductions in male HPV infection will ultimately lead to reduction in HPV infection and disease in females. This is especially true for countries such as the US where less than 50% of females completed the three dose sequence (currently 17% in the US). In most countries where there is not mandatory HPV vaccination it is doubtful that 75% of females will complete the three dose series of HPV vaccine in the coming decades. With a low uptake of vaccine in females, male vaccination becomes an important part of establishing herd immunity and reducing infection and disease caused by HPV in females, and under this scenario is cost-effective.

To achieve public health benefit and opportunity the HPV vaccine presents, both sexes should be vaccinated. Men should have the opportunity to directly benefit from the newly licensed vaccine for boys.
VACCINATION OF MALES: YES OR NO?
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- Genital warts
- Cost-effectiveness
- Disease burden
- Disease prevention
- Coverage
- Public health stewardship
- Preadolescent
- Herd-immunity
- Cross-protection
- Pan-vaccination

IS TYPE REPLACEMENT SOMETHING TO WORRY ABOUT: YES OR NO?
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With the advent of prophylactic HPV vaccination there has been a concern that the gradual decrease in the incidence and prevalence of infections by the vaccine-targeted HPV types may create the opportunity for other HPV types to become more common by taking up the ecological niches previously occupied by HPVs 16 and 18. A similar concern exists for HPVs 6 and 11, which are also targeted by a quadrivalent vaccine. These concerns have prompted the initiation of HPV infection surveillance studies in different populations to define the distribution of genital HPV types at baseline, prior to deploying large-scale HPV vaccination. While a biological precedent exists for the phenomenon of “type replacement” (e.g., shifts in serotype distribution post-pneumococcal vaccination) there is no empirical basis for a similar concern in the case of HPV vaccination. First, HPVs are DNA viruses with very low mutation rates, unlike the situation with pneumococci, which are highly adaptable to changes in immune status in populations. Second, the proportions of women (and men) that are susceptible to being infected with HPV types not targeted by vaccination are already very high and thus, there is no indication that prevalent infection by HPVs 16 and 18 constrains or restricts ecological niches available for other HPV types. Finally, if these two types truly exert an inhibitory effect on the ability of other HPV types to infect the genital mucosa one would expect the rate of coinfections of any non-16 and non-18 types in the presence of either HPV 16 or 18 to be lower than that expected by chance alone, based on the individual prevalences of all types. Epidemiological studies conducted in different populations have consistently shown that the observed rate of coinfections by any two types is consistently greater than the expected value assuming independence of distributions.
There is concern that the elimination of HPV types 16 & 18 that are included in both the quadravalent and the bivalent vaccines may affect the spread or pathogenicity of non-vaccine HPV types. There is as yet no evidence suggesting that there will be replacement.

It is important to remember that papilloma viruses are DNA viruses that use the replication enzymes of the host cell that have a low error rate. The diversity of the papillomaviruses has required an evolution over many millions of years. That immune selection would result in emergence of new variants of HPV is therefore unlikely to occur in the foreseeable future. If elimination of some HPVs would result in increase in other HPVs, the different HPVs would need to be competing with each other. However, data from cell biological and epidemiological studies have not found any evidence to indicate that the presence of pre-existing cervicovaginal HPV infection increases the risk of other HPV genotypes in men or women and cross-protection after natural infection appears to be limited. This implies that competition is unlikely. On balance, the available evidence would indicate that HPV infections are independent of each other, suggesting that genotype replacement is not probable.

However, only in large long-term studies and post vaccine surveillance will this question be answered.
HPV TESTING AND CERVICAL CYTOLOGY SHOULD BE COMBINED IN PRIMARY CERVICAL SCREENING
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In 2002 the American Cancer Society (ACS) and in 2003, and again in 2009, the American College of Obstetricians and Gynecologists (ACOG) recommended that one option for cervical screening of women age 30 and over be to screen with a Pap and an HPV test (cotest), and to not screen women negative both tests more often than every 3 years (routine screening in the US traditionally has been annual). One large health provider (Kaiser Permanente Northern California) reported in 2009 on the findings from over 800,000 cotests on over half a million women. The overall rate of HPV positive/Pap negative women was 3.99%, which is not burdensome. HPV testing is approximately 92-96% sensitive for CIN 3 and cervical cancer in most studies. Adding the Pap has consistently been shown to increase sensitivity of combined testing to nearly 100%. Recent ACOG Guidelines (November 2009) recommend that women having three consecutive normal Pap test results can now be screened every three years, the same recommendation as for women with a negative Pap/negative HPV cotest result. Prolonging screening intervals, whether based on cytology alone, or combined with HPV testing, benefits most women by reducing the risk of detection of transient HPV-induced events not destined to become CIN 3, AIS or cervical cancer. However, increasing the interval to 3 years in the setting of opportunistic screening, such as exists in the US and in many other countries, may result in irregular screening for many women at intervals beyond the recommended 3 years, thereby providing significant reduction in protection in a cytology-only based program. Cotesting has been demonstrated to reduce the risk of missed CIN 3+ to approximately 1/1000, offering the reassurance women require that no significant precancer or cancer is present either now, nor will likely occur well beyond the recommended 3 year interval. While testing for HPV without accompanying cytology may be the best choice in some settings, for countries without an organized screening structure and with the resources, cotesting will dominate the screening options.

HPV TESTING: WHICH TEST IS THE BEST CHOICE OF HPV TESTING TECHNOLOGY FOR CLINICAL PRACTICE
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The goal for using any screening test is to stratify populations into low- and high-risk for the disease of interest, with appropriate clinical actions/responses such as changing screening intensity, diagnostic evaluation, or treatment linked to those risks. Based on the causal role of persistent cervical infections by carcinogenic human papillomavirus (HPV) in the development of cervical cancer and its immediate precursor lesions, HPV testing is now being introduced with and without cervical cytology for primary cervical cancer screening. Carcinogenic HPV testing is more sensitive for cervical precancer and cancer than cervical cytology. Correspondingly, a negative carcinogenic HPV testing provides more reassurance against cervical precancer and cancer than cervical cytology, and therefore safely permits longer intervals between screens. However, among the carcinogenic HPV-positive women, few will have concurrent clinically-relevant disease, creating a clinical dilemma on how to identify the subset of women that require further immediate clinical attention such as colposcopy. Several approaches have been suggested to improve the predictive value for disease among the carcinogenic HPV-positive women. One approach is the use of HPV genotyping for identification of the most risky HPV genotypes (e.g., HPV16, HPV18, and perhaps HPV45) and to identify women with carcinogenic HPV-genotype specific persistence. However, the theoretical advantage of measuring approximately 15 individual HPV genotypes must be weighed against the error of 15 individual measurements. In epidemiological studies, we have found that detection of HPV16 and HPV18 to be useful. However, the individual detection of other, less carcinogenic HPV genotypes tends to be less useful because pooling of the other HPV genotypes has proven to be a good-to-excellent proxy for detecting short-term carcinogenic HPV genotype-specific persistence. Thus, detection of a pool of HPV genotypes, with limited HPV genotyping for detection of the most risky HPV genotypes, might provide the optimal balance for good clinical risk stratification.

Reference List


DE 2-3a

SCREENING IN YOUNG ADULTS: IS IT NECESSARY? (AGAINST THE MOTION)

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- Is screening ever a necessity?
- Before approaching a healthy individual, a clinician should be confident that the balance of benefits versus harms are strongly in favour of screening
- Protection against cervical cancer - incidence of cancer and relative protection offered by screening
  - Cervical cancer always rare in young adults, but incidence increasing due to earlier age at first pregnancy
  - Case-controls studies show relative protection to be less in young women
- Reassurance
- Anxiety and other psycho-social morbidity associated with abnormal screen result
- Physical trauma of treatment of cervical intraepithelial neoplasia
- Very little of which would progress to cancer whilst still young
- Possible increase in risk of preterm delivery in subsequent pregnancies
- When should screening first be offered? Any policy will be somewhat arbitrary.
- Precise balance is unknown, but harms seem to outweigh benefits. Screening in young adults is certainly not a necessity.

DE 2-3b

SCREENING OF YOUNG ADULTS: IS IT NECESSARY?

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SCIENTIFICALLY? UNEQUIVOCALLY NO:
1) Cancer rates rates under age 21 are so low that screening would not be justifiable if screening was protective, which it does not appear to be
2) Cancer rates with and without screening are virtually identical under the age of 25 and very similar between 25 and 30.
3) Countries with the lowest cervical cancer incidence start screening at age 25-30
4) Screening means treatment
5) Treatment means adverse obstetrical performance

POLITICALLY? ABOLITION OF SCREENING OF YOUNG ADULTS IN THE US WILL NOT BE POSSIBLE WITHOUT REFORM OF PERVERSE FINANCIAL INCENTIVES AND THE US LEGAL SYSTEM.
1) Insuperable forces are arrayed against abolishing screening under the age of 25
   a) US legal system incentivizes optimizing cancer prevention at the expense of prematurity
   b) US legal system “community standard of care” principle prevents gradual improvement
   c) Perverse financial incentives rule
2) Therefore it is incumbent on us to work to try to minimize harm of continued screening of young women
   a) Stop recommending screening under the age of 21 under any circumstances (ASCCP)
   b) Stop recommending annual screening under the age of 30 (ACS)
   c) Stop colpo for the first ASC or LSIL under age 25
   d) Stop LEEP for ECC = CIN 1 under age 25

CONCLUSION:
Absent the ability to change reimbursement or the US legal system, working to minimize the harms of screening of young adults is ethical and may be beneficial. “First do no harm”.
Debate points:
- The positive predictive value of both cytology and HPV DNA testing decreases considerably when women are vaccinated against HPV16/18 infection. This causes a decrease in the efficiency of cervical cancer screening.
- HPV DNA screening is more attractive than cytological screening in a vaccinated population because 1) the HPV DNA test is an objective test and 2) HPV DNA screening is more cost-effective.
- HPV DNA screening gives the possibility to reduce the number of screening rounds.
- Cytology can be used as a triage instrument for HPV DNA positive women and/or in the follow up of HPV positive cytologically normal women.
- A reduced form of cervical cancer screening will remain cost-effective in the future, even if the protection of HPV vaccines is extended to other HPV types (e.g. due to cross-protection or the development of polyvalent vaccines).
GENITAL WARTS: ABLATION-VS. CONSERVATIVE THERAPIES

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Keypoints of ablative therapies

Advantages
- Ablative therapies are physician-applied therapies, comprising electrocautery, CO2-Laser, scissor-snip excision, curettage and cryotherapy
- Success of ablation is independent of the clinical picture of genital warts
- Ablative therapies have an immediate effect
- Duration of treatment is short
- Ablative therapy is particularly attractive for men. Most men do not succeed with local therapy
- Neither age of lesions nor age of patients has any influence on the effect of ablation.
- Ablation is efficacious in both immunocompetent and immunodeficient patients
- There is no contraindication for pregnant women and children
- In case of a lesion at risk excision allows histologic examination of the whole tumor (i.e. verrucous carcinoma vs. giant condyloma acuminatum)

Disadvantages
- Anaesthesia is always necessary
- Peri- and postoperative pain may occur
- Inadequately performed ablative therapy may cause continuous harm
- If lesions are only partially ablated there is a high risk of recurrence
- Combination of ablation and adjuvant immunotherapy (i.e. imiquimod 5 % cream) lowers the risk of recurrence significantly

GENITAL WARTS: ABLATION VS. CONSERVATIVE THERAPIES

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The natural history of external genital warts is still obscure in many ways. We do know that a mean of few months may pass between the acquisition of a low risk HPV and the development of warts. We also know that some patients without treatment will get clear their warts. Patients who do clear their warts do not necessarily have antibodies against the specific HPV. There are few comparative studies of conservative (podophyllotoxin or imiquimod) vs ablative therapy (cryotherapy, laser, electrocautery, excision...). And the studies are quite difficult to interpret because of their various inclusion criteria and endpoints evaluation. Conservative therapy should be part of the management for most patients. They are less expensive and cost less in terms of human resources and health care cost. No cost/effectiveness data is available to help choose the right choice of conservative therapy or the right mixture of conservative and ablative. Some patients will not respond well to conservative therapy especially those with important immune suppression. Ablative therapy have the potential of harming the patients and should be used with caution since disappearance of lesions does not necessarily mean that the patient has been cleared of their warts.
The traditional morphology-based model of stepwise progression from Normal to CIN1 to CIN2 to CIN3 to cancer is most likely incorrect, yet histopathology remains the foundation of clinical care. In the U.S. and Europe, a diagnosis of CIN2 + is the clinical threshold for therapy. However, CIN2 as a separate diagnostic category remains a clinical enigma, given its poor reproducibility and evidence of significantly more regression of CIN2 compared to CIN3. Pathologists do not necessarily agree on even the conceptual definition of whether a CIN2 diagnosis should be considered as a low-grade or high-grade lesion. Women with CIN2 are neither younger than women with CIN3, nor is CIN2 a more common diagnosis. Both of these findings are the opposite of what would be expected if CIN 2 were indeed a precursor of CIN 3. Furthermore, the majority of women with a routine CIN2 diagnosis on biopsy are not subsequently diagnosed as CIN2 based on the review of a subsequent LEEP. In contrast, adjudicated or consensus diagnoses of CIN2 are much more likely to have a subsequent LEEP diagnosed as ≥CIN2 or ≥CIN3 and this outcome is strongly correlated with the presence of HPV16, high grade cytology and high grade colposcopic impression. Thus, CIN2 diagnoses may represent an equivocal diagnosis rather than a separate biological stage in cancer development. In its heterogeneity, CIN2 may be in some ways conceptually analogous to an interpretation of ASCUS on cytology. In our opinion, the bulk of the evidence strongly suggests that CIN2 is not a real disease state, but a misclassification of biologic CIN3 or CIN1.

In early-stage cervical cancer, when pelvic lymph nodes are negative, surgical treatment gives a very good survival rate ranging between 95 to 98%. But loss of fertility is a serious consequence of the classical surgical approach: Radical hysterectomy with pelvic lymphadenectomy. As a consequence, fertility-preserving approaches have been proposed and accepted internationally.

For FIGO stage IA1 without lympho-vascular space involvement, cold knife conisation is now recommended as a fertility-preserving treatment, since the rate of parametrium and pelvic lymph node involvement is negligible.

In patients with FIGO stages Ia2 and small IB1 disease, three fertility-preserving treatments have been proposed in the last decade:

1) Radical vaginal or abdominal trachelectomy with pelvic lymphadenectomy,
2) Neo-adjuvant chemotherapy followed by pelvic lymphadenectomy and cervical conisation,
3) Pelvic lymphadenectomy with simple vaginal trachelectomy (without parametrectomy),
4) Large conisation with pelvic lymphadenectomy (or sentinel nodes only).

Oncological results from the literature show that the recurrence rate is comparable to a radical hysterectomy with pelvic lymphadenectomy: around 3.3% for radical trachelectomy.

Obstetrical results from the literature confirm that pregnancies are possible after conservative treatments and, except for a higher rate of prematurity, and in a few centers second trimester loss, pregnancies are normal.

We can conclude that the fertility-preserving surgical techniques fulfill the requirements of radical treatment of invasive cervical cancer. The complication rate is low, the cumulative multicenter oncologic and reproductive outcome are very encouraging. It is evident though that pregnancies are at high risk.
DE 3-4a

ASSESSING RISK PROFILE: IS IT OF VALUE IN CLINICAL PRACTICE?

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- Individual behavioral and genetic risk of acquiring HPV, of HPV persistence, and of HPV-related transformation
- Tests determining the risk of prevalent disease, future disease
- Risk profile determines management decision: treatment vs. conservative management
- No fixed threshold; depends on healthcare setting, age, reproductive history, etc.
- Expectant management requires high compliance
- Risk profiles may be important to assist management decisions
- Standardization of risk assessment and adherence to guidelines is very important

DE 3-4b

ASSESSING RISK PROFILE IN MANAGEMENT: IS IT OF VALUE IN CLINICAL PRACTICE?

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Key Words:
Management, patient, clinical practice, CIN, risk profile, multinomial regression analysis, disease progression, algorithms, cytology, colposcopy, histology, gold standard
MANAGEMENT: MEDICOLEGAL CONTROVERSIES

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Key Words:
- HPV
- Infection
- Transmission
- Disease
- Cervical cancer
- Warts
- Liability
- Causal relationship
- Litigation
- Reimboursement

MEDICO LEGAL CONTROVERSIES

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Every country has its own judicial framework to solve medico legal problems. In France Doctors’ prosecution can occur towards several courts of justice pending where the complaint is lodged. It may be:

- Penal jurisdiction with eventual condemnation for unintentional injury
- Civil jurisdiction when compensation is required
- Administrative court if medical accident occurs in a public hospital
- Conseil de l'ordre which is the official French medical organization with a special jurisdiction to consider breach on deontology code.

Obligation for doctors is an obligation of means but not that of results. There is also the Kouchner law of 2002 which is an obligation of information of the patient, a free access to his (her) dossier and an agreement for treatment.

More recently was created a national office to compensate for medical accident without fault (ONIAM).

In case of prosecution, the judge will name a medical expert to have specific information on the problem before giving his sentence. On this occasion, expert will find useful every reference to the guidelines available from national health agencies or scientific societies.

Cervico vaginal pathology is not so often concerned by medico legal problems far after obstetrical or anesthetic accidents but one find judicial proceedings mainly after:

- Misdiagnosis of cancer by cytology, colposcopy or biopsy
- Misbehavior of treatment
- Complication of treatment
Background: The World Health Organization (WHO) issues recommendations for vaccine use in national immunization programs. These recommendations strongly influence decision makers in low- and middle-income countries and organizations that subsidize vaccines for low income countries such as the Global Alliance for Vaccines and Immunization (GAVI).

WHO Recommendation on HPV Vaccine Use: In April 20091, the World Health Organization recommended routine use of HPV vaccine in young adolescent girls for prevention of cervical cancer in countries where cervical cancer prevention is a priority, delivery is feasible and sustainable, and cost-effectiveness has been considered. WHO provided advice on characteristics of vaccine target populations, vaccine administration, interchangeable use of the two vaccines, vaccine product selection, delivery strategies, monitoring vaccination programme impact, integration of vaccination and cervical cancer screening, and education of vaccination candidates and parents.

Priorities for HPV vaccine implementation: Since this recommendation was released, WHO has been taking several steps to support programmes in low- and middle-income countries considering vaccine introduction. WHO continues to monitor post-marketing vaccine safety at a global level. WHO is also developing general guidance about how low- and middle-income countries might measure and monitor coverage, safety, and biological outcomes in vaccinated populations, particularly ways that are feasible, affordable, and sustainable and capitalize on existing immunization infrastructure. WHO is reviewing experience, and operations research on delivery methods in low- and middle-income countries as relevant to the introduction of HPV vaccine.

Objectives: Although vaccines are licensed with common indications in Europe, national vaccination programmes differ among states: different schedules, methods for vaccination coverage assessment and adverse events surveillance are adopted, so making comparisons between countries quite difficult. In 2006-2008, a European project, the Vaccine European New Integrated Collaboration Effort (VENICE), sponsored by EC-DG SANCO, was carried out with the aim of promoting the collection and dissemination of knowledge and best practice relating to vaccination through the creation of a collaborative European network of experts working in immunisation programmes.

Methods: A network of experts from 27 European Union member states and two EEA/EFTA countries was established; a functioning website was developed and web based surveys were used to collect, share and spread information among member states.

Conclusions: Eight web-based surveys were performed with a high participation rate. Information on individual states immunisation programmes, vaccine coverage assessment, adverse events following immunisation surveillance systems, national seasonal influenza vaccination strategy in Europe were collected. The network was also used to monitor in real time the introduction at national level of two new vaccines, the human papillomavirus and rotavirus vaccines, and to evaluate the rational approach to these vaccination policy decision-making processes.

Regarding HPV vaccination, two surveys were carried out in 2007 or 2008 to collect information about the status of its introduction in the national immunisation programmes and about studies and activities carried out, in each country, to support the decision process. An HPV vaccination updated survey was also carried out at the end of 2009, through a new project, named VENICE II, sponsored by ECDC, which started in December 2008 in order to maintain the potentiality of this true working network.
HPV DNA SCREENING
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Compared to conventional cytology, HPV-based cervical screening is known to increase sensitivity for detection of high-grade CIN and to convey an extended duration of the protective effect against CIN3 among women testing negative. The longitudinal performance of HPV DNA testing has been evaluated in several large HPV DNA screening trials. The high sensitivity of HPV DNA testing has unequivocally established that cytological screening among HPV DNA-negative women is not cost-effective and that screening algorithms with cytological screening being restricted to HPV-positive women should be contemplated.

Algorithms on how to triage HPV DNA-positive, but cytology-negative women are still being researched. Follow-up of these women with repeat HPV DNA testing and referral of women with HPV persistence has been shown to be feasible, but may require lengthy follow-up which is a burden for these women. Algorithms where these women are not followed up may be still cost-effective if HPV testing has low cost, but will not allow extension of screening intervals. We propose the use of systematic biobanking of liquid-based cytology samples, as this will allow assessing whether HPV positivity represents HPV persistence by analysis of archival samples from previous screening rounds.

With the current level of evidence, where the use of HPV DNA screening is established as an effective and safe option, the implementation research should primarily use randomized healthservices studies (RHS) to distinguish between the different screening options available. Possible strategies and suitable study designs for their implementation and evaluation will be discussed.

HOW STRONG IS THE EVIDENCE FROM THE RANDOMIZED CONTROLLED TRIALS COMPARING HPV TO CYTOLOGY SCREENING?
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Objectives: Eight randomized controlled trials (RCT) were undertaken comparing HPV testing with cytology in cervical screening, with conflicting conclusions. While reports from most RCTs concluded that HPV testing is more effective than cytology, and that the excess false positive tests can be reduced, others were more cautious. In order to reconcile these results, we performed a structured review of the RCTs.

Methods: We searched PubMed for reports on the RCT design, rates of test-positivity and CIN from both arms of the trial, and quantitative analyses of triage strategies aimed at improving specificity of HPV testing. We calculated the relative CIN detection rates and various indicators of side effects for HPV testing compared with cytology screening using the intention-to-treat approach. For the trial reporting long-term cervical cancer mortality data we evaluated the assumption of randomization producing balanced groups.

Conclusions: Firstly, the detection rate of CIN 3+ at baseline was significantly increased only in two of the RCTs (CIN 3+ data was not presented for one RCT). Two RCTs showed an approximately 50% reduction in CIN 3+ detection in the subsequent screening round, but changes in the screening modalities from the baseline to the subsequent round per se favored HPV testing. These data do therefore not show the expected effect of a switch from continued primary screening with cytology to continued primary screening with HPV testing. Secondly, we calculated that for each extra detected CIN 2+ with HPV testing, 7 extra women experienced a false-positive test compared with cytology at ≥ASCUS threshold, this number being 49 in women above 35 years in one RCT. Because of the way positive screening tests were defined in most reports, side-effects induced by follow-up with repeat testing were effectively ignored in evaluation of strategies aimed at improving the specificity of HPV testing. When repeated tests were included, only strategies defining viral load at ≥ 2 pg/ml reduced the side effects of HPV screening, whereas strategies combining primary HPV screening with cytology triage did not. Thirdly, the better mortality protection with HPV testing than cytology found in one trial probably in part resulted from imbalances unrelated to screening tests.
CHARACTERISTICS OF 44 CERVICAL CANCERS DIAGNOSED FOLLOWING PAP NEGATIVE HPV
POSITIVE SCREENING IN ROUTINE CLINICAL PRACTICE

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Objective: To characterize the cancers diagnosed following a Pap negative HPV positive screen in routine clinical practice.

Methods: Clinical Practice Guidelines for cervical cancer prevention in our organization recommend general population screening with combined Pap and HPV testing for women 30 and over. Specimens for HPV testing are collected into STM tubes and tested for high-risk HPV using Hybrid Capture II (Qiagen). Conventional Pap smears are used. Our Regional lab has been repeatedly honored for excellence in cytology by the American College of Pathology. Data was collected as part of our ongoing quality assurance and practice management activities, and analyzed and reported in accordance with our IRB approval for this work.

Results: During the study period January, 2003 through January, 2009, 44 women were diagnosed with primary invasive cervical cancer following one or more Pap negative HPV positive screens. One Pap negative HPV positive screen preceded the diagnosis in 27 women, two Pap negative HPV positive screens preceded the diagnosis in 14, and three Pap negative HPV positive screens preceded the diagnosis in 3 women. Among women so diagnosed, 20 were age 30-39, 16 were age 40-49, 5 age 50-59 and 3 women were age 60 and above. Histology included 16 pure squamous cancers, and 28 with a glandular component, including one adenosquamous tumor and one “collision tumor” (separate invasive adenocarcinomas and squamous carcinomas). FIGO Stage was IA in 11 women, IB in 31 women and IIA in 2 women. Stage distribution and age distribution were similar for squamous and glandular lesions. Treatment included a pelvic node dissection in 29; 2/29 (6.9%) had positive nodes.

Conclusions: HPV testing contributes to cervical cancer detection in women with negative Pap tests. Most women in this cohort have early stage, node negative, treatable and potentially curable disease. Glandular lesions predominate as would be expected. Endocervical curettage may prove to be an essential part of the evaluation of women undergoing colposcopy for evaluation of Pap negative HPV positive screening results. The majority of women were diagnosed after a single Pap negative HPV positive test, suggesting that for women with cancer, effective triage would be preferable to retesting in one year as currently recommended.

PRIMARY HPV TESTING IN A CANADIAN ORGANIZED SCREENING PROGRAM

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Objectives: The British Columbia Cervical Cancer Screening Program (BCCCSP) is conducting the first large randomized controlled trial (RCT) evaluating primary HPV testing followed by cytology triage of HPV positives in a North American population based screening program. Goals: establish efficacy of high-risk HPV (HR-HPV) testing followed by liquid based cytology (LBC) triage of HPV-positives for cervical screening compared to LBC alone in an organized cervical cancer screening program; establish appropriate screening interval for HPV-negative women; determine cost-effectiveness.

Method: RCT comparing LBC to HPV testing as primary screening for cervical cancer. 33,000 BCCCSP women aged 25 to 65 randomly assigned to 1 of 3 study arms:

Control: LBC testing. Negatives screen again at 2 and 4 years. Colposcopy referral at ≥LSIL or HPV-positive.

Safety-Check: HR-HPV testing. Exit screen at 2 years with LBC. HPV-positives undergo reflex cytology testing and managed same as intervention arm.

Intervention: HR-HPV testing. Negatives exit screen at 4 years. HPV-positives undergo reflex cytology testing. Exit colposcopy referral at ASC-US threshold or HPV-positive.

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

Conclusions: By August 24 2009, results available for 6698 subjects. Control: 94.9% LBC negative; 1.1% had high grade squamous intraepithelial lesions (HSIL) on LBC, and highest HSIL rates in those 25-29 yrs (2.9%). Safety and Intervention: 91.4% and 92.1% hr-HPV negative respectively. Highest hr-HPV positivity rate in those 25-29 yrs (23.8% and 25.2%) and lowest in those 60-65 yrs (3.0% and 3.0%). Demographics equally distributed in the 3 arms indicating successful randomization.

The trial will demonstrate if HPV testing as primary screening in an organised screening program will enhance cervical cancer precursor detection, allow for extension of the screening interval, and be cost-effective.
EVALUATION OF HUMAN PAPILLOMAVIRUS DNA DETECTION TEST IN PARALLEL WITH CYTOLOGY TEST IN 25000 SELECTED WOMEN UNDER HPV SCREENING PROGRAM FOR CERVICAL CANCER.

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6 Hospital Universitario de Salamanca, Spain. 7 Genómica S.A.U, Coslada, Madrid, Spain.

Introduction: Infection with human papillomavirus (HPV), especially high-risk (HR) HPV types, is necessary for the development of cervical cancer, the second-most prevalent cancer in young women around the world. As a part of prevention and detection screening program in Castilla y Leon region from Spain we have studied the presence of HPV by detection of the different genotypes together with the detection of cytological abnormalities among random women population.

Methods: A group of more than 25000 women aged 35-64 were under screening routine using an array-based HPV genotyping test CLART®-HPV (GENOMICA) and cervical smear test (Papanicolau test) in screening performed between October 2008 and April 2009 were studied retrospectively.

Conclusions: Among a group of n=25266 women studied between the age of 35 and 64 years, 1.6% presented any cytological abnormalities and 6.33% presented HPV DNA positive detection, being HPV testing more sensitive than cytology. Analyzing the relation between the different HPV genotypes identified from a subset of 2129 women who showed positive cytologies we determined that the most prevalent HPV genotype identified is HPV16, detected in 11.93% of the positive cytologies and in 0.96% of total women screened, meaning a low presence of this genotype in general population in order to assess massive vaccination programs. The second HR-HPV type (18) showed a prevalence of 2.91% of positive cytologies. The rest of HPV types detected (6- 89) shows a prevalence ranging from 0.09 to 8.12% of positive cytologies. Analyzing the presence of two or more HPV genotypes we found a rate of 21% of coinfections versus a rate of 79% of presence of unique genotypes, and we detected a rate of 6.76% detections of any type of HPV in negative cytologies versus 1.16% HPV negative detections in positive cytologies. Cytology tests followed by HPV detection and genotyping using the CLART®-HPV assay allowed an optimal identification of women at risk of development of cervical cancer, meaning a reliable tool for monitoring the prevention and detection of HPV and for assessing the implementation of massive vaccination programs from Public Health Care Administrations.

PILOT PROJECT ON HPV TESTING AS PRIMARY TEST FOR ALL INVITED WOMEN WITHIN SELECTED ORGANIZED SCREENING PROGRAMS IN NORTH-EAST ITALY

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Objectives: To evaluate the feasibility of utilizing HPV test for high-risk types as primary test in organized screening for cervical cancer. Women and health professional needs have been taken into account in planning organizational procedures and written information to women.

Methods: In Italy women 25-64 years old are invited for a new screening episode every 3 years. The pilot program will finally involve five screening programs in two areas of the Veneto region, for a total of 311.810 target population. Two samples are being obtained for all screened women; for conventional cytology and for HPV test (conventional cytology only for virgin women). Cells collected in STM medium are centrally analysed by Hybrid Capture 2 (HC2, Digene, Qiagen, high risk probe set) assay, according to the manufacturer’s instructions (cut-off 1 pg/ml). Women with negative HC2 result receive a letter with invitation for the next screening episode. For women with positive HC2 result, the Pap smear is stained and read according to the Bethesda 2001 system; if ASC-US or more severe, women are referred to immediate colposcopy; if less severe than ASC-US, they are invited to repeat HPV and Pap tests 12 months later. The health professionals involved in the screening are being specifically trained. Written information to women were prepared, taking into account the results of a previous qualitative research that had explored women’s perceptions and information needs regarding HPV testing for triage for abnormal cytological results.

Conclusions: The project started in April 2009 in one screening centre, and will progressively involve the other centres. Preliminary data relative to 2304 invited women, indicate an overall attendance of 42.4%, comparable to that registered when using cytology as primary test. Invited women are demonstrating good acceptance of the new screening methodology, and the continuous interplay among all the health professionals involved in the screening program allows prompt discussion and resolution of the new organizational challenges. Positivity for high-risk HPV sequences has been detected in 6.8% of the tested women, and high-grade squamous intraepithelial lesions have been diagnosed in 22 women (2.8%); (1 HG-SIL, 21 LG-SIL).
MEXICAN CERVICAL CANCER SCREENING STUDY II (MECCS II)

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Objective: Determine the comparative sensitivity and specificity for primary computer assisted liquid-based cytology with two primary HPV testing technologies.

Methods: The MECCS II trial was conducted in Patzcuaro and Zitacuaro, Michoacán, Mexico. Women ages 30-50, non-pregnant, varied histories of screening, no history of hysterectomy or pelvic irradiation participated. All women had a self-sample obtained with the POI/NIH brush placed in PreservCyt (PC) and a direct sample for ThinPrep cytology read using the Hologic imager protocol. Two additional direct samples were placed alternatively by study number in PC or direct to Gen-Probe transport media (GPTM). The self samples were tested with HC-II, and the Gen-Probe Aptima HPV assay (AHPV), the direct to PC with HC-II and AHPV and the direct to GPTM with the AHPV. Subjects positive on any test were recalled. At the 2nd visit VIA was done to rule out large (>3 quadrants) pre-invasive disease or cancer, colposcopy and biopsy followed using the POI directed and random biopsy protocol (>5 biopsies/patient). All HC-II positive subjects eligible by VIA triage above were treated with Cryotherapy. This report will focus on the results of the direct testing (cytology, HC-II, and AHPV). 2057 patients have complete results.

Conclusions: Mean age (SD) = 39.1(6.0); 8.8% were smokers; and 84.5% were married/cohabiting. Cytology ≥ ASCUS 7.7% (159/2057); ≥ LGSIL 1.8% (36/2057); and ≥ HGSIL 0.5% (10/2057). Final biopsy ≥ CIN2 = 2.0% (41/2057) and; ≥ CIN3 = 0.7% (15/2057). HC-II and AHPV were positive in 8.9% (182/2057) and 8.5% (174/2057) respectively. The sensitivity of ThinPrep ≥ ASCUS, HC-II (at 1Pg), and AHPV for ≥ CIN2 was 75.6%, 80.5%, and 78.1% respectively (N.S); for ≥ CIN3 it was 86.7%, 100% and 100% respectively (N.S.). The specificity of ThinPrep ≥ ASCUS, HC-II (at 1Pg), and AHPV for ≥ CIN II was 93.7%, 92.6%, 93.0% respectively (N.S.); for ≥ CIN3 was 92.9%, 91.8%, 92.2% respectively, (N.S.). PPV were 19.5, 18.1 and 18.4 and the NPV were 99.5, 99.6 and 99.5 respectively. These data demonstrate diagnostic equivalence of the technologies reported.

EFFICIENCY OF PRIMARY SCREENING WITH PAP-SMEARS AND HIGH-RISK HPV TEST OF WOMEN 50 YEARS OR MORE

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Objectives: The aim of the present study was to compare the efficiency of primary screening with Pap-smears and a high-risk HPV test of women 50 years or more.

Methods: The data base for cytological screening at the Department of Pathology and Cytology, University Hospital of Uppsala, Sweden was examined during the year 2006. Primary screening with a high-risk HPV test (HC2) was performed during the years 2007-2009 by offering a self sampling device at home (QvintipR) for women 50-65 years, who had not participated in the organised screening for 6 years or more. Abnormal smears, ASCUS-CIN3 alterations occurred in 4.5% of smears collected in women 50-65 years. The high-risk HPV test was positive in 4.1%. With primary HPV testing CIN2-3 lesions was obtained in 0.68% (9/1329) of the total number of HPV analyses, whereas with Pap-smear screening the corresponding figure was 0.25% (15/5943). With primary HPV testing CIN2-3 lesions occurred in 16.4% (9/55) of the total number of positive high-risk HPV tests. With Pap smear screening the corresponding figure was 5.6% (15/268).

Conclusions: When primary screening with a high-risk HPV test is compared with primary Pap smear screening in women 50 years old or more, the primary HPV test seems to be more effective to detect women with CIN2-3 lesions.
**AGE-SPECIFIC PERFORMANCE OF HPV DNA TEST IN PRIMARY CERVICAL SCREENING**

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Objectives: To study the age-specific performance of primary HPV DNA test with cytology triage in cervical cancer screening.

Methods: Women aged 25-65 years invited for routine cervical cancer screening were randomised to primary HPV DNA screening (n=54 207 invitations) followed by cytology triage for HPV DNA-positives, and to conventional cytology (n=54 218) screening. A Hybrid Capture 2 assay with the probes for 13 high-risk HPV types was used and samples were classified positive if the rlu ratio was ≥ 1.00. In both arms, cytology results of LSIL or worse triggered a referral for colposcopy.

Conclusions: The referral rate for colposcopy was 1.2% overall but women < 35 years were referred more often in the HPV vs the conventional screening arm (RR = 1.27, 95% CI; 1.01-1.60). The relative detection rates for CIN 1, CIN 2, and CIN 3+ in the HPV vs conventional screening arm were 1.44 (95% CI; 0.99-2.10), 1.39 (95% CI; 1.03-1.88), and 1.22 (95% CI; 0.78-1.92), respectively. The specificity of the HPV DNA test with cytology triage was similar to that of cytological screening (99.2% vs. 99.1% for CIN 2+) whereas the specificity of a single HPV DNA test was clearly inferior (93.0% for CIN 2+). Among women ≥ 35 years, the HPV DNA test with cytology triage tended to have higher specificity than conventional screening. The PPVs for HPV DNA screening were consistently higher than those for cytology screening. In both arms, the test specificities increased with increasing age, whereas the highest PPVs were observed among the youngest women.

Primary HPV DNA screening with cytology triage is more sensitive than conventional cytology. Among women ≥ 35 years, it is also more specific and decreases colposcopy referrals and follow-up tests. Long-term follow-up is needed to ultimately decide whether to use HPV DNA screening in women < 35 years, as well as use of regular HPV DNA screening without cytology triage at any age. It is likely that these interventions would lead to increased detection of mild lesions and consequently the possible adverse effects of overtreatment have to be evaluated.

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**HPV TESTING ON SELF-COLLECTED CERVICO-VAGINAL LAVAGE SPECIMENS IS AN EFFECTIVE SCREENING METHOD FOR NON-ATTENDEES OF CERVICAL SCREENING**

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Objectives: In countries with population-based cervical screening programmes more than half of cervical carcinomas are diagnosed in women who do not participate (i.e., ‘non-attendees’). We assessed whether offering self-sampling of cervico-vaginal material for hrHPV testing on non-preserved samples is an effective screening method for women not complying to the invitation and recall of the Dutch regular cervical screening programme.

Methods: In this cohort analytical study 27,792 women aged 30 to 60 years registered as screening non-attendee in 2005 were offered Hybrid Capture 2 (HC2) hrHPV testing on self-collected cervico-vaginal specimens using a lavage device (self-sampling group). Another, randomly assigned group of 281 non-attendees received a second re-invitation for conventional cytology (recall control group). Referral of self-sampling responders to the general practitioner was based on hrHPV test results of the self-sampling women who were screened in the prior round (43%).

Conclusions: Offering self-sampling by sending a device for collecting cervico-vaginal specimens for hrHPV testing to non-attendees is a feasible and effective method to increase the coverage of the screening programme. Both the response rate and the yield of high-grade lesions argue for implementation of this method for non-attendees of the regular screening programme.
**SS 2-2**

HPV HIGH-RISK GENOTYPE DISTRIBUTION IN A SCREENING POPULATION: RESULTS FROM THE ATHENA TRIAL

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Objectives: The distribution of high-risk (HR) HPV genotypes depends in part on the demographics, cytologic characteristics, and vaccination status of the studied population. The goals of this study were to determine in a largely un-vaccinated population (i) the distribution of HR genotypes, both as single infection as well as multiple infections; (ii) the correlation between genotype and high-grade cervical disease (CIN2 and greater).

Methods: The ATHENA trial is an HPV screening study aimed at determining the effectiveness of HPV testing as part of a cervical cancer screening program. 47,000 women were recruited from 62 clinical sites across the United States. Cytology specimens were analyzed at four study laboratories by a research use only Linear Array® Test for 16 HR HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 73, and 82). All women with a positive HPV test result were referred for colposcopy and biopsy. Cervical biopsies were read by community pathologists as well as by a central pathology review (CPR) panel. The CPR result was designated as the study endpoint. Prevalence of each of the HPV genotypes was calculated as well as the association between genotype and CIN2+ status.

Results: Preliminary analysis from 5,607 HPV positive women with CPR results demonstrated that HPV 16 had the highest rate of single infection regardless of cytology result. HPV 52 was the second most common genotype among women with normal as well as with ASCUS cytology, but was relatively uncommon in women with >ASCUS. HPV 16 had the highest association with CIN2+ status in all cytology groups. HPV 31 was also associated with CIN2+ independent of cytology result. HPV 18 was highly associated with CIN3+, particularly in women with >ASCUS cytology.

Conclusions: The ATHENA trial supports the importance of identifying not only pooled HR HPV infection, but also specific infection with HPV 16 as well as HPV 18. Determining risk for developing CIN2+ over three years of follow-up in the ATHENA trial will advance our understanding of disease associated with individual genotypes as well as with pooled HR HPV infections.

**SS 2-3**

HIGH RISK HPV DNA TESTING AS A PRIMARY SCREENING TOOL IN A POPULATION WITH A HIGH INCIDENCE OF HUMAN IMMUNODEFICIENCY VIRUS

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Objectives: Human papillomavirus (HPV) DNA testing is widely used as part of primary screening in the developed world. In developing countries with a high prevalence of Human Immunodeficiency Virus (HIV) both squamous intraepithelial lesions (SIL) and cervical cancer is more prevalent and screening thus more important. In South Africa HIV prevalence in females peaks at age 25 to 29 years at 32.7%, with a range of 14.1 to 32.7% in females aged 20 to 49 years(1).

Methods: We report the findings of a population based HPV DNA screening study performed in primary health care clinics in the Pretoria region of South Africa (n=629). HPV DNA genotyping was performed on cervical swab and tampon collected specimens using the Linear Array HPV Genotyping test (Roche Molecular Systems®). In this population 53% (n=334) tested positive for high risk HPV types and 37.5% (n=236) for the “top 6” cervical cancer causing types (HPV types 16, 18, 31, 33, 35 and 45) in this region.

Conclusions: We found that screening for high risk HPV DNA is not a good screening tool in our population because more than half of our population tested positive. Screening for the “top 6” cervical cancer causing types may be a more useful. We suspect that the high HPV prevalence can be explained by the high prevalence of HIV and AIDS in this population.

IS HPV GENOTYPING MEANINGFUL FOR THE TRIAGE OF HIGH-RISK HPV POSITIVE WOMEN?

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**Objective:** Because of its marked higher sensitivity for CIN2+ hrHPV testing is now being considered as an alternative for cytology-based cervical cancer screening. A concern is, however, that the lower specificity of the hrHPV test for CIN2+ may lead to a higher number of unnecessary referrals for colposcopy and over-treatment. To solve this problem reflex cytology as a triage tool for hrHPV DNA positive women has been advocated. Still, there is no agreed management protocol for hrHPV positive, cytologically normal women, who typically form the largest group of hrHPV positive women. Viral genotyping has recently been recognized as a complementary triage tool since the risk of CIN2+ varies across types, with particularly HPV16 and 18 conferring an excess risk of CIN2+. Using data from two large population-based screening trials (i.e. POBASCAM and VUSA-SCREEN) executed with combined cytology/hrHPV testing we aim to identify the best triage strategy for hrHPV positive women in the setting of a screening programme. Here, data of VUSA-SCREEN are presented.

**Methods:** Women (n=25,871; aged 30-60 years) participating in VUSA-SCREEN were tested by cytology and for hrHPV presence (HC2 assay), with GP5+/6+-PCR-based genotyping being performed in case of hrHPV positivity. Twelve potential triage strategies for hrHPV positive women (n=1318) were evaluated, involving cytology, genotyping and combinations thereof at baseline and/or a follow-up visit at 12 months. Primary outcome measure was the number of CIN3+ detected cumulatively in 3 years. Amongst the various triage strategies reflex cytology testing at baseline followed by cytology and genotyping for HPV 16/18 at follow up of cytology negative women at baseline was one of the three strategies, which might be considered for implementation.

**Conclusions:** Cytology triage at baseline and combined HPV16/18 genotyping and cytology at follow-up is an attractive triage alternative for evaluating hrHPV positive women. The projected extension of the screening interval with one or two years makes implementation of HPV attractive in terms of costs, detection of CIN3+ and number of life years gained.

DETECTION OF PERSISTENT CIN2+ AFTER TREATMENT: ROLE OF HPV 16 GENOTYPING

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**Objectives:** HPV testing has been included in many international management guidelines for treatment follow-up. However, the role of genotyping in this setting has been poorly investigated. The objective of this study was to identify the viral pattern in persistent high grade SIL after CIN2+ conservative treatment.

**Methods:** 134 CIN2+ patients treated by LEEP or laser conization were tested for HR-HPV with AMPLICOR HPV test (Roche Diagnostics) at baseline, just before the excisional treatment, and at the follow-up visits after treatment; in case of positivity of any of these tests, genotyping was performed using LINEAR ARRAY (Roche Diagnostics).

**Results and Conclusions:** The median age of the patients was 37 (range 21 - 67) years. 85 out of 134 cases (64.4%) were HPV 16 positive at baseline, 33/85 cases (38%) in association with other HPV types. As early as three months after treatment 9 cases (6.8%) showed persistent CIN2+; all the persistent cases had HPV 16 at both baseline and three months after treatment. 7 out of 9 persistences had HPV 16 as single infection at baseline. In relation to margin status, only 5 cases out of the 9 persistent cases had positive margins on the cone. Conversely, CIN2-3 cases that were HPV 16 negative at baseline had no persistence at 12 months after treatment. These results suggest a clinical role for HPV 16 genotyping to detect persistent disease after treatment of CIN2-3; a single HPV16 genotype assay as early as three months after treatment correctly identified persistent disease and was a stronger predictor of persistence than positive margins on the cone.
Virtually all cases of cervical cancer are caused by persistent infections with a restricted set of human papillomaviruses (HPV). Some HPV types, like HPV16 and HPV18, are clear and powerful carcinogens. However, the categorization of the most weakly carcinogenic HPV types is extremely challenging. The decisions are important for screening test and vaccine development. This presentation will summarise the process recently taken by a World Health Organization International Agency for Research on Cancer (IARC) Monographs Working Group to re-assess the carcinogenicity of different HPV types.

**Objectives:** The AS04-adjuvanted human papillomavirus (HPV)-16/18 vaccine (Cervarix®; GlaxoSmithKline Biologicals) was shown to have prophylactic efficacy against HPV-16/18 infections and cervical intraepithelial neoplasia (CIN)2+ associated with the most common oncogenic HPV types. We evaluated type-specific cross-protective vaccine efficacy (VE) against 6-month persistent infection and CIN2+ associated with non-vaccine oncogenic HPV types in a population approximating young girls prior to sexual debut.

**Methods:** In this study (NCT00122681), women aged 15-25 years were randomised (1:1) to receive HPV-16/18 vaccine (n=9,319) or hepatitis A vaccine (control; n=9,325) at months 0, 1 and 6. Cervical samples were collected every 6 months for HPV DNA testing; gynecological and cytopathological examinations were performed every 12 months. CIN2+ analyses considered detection of HPV DNA in the lesion independently of other types. VE analyses were performed in the TVC-naïve, a TVC subset including women who received ≥ 1 vaccine dose (92% received 3 doses) (HPV-16/18 vaccine, n=5,822; control, n=5,819), with normal cytology, seronegative for HPV-16/18 and DNA negative for 14 oncogenic HPV types at baseline. Mean (SD) follow-up was 39.5 (9.0) months after first vaccination.

**Conclusions:** VE (96.1% CI; p-value) against CIN2+ was 98.2% (89.1, 100; p<0.0001) for HPV-16, 100% (61.3, 100; p=0.0002) for HPV-18, 72.3% (19.1, 92.5; p=0.0065) for HPV-33 and 100% (78.3, 100; p<0.0001) for HPV-45 (0 vs 5 cases). VE against 6-month persistent infection was 93.3% (89.6, 95.9; p<0.0001) for HPV-16, 92.5% (85.9, 96.5; p<0.0001) for HPV-18, 77.5% (66.1, 85.5; p<0.0001) for HPV-31, 43.5% (18.6, 61.2; p=0.0008) for HPV-33 and 81.4% (64.3, 91.2, p<0.0001) for HPV-45. VE against CIN2+ irrespective of HPV DNA type in the lesion was 70.2% (54.7, 80.9; p<0.0001). The extended protection against HPV-31, -33 and -45, observed across virological and clinical endpoints, is anticipated to contribute to clinically meaningful reductions in the overall incidence of cervical cancer and pre-cancer and suggests favourable implications for public health and potential cost benefits of the vaccine.
PATRICIA PHASE III TRIAL: EFFICACY OF THE AS04-ADJUVANTED HPV-16/18 VACCINE WOMEN AGED 18-25 YEARS

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Objectives: The AS04-adjuvanted human papillomavirus (HPV)-16/18 vaccine, Cervarix® (GlaxoSmithKline Biologicals), shows high prophylactic vaccine efficacy (VE) against cervical intraepithelial neoplasia (CIN)2+ associated with HPV-16/18. We evaluated the efficacy of the vaccine in the subset of 18-25 year old women from the PATRICIA study.

Methods: In this study (NCT00122681), 18,644 women aged 15-25 years were randomised (1:1) to receive HPV-16/18 vaccine or hepatitis A vaccine as control at Months 0, 1 and 6. Baseline HPV DNA status and serostatus were assessed by PCR and ELISA, respectively. Cervical samples were collected every 6 months for HPV-DNA typing and every 12 months for gynaecological/cytopathological examinations. We report VE against CIN2+ associated with HPV-16/18 by DNA/serostatus in women 18-25 years in the total vaccinated cohort (TVC), i.e. women who received ≥1 dose (N=12,665).

Conclusions: VE (96.1%CI) against HPV-16/18 CIN2+ in baseline HPV DNA-negative/seronegative women was 93.5% (78.8-98.8; p<0.0001) in the pre-defined primary analysis and 97.7% (85.6-100; p<0.0001) using the HPV Type Assignment Algorithm (TAA), an analysis that assigns probable HPV causality in lesions containing multiple HPV types. VE in baseline HPV DNA-negative women regardless of serostatus was 89.5% (74.9-96.5; p<0.0001) and 96.2% (85.0-99.6; p<0.0001) using the HPV TAA. Overall VE against HPV-16/18 CIN2+ (regardless of baseline HPV DNA and serostatus) was 93.4% (15.0-57.2; p=0.0017) and 41.6% (17.3-59.1; p=0.001) using the HPV TAA. Cervarix® showed high and significant efficacy against HPV-16/18 CIN2+ in 18-25-year-old women with no evidence of current infection with a vaccine type. These data are similar to data in young women 15-17-years of age from the same study.

QUADRIVALENT HPV (TYPES 6/11/16/18) VACCINE: END-OF-STUDY EFFICACY AGAINST HPV6/11/16/18-RELATED PERSISTENT INFECTION AND DISEASE IN WOMEN AGED 24 TO 45

Ferris D, for the FUTURE Iii Steering Committee

Objectives: Prophylactic administration of quadrivalent HPV (types 6/11/16/18) L1 virus-like-particle (VLP) vaccine (qHPV) is highly effective in preventing HPV6/11/16/18-related cervical and genital disease in adolescent and young adult women. Previous analyses of Merck protocol 019 (mean 2.2 years follow-up per subject) have also demonstrated qHPV vaccine efficacy in women aged 24-45. Vaccine efficacy in this population of adult women against the incidence of vaccine type related cervical intraepithelial neoplasia (CIN) or external genital lesions (EGL) was 92.4% (95% CI: 49.6, 99.8). As this clinical trial has now ended, an end-of study efficacy analysis is possible. Therefore, we evaluated the efficacy of quadrivalent HPV vaccine against CIN or EGL (includes vulvar or vaginal intraepithelial neoplasia [VIN and VaIN] and condyloma) in women aged 24-45 through the end-of-study.

Methods: An international, placebo-controlled, multi-center study of qHPV vaccine (GARDASIL™, Merck & Co., Inc., Whitehouse Station, NJ) was conducted. This study enrolled 3819 24-45 year old women with no history of cervical disease in the past 5 years, LEPP, hysterectomy, or genital warts. Women received quadrivalent vaccine or placebo at day 1, and months 2 and 6. Ascertainment of HPV-related cervical and genital disease was accomplished via Pap testing, genital inspection and cervicovaginal sampling (conducted every 6 months). Analyses were conducted in a per-protocol population (women who received 3 doses of vaccine/placebo within 1 year of enrollment, were naïve to the relevant HPV types at day 1, and remained free of infection through the completion of the vaccination regimen). Mean follow-up time per subjects was 3.8 years, representing an additional 1.6 years of follow-up when compared to earlier analyses.

Results: The efficacy of quadrivalent HPV vaccine in the prevention of HPV6/11/16/18-related CIN or EGL was 95.7% (95% CI: (73.4, 99.9) (23 placebo cases versus 1 vaccine case). The one case among vaccinees was an HPV16-related CIN2 which was included in the original analysis. No new CIN or EGL cases were seen over the additional follow-up. Efficacy against HPV6/11/16/18-related persistent infection was 89.6% (95% CI: 79.3, 95.4) (85 placebo cases versus 9 vaccine cases).

Conclusions: These data demonstrate that qHPV vaccine is highly effective in preventing HPV6/11/16/18-related persistent infection, CIN and EGL in women aged 24 to 45 naïve to vaccine HPV types.

Merck & Co., Inc. funded the study from which these data are derived.
ANAMNESTIC RESPONSE TO NON-VACCINE TYPES ELICITED BY A FOURTH DOSE OF THE HPV-16/18 AS04-ADJUVANTED VACCINE

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Objectives: The HPV-16/18 AS04-adjuvanted vaccine protects against vaccine and non-vaccine HPV types. We report the anamnestic response against HPV-31 and -45, the most common non-vaccine types associated with cervical cancer.

Methods: Healthy women (15-25 y) received 3 doses of vaccine or placebo in an initial, double-blind, randomised study (NCT00689741). In the present study (NCT00546078), women in the vaccine group received a 4th dose ~7 y later. Anti-HPV-31 and -45 antibody titres, T-cell and B-cell responses were measured by ELISA, intracellular cytokine staining and ELISPOT, respectively. The primary immunogenicity analyses were in the according-to-protocol cohort (N=59); T-cell and B-cell responses were evaluated in a subset (N=30).

Conclusions: Immune response against HPV-31 and -45 was sustained 7 y after the primary vaccination course (Table). Geometric Mean Titres rose within 7 d after the 4th dose (Table) to above the peak response in the initial study. The proportion of T-cell and B-cell responders was high before the 4th dose and rose at M1 (Table). The geometric mean of specific CD4 T-cells was 1404 and 971 per Mio of CD4+ T cells respectively for HPV-31 and -45. Corresponding values for specific memory B-cells were 368 and 282 per Mio of memory B cells. In conclusion, a high humoral and cell-mediated immune response against HPV-31 and 45 was sustained for ~7 y after a 3-dose course of the HPV-16/18 AS04-adjuvanted vaccine, and a 4th dose induced a strong anamnestic response.

**Table:**

<table>
<thead>
<tr>
<th></th>
<th>HPV-31</th>
<th>HPV-45</th>
</tr>
</thead>
<tbody>
<tr>
<td>GMT (EL.U/mL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PRE</td>
<td>209.4</td>
<td>192.9</td>
</tr>
<tr>
<td>D7</td>
<td>2228.2</td>
<td>2530.5</td>
</tr>
<tr>
<td>M1</td>
<td>3630.8</td>
<td>4253.8</td>
</tr>
<tr>
<td>M7</td>
<td>1193.8</td>
<td>1503.1</td>
</tr>
<tr>
<td>T-cells (% responders*)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PRE</td>
<td>78.3</td>
<td>59.1</td>
</tr>
<tr>
<td>M1</td>
<td>95.2</td>
<td>95.2</td>
</tr>
<tr>
<td>M7</td>
<td>73.1</td>
<td>61.5</td>
</tr>
<tr>
<td>B-cells (% responders#)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PRE</td>
<td>45.8</td>
<td>45.8</td>
</tr>
<tr>
<td>M1</td>
<td>95.8</td>
<td>95.8</td>
</tr>
<tr>
<td>M7</td>
<td>70.0</td>
<td>80.0</td>
</tr>
</tbody>
</table>

PRE: ~7 y after primary vaccination course, before 4th dose

* >500 specific CD4 T-cells expressing ≥2 of 4 immune markers

**Table:**

**UPDATE ON GARDASIL® (QUADRIVALENT HUMAN PAPILLOMAVIRUS [HPV] 6/11/16/18 VACCINE) CLINICAL TRIAL EFFICACY RESULTS**

Marc Steben

Background and aims: At licensure (2006), GARDASIL was shown to prevent HPV16/18-related high-grade lesions (CIN2/3 and AIS) with up to two-year follow-up (protocols 013/015, women aged 16-26). Here we present end-of-study vaccine efficacy (VE) for up to four years; VE for women aged 24-45 (protocol 019), men aged 16-26 (protocol 020), and long-term VE for the HPV16 monovalent prototype-vaccine (protocol 026).


Results: In the per-protocol-population of women aged 16-26, end-of-study VE for HPV16/18-related CIN2/3 or AIS was 98% (95%CI:94-100); VE for HPV6/11/16/18-related condyloma, VIN1-3, and VaIN1-3 was 99%, 100% and 100%, respectively. In PCR-negative subjects for HPV6/11/16/18/31/33/35/39/45/51/52/56/58/59 pre-vaccination, Gardasil significantly reduced CIN2-3/AIS associated with the 10 non-vaccine HPV types which cause ~20% of cervical cancers. In women aged 16-26 who had cleared a previous infection with one of the vaccine-HPV types at the time of vaccination, Gardasil recipients were protected against recurrence of disease from that type, unlike placebo recipients. Among women aged 24-45, per-protocol VE for any HPV6/11/16/18-related disease was 92% (95%CI:50-100). In men aged 16-26, VE against any HPV6/11/16/18-related external genital lesion in the per-protocol-population was 90% (95%CI:69-98). In the extended follow-up of 16-23 year old women up to 9 years after vaccination with the HPV16 monovalent prototype-vaccine, per-protocol VE against HPV16 CIN was 100%.

Conclusions: Disease prevention remains the most important measure of long-term VE. Vaccination with GARDASIL is expected to reduce significantly the burden of cervical and other cancers, dysplasia, and genital warts in women and men.
QUADRIVALENT HPV 6/11/16/18 VACCINE EFFICACY AGAINST PERSISTENT INFECTION OR DISEASE IN SUBJECTS WITH PRIOR VACCINE HPV TYPE INFECTION

Riethmuller, D, for the Quadrivalent HPV Vaccine Investigators

Department of Gynecology and Obstetrics, Emory University School of Medicine

Objectives: In the international clinical program for the quadrivalent HPV (types 6/11/16/18) vaccine (qHPV), 15% of women had evidence of past cleared infection with one or more vaccine HPV types (seropositive and DNA negative) at the time of vaccination. Previous analyses in women 16-26 years old have demonstrated that no qHPV vaccine recipients who were seropositive and DNA negative at enrollment for a vaccine type were diagnosed with disease related to the HPV type which they had previously cleared. Therefore, the vaccine may prevent recurrence or reactivation of infection or disease with vaccine HPV types. However, in the placebo group 15 subjects developed disease related to previously cleared vaccine types. Here we present a similar analysis in women aged 24-45 enrolled in a large clinical trial of the qHPV vaccine.

Methods: An international, placebo-controlled, multi-center study of qHPV vaccine (GARDASIL™, Merck & Co., Inc., Whitehouse Station, NJ) was conducted. This study enrolled 3819 24-45 year old women with no history of cervical disease in the past 5 years, LEEP, hysterectomy, or genital warts. Women received quadrivalent vaccine or placebo at day 1, and months 2 and 6. Ascertainment of HPV-related cervical and genital disease was accomplished via Pap testing, genital inspection and cervicovaginal sampling (conducted every 6 months). Analyses were conducted in a population of women who were seropositive and PCR negative to ≥1 vaccine HPV type at enrollment. Mean follow-up time per subject was 3.8 years.

Results: There were 5 and 15 cases of persistent infection with vaccine HPV types in the vaccine and placebo groups, respectively. There were no cases of CIN or EGL in either the vaccine or placebo groups. Vaccine efficacy against persistent infection was 66.8% (95% CI: 3.8, 90.5). Vaccine efficacy against persistent infection in women aged 35-45 was 81.3% (95% CI: 14.4, 98.0) (11 placebo cases vs. 2 vaccine cases).

Conclusions: Vaccination with the qHPV vaccine is associated with a lower incidence of reactivation/recurrence of persistent infection related to vaccine HPV types in women aged 24-45.

Efficacy of HPV-16/18 AS04-ADJUVANTED VACCINE AGAINST ABNORMAL CYTOLOGY, COLPOSCOPY REFERRALS AND CERVICAL PROCEDURES

Paavonen J on behalf of the HPV PATRICIA Study Group

University of Helsinki, department of obstetrics and gynaecology, Helsinki, Finland

Objectives: The AS04-adjuvanted human papillomavirus (HPV)-16/18 vaccine (Cervarix®; GlaxoSmtihKline Biologicals) shows high prophylactic vaccine efficacy (VE) against cervical intraepithelial neoplasia (CIN)2+ associated with HPV-16/18. We present VE against abnormal cytology (low / high grade squamous intraepithelial lesions [LSIL / HSIL] or atypical squamous cells of undetermined significance [ASCUS]) and reduction both in colposcopy referrals and cervical excision procedures.

Methods: In this study (NCT00122681), women aged 15-25 years were randomised to receive HPV-16/18 vaccine (n=9,319) or hepatitis A vaccine (n=9,325) at Months 0, 1 and 6. Cervical samples were collected every 6 months for HPV DNA typing; gynecological and cytopathological examinations were performed every 12 months. Women were referred for colposcopy and treatment according to the protocol pre-defined algorithm. VE was reported for the total vaccinated cohort (TVC)-naïve (women who were HPV-16/18 seronegative, HPV DNA-negative for 14 oncogenic types, with normal cytology at baseline, who received ≥1 vaccine dose).

Conclusions: In the TVC-naïve, VE (96.1% CI; p-value) against HSIL and LSIL associated with HPV-16/18 was 93.4% (54.1, 99.9; p=0.0003) and 92.6% (87.8, 95.8; p:<.0001), respectively. VE against ASCUS associated with HPV-16/18 was 88.2% (80.7, 93.2; p<0.0001). VE against HSIL, LSIL and ASCUS irrespective of HPV DNA results was 53.7% (4.6, 78.9; p=0.0192), 23.9% (13.9, 32.9; p<0.0001), and 19.7% (9.0, 29.1; p=0.0002), respectively. Reductions in colposcopy referrals and cervical excision procedures were 26.3% (14.7, 36.4; p<0.0001) and 68.8% (50.0, 81.2; p<0.0001), respectively. Vaccination of women naïve for oncogenic HPV types (approximating the target population of current organised adolescent vaccination programmes) with AS04-adjuvanted HPV-16/18 vaccine significantly reduced cytological abnormalities, with a corresponding reduction in colposcopy referrals and cervical excision procedures. VE was greatest against the highest grade lesions. These results suggest the potential public health and cost benefits of this vaccine.
Efficacy, Immunogenicity and Safety of HPV-16/18 AS04 Adjuvanted Vaccine in Japanese Women; Final Analysis at Month 24

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3 GlaxoSmithKline Biologicals, Rixensart, Belgium, 4 University of Tsukuba, Ibaraki, Japan

Objectives: Primary objective was to demonstrate the efficacy of the HPV-16/18 AS04-adjuvanted vaccine against persistent infection (6-month definition) associated with HPV-16/18 in women who were seronegative at Month 0 and DNA negative at Month 0 and Month 6 for the corresponding HPV type. Secondary objectives included immunogenicity and safety (up to 24 months).

Method: Phase II double-blind randomized controlled study with HPV-16/18 AS04-adjuvanted vaccine, conducted in healthy women aged 20-25 years in Japan. A total of 1,040 healthy women aged 20-25 years were vaccinated at 13 study centers between April and October 2006; 519 women in the HPV group (HPV) and 521 women in the hepatitis A control group (HAV). Duration of study was approximately 24 months for each subject. Vaccines were administered intramuscularly at months 0, 1 and 6. Blood samples were collected at 0, 6, 7, 12, 18 and 24 months and the investigator obtained cervical specimens every 6 months up to 24 months. Vaccine efficacy was analysed in the according to protocol cohort for efficacy (ATP-E=1002; HPV=501, HAV=501);

Conclusions: Vaccine efficacy against persistent infection (6-month definition) with HPV-16/18 was 100% [95.5% CI: 71.3, 100, p<0.0001], with no case in the HPV group versus 15 cases in the HAV group. The vaccine induced peak anti-HPV-16 and HPV-18 antibody titers at Month 7. All HPV vaccine recipients were still seropositive at Month 24, with sustained antibody response. Vaccine safety was similar in both treatment groups in terms of medically significant AEs, SAEs and pregnancy outcomes. Overall we can conclude that, in the study population of 20-25 year old Japanese females, the HPV-16/18 AS04-adjuvanted vaccine showed 100% protection against persistent infection with HPV-16/18 (6-month definition), high immune response and a favorable safety profile.

Impact of Gardasil® in Women Who Have Undergone Definitive Therapy

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2 Microbiology and Infectious Diseases Department, Royal Women’s Hospital and Department of Obstetrics and Gynecology, University of Melbourne, Melbourne, Victoria, Australia;
3 Austria Department of Obstetrics and Gynecology, University Central Hospital, Helsinki, Finland;
4 Departments of Family Medicine and Obstetrics and Gynecology, Medical College of Georgia, Augusta, GA, USA;
5 Merck Research Laboratories, West Point, PA USA

Objective: Prophylactic HPV vaccination is highly effective in preventing pre-cancerous lesions and genital warts (GW). It is not known if women with a history of cervical, vulvar, or vaginal pre-cancers (CIN, VIN, VaIN) or GWs will benefit from vaccination. We report vaccine efficacy for these endpoints in women who underwent excisional therapy in the context of 2 randomized clinical trials.

Methods: 17,622 women aged 16-26 were enrolled in 1 of 2 trials (protocol 013 and 015). Vaccine or placebo was given at Day 1, Month 2 and 6. Pap testing occurred at Day 1 and every 6-12 months. Definitive therapy referral was per standard of care. This intention-to-treat analysis identified women who underwent excisional therapy for CIN, VIN, VaIN or GWs. Case counting began after the excisional therapy.

Conclusions: Within an average of 3.6 years, in the combined trials, 587 vaccine recipients and 763 placebo recipients underwent cervical definitive therapy. Vaccine efficacy for any CIN1 or worse post-definitive therapy was 47% (95%CI: 17-66). In protocol 013, 222 vaccine recipients and 306 placebo recipients were treated for VIN1-3, VaIN1-3 or GWs. Vaccine efficacy for these endpoints post-therapy was 44% (95%CI: 14-64). Efficacy for endpoints associated with HPV6/11/16/18 was 74% for CIN (95%CI: <0, 97) and 79% for VIN1-3, VaIN1-3 or GWs (95% CI: 53-92). Our data suggest that offering HPV vaccine to women who have undergone cervical conization or treatment for VIN, VaIN or GW will benefit from vaccination through prevention of recurring disease.
IMPACT OF GARDASIL® ON INCIDENCE OF CIN, EGL, ABNORMAL PAP TESTS AND CERVICAL PROCEDURES

Ole-Erik Iversen, University Hospital, Bergen, Norway

Background: Prophylactic administration of GARDASIL® is up to 100% effective in preventing HPV16/18-related CIN2/3 and AIS. We report the impact on the incidence of any CIN2/3 or worse, external genital lesions (genital warts, VIN1-3, or VaIN1-3), abnormal Pap tests, and procedures, regardless of causal HPV type.

Methods: A total of 17,622 women were enrolled in 2 Phase 3, randomized, placebo-controlled trials (FUTURE I and II). Vaccine or placebo was given at Day 1, Month 2 and 6. Subjects underwent cervicovaginal sampling at Day 1. Pap testing occurred at Day 1 and every 6-12 months for up to 48 months. Colposcopy referral was Pap algorithm/HPV test-based. Definitive therapy referral was algorithm-based, using generally accepted standards of care. We estimated number of events prevented annually per 100,000 vaccinated women, in terms of risk difference based on subtracting the rate in the vaccine arm from the rate in the placebo arm in: 1) an unexposed population that approximates sexually naïve females; 2) a mixed population of HPV-exposed and unexposed women; and 3) the population of women already exposed to HPV.

Conclusions: After an average follow-up of 3.6 years, significant reductions in the number of lesions, Pap tests, colposcopy, cervical biopsy, and definitive therapy were observed (See Table). Our data suggest that whether we offer HPV vaccination to a population of women who are HPV naïve, a mixed population of naïve and exposed, or a population of previously exposed women (data not shown), we could expect to have similar public health impacts in terms of disease reduction in the years immediately following vaccination.

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>Number of cases prevented annually per 100,000 vaccinated women</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any CIN 3 or AIS</td>
<td>0.17 (0.05, 0.28)</td>
</tr>
<tr>
<td>Any Pap abnormality</td>
<td>1.38 (0.76, 1.99)</td>
</tr>
<tr>
<td>Any Colposcopy</td>
<td>1.35 (0.80, 1.90)</td>
</tr>
<tr>
<td>Any Cervical Biopsy</td>
<td>1.30 (0.50, 1.81)</td>
</tr>
<tr>
<td>Any Cervical Definitive Therapy</td>
<td>0.58 (0.36, 0.80)</td>
</tr>
<tr>
<td>Any EGL (GW, VIN1-3, VaIN1-3)</td>
<td>1.02 (0.82, 1.22)</td>
</tr>
<tr>
<td>Any EGL procedure</td>
<td>1.17 (0.60, 1.74)</td>
</tr>
</tbody>
</table>

CERVICAL CANCER RISK AMONG NON-VACCINATED WOMEN WITH VARYING COVERAGE OF HPV-16/18 VACCINATION IN THE NETHERLANDS

Bogaards J1, Xiridou M2, Coupé V1, Meijer C1, Wallinga J2, Berkhof J1

1 VU University Medical Centre, Biostatistics Unit, Amsterdam, The Netherlands
2 National Institute of Public Health and the Environment, Bilthoven, The Netherlands
3 VU University Medical Centre, Department of Pathology, Amsterdam, The Netherlands

Objectives: In 2009, HPV-16/18 vaccination of pre-pubescent girls has been added to the Dutch national immunization program. Attendance rate in the first year of vaccination was disappointingly low: about 50% of eligible girls were vaccinated. If vaccination coverage of future cohorts remains low, the indirect protective effect of HPV-16/18 vaccination (due to herd immunity) becomes a prominent aspect of cervical control. Our primary objective, therefore, is to provide estimates of the lifetime risk of cervical cancer among non-vaccinated women in a screened population with varying coverage of HPV-16/18 vaccination.

Methods: We developed an individual-based competing risk model that describes the relation between fourteen high-risk HPV types and cervical disease. The model allows the occurrence of multiple type-specific infections, each giving an independent risk of developing cervical lesions and cancer. Infection risks of each HPV type are informed by the force of infection, obtained from a type-specific compartmental transmission model calibrated to pre-vaccine prevalence of HPV infection.

The lifetime risk of cervical cancer for a non-vaccinated individual was calculated by assuming that the current practice of screening (7 rounds at 5 year intervals, starting at age 30 with cytology as primary instrument) would not change over time. Herd immunity was considered by calculating the expected force of infection that a woman will experience in her lifetime, depending on her particular birth cohort and the population coverage of HPV-16/18 vaccination.

Conclusions: Our study suggests a considerable reduction of cervical cancer risk among non-vaccinated women if HPV-16/18 vaccination coverage is above 50%. Girls born at the time the vaccination campaign is initiated will experience almost full benefit of reduced exposure to high-risk HPV during their lifetime. The total number of cervical cancer cases averted due to indirect protective effects of HPV-16/18 vaccination will be highest at around 70% coverage, even though the individual risk continues to decrease with increasing coverage.
THE EFFECT OF HPV VACCINATION ON INFECTION IN PARTNERSHIPS

Burchell AN\textsuperscript{1}, Tellier PP\textsuperscript{1}, Coutlée F\textsuperscript{2}, Hanley J\textsuperscript{1}, Franco EL\textsuperscript{1}.

\textsuperscript{1}McGill University. \textsuperscript{2}Centre Hospitalier de l'Université de Montréal. Both in Montreal, Quebec, Canada.

Objectives: To describe the effect of vaccination with Gardasil\textsuperscript{TM} on prevalence of vaccine-preventable types (6, 11, 16, 18) in young women and their male partners.

Methods: Couples attending a university or junior college in Montreal, Canada, were recruited for the HITCH Study (HPV Infection and Transmission among Couples through Heterosexual activity). Female vaccination status and sexual histories were obtained by self-report. To date, Gardasil\textsuperscript{TM} is the only HPV vaccine approved in Canada; therefore all self-reported HPV vaccination was assumed to be with the quadrivalent vaccine. Self-collected vaginal swabs and clinician-collected swabs of epithelial cells from the penis and scrotum were tested for type-specific HPV-DNA using the Roche Linear Array. Data from 322 couples enrolled between 05/2005 and 02/2009 were analysed using descriptive statistics and logistic regression. Results are reported as odds ratios (OR) with 95\% confidence intervals (CI). 9\% (28/322) of women reported vaccination at enrolment. Compared to unvaccinated women, vaccinees were younger and their male partners were younger and less sexually-experienced; however, women's lifetime number of sex partners did not vary by vaccination status. Infection with vaccine-preventable types was 3 times less common in vaccinated women and their partners (7\%) compared to unvaccinated women and their partners (22\%). After adjustment for women's and men's lifetime number of partners, female vaccination offered a 10-fold protective effect against infection in women (OR = 0.10, 95\%CI 0.011-0.95) and a 2.7-fold protective effect against infection in men (OR = 0.37, 95\%CI 0.083-1.6), although there was inadequate precision to conclude that the latter was not due to chance.

Conclusions: To our knowledge, these are the first data on HPV vaccination in couples. The preliminary findings suggest that female vaccination prevents transmission to men; this requires confirmation in a larger sample. In settings where vaccine coverage is not high, post-licensure studies of vaccine effectiveness should collect information on partner characteristics.

ISSUES TO CONSIDER FOR HPV VACCINE IMPACT MONITORING IN LOW RESOURCE SETTINGS

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Objectives: Human Papillomavirus vaccine implementation provides a unique opportunity for primary cervical cancer prevention as part of a coordinated strategy to decrease cervical cancer. Evaluating its impact is crucial to assess effectiveness, and encourage future funding mechanisms. Particularly in low resource settings, new strategies to assess HPV vaccine impact monitoring and coverage are necessary because of the target age range, the need for multiple doses, and the length of time required to document cervical cancer prevention.

Methods: Multiple discussion forums are being utilized to discuss issues pertinent to HPV vaccine monitoring in low resource settings. Examples include a CDC led satellite symposium to discuss surveillance at the International Human Papillomavirus Meeting in Malmo, Sweden; ongoing series of phone calls and meetings of international experts in HPV laboratory tests, HPV disease manifestations, epidemiology, cancer prevention, reproductive health, vaccine coverage and vaccine safety coordinated by the World Health Organization; and online discussions in HPV Vaccine Global Community list serve. Guidance from these sources as well as published literature informs planning for HPV Vaccine Impact Monitoring.

Conclusions: In general, HPV vaccine monitoring encompasses safety monitoring, vaccine coverage monitoring, and vaccine effectiveness monitoring. Minimal safety monitoring should include reporting of adverse events. For program evaluation purposes, coverage monitoring could be done by delivery strategy. For HPV vaccine effectiveness, the prevention of cervical cancer is a long term biologic outcome. More proximal biologic endpoints such as vaccine specific HPV DNA infection or HPV types in precancerous lesions could be utilized for more rapid assessment of vaccine efficacy. Using opportunistic targeted populations for impact monitoring will likely be necessary, and may occur at sentinel sites covered by cancer registries or precancerous lesion registries. Age of the populations sampled will be dependent on whether proximal or distal vaccine impact is being assessed.

This paper will compile information from the above resources to discuss issues around HPV vaccine monitoring, especially pertaining to HPV vaccine use in low resource settings.
HPV VACCINATION AT THE POPULATION LEVEL: EXPECTED OUTCOMES

Suzanne M Garland

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2 Department of Obstetrics and Gynaecology, University of Melbourne,
3 Murdoch Childrens Research Institute, Parkville, Victoria, Australia

Inaugural and Past President of AOGIN

Objective: Prophylactic vaccines, the bivalent (protects against 16 and 18 the cause of 70% of cervical cancers worldwide) and the quadrivalent (also protects against ~90% of genital warts as has 6 and 11 protection too) are both licensed in over 100 countries worldwide. Licensure however does not necessarily translate to HPV vaccine provision through public sector programs. It is essential that HPV vaccines are provided to the resource poor countries where the greatest burden of disease resides. Input from groups such as WHO and GAVI will be important in ensuring appropriate pricing and coverage to these countries.

Various strategies for appropriate vaccine coverage include school-based approaches, community health centres, and ultimately the potential for utilizing the infrastructure of the EPI program.

Methods: An example of a successful school-based program is Australia, where the quadrivalent vaccine was registered in June 2006 for females 9-25 years, and males 10 to 15 years. A Federal Government funded school-based HPV vaccination programme which is ongoing for all 12 year old females, began in April 2007, with a general practitioner 2-year catch up program for females 13-26. The bivalent vaccine was registered in Australia, May 2007 for use in females aged 10-45 years, being the first country to approve an HPV vaccine for those >27.

A National HPV Vaccination Program Register has also been established, to measure coverage, maintain records of recipients should a booster dose of vaccine be required, and provide documented vaccination status for assessments of vaccine effectiveness at a population level, with linkages to cytology registers.

Results: To date, state health department records indicate after the first 2 years of this school-based program an estimated uptake of complete HPV vaccine schedule of 3 doses at 75 to 80%, with slight variations by State and Territory. Already in sexual health centres, a decline in genital warts is being seen in young women <27 years of age, with an impact on heterosexual males <27 years (evidence of herd immunity), but not that in those men having sex with me. We expect the next changes will be in Pap cervical cytology abnormality rates, followed by high-grade dysplasias and ultimately by cancers (cervix, HPV-related vulvar, vaginal, anal and head and neck.)

A SUMMARY OF THE POST-LICENSURE SURVEILLANCE INITIATIVES FOR GARDASIL/SILGARD®

PART I- IMPACT STUDIES

Kjaer S,1 Bonanni P,2 Cohet C,3 Latham NB,4 Reisinger K5 and Haupt RM6

1 University of Copenhagen, Copenhagen, Denmark; 2 University of Florence, Florence, Italy; 3 Sanofi Pasteur MSD, Lyon, France; 4 Centre of Vaccinology, Geneva, Switzerland; 5 Primary Physicians Research, Pittsburgh, Pennsylvania, USA; 6 Merck Research Laboratories, West Point, Pennsylvania, USA

Objectives: Because of its expected public health benefit on reduction of cervical cancer and other HPV-related diseases, GARDASIL® has been rapidly implemented in the routine vaccination programs of several countries. It is therefore essential to assess its impact through post-licensure surveillance programs. Here we present a summary of the post-licensure effectiveness studies being conducted in collaboration with the vaccine’s manufacturers and marketers (Merck and Co., Inc., Sanofi Pasteur MSD) as well as other known independent initiatives in Europe, Canada, and Australia.

Methods: We will describe the following:
1) A study of the long-term effectiveness in preventing cervical, vulvar, and vaginal cancers and related pre-cancers caused by HPV6/11/16/18, and those caused by non-vaccine types, among Nordic women who participated in FUTURE II; 2) A Vaccine Impact in Population (VIP) Study that will assess the overall and age-stratified incidence of disease from 1-3 years before the introduction of GARDASIL in the Nordic region, until 5 years after the introduction of GARDASIL; and 3) A 10 year effectiveness study to provide the first long-term data among adolescents. We will also describe other surveillance efforts in Europe, Canada and Australia.

Conclusion: The surveillance efforts for GARDASIL represent one of the most comprehensive vaccine surveillance programs to date, reflecting the public health authorities and manufacturers intentions to enable early access to an intervention that is expected to prevent cervical cancer, while taking the necessary steps to monitor its long-term effectiveness.
A SUMMARY OF THE POST-LICENSURE SURVEILLANCE INITIATIVES FOR GARDASIL/SILGARD® 
PART II- SAFETY STUDIES

Bonanni P,1 Kjaer S,2 Cohet C,3 Latham NB,3 Lambert P-H,4 Reisinger K5 and Haupt RM6
1 University of Florence, Florence, Italy; 2 University of Copenhagen, Copenhagen, Denmark; 3 Sanofi Pasteur MSD, Lyon, France; 4 Centre of Vaccinology, Geneva, Switzerland; 5 Primary Physicians Research, Pittsburgh, Pennsylvania, USA; 6 Merck Research Laboratories, West Point, Pennsylvania, USA

Objectives: Due to the potentially widespread use of GARDASIL (over 50 million doses distributed globally as of June 2009), it is important to monitor its safety in vaccinated populations. Accurate post-licensure safety assessment relies on the continued collection, management, and assessment of safety data by both the vaccine’s manufacturer and regulatory authorities such as the FDA and the European Medicines Agency (EMEA). Here, we describe post-licensure initiatives that will assess the safety of GARDASIL in the general population.

Methods: Here we describe the following:
1) a post-licensure surveillance program among ~44,000 females in a US managed care organization;
2) the pregnancy registry for Gardasil, which is based on post-licensure reports of pregnancy exposures that are spontaneously reported to the company;
3) a study to examine potential HPV type replacement in Italian women; and
4) a study of GARDASIL and autoimmune diseases using a specific Information System. We will also describe other surveillance efforts in Europe, Canada and Australia.

Conclusion: Vaccines are introduced via population-based vaccination programs as soon as their efficacy is shown to be higher than any potential risk. The long-term effectiveness and full safety profile of any new healthcare intervention or technology is obviously not fully known until it can be monitored through long-term post-licensure surveillance programs. The surveillance efforts for GARDASIL represent one of the most comprehensive vaccine surveillance programs to date.

HUMAN PAPILLOMAVIRUS VACCINATION PROGRAM IN PREadoLESCENTS IN THE REGION OF MURCIA (SPAIN)

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Introduction. The human papillomavirus vaccine was introduced in the Spanish Autonomous Communities in 2008, on the basis of consensus between them and the Ministry of Health, in application to girls between 11-14 years of age. Objectives. We present the experience with the vaccine in an Autonomous Community (Murcia) with a population of 1.4 million inhabitants, including 8500 and 8400 girls aged 13 and 14 years, respectively.

Methods. From March 2008, population recruitment strategies were designed for the 1995 cohort, involving the mailing of personalized letters using the computer-based regional vaccination registry; talks with school directors and parent associations; seminars with primary care physicians and nurses; the distribution of leaflets and posters; and specific publicity campaigns in the communications media. The girls in this cohort were rewarded with a pin for each of the three vaccine doses. Vaccination was carried out in the schools starting in October 2008, administering three bivalent vaccine doses. Coverage of the first, second and third doses was 92.84%, 91.01% and 90.86%, respectively. The decision to vaccinate the 1994 cohort was taken in February 2008, two weeks before notification of an incident related with the safety of the tetravalent vaccine occurring in Valencia. Due to a lack of time to design a specific recruitment campaign for this cohort, the decision was taken to perform vaccination in the primary care centers starting in April 2009, and a personalized letter was mailed to this cohort of girls. Coverage of the first and second doses was 47.62% and 44.59%, respectively - evaluation of the third dose being pending. In view of the lower coverage with respect to the 1995 cohort, in September a repeat recruitment campaign was designed based on the mailing of letters with informative leaflets and SMS messages.

Conclusions. The anticipative design of vaccination campaigns, and vaccination in schools, are key elements for ensuring optimum coverage among preadolescents. The appearance in the communications media of news relating to the safety of the vaccines may cause adherence to decrease.
THE TRUE MEANING OF BETHESDA SYSTEM DIAGNOSES: USING CYTOLOGY AND HPV TESTING TO PREDICT CIN3

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Historically, cervical cytology has been viewed as a screening test, rather than an accurate diagnostic reflection of the true cervical disease state. Yet the diagnostic terminology used to communicate Pap cytology findings is meant to reflect the probable underlying histology. While the Pap cytology is correlated with the eventual results of the colposcopic biopsy, colposcopic biopsy has longed been viewed as the superior gold standard for predicting the severity of cervical neoplasia in order to guide therapy and patient management. Furthermore, in the United States under CLIA, cytologic-histologic correlation is required as a quality assurance measure, which implies one can discern by making subjective morphologic comparisons, the accuracy of one modality vs. the other.

Data developed within the last decade challenges all of the above dogma. The errors in cytologic, colposcopic and histologic assessment have been documented and there is significant variation in each step and assessment. In light of the fact that no one test in the pre-therapeutic screening paradigm can be considered a gold standard, the true meaning of a cytologic interpretation using the Bethesda system classification can be viewed in a different way. Each Bethesda system diagnosis, especially when correlated with knowledge of the patient’s HPV status (using a validated test) is best viewed as a predictor of risk of prevalent CIN3. The same principals can be applied to the biopsy results using essentially identical Bethesda System terminology. Finally, with our current understanding, neither the cytologic interpretation, nor the histologic interpretation of the biopsy can be considered a gold standard. Instead the most severe reading most accurately governs the risk, thereby directing patient management.

UPDATE ON ASCCP GUIDELINES - HOW WILL THEY AFFECT CLINICAL MANAGEMENT

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Widespread HPV vaccination will leave behind more equivocal and less predictive cervical lesions and affect all parts of the cervical cancer prevention program from screening to posttreatment. How this will change guidelines as women receiving the human papillomavirus (HPV) vaccine as adolescents attain the age of cervical cancer screening has been the subject of much debate, because changes will depend on a number of factors that cannot be predicted with surety at this date. These include: 1). The percent of each age cohort receiving the vaccine. 2). Whether screening guidelines will change for all women of an age cohort or only for women who can prove they have received the vaccine, and if so, whether the entire series or only partial? 3). Will vaccine efficacy fall with time, and if so, how effective will programs be that advocate boosting? Shorter protection would lessen the effect of the HPV vaccine on screening. 4). Will primary screening guidelines for women vaccinated after onset of sexual activity differ from those vaccinated before this occurs? 5) Will the age of onset and the frequency of screening be increased as risk decreases and in the interest of cost-effectiveness? Presuming high vaccination coverage and long-term protection, guidelines for the management of women with abnormal cervical cytology will likely change to reflect the decreasing prevalence of HPV 16 and 18 in these age cohorts, resulting in decreased risk of abnormal cervical cytology, particularly high-grade squamous intraepithelial lesion (HSIL), and the decreased likelihood of progression of high-grade lesions. A result of removing a majority of the most abnormal lesions will be the reduced predictive values of all the present modalities used in screening and management, including cytology, colposcopy, and pooled panel HPV testing. Testing for HPV 16 and 18 in certain management situations will likely continue, but may become increasingly less important as these viral types become less prevalent. Hence, finding markers that predict risk for women with cervical abnormalities caused by high-risk types still prevalent following HPV vaccination will be increasingly important. A number of potential management algorithms that may evolve in the era of HPV vaccination will be discussed and compared to present American Society for Colposcopy and Cervical Pathology (ASCCP) guidelines for the management of women with abnormal cervical cytology and histology.
Vaccination against the two most important HPV types, 16 and 18, is very efficacious in preventing HPV infections and related cancer precursors. Many countries have now implemented HPV vaccination programs. However, current vaccines can only prevent up to 70% of cervical cancers if widespread vaccination is achieved before onset of sexual activity. Thus, screening remains a very important component of cervical cancer prevention both in vaccinated and unvaccinated populations. Recently published randomized trials have demonstrated that HPV DNA testing can be efficiently used in primary cervical cancer screening.

Several challenges need to be addressed when restructuring current cytology-based screening programs:

1) While HPV DNA testing has very good sensitivity for detecting cancers and cancer precursors and its high negative predictive value allows extending screening intervals, it cannot discriminate between an innocuous transient infection and a prevalent high grade lesion. A good triage strategy needs to be developed to decide who among the HPV positive women needs to undergo further evaluation.

2) It has been demonstrated that up to 50% of prevalent precancers may be missed using conventional colposcopy-biopsy procedures. New clinical algorithms as well as biomarker discovery and validation studies need to address these limitations of disease ascertainment.

3) Vaccination against HPV16 and 18 greatly reduces the number of cancer precursors, while low grade abnormalities, frequently caused by other types, are less affected. As the signal to noise ratio is reduced, it is important to identify new biomarkers with strong discriminatory values.

Objectives: Atypical cellular changes reflecting immature metaplasia, inflammation and atrophy can be diagnostically challenging in small cervical biopsies when evaluating for high-grade (HG) cervical intraepithelial neoplasia (CIN). Such changes may mimic HG dysplasia and can lead to unnecessary cervical excision. The aim of this study was to retrospectively re-evaluate CIN2 lesions diagnosed on cervical biopsies in patients with no residual HG lesion in subsequent cervical excision, utilizing immunohistochemical (IHC) stains.

Methods: 211 cervical biopsies from 151 patients with histologic diagnosis of CIN2 who underwent cervical excision (142 LEEP and 9 cone) were identified from 2000 to 2007. CIN2 was present in cervical excision in 89 (59%) patients (group 1, 130 biopsies) and not found in 62 (41%) (group 2, 81). Tissue blocks of 72/81 cervical biopsies (group 2) were stained for 3 IHC markers: p16INK4a (CINtec, MTM) and proliferation markers Ki-67 (DAKO) and proExC (BD). H&E and immunostained slides were independently reviewed by two pathologists. Nuclear staining for Ki-67 and proExC in cells occupying > lower 1/3 of the epithelium, and diffuse nuclear/cytoplasmic staining for p16 was interpreted as positive for CIN2, and negative otherwise. The histologic diagnosis of CIN2 (group 2) was supported by 3 stains in 61% (44/72) cervical biopsies and by 2 (p16-) in 15% (11/72). In 24% (17/72) stains failed to support the presence of CIN2. Overall (groups 1&2) CIN2 was accurately diagnosed in 88% (185/211) and overdiagnosed in 8% (17/211) cervical biopsies (13 patients). In majority of patients the colposcopic impression (9/13) and ThinPreps (11/13) did not favor HG lesions.

Conclusions:
1) In 26% (55/211) of biopsies the absence of CIN2 in subsequent excision may be due to small lesion size with complete removal by colposcopic biopsy, or regression.
2) In 8% cervical biopsies the difficult to grade lesions were overdiagnosed as CIN2.
3) Immunostains for p16, Ki-67 and ProExC can be useful in separating CIN2 lesions from its benign mimickers.
4) P16 expression was present in 80% (44/55) of CIN2 lesions, compatible to reported rate in the literature. Whether the absence of p16 co-expression with proliferation markers reflects less potential for progression needs to be further investigated.
OBJECTIVES: Although on a three year base participation rates in prevention exams equal those in Great Britain or the Netherlands cervical cancer rates in Germany are still considerably higher. Therefore the need for the introduction of validated new techniques with higher sensitivity and at least the same specificity has been postulated. Since 2005 several studies have shown a significantly increased sensitivity for biopsy confirmed HSIL of the computer-assisted ThinPrep Imaging System (TIS) compared to conventional cytology (CC) and even to manually read ThinPrep thinlayer cytology (TP). This improvement was achieved without a loss in specificity and with a significant gain in productivity. The professional association of gynaecologists (BVF) of the southwestern regions of Rhineland-Palatinate and Saarland have therefore conducted a randomized trial to compare CC with a combination of TP and TIS.

METHODS: Between August 2007 and October 2008 21.081 women attending routine screening at 20 office-based gynaecologists have been recruited. Weekly randomization allocated them to CC or TP/TIS. 20.607 women (97.75%) were finally included. The evaluation of smears was only performed by experienced cytotechnicians (>2.000 slides in each technique). All women with cytologic abnormalities (≥Pap III = ASC-H/LSIL/HSIL) were invited for expert colposcopy including biopsy if indicated. The trial outcome was the detection of histologically confirmed CIN2+ lesions. 43% of the participants were examined with CC and 57% with TP. The colposcopy rate reached 90% among women with >Pap IVa (>CIN 3), while it was lower in women with minor abnormalities. TP and TIS (combined) detected more than three times more abnormal cytologic findings (Pap III / IIId and Pap IVa) than CC.

CONCLUSIONS: In the first randomized direct to vial study computer-assisted thinlayer cytology under field conditions in Germany had a more than three times higher finding rate for cytological abnormalities than conventional cytology. Final results of statistical analysis will be presented.
USE OF AUTOMATED IMAGE ANALYSIS TO DISCRIMINATE CERVICAL NEOPLASIAS

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Objectives: The objective of this study is to examine the accuracy of an automated cervical image analysis system to differentiate high-grade cervical lesions (≥ CIN2, cervical intraepithelial neoplasia) from normal and low-grade lesions.

Methods: A convenience sample of 99 women underwent colposcopy and electrosurgical loop excision procedures. Stereoscopic digital cervical images were acquired throughout the colposcopic examination and after excision. Cervical images were analyzed using multi-step algorithms designed to analyze cervical anatomy, acetowhite epithelium and blood vessels. Linear discriminant analysis was applied to differentiate high-grade lesions from normal and low-grade lesions. Sensitivity and specificity were calculated using cervical image annotations and histopathology as the criterion standards.

Conclusions: The automated image analysis system had a sensitivity and specificity of 92% and 89%, respectively, in discriminating high-grade lesions (≥ CIN2) from normal and low-grade lesions. The receiver operating characteristics area under the curve was 0.94. For comparison, the colposcopic impression had a sensitivity and specificity of 92% and 33%, respectively, compared with histology. We conclude that a computer-based image analysis system that detects and discriminates cervical cancer precursors from less severe neoplasias may be a useful adjunct for colposcopy.

MOLECULAR CYTOLOGY WITH FISH AS A COMPLEMENT TO HPV DNA TESTING

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Objectives: To assess the correlation between accumulation of chromosomal alterations in CIN 3 and invasive cervical cancer lesions and the HPV status in corresponding liquid based cervical cytology.

Methods: We examined 145 FFPE cervical biopsy samples from patients with CIN 3 (n=100), invasive cervical cancer (n=25) and normal histology (n=20) using the 3 component Cervical FISH assay (Abbott). The test simultaneously detects 13 different HPV types (16, 18, 26, 31, 33, 35, 39, 45, 52, 53, 56, 59, 66) with a biotinylated probe and numerical alterations on chromosome regions 3q26 (TERC) and 8q24 (MYC) with fluorescent labelled probes. Cervical FISH result were considered positive when at least 5 cells with an unequivocal HPV FISH signal were detectable, and at least 4 of them had chromosomal alterations (gain of TERC and / or MYC).

In 132 / 145 a BD SurePath liquid based cervical cytology sample taken before the biopsy was available. Real-time quantitative PCR was used to estimate the normalized viral load of HPV 16E7, 18E7, 31E6, 33E6, 35E6, 39E7, 45E7, 51E6, 52E7, 53E6, 56E7, 58E6, 59E7, 66E6 and 68E7 in liquid based cytology. Viral loads were expressed as the number of HPV copies/cell. For samples infected with multiple HPV types the type with the highest viral load was used to correlate with the biopsy result.

Conclusions: There was no copy gain for TERC and / or MYC in disease negative (HPV positive) biopsies. FISH testing revealed that the gains in chromosomal regions 8q24 and 3q26 were observed in all cases of cervical cancer, 63% of CIN3 cases, and in none of normal histology cases. The Cervical FISH assay might be useful in assessing risk of progression from CIN 3 to invasive cancer. The detection of TERC/MYC by FISH is technically robust, sensitive, and easy to evaluate.
SIGNIFICANTLY IMPROVED PREDICTIVE VALUE PROVIDED BY PROEXCTM STAINING OF PAP SMEARS DIAGNOSED AS HSIL OR ASC-H WARRANTS LEAP TO LEEP

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Objectives: Management guidelines now allow for a “leap to LEEP” in women with a pap smear diagnosis of high grade dysplasia (HSIL). This change was due to an improved positive predictive value (PPV) of HSIL (70-80) provided by liquid-based paps sufficient to bypass inefficient colposcopy. In our experience, however, the consequence has been a reduction in HSIL and a doubling of ASC-H (atypical squamous cells, cannot exclude HSIL) diagnoses. Our clinicians are also hesitant to leap to LEEP because of significant false positive rates. HPV testing is not useful in these cases because the PPV is no better than cytology. Our objective was to test whether a specific marker of neoplastic transformation, such as ProExCTM, provides sufficient PPV to warrant “leap to LEEP”

Methods: SurePathTM cervical pap smears diagnosed as either ASC-H (n=136) or HSIL (n=118) were immunostained for ProExC using a Ventana Benchmark XT. At least 5000 epithelial cells were required on each slide to be adequate for staining and outcome required either biopsy proven high grade dysplasia (CIN2+) or at least three years of negative followup. Biopsies were also stained for ProExC and a “Consensus Diagnosis” between gynecologic pathologists (TM and RK) provided the gold standard outcome. Stained pap smears were scored by a cytopathologist (TM) and resident (AS and CR). Because of the difference in CIN2+ prevalence in ASC-H and HSIL, these groups were analyzed separately. Predictive values were calculated and associations tested for by Chi-square.

Conclusions: We observed excellent agreement between pathologists scoring ProExC (ASC-H kappa statistic 0.59; HSIL kappa 0.61). Discordant scores were primarily in paps with fewer than 10 positive cells (52% discordance compared to 3-5% in negative or abundant cases). Chi-square analysis revealed an association between ProExC staining in both ASC-H (p<0.0001) and HSIL (p<0.0001) compared to CIN2+ outcome. The prevalence of CIN 2+ in the ASC-H group was 37% with staining yielding a PPV of 73, NPV 62, and likelihood ratio of 1.8. The prevalence of CIN 2+ in HSIL was 81% with PPV of 95, NPV 78, and likelihood ratio of 4.5. We conclude ProExC staining may strengthen clinical confidence when leaping to LEEP and may warrant LEEP in ASC-H.

NOVEL HISTOCHEMICAL STAIN FOR CERVICAL NEOPLASIA DIAGNOSIS IN BIOPSIES AND CYTOLOGICAL SPECIMENS

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Objectives: The introduction of a new histochemical stain (CellDetect®, Zetiq Technologies) that claims to differentiate between cervical neoplastic cells (cytoplasm stain pink/purple) and normal cells (cytoplasm stain green), while enabling concomitant morphological classical analysis, may represent a significant advance in diagnosis. The objective of the current study was to validate the use of the new CellDetect® method as an adjunct to the diagnosis of cervical neoplastic processes, applying to both cervical biopsies as well as to cervical cytology.

Methods: Two-stage study was performed, the first, examined biopsy samples, while the second focused on cytological samples. In the “biopsy stage”, 60 samples (prepared from paraffin blocks of cervical biopsies) with a previous pathologic diagnoses of normal squamous epithelium (n=20), CIN-3 (n=20), and squamous cell carcinoma (n=20), were independently re-evaluated by 2 pathologists after staining with the novel CellDetect® method. The diagnoses following the CellDetect® staining were compared to the H&E-based diagnosis. In the “cytology stage”, 64 liquid-based cytological samples were obtained from subjects undergoing cervical biopsy. Each sample was stained by both, the classical Pap’s and the novel CellDetect® method, concomitantly with HPV-typing. The 2 slides from each case were blindly examined and the cytologic interpretations were compared to the histological diagnosis of the case.

Conclusions: Cervical neoplasia could be diagnosed and CIN could be graded following the CellDetect® staining by the laminar vertical extent of purple cells through the depth of the epithelium, in addition to the classical morpho-cytological criteria for these grades. High level of agreement (95-100%) was noted between the 2 pathologists in assigning diagnoses based on the CellDetect® method. Similarly, in the “cytology stage”, the sensitivity and specificity of the CellDetect® method were found to be higher compared to the other methods as follows: CellDetect® test - 84% & 86%; Pap’s - 60% & 78%; HPV-typing - 88% & 36%, respectively. The performance of the CellDetect® technology was comparable and even superior to current methods used in cervical diagnosis. The CellDetect® method is simple to read (green vs purple) and offers a new dimension to the diagnosis and grading of cervical neoplasia. The novel staining has a potential to be used in diagnosis as well as in screening for cervical dysplasia.
**SS 7-7**

**CYTOACTIV® - PROGNOSTIC SIGNIFICANCE OF L1 CAPSID PROTEIN DETECTION IN A INTERNATIONAL MULTICENTER STUDY OF 3000 EARLY DYSPLASTIC LESIONS**

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**Background:** Data from ALTS confirmed that HPV DNA testing is not a useful triage strategy in low grade SIL (LSIL) cases and that it is still unresolved if cervical intraepithelial neoplasia grade 2 (CIN 2) represents low grade or high grade lesions. We and others have shown the prognostic significance of HPV L1 capsid protein detection with cytoactiv on HPV high risk associated mild and moderate dysplastic lesions.

In short, HPV L1 capsid protein negative early dysplastic lesions are significantly more likely to progress to histologically confirmed CIN 3 lesions than are L1 positive cases.

**Objective:**

1.) to validate the prognostic relevance of HPV L1 capsid protein detection for early dysplastic lesions.

2.) to evaluate the impact of different preparation techniques (conventional Pap smear versus FDA approved LBC) on the sensitivity of L1 detection and its prognostic significance.

**Material and Methods:** Until June 2008, study centers located in Germany, USA, Sweden, Italy, Switzerland, and Australia contributed 3000 randomly selected cases of HIV negative, non-pregnant, non HPVL1 vaccinated women reported as LSIL (internationally) or as group IIID (Germany) with subclassification into mild (LSIL) or moderate (HSIL) dysplasia.

Follow up will be until June 2010.

**Results and Conclusion:** 53-85% of the LSIL and 23-50% of the HSIL cases have been positive for L1 capsid protein detection. L1 positive mild and moderate dysplasias, reflecting productive HPV infection, showed a low risk of progression. L1 negative early dysplastic lesions, as non-productive infections or precancerous lesions, showed a high progressive potential, as high as expected for severe dysplasias.


**SS 7-8**

**DUAL STAIN FOR P16 / KI-67 CO-EXPRESSION AS A HIGHLY EFFICIENT TOOL TO TRIAGE PAP NEGATIVE, HPV POSITIVE CERVICAL CANCER SCREENING RESULTS**

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**Background:** Primary screening using co-testing for Pap cytology and HPV has been proposed as a potential way to increase the sensitivity for the detection of high-grade cervical dysplasia in women aged 30 years and older. However, to detect CIN2+ with high sensitivity, HPV co-testing requires the follow-up of women tested negative for Pap abnormalities, but positive for HPV. As the number of underlying disease cases is low, it is highly desirable to have a diagnostic test that allows for the efficient triage of Pap negative, HPV positive screening test results.

p16 has been shown to be strongly over-expressed in dysplastic cervical tissues. As the simultaneous expression of both p16 and proliferation markers such as Ki-67 within the same cell should mutually exclude each other under normal physiological conditions, the detection of epithelial cells in cervical cytology specimens co-expressing both markers should indicate cell cycle deregulation and may be used as a biomarker for dysplasia independent from morphology interpretation.

**Objectives and Methods:** We assessed the performance of an immuno-cytochemical dual stain (CINtec® PLUS, mtm laboratories) simultaneously detecting both p16 and Ki-67 over-expression in a cohort of more than 4,500 women aged 30 and older and participating in a prospective screening study with Pap cytology (ThinPrep®) and hc2 HPV testing. Follow-up results were collected during repeat visits for any positive Pap or HPV test result. HGCIN as confirmed on biopsy was used as study endpoint. Results: Sensitivity of baseline CINtec PLUS Dual stain testing for the detection of CIN2+ (CIN3+) developing during a ≥2 years follow-up period of Pap negative, HPV positive women was 87% (92%). Negative CINtec PLUS test results at baseline had a NPV of > 97% for the development of CIN2+ during follow-up.

**Conclusions:** The detection of co-localization of p16/Ki-67 expression in cervical cells identifies women that may benefit from immediate colposcopy, whereas negative test results (70% of all cases tested Pap negative, HPV positive in women aged 30 and older) may exclude HGCIN with a high NPV.
UTILITY OF DUAL-STAIN FOR P16 AND KI-67 IN THE INTERPRETATION OF ABNORMAL PAP CYTOLOGY RESULTS: A PROSPECTIVE STUDY

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Objectives: p16 has been found to be strongly over-expressed in nearly all high-grade pre-cancerous and cancerous cervical lesions and may serve as a surrogate marker for the transforming activity of high-risk HPV. As over-expression of the cell-cycle regulatory protein p16 in cells with intact cell cycle regulation should prevent those cells from proliferating, we further tested the hypothesis that the detection of individual cells simultaneously co-expressing p16 protein and proliferation marker Ki-67 can be used as an indicator for the presence of cervical dysplasia. To accomplish this goal we initiated a large prospective study on cervical cytology specimens showing Pap abnormalities.

Methods: Residual materials from liquid-based cytology specimens of women attending cervical cancer screening at a major tertiary hospital and reference center over one year period were used for the analysis. Specimens with any abnormal Pap cytology result (ASC-US+) were included. For each case, an additional ThinPrep™ slide was prepared and immuno-stained using a prototypic dual staining reagent kit (CINtec© Cytology, Dual stain) for the simultaneous detection of p16 and Ki-67 expression on the same slide. The presence of one or more individual cells co-expressing both p16 and Ki-67 were interpreted as “positive” test result. Follow-up biopsy and HPV results were obtained.

Conclusions: 1. Sensitivity of the Dual stain was 87.0% for CIN2+ and 95.7% for CIN3+, with specificity of 89.8% for non high-grade CIN. 2. In ASC-US/ASC-H/LSIL categories with positive HPV results, a positive Dual stain result identified all CIN3+ cases at high levels of specificity (up to 80%). 3. Initial results from a first set of cytology specimens subjected to simultaneous p16/Ki-67 dual staining and with biopsy follow-up (n=661) indicate both high sensitivity and specificity of this novel screening approach to detection of CIN2/3+ on biopsy follow-up. 4. Results showing high specificity rates for the Dual stain support this approach as an enhancement for detecting histopathological CIN2/3+.

TRIAGE OF HPV POSITIVE WOMEN BY NON-MORPHOLOGICAL METHODS

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Results from several randomized trials argue for the implementation of HPV testing to increase the effectiveness of cervical screening programs. Yet, additional triage tests are required to differentiate among HPV-positive women those who have or develop CIN2+ and need referral for colposcopy, from those who have transient HPV infections and should be referred to the next screening round. Presently, reflex cytology and viral genotyping (HPV16/18) have been advocated by the American Society for Colposcopy and Cervical Pathology (ASCCP) as valuable triage tools for HPV-positive women. However, cytology is subjective with a considerable range of insecurity and non-16/18 high-risk HPV-positive women can also have or develop CIN2+. Moreover, cytology as triage tool is unsuitable for self-collected specimens, a screening approach, which nowadays get more attention to improve screening compliance. In recent years, many efforts have been put in identifying novel (molecular) biomarkers, both of viral (eg. E6/E7 mRNA) and non-viral (eg. p16INK4a) origin, for risk stratification of HPV-positive women. Biomarkers that allow detection of molecular events that are essential for cervical carcinogenesis are likely most promising to improve the detection of clinically relevant CIN2+ lesions. We recently identified two genes, i.e. CADM1 and MAL, which not only displayed a functional role in HPV-mediated transformation, but also were frequently affected by epigenetic silencing via promoter methylation in cervical carcinomas and high-grade CIN lesions. Data from recent studies performed on large cohorts of screening responders (i.e. POBASCAM) and non-responders (i.e. PROHTECT) indicate that methylation analysis for CADM1 and MAL in combination allow stratification of HPV-positive women for risk of CIN2+, both on physician-taken and self-sampled smears, with sensitivities for CIN2+ exceeding those of cytology. Therefore, these methylation markers are potentially valuable alternative triage tools for hrHPV positive women.
**COMPARISON OF HPV HIGH RISK ASSAYS FOR HIGH GRADE CIN IN WOMEN FOR ABNORMAL SMEARS**

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**Objectives:** The aim of this study was to compare the diagnostic accuracies of several tests for the detection of advanced disease (CIN2+) in a population referred to colposcopy because of abnormal cytology.

**Methods:** 133 women were included in four referral gynecologic clinics in Marseilles (France) between March 2007 and June 2008. Five HPV tests were assessed in addition to colposcopy: Hybrid Capture 2 (HCII) (Digene), Papillocheck (Greiner), Abbott RealTime HR HPV (RT HR HPV) (Abbott), Linear Array (Roche), and EasyQ HPV (Biomérieux).

**Results:** 36 (34%) women had CIN2+; among them 6 (6%) had CIN3+ disease. For CIN2+ detection, all tests had comparable sensitivities except EasyQ HPV test which tends to had a lower sensitivity: HCII 94%, Papillocheck and LA 92%, RT HR HPV 89%, and EasyQ HPV 75% (P not significant). On the other hand, EasyQ HPV specificity tends to be higher than the one of the other tests: Papillocheck 49%, HCII and LA 48%, RT HR HPV 31%, and EasyQ HPV 65% (P<0.08 for the least significant comparison). Positive and negative predictive values were comparable for all tests: from 84% to 94% for NPVs, and from 47% to 52% for PPVs.

**Conclusions:** All tests had comparable diagnostic values for CIN2+ detection, although DNA based tests seem to be more sensitive and RNA based assay more specific. The use of a combination of these tests as an alternative to colposcopy should be of interest. A complementary analysis of HPV genotypes detected by these tests is conducted in order to improve the interest of using such tests in laboratory routines.

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**COMPARISON OF HPV DETECTION TECHNOLOGIES; HYBRID CAPTURE 2 (QIAGEN), FULL-SPECTRUM HPV (GENOID), GENOID MOLECULAR BEACON REAL-TIME HPV ASSAY WITH GENOTYPING BY LINEAR ARRAY (ROCHE) AND GENOID HPV ELISA GENOTYPING ASSAY IN AN IRISH COLPOSCOPY POPULATION**

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**Objectives:** Cervical screening programmes are moving towards HPV testing as part of the screening process and as triage for colposcopy. We evaluated 3 HPV detection and 2 genotyping methods on liquid based cytology specimens from a colposcopy population. This study forms part of the AutoCast Consortium funded under the EU 7th framework and is supported by the Irish Cervical Screening Research Consortium CERVIVA formed under the Health Research Board.

**Methods:** Cytology specimens from 150 women with greater than 2 persistently abnormal smears were recruited through the Coombe Women and Infants University Hospital, Dublin. Smears were taken at first visit and prior to any procedure. Patients were examined colposcopically and a biopsy or Lletz performed. Cytological diagnoses were made using BSCC guidelines. HPV DNA was detected by Hybrid Capture (hc2) for 13 high-risk HPV types, Full-Spectrum HPV (FS-HPV) for 49 high and low-risk types and Molecular Beacon Real-Time HPV assay (MB-RTHPV) for 16 high and low-risk types. HPV genotyping was performed using Linear Array HPV Assay (LA) and HPV ELISA assay. Histology results were available for N=93 hc2 cases, N=81 FS-HPV cases and N=82 MB-RTHPV cases.

The sensitivity of the assays for CIN2+ cases were, 96%, 98% and 94% for hc2, FS-HPV and MB-RTHPV assays with positive predictive values (PPV) of 64%, 64% and 68% and negative predictive values (NPV) of 75%, 86% and 77% respectively. The most common HPV genotypes were 16, 33, 58, 31, (18 and 51), 66, 39 and 56 for the LA assay versus 16, (33, 31, 51), 58, (66 and 39), 56, 18 by HPV ELISA.

**Conclusions:** The FS-HPV and MB-RTHPV show comparable sensitivity and have a similarly high PPV as the hc2 assay for HPV detection in CIN2+ patients, while the FS-HPV has a higher NPV. HPV genotype distribution was similar for all types with the exception of HPV18 which was detected more frequently using LA.
**COMPARISON OF PAPILLOCHECK® DNA MICRO-ARRAY HPV DETECTION ASSAY WITH HC2 AND PCR ENZYME IMMUNOASSAY (GP5/6+)**

HIBBITTS S, JONES J, PEEVOR R, SALEEM A, POWELL N, TRISTRAM A, FIANDER A

HPV Research Group, Cardiff University, Cardiff, UK

**Objectives:** To evaluate the PapilloCheck® micro-array assay (PapilloCheck®) for detection of HPV in comparison with Hybrid Capture II (hc2) and PCR-enzyme immunoassay (PCR-EIA) in different sample sets.

**Methods:** The HPV tests were applied to samples from 3 groups:
1. A cytologically defined population (n=878)
2. Cases with reported dyskaryotic cytology and an initial HR HPV negative result (n=52 out of 219 in a population of 10,000)
3. Women attending colposcopy for LLETZ treatment of biopsy proven high grade CIN with smears collected at treatment with a follow-up sample collected at 6 months (n=195 paired samples).

**Results:** In group 1, 674 out of 878 samples gave a consistent result (76.8%; 95% CI 73.83-79.52%) on all 3 platforms and the genotype results obtained by PapilloCheck® and PCR-EIA were 94% consistent (95% CI 92.1-96.4%). There was no statistically significant difference between the performance of each assay when HR HPV positive samples were linked with clinical result (cytology and histology grade) although PapilloCheck® detected the highest number of HR HPV infections in samples with histology confirmed as CIN1, CIN2 and CIN 3 (76.6%, 85% and 91.7% respectively).

In group 2, direct repeat HPV PCR-EIA identified 24% (n=12/51) of samples positive for HR HPV. Re-extracted DNA and PCR-EIA increased detection to 41.2% (n=21/51) and PapilloCheck® detected 78.4% (n=40/51). HR HPV detection by PapilloCheck® was significantly higher compared with the other methods of re-analysis.

Analysis of the group 3 samples by PapilloCheck® and PCR-EIA is currently ongoing and updated results from this cohort will be presented.

**Conclusions:** In groups 1 and 2 PapilloCheck® proved to be a sensitive, reproducible, robust molecular assay for HPV genotyping with the potential for high throughput of specimens in a clinical setting. In addition, results from group 2 highlighted that up to 78% of samples with dyskaryotic cervical cytology that test negative for HPV can be found to be HPV positive on re-analysis. The reliance on a single negative HPV test result could lead to missed HPV related disease in a subset of patients, the number dependant on which HPV test is performed.

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**COMPARISON OF HPV DNA DETECTION TECHNOLOGIES; HYBRID CAPTURE II (QIAGEN), CERVISTA™ HPV HR (HOLOGIC UK LTD) IN A NORTHERN IRISH SCREENING POPULATION**

Keegan H 1, Pilkington L 1, Jamieson J 2, Wilson RT 2, Carson J 2, Martin CM 1, O’Leary JJ 1

1 Department of Histopathology, University of Dublin, Trinity College, and Coombe Women and Infants University Hospital, Dublin 8. Ireland
2 Department of Cytopathology and Molecular Pathology, Antrim Area Hospital, Antrim, Northern Ireland

**Objectives:** Cervical screening programmes worldwide are moving towards HPV DNA testing as part of the population screening process. We evaluated 2 HPV detection methods on ThinPrep Pap Test specimens from a cervical screening population.

**Methods:** Cervical smear specimens from 331 women were recruited through the Antrim Area Hospital, Antrim and the residual PreservCyt was used for HPV testing. Cytological diagnoses were made in accordance with UK National Health Service Cervical Screening Programme (NHSCSP). HPV DNA was detected by Hybrid Capture (hc2) for 13 high-risk HPV types and Cervista HPV HR (Cervista) for 14 high-risk types including HPV66, which is not included in the hc2 assay. The Cervista assay includes three different HPV specific master mixes one of which (Mix 1) contains probes for HPV66, HPV56 and HPV51. HPV66 and 51 are the joint third most common HPV types in an all Ireland study of HPV prevalence.

**Results:** The prevalence of HPV was 23% by hc2 and 22% by Cervista. The concordance rate was 87%. A discordant result is a positive result by one assay and negative by the other test. Using detection of HPV in specimens with mild or greater abnormalities as the true positive, there was no significant difference in the sensitivity (P=0.148) or specificity (P=0.918) of the tests.

**Conclusions:** There was a high rate of concordance between the hc2 assay and Cervista despite the absence of HPV66 from the hc2 assay mix. This may be due to cross-reactivity of HPV66 with other HPV probes contained in the hc2 assay. There was no statistical difference in the sensitivity or specificity of either assay Further studies containing greater numbers of cases with severe abnormalities will be performed.

This study was sponsored by Hologic Inc. and forms part of the Irish Cervical Screening Research Consortium CERVIVA funded by the Health Research Board, Ireland.
FOLLOW-UP AFTER CONIZATION: WHEN DOES HPV TEST WORK THE BEST?

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Introduction: HPV test has proven to be excellent as a follow-up method of conization. However, when practiced too soon after the surgical procedure HPV test often remains positive; conversely, if proposed too late, many patients will be lost to follow up.

Objectives: determine the best date to perform HPV testing after conization.

Material and Methods: From October 2000 to October 2007, 582 conizations were performed. Hybrid Capture 2 (Qiagen) HPV test was taken most often just before the surgical procedure in the operating ward and in some cases a few weeks before. A follow up visit was proposed to the patients after 3 to 6 months in order to obtain Pap smear and HPV testing; 351 controls only were performed, as many patients were seen by their private doctor and no HPV test was obtained.

Results: They are shown in tables I and 2.

<table>
<thead>
<tr>
<th>* CIN</th>
<th>N</th>
<th>HPV - 3 months</th>
<th>N</th>
<th>%</th>
<th>N</th>
<th>%</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>CIN2</td>
<td>91</td>
<td>23</td>
<td>56%</td>
<td>39</td>
<td>61,5%</td>
<td>28</td>
<td>70,5%</td>
<td></td>
</tr>
<tr>
<td>CIN3</td>
<td>366</td>
<td>98</td>
<td>68,8%</td>
<td>114</td>
<td>69,1%</td>
<td>95</td>
<td>77,8%</td>
<td></td>
</tr>
<tr>
<td>CIN2+</td>
<td>457</td>
<td>121</td>
<td>65,8%</td>
<td>153</td>
<td>63,5%</td>
<td>123</td>
<td>75,5%</td>
<td></td>
</tr>
</tbody>
</table>

Table I Results of HPV testing, according to delay after conization

In table 2 the influence of the viral load appears low.

<table>
<thead>
<tr>
<th>Viral load</th>
<th>N cases</th>
<th>N - at 12 months</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-99 RLU</td>
<td>145</td>
<td>112</td>
<td>77.25</td>
</tr>
<tr>
<td>100-999 RLU</td>
<td>169</td>
<td>128</td>
<td>75.74</td>
</tr>
<tr>
<td>&gt; 1000 RLU</td>
<td>52</td>
<td>36</td>
<td>69.24</td>
</tr>
</tbody>
</table>

Table 2 Rate of - at 12 months according to the initial viral load

Comments: There is no significant difference at 3, 6 and 12 months. It justifies the first control at 3 months: so we can decrease the rate of lost to follow-up and treat without delay a synaechia of the cervical os. The viral load seems to have a little influence.

Conclusion: HPV testing 3 months after the conization is efficient, avoids poor follow up, and allows to detect ant treat complications.

HPV DNA VIRAL LOAD AND E6 PROMOTER METHYLATION IN LIQUID-BASED CYTOLOGY SAMPLES STRATIFIED BY DISEASE STAGE

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3 Division of Cancer Studies and Imaging, University of Manchester, Manchester M13 0JH, UK

Objectives: Vaccine-incorporated HPV genotypes account for ca. 70% of cervical cancers and so disease burden due to other oncogenic types, and vaccine types in unvaccinated women, will remain significant for decades. Delineation of epigenetic markers of cervical disease is critical to improve prognostic tools. Our objectives were to determine DNA viral load (VL) and 3'L1-LCR-E6 promoter methylation status in liquid-based cytology samples (LBC) stratified by cervical disease grade.

Methods: LBC (n=260) were selected, based on monospecific infection with HPV 16 (n=168), 18 (n=20), 31 (n=53) or 45 (n=19), from a cohort of 4,162 women undergoing cervical screening. LBC were grouped into Normal and Low Grade (NLG: Normal and borderline cytology) and High Grade (HG: Moderate or Severe dyskaryosis). Where data were available, 12% of NLG and 87% of HG LBC were confirmed as ≥ CIN2. Mildly dyskaryotic samples were histologically heterogeneous and excluded to create a clear differentiation between the NLG and HG groups. HPV VL (DNA copies per cell) was determined using Taqman™ real-time PCR for HPV 16, 18, 31, or 45 and the cellular gene, GAPDH, and HPV 16 3'L1-LCR-E6 promoter methylation status was determined by Biotage™ pyrosequencing.

Conclusions: Although DNA VL data overlapped, significant differences were found between NLG and HG LBC for HPV 16 (median NLG VL = 1.52 [IQR 0.01 - 17.87], HG = 31.40 [6.28 - 104.33]; Mann Whitney U test, p<0.001) and HPV 31 (NLG = 1.62 [0.04 - 17.44], HG = 21.33 [2.68 - 86.63]; p<0.01) and the data for HPV 18 (NLG = 0.12 [0.01 - 3.24], HG = 1.06 [0.28 - 8.20]; p=0.081) and HPV 45 (NLG = 1.11 [0.01 - 9.99], HG = 31.97 [18.86 - 32.18]; p=0.162) suggests a trend in the same direction. These differences were reduced or eliminated when mildly dyskaryotic samples were included in the NLG group. For HPV 16 and 31 a trend was found between DNA VL and individual LBC grade (Kruskal-Wallis test, p<0.001). HPV 16 3'L1-LCR-E6 promoter methylation data will be presented. LBC are convenient for study but it remains to be seen whether inherent sampling heterogeneity and poor histology grade specificity hinder their utility in evaluating prognostic markers.
**THE EUROPEAN REALTIME HIGH RISK HPV EXPERIENCE: RESULTS EVALUATION OF THE ABBOTT REALTIME HIGH RISK HPV ASSAY IN A PRIMARY SCREENING SETTING**

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**Objectives:** It is essential that new Human papillomavirus (HPV) tests are evaluated within population-based screening settings. This study investigates the longitudinal clinical performance of Abbott RealTime high-risk HPV assay within a primary screening setting.

**Methods:** From Swedescreen, a randomized-controlled trial of HPV-based screening of women 32-38 years nested within the Swedish cervical screening program, baseline cervical samples from women who developed cervical intraepithelial neoplasia 2 or worse (CIN2+) during 5 years of follow-up (n=197) and a population-based sub-sample (n=788) were selected for HPV testing with three HPV tests. GP5+/6+ PCR detects 14 individual high-risk HPV types, Abbott RealTime detects the same 14 high-risk HPV types and simultaneously differentiates between HPV 16, 18 and 12 non-HPV 16/18 types and the Hybrid Capture II (HCII) assay detects 13 high-risk HPV types without individual typing. Kappa statistics, sensitivity (ability to detect disease), and positive predictive values (PPV, a measurement of unnecessary follow-up), were calculated with both CIN2+ and CIN3+ as outcomes.

**Conclusions:** Test agreement between Abbott RealTime and GP5+/6+ PCR was better for both a randomly selected population-based sample and among women with CIN2+ (kappa values 0.82 and 0.66 respectively) than between Abbott RealTime and HCII (kappa values: 0.71 and 0.60 respectively). Abbott RealTime was not inferior at detecting CIN2+ or CIN3+ compared to GP5+/6+ PCR or HCII (non-inferiority test defined as sensitivity of Abbott RealTime should not be <90% of performance of GP5+/6+ PCR or HCII, p-values <0.007). The PPV was similar for the three tests with both CIN2+ and CIN3+ as endpoint (range 14.9-16.1% and 9.7-10.6% respectively). Both GP5+/6+ PCR and Abbott RealTime identified women at increased risk of CIN2+ and CIN3+ due to infection with HPV 16 and 18 compared to women infected with other high-risk HPV types. In conclusion, Abbott RealTime high-risk HPV assay has an acceptable sensitivity and PPV to detect CIN2+ and CIN3+ in primary screening in a population with a high-risk HPV prevalence as in the Swedescreen trial, and the assay has the ability to identify women at increased risk of CIN2+ and CIN3+ due to infection with HPV 16 and 18.

**BIOMARKERS- USING AN IMPROVED GOLD STANDARD**

Wentzensen N

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Determining the cervical disease gold standard is important for screening programs, clinical management, natural history studies, and biomarker validation. The current disease gold standard is based on identification of women with abnormal screening results, referral to colposcopy and biopsy, and morphological evaluation of cervical tissue obtained from biopsies or excisional treatment. Problems to correctly identify the disease gold standard can occur at several levels. First, an imperfect screening test may not refer all women with disease for further workup. Second, colposcopy has been shown to be unreliable with missed disease in up to 50% of prevalent lesions. Third, histological evaluation of tissue specimens may be challenging, especially in transitional categories such as CIN1 and CIN2.

While failure to detect prevalent disease in screening programs is usually compensated by frequent screening, it can pose a challenge for biomarker validation studies. New biomarkers with performance exceeding that of the gold standard may produce false false-positive results, as has been demonstrated for HPV-based screening. The problems of ascertaining the cervical disease gold standard can be addressed by accounting for referral bias, by increasing the number of biopsies taken during colposcopy including biopsies from visually normal areas, and by conducting expert histology reviews and using biomarker-adjudicated histology endpoints.
Infections by high risk HPV types are widespread among women and men. The vast majority of these infections are transient and regress spontaneously; however at distinct epithelial sites some of these infections may become persistent and eventually progress to invasive carcinomas. Biomarkers that help to identify HPV-infected epithelial cells that will undergo neoplastic progression are important diagnostic tools in cancer early detection, diagnosis and prevention. Conceptually, these markers should unequivocally indicate molecular signatures that trigger neoplastic transformation among the many infected cells. Recent research revealed that differential expression of viral genes during its replication cycle and HPV-induced neoplastic transformation is triggered by differential methylation of certain CpG dinucleotides within the HPV genome. Biomarkers that indicate the activation of the transforming mode of viral gene expression have substantial conceptual advantages in comparison to the mere detection of persistent HPV infections.

At the conference we will discuss how the viral life cycle is regulated by epigenetic modification and how this relates to the expression of viral and cellular markers that indicate neoplastic transformation. A particular focus will be put on the conceptual and clinical use of p16INK4a, a cellular marker that specifically highlights the transforming mode of viral gene expression.

**ONE HPV VIRUS, ONE LESION AS DETERMINED BY LCM/PCR TECHNOLOGY**

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(1): DDL Diagnostic Laboratory, Voorburg, The Netherlands, (2): GlaxoSmithKline Biologicals, Rixensart, Belgium

**Background:** In 20-40% of CIN lesions and in 4-8% of cervical carcinoma specimens multiple HPV genotypes have been detected by PCR on DNA isolated from whole tissue sections. Whether these HPVs are causally related to CIN lesion present in the tissue is difficult to investigate by existing hybridization technologies like Insitu DNA Hybridization. Applying Laser capture microdissection (LCM) in combination with a sensitive PCR-based HPV genotyping system can significantly increase the specificity of relating HPV types to specific lesions.

**Objective:** To investigate in CIN lesions whether one virus or more viruses are present in one lesion.

**Materials and Methods:** After examination of Haematoxylin Eosin slides, selected regions were isolated by LCM (Zeiss P.A.L.M.) and were analyzed by the broad spectrum HPV SPF10 PCR/ LiPA25 HPV genotyping system version 1 (1*). HPV genotypes detected in LCM captured lesions were compared to results in 37 whole tissue sections CIN1, 2 and 3 lesions with multiple HPV types.

**Results:** From the 37 cases with multiple HPV in the whole tissue section, using LCM, 575 lesional areas were removed with an average area size of ~30,000 um2 and tested for the presence of HPV. On LCM area level single HPV positivity was found 70.5% of the lesional areas, multiple HPV types were detected in 5.4% of areas and 24.1% of areas were HPV negative. On case level, 93% (n=13) of CIN1 cases were positive and 7% (n=1) was HPV negative by LCM. 100% (n=23) of CIN2/3 cases could be assigned to a single HPV genotype by LCM.

**Conclusion:** The LCM/PCR technique is a highly accurate method providing a high resolution HPV genotyping and allows for assignment of a specific HPV type to one CIN lesion.

(1*) SPF10 HPV LiPA25, version 1 and SPF10 HPV DEIA are manufactured by Labo Biomedical Products (Rijswijk, The Netherlands) based on licensed INNOGENETICS SPF10 technology.
The severity of cervical neoplasia is generally assessed on the basis of cell morphology, either by cytology, or by pathology-assessment following the inspection of tissue sections stained with haematoxylin and eosin. Disease grading depends on the extent to which cell divisions extend above the basal layer, and on other morphological criteria such as nuclear/cytoplasmic ratio and the presence of cytopathic features of HPV infection such as koilocytes. As cervical neoplasia and cervical cancer are generally caused by papillomavirus infection, the alternative is to implement a ‘molecular pathology’ scheme in which the de-regulation of viral and cellular gene expression is directly assessed at the level of protein expression. To some extent, this approach is already in use with markers such as p16 (which is thought to detect de-regulated E6 and E7 and thus most CIN3), and MCM, which detects and localizes cells expressing E7 irrespective of disease grade. By supplementing such ‘E7’ staining with easily detectable markers of virus infection such as E4 and L1, we are starting to develop a molecular classification of early cervical disease based on the extent of de-regulation of viral gene expression. It appears that this approach may be able to distinguish viral and non-viral CIN1, and allow division of the CIN2 group into categories based on the extent to which E7 expression extends into the upper epithelial layers and on the loss of HPV late gene expression. Molecular pathology methods have the potential to eliminate the subjectivity of interpretation that confounds the pathology and cytology-based evaluation of cervical neoplasia.

Many studies assessed biomarker expression in cervical cytological samples or histological specimens, demonstrating that biomarker overexpression or downregulation correlates with the degree of cervical abnormality. However, several shortcomings of immunochemistry, such as the lack of standardized methodology, interobserver variation and the absence of systems for automatic interpretation, hamper the determination of its clinical role and the implementation in a screening setting. Recently, FCM has been applied on LBC specimens for quality control purposes (Polina et al., 2008) or to detect p16\textsuperscript{INK4a} and cell cycle markers (Ling et al., 2008). These studies suggest the conceptual feasibility of FCM in a LBC setting. However, in almost all published studies specimens were filtered to remove clusters. Removal of cell clusters does not seem a reliable approach because of their diagnostic relevancy, while desegregation techniques are not straightforward. The epithelial character of these cells makes desegregation very difficult, especially if cell integrity is important. Our experiments based on p16\textsuperscript{INK4a} showed that the fluorescence contrast between biomarker positive and biomarker negative cells was minor. In this context, the choice of fluorochromes appeared to be decisive and primary labeling of the antibodies was time-consuming and expensive. The most important disadvantage of the FCM technique for cervical screening is the analysis and preparation time.

In conclusion, flow cytometry analysis of cervical samples, although theoretically interesting, is hampered by technical, methodological, logistical and conceptual problems, making it unfit for high throughput settings.
A NOVEL CYTOLOGY-BASED BIOMARKER ASSAY TO IMPROVE THE DETECTION OF CIN2+ DISEASE


BD Diagnostics - Women's Health and Cancer, Durham, NC, USA

Objective: To develop an improved cervical cancer screening test that combines protein biomarker immunocytochemistry with a Papanicolaou (Pap) counterstain to assist with the identification of abnormal cells on BD SurePath liquid-based cytology slides.

Methods: BD ProEx C is a commercially available biomarker cocktail containing antibodies to minichromosome maintenance protein 2 (MCM2) and topoisomerase II± (TOP2A) with both immunocytochemistry (ICC) and immunohistochemistry applications. These S-phase proteins are upregulated upon cell cycle dysregulation resulting from persistent HPV infection. In cervical cytology, BD ProEx C has been used as a reflex test for borderline cases, with research studies showing an improvement in the positive predictive value for CIN2+ over cytology alone. Given the ability of the biomarkers to assist in the detection of true cervical disease, we set out to design an assay to be utilized for cervical screening that would incorporate both the standard Pap stain and protein biomarker detection concurrently on a single slide. This involved development of low heat antigen retrieval methods for retention of cellular morphology, antibody selection to allow high specificity, and optimization of Pap staining. This novel assay, the BD SurePath Plus test, was performed on standard BD SurePath specimens using an integrated staining instrument. Briefly, cell pellets were resuspended and deposited on slides. Protein biomarker staining for S-phase proteins MCM2 and MCM7 was performed using an optimized ICC assay, followed by a Pap counterstaining procedure. To evaluate the performance of the new assay, two slides were prepared for each case, one processed for a traditional SurePath Pap test and the other for the BD SurePath Plus test. Preliminary results have validated the feasibility of the novel assay concept and performance data will be presented.

Conclusions: The BD SurePath Plus test integrates the detection of the MCM2 and MCM7 biomarkers with Bethesda classified cellular morphology on a single cytology slide. The brown ICC staining assists with both the localization of potentially abnormal cells and with the cytological assessment of borderline cases, by detecting those cells with aberrant S-phase induction resulting from persistent HPV infection.

3q26 AMPLIFICATION IS RARELY PRESENT IN WOMEN WHOSE LSIL CYTOLOGY DOES NOT REPRESENT CIN 2+ DISEASE.

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University of Missouri-Kansas City School of Medicine, Departments of Community and Family Medicine1, Obstetrics and Gynecology1, Pathology2, Kansas City, MO USA. 3Ikonisys, Inc, New Haven, CT.

Objective: 10-17% of women with LSIL cytology truly have CIN 2+ disease at colposcopically directed biopsy and 20% of the CIN 2+ lesions derive from women with LSIL cytology. No molecular marker has yet been able to triage LSIL cytology effectively. If possible, the triage would spare women the referral to colposcopy. Irreversible chromosomal damage occurs during oncogenesis. Increasing cervical dysplastic severity occurs with increasing amplification of the 3q26 chromosomal region. The purpose of this study is to evaluate the test characteristics of 3q26 amplification in women whose routine cytology is reported as LSIL with emphasis on the negative predictive value for reassurance.

Methods: We conducted a retrospective study using the available SurePath™ liquid cytology LSIL archival samples from women 17-59 years old which were linked to colposcopically directed biopsy samples taken on average 36 days after cytology sampling (3-90 day range). Nuclei from the LSIL samples were hybridized with a single-copy probe for the chromosome 3q26 region and a control probe for the centromeric alpha repeat sequence of chromosome 7, using standard FISH methods. Amplification was defined as five or more signals present in at least 2 cells.

Results: Of the 68 paired cytology/biopsy samples, 3q26 amplification occurred in 40% of the women with CIN 2+ disease (sensitivity 95% CI: 12, 74). There was no amplification in 91% of women with less than CIN 2 disease (specificity 95% CI: 81, 97); and the negative predictive value was 90% (79, 96).

Conclusions: The lack of 3q26 amplification in women with screening cytology LSIL results offers reassurance that CIN 2+ disease has not developed. Future prospective studies are ongoing.
Objective: To assess the value of p16\textsuperscript{INK4a} immunostaining to aid accurate diagnosis of CIN2/3 lesions in routine histopathology

Methods: We collected 454 biopsies of cervical lesions, and assessed whether the use of p16\textsuperscript{INK4a} immunohistochemistry can increase reproducibility and accuracy of CIN lesion grading. First, we used a (randomly) selected subset of 54 samples to evaluate the value of p16\textsuperscript{INK4a} immunostaining to reduce inter-observer variation in CIN diagnoses between three pathologists. The inter-observer agreement was calculated using kappa statistics. Subsequently, in the total cohort we compared both the routine (original) diagnoses and the consensus (review) diagnoses of two histopathologists based on H\&E-staining, to lesion grading on H\&E-stained slides by a single histopathologist using p16\textsuperscript{INK4a}-stained slides as an adjuvant marker (p16-supported diagnoses). Again, kappa statistics were used to evaluate the concordance between the routine diagnoses, the consensus (review) diagnoses and the p16- supported diagnoses.

Conclusions: Our results show that the agreement between different pathologists, for evaluating H\&E-stained slides, ranged from fair (kappa 0.41 (95% CI 0.16-0.60)) to moderate agreement (kappa 0.53 (95% CI 0.31-0.70)). However when scoring was based on p16\textsuperscript{INK4a}-stained slides, the concordance improved significantly for all pathologists (mean kappa 0.87 (95% CI 0.74-0.93)). Furthermore, results from the analysis on the total cohort show that the routine registered diagnoses of high-grade CIN lesions, as made in our lab setting, is nearly as accurate as both the consensus diagnoses made by expert pathologists (kappa 0.84 (95% CI 0.76-0.91)) and the p16-supported diagnoses of a single pathologist (kappa 0.71 (95% CI 0.66-0.77)). However, considering the speed of the diagnostic process, the increase in reproducibility and the ease of grading by the use of p16\textsuperscript{INK4a} immunohistochemistry, we advise to use a p16\textsuperscript{INK4a}-stained slide complementary to routine H\&E staining, as an alternative to histology review.
Monitoring cervical screening is recommended by European Guidelines for quality assurance in cervical cancer screening. In order to compare performance indicators in different European countries standardised tables of aggregated data were collected from 15 European national or regional cervical screening programmes and key performance indicators computed as reported in EU Guidelines, 2nd edition.

Cytological results varied widely between countries, both in the total proportion of abnormal tests (from 1.2% in Germany (Mecklenburg-Vorpommern) to 11.7% in Ireland-Midwest Region) and as for their distribution by grade. Referral rates for repeat cytology (ranging from 2.9% of screened women in the Netherlands to 16.6% in Slovenia) and for colposcopy (ranging from 0.8% in Finland to 4.4% in Romania-Cluj) and the Positive Predictive Value (PPV) of colposcopic attendance (ranging from 8% in Romania-Cluj to 52% in Lithuania) were strongly influenced by management protocols, in particular for ASCUS and LSIL cytology. However, cytology-specific PPV also showed remarkable variability. The detection rate of CIN2+ histology ranged from <0.1% of screened women in Poland to >1% in England and Denmark. Low attendance for colposcopy after referral was observed in some east-European countries. Overall, quality was better in countries that have operated organised programmes for a longer time, plausibly as a result of long-lasting monitoring and quality assurance activities.

These comparisons may be useful for improving the performance of cervical screening and will be even more needed if new screening technologies and vaccination for Human Papillomavirus are introduced. Therefore, the availability of these data, the first comparing European countries, represents progress. Nevertheless, there is a clear need to standardise the cytological and histological classifications used in screening, as well as data registration systems across Europe.

**Objectives:** A comprehensive audit of the Swedish cervical cancer screening program to set standards for routine monitoring to improve the effectiveness. We evaluated risk factors for developing cancer and whether early diagnosis by screening also implies improved survival.

**Methods:** Nationwide case control study linking the population, cancer, morphology and causes-of-death registers. All 1230 cervical cancer cases in Sweden during three years were reviewed and classified by age, stage and histopathological type. Five population controls per case. Screening history obtained from routine databases.

**Results:** Absence of Pap smear within recommended 3-5 year interval increased the risk for cervical cancer in ages 30-65 (OR 2.52, 95%CI 2.19-2.91). Risk was likewise increased after the age of 65 if no smear within 6 years (2.79, 1.89-4.11) and for stage IB+ cancer in young women age 27-29 (2.78, 1.01-7.69) if no smear within 3 years. Risk was also increased for stage IB+ adenocarcinomas (1.56, 1.10-2.22). Within 5 years of diagnosis 372 women died, 50% were over 65 without a smear within 6 years. 18% of women who died had a normal pap smear within recommended intervals. Survival was 57% for symptom-detected non-screened cases, 74% for symptom-detected interval cases. Screen-detected cases survived 94% irrespective of previous history. An abnormal smear in the last two screening rounds implied increased cancer risk, especially if not assessed with biopsy, but survival was excellent in these cases. Survival was well related to FIGO stage at diagnosis. Low stages with excellent survival were seen in screen-detected cases, whereas aggressive interval cancer cases were rare. Survival by stage was equal for all histological types except small cell cancers.

**Conclusion:** The risk of incident cervical cancer including adenocarcinomas is increased in women not adhering to screening intervals at all ages from 27 and up, and in older women without a Pap smear within six years. Few deaths remain to be prevented in the screened population. Increased coverage, especially of risk groups in the older population is a priority. Women who fear that an invasive cancer may be detected can be encouraged to participate.
EFFECTIVENESS OF CERVICAL SCREENING IN YOUNGER AGES: DATA FROM THE DUTCH SCREENING PROGRAMME

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Objectives. It has been previously shown that the 10-year cumulative cervical cancer incidence after three consecutive negative primary smears was similar in women aged 30-44 years, CIR: 41 (95% CI: 33-51) per 100,000, and in women aged 45-54 years, CIR: 36 (95% CI: 24-52) per 100,000 (P=0.48), suggesting that further screening is as warranted in this younger as in the older group. Based on this analysis, we aimed to further explore the effectiveness of screening in younger women, including those aged <30 years, in whom previous data on screening effectiveness has given equivocal results.

Methods. Information on all cervix uteri examinations in the Netherlands was retrieved from the nationwide registry of histo- and cytopathology (PALGA). Because the identification string was not always unique, women with 0.5% most common surnames were excluded from the analysis. Women with completely negative screening histories were followed-up after the 3rd consecutive negative smear. The follow-up was ended at the cancer diagnosis, and left-censored at the beginning of 1994 and right-censored at the end of 2002. 95% CI were estimated by a non-parametric Kaplan-Meier estimator. Statistical significance of the CIR by age group was tested by Poisson regression.

Conclusions. From 126,748 women aged 30-34 years at the time of the third consecutive negative primary smear, 21 were diagnosed with cervical cancer during the first 10 years of follow-up, with a CIR of 36 (95% CI: 23-58) per 100,000. The CIR in these women was similar to the CIR observed in older women: 39 (95% CI: 26-59), 45 (95% CI: 32-61), 38 (95% CI: 22-66), and 33 (95% CI: 21-53) per 100,000 in women aged 35-39, 40-44, 45-49, and 50-54 years, respectively, at the time of their third consecutive negative smear. These data will be supplemented with the observed 10-year CIR for women aged 20-29 years.

VACCINATION AGAINST HUMAN PAPILLOMAVIRUS TYPE 16 FOR VULVAR INTRAEPITHELIAL NEOPLASIA GRADE 3

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6 ISA Pharmaceuticals, Bilthoven, The Netherlands.

Vulvar intraepithelial neoplasia (VIN) is a chronic disorder caused by high-risk types of human papillomavirus (HPV), most commonly HPV type 16 (HPV16). Spontaneous complete regression occurs in less than 1.5% of patients and the rate of recurrence after treatment is high. To investigate the immunogenicity and efficacy of an HPV16 E6/E7 synthetic long-peptide vaccine (HPV16-SLP®) 20 women with HPV16 + VIN grade 3 (VIN3) were vaccinated 3-4 times and clinical as well as HPV16-specific T-cell responses were assessed.

The most common adverse events were local swelling and fever but these did not exceed grade 2 of CTC. At 3 months after the last vaccination, 12 out of 20 patients (60%; 95%CI, 36-81) displayed an objective clinical response and reported relief of symptoms. Five women had a complete regression of their lesion and HPV16 was no longer detectable in four of them. Importantly, the number of clinical responses rose onto 12 months of follow-up. At that point, 15 of 19 patients displayed a clinical response (79%; 95% CI, 54 to 94), with a complete regression in 9 of 19 patients (47%; 95% CI, 24-71). These complete regressions were durable and maintained for the rest of the current follow-up period (24 months). Immunomonitoring revealed that all patients had vaccine-induced T-cell responses. Post-hoc analyses revealed that the group of patients with a complete regression had significantly stronger interferon gamma (IFNg)-associated proliferative CD4+ T-cell response and a broader CD8+IFNg+ T-cell response than the group of patients with a partial or no clinical response.

In conclusion, clinical responses in women with HPV16 + VIN3 can be achieved by vaccination with HPV16-SLP®. A complete regression of the lesion appears to be correlated with induction of a strong and broad HPV16-specific effector type T-cell response.
HUMORAL IMMUNE RESPONSES TO HUMAN PAPILLOMAVIRUS L2 INDUCED BY NATURAL INFECTION OR VACCINATION

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Objectives: Human papillomavirus (HPV) infection is associated with an L1-specific antibody response. However the humoral immune response to minor capsid antigen L2 is less well characterized and is of interest for the design of vaccine trials using this antigen.

Methods: We have explored the humoral immune response to HPV16 L2 in the CATCH population-based study sampling women over the age of 25 in a rural Indian setting in which a 10.3% prevalence of high risk HPV infection was observed. Sera from women in the CATCH study were screened first by HPV16 L2 ELISA, and candidate positives were verified by Western blot analysis using HPV16 L2 expressed in 293T cells. HPV16 L2-specific antibodies were only verified in 1 of 850 patients tested, suggesting that these responses are infrequent compared to L1-specific antibodies. Animal studies indicate that vaccination with L2 is a viable approach to prevent HPV infection and thereby potentially reduce the incidence of cervical cancer. A fusion protein comprising HPV16 L2, E6, and E7 is a candidate combination preventive and therapeutic HPV vaccine, and this is the only L2-based vaccine that has been tested in patients. The L1- and L2-specific and neutralizing serum antibody titers generated by vaccination three times at monthly intervals with HPV16 L2E7E6 were compared in two studies: a phase I randomized double-blind placebo controlled dose escalation trial in 40 healthy volunteers and a phase II trial of HPV16 L2E7E6 at the maximum dose in 29 women with high-grade anogenital intraepithelial neoplasia (AGIN). Vaccination of healthy volunteers induced L2-specific serum antibodies that were detected 1 month after the final vaccination. There was a significant trend to seroconversion for HPV18 and HPV18 neutralizing antibodies with increasing vaccine dose. Seroconversion for HPV18 neutralizing antibodies showed a significant positive trend with increasing dose and was associated with seroconversion for HPV16 neutralizing antibodies. However, AGIN patients responded less effectively to vaccination than healthy patients for induction of HPV16 L2-specific antibody and proliferative responses.

Conclusions: Naturally infected patients rarely produce L2-specific antibody responses. Vaccination of healthy volunteers three times with 0.5mg HPV16 L2E7E6 at monthly intervals induced L2-specific serum antibodies that neutralized across papillomavirus species. L2-antibody responses to three 0.5mg HPV16 L2E7E6 immunizations were infrequent in AGIN patients. The weak L2-antibody responses to HPV16 L2E7E6 vaccination suggest the need for an adjuvant.

T-CELL RESPONSES TO PROPHYLACTIC HPV VACCINES GARDASIL AND CERVARIX IN A LONGITUDINAL STUDY

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3 DKFZ Heidelberg, Germany

Objectives: Two prophylactic HPV vaccines have been developed and their immunogenicity and clinical efficacy was extensively investigated. The quadrivalent vaccine Gardasil contains virus-like particles (VLP) of HPV types 6, 11, 16, and 18 adjuvanted with aluminum salts. Cervarix contains VLP of types 16 and 18 together with the TLR 4 stimulating adjuvant AS04. Both vaccines showed comparable efficacy in phase II/III clinical trials in protection from infection with vaccine-type HPV and dysplasia. T helper cells are important for B cell differentiation, sustained memory, activation of recall reactions, and thus anamnestic responses. Only few reports on cellular immune responses to the prophylactic HPV vaccines are available to date.

Methods: In 36 vaccinated individuals, blood samples taken before, after each vaccination, and 6 months after the last vaccine dose were analysed by ex vivo flow cytometry. L1-specific T cell frequencies were identified by intracellular staining for CD4, CD154, IL-2, IL-4, and IFN-y. T helper cell responses to the vaccine HPV types were readily detectable after the first vaccine dose. There was a trend for higher T cell frequencies after Cervarix for both HPV16 and HPV18 L1. Cross-reactive T cell responses to HPV31 L1 and HPV 45 L1 were induced by both vaccines again with higher frequencies for Cervarix. Unexpectedly, also HPV6 and HPV11 L1-reactive T cells were induced after Cervarix at comparable frequencies as in Gardasil vaccinees. Sequence homology of the L1 of HPV types is high and conserved MHC class II-restricted epitopes can be found that allow for T helper cell cross-reaction.

Conclusion: To date clinical efficacy of both HPV vaccines is comparable. T helper responses to high-risk HPV types are higher for Cervarix. Analysing T cell responses may help understand mechanisms of vaccine cross protection. Any difference in immunogenicity of the two vaccines is interesting for the evaluation of sustained immunity.
## SS 11-5

**HPV-16/18 AS04-ADJUVANTED VACCINE ADMINISTERED AS A 2-DOSE SCHEDULE COMPARED WITH THE STANDARD 3-DOSE SCHEDULE**

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**Objectives:** The HPV-16/18 AS04-adjuvanted vaccine (Cervarix®, GlaxoSmithKline Biologicals) is highly immunogenic using its licensed vaccination schedule. This study (NCT00541970) evaluated 2-dose (2D) schedules using the current vaccine formulation (20 μg of each antigen [HPV-16 and 18]; 20/20) or an alternative formulation (40 μg of each antigen; 40/40), compared with the standard 3-dose (3D) schedule.

**Methods:** Healthy females were randomised and stratified by age (9-14, 15-19, 20-25y) to receive HPV-16/18 vaccine (20/20 Months (M) 0,1,6) (n=239), 40/40 M0,6 (n=241), 40/40 M0,2 (n=240) or 20/20 M0,6 (n=240). HPV-16/18 antibodies were assessed 1M after the last active dose. Reactogenicity was assessed for 7 days after each dose and safety to M7.

**Conclusions:**

<table>
<thead>
<tr>
<th>Dose/schedule</th>
<th>GMT, EL.U/ml (95% CI)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HPV-16</td>
</tr>
<tr>
<td>20/20 M0,1,6</td>
<td>13165 (11834-14645)</td>
</tr>
<tr>
<td>40/40 M0,6</td>
<td>11204 (10049-12490)</td>
</tr>
<tr>
<td>40/40 M0,2</td>
<td>5692 (5148-6294)</td>
</tr>
<tr>
<td>20/20 M0,6</td>
<td>8093 (7275-9002)</td>
</tr>
</tbody>
</table>

*values are adjusted for age

For HPV-16, the 3D schedule was superior (LL of the 95% CI GMT ratio [2D/3D] was <0.5) to 40/40 M0,2 but not to 40/40 M0,6 or 20/20 M0,6. For HPV-18, 3D was not superior to any of the 2D schedules. In the cohort aged 9-14 y, for both HPV-16 and HPV-18, each 2D schedule was non-inferior to 3D in 15-25 y (UL of the 95% CI for GMT ratio [3D/2D] was <2). All formulations had acceptable reactogenicity and safety profiles. These results indicate that the HPV-16/18 vaccine on a 2-dose schedule is immunogenic and safe in 9-14 year old girls. Further investigations are warranted to evaluate kinetics and persistence of the immune response as well as cross-protection induced by a 2 dose schedule.

## SS 11-6

**ANAMNESTIC RESPONSE ELICITED BY A FOURTH DOSE OF THE HPV-16/18 AS04-ADJUVANTED VACCINE IN YOUNG WOMEN**

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**Objectives:** The HPV-16/18 AS04-adjuvanted vaccine (Cervarix® GlaxoSmithKline Biologicals) is administered in a 3-dose schedule. While a booster is not required, we present a study (NCT00546078) evaluating immune memory induced by a 4th dose of vaccine given ~7 years after the initial vaccination course.

**Methods:** Women were randomised in an initial double-blind study to receive 3 doses of HPV-16/18 vaccine or placebo at months 0, 1 and 6 (NCT00689741). Approximately 7 years later, the vaccine group received a 4th dose of HPV-16/18 vaccine (4th dose [4D] group), while the placebo group received a 1st dose of a 3-dose vaccine course (primary vaccination [PV] group). GMTs of anti-HPV-16/18 antibodies were measured by ELISA. Primary analysis was performed on the according-to-protocol for immunogenicity cohort (4D group: N=61; PV group: N=45).

**Conclusions:** Women in the 4D group were all seropositive for anti-HPV-16 and -18 prior to the 4th dose, with GMTs of 720.7 and 502.9 EL.U/mL, respectively. GMTs were 8.2 and 7.8-fold higher 7 days after 4th dose (5894.9 and 3916.2 EL.U/mL) and 21.4 and 16.6-fold higher (15410.7 and 8362.7 EL.U/mL) after 1 month, respectively. Equivalent values 1 month after 1st dose in the PV group were 1231.1 and 442.0 EL.U/mL. A high proportion of the 4D group had >500 HPV-16/18 specific CD4 T cells/million before 4th dose: 89.9% and 63.0% for HPV-16 and -18, respectively, rising to 100% and 96.0% 1 month after 4th dose with a 1.7-fold increase in cell number. Similarly, before the 4th dose, 74.1% and 77.8% had memory B cells against HPV-16 and -18 respectively, rising to 100% 1 month after 4th dose. The safety profile of the 4th dose was similar to that of a first dose. In conclusion, women showed a high and sustained immune response ~7 years after 1st dose of HPV-16/18 AS04-adjuvanted vaccine, and a rapid and strong anamnestic response induced by a 4th dose.

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PREVALENCE, INCIDENCE AND PERSISTENCE OF GENITAL HUMAN PAPILLOMAVIRUS (HPV) INFECTION IN WOMEN BETWEEN THE AGES OF 25 AND 45

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\textbf{Background:} A prophylactic quadrivalent HPV vaccine could benefit adult women if they are susceptible to incident genital HPV infections and are acquiring new infections with vaccine HPV types. This report presents baseline and prospective data from a randomized, double-blind, placebo-controlled trial of the safety, immunogenicity and efficacy of the quadrivalent HPV (type 6/11/16/18) vaccine in women ages 24 to 45.

\textbf{Methods:} We present the results of an epidemiologic analysis of 3,730 women enrolled in a quadrivalent HPV vaccine efficacy trial between 6/18/2004 and 4/30/2008. Subjects were enrolled from 7 countries (Colombia, France, Germany, Philippines, Spain, Thailand, and the United States) through community and academic health centers and primary health care providers.

\textbf{Results:} Average baseline prevalence of anogenital infection and/or seropositivity was 32.8\% for $\geq 1$ vaccine HPV types, and 0.3\% for all vaccine HPV types (vaccine and placebo arms). Incidence of anogenital infection with any vaccine HPV type was $\sim 10.5\%$ (placebo arm). The rate of persistent infection was $\sim 5\%$ over a 30-month period among women in the placebo arm naïve to the relevant type at baseline. Predictors of incident infection included younger age, marital status other than first marriage, higher number of lifetime and recent sex partners and Chlamydia/gonorrhea infection at baseline.

\textbf{Conclusions:} These findings indicate that women up to age 45 could benefit from administration of the quadrivalent HPV vaccine, as they are acquiring anogenital infections with vaccine HPV types. Future study concerning incident and prevalent HPV infection among women over age 25 would be warranted.

\textbf{Concurrent Infection with Multiple HPV Types}

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for the IARC HPV Prevalence Surveys (IHPS) Study Group

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\textbf{Objectives:} To understand viral interactions and cross-reactivity of natural or vaccine-induced responses, we investigated whether multiple human papillomavirus (HPV) infections, particularly certain combinations of types, have the tendency to cluster together.

\textbf{Methods:} Cervical cell samples were collected from women in the framework of the International Agency for Research on Cancer HPV Prevalence Surveys. Women with a cytology diagnosis of high-grade squamous intraepithelial lesion or worse were excluded, leaving 13,961 women for this analysis. HPV DNA was assessed using a general GP5+/6+ primer-mediated PCR. HPV genotyping was performed by using enzyme immunoassay (EIA) or reverse-line blot analysis (RLB). Logistic regression with type-specific HPV positivity as an outcome was used, adjusted for age, study area and lifetime number of sexual partners. Woman-level random effects were added to represent unobservable risk factors common to all HPV types.

\textbf{Conclusions:} Observed-to-expected (O/E) ratio for infection with two HPV types was 1.20 [95\% credible interval (CI), 1.14-1.26] and for $\geq 3$ types was 1.02 [95\% CI: 0.91-1.12], with the best possible adjustment. Among combinations of specific HPV types, the tendency to cluster increased with the genetic similarity of the L1 region. High O/E ratios were observed for closely homologous types, including HPV33/58, 18/45, 33/35, and 31/35. The excess of multiple infections, however, was clearly evident only when EIA, and not RLB, was used as the genotyping method. The different results by genotyping method suggest that the apparent clustering of HPV infections is an artifact of the measurement process. Further investigation is required to evaluate other widely used HPV detection methods.
Epidemiological Study on the Prevalence of HPV Infection of Women in Portugal - A CLEOPATRE Study

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Information on HPV prevalence and distribution of HPV types is scarce in Southern-Europe; this is the first comprehensive study carried out in Portugal.

Objectives: To assess the overall and age-stratified prevalence of HPV infection, and the type-specific distribution in the general female Portuguese population.

Methods: This cross-sectional population-based study was conducted across the five Regional Administrations of Health in Portugal. From February 2008 to March 2009, LBC samples were collected from women ranging from 18-64 years and sent to central laboratories for cytological diagnosis and HPV genotyping (CLINICAL ARRAY HPV 2, Genomica). Descriptive and inferential analyses were performed using SPSS program.

Conclusions: Of the 2326 women included, 2315 had valid cytological results: 93.8% normal cytology, 2.5% ASC-US, 0.1% ASC-H, 3.2% LSIL, 0.2% HSIL, and 0.3% AGC. Overall, crude HPV prevalence was 19.4%. HPV prevalence was highest in women younger than 24 years (20–24 years: 28.8%, 95% CI 25.4–32.2; 18–19 years: 27.0%, 95% CI 21.8–32.3), and then decreased, without a second peak in older age. Among positive women, 36.6% had multiple infections and 57.0% harboured a HR-HPV type. The ten most common HR types were HPV 16 (19.7%), 31 (11.8%), 53 (11.8%), 51 (9.8%), 66 (8.6%), 52 (8.0%), 58 (6.9%), 59 (6.7%), 18 (4.4%), and 56 (4.0%). HPV 16 was the most frequent genotype in all ages, except in women between 30–59 years. HPV increased significantly with the degree of cervical neoplasia (16.5% - normal cytology, 16.7% - AGC, 42.1% - ASC-US, 100% - ASC-H, 81.1% - LSIL, 100% - HSIL). HPV 16 was the most frequently detected genotype in all lesions, except in AGC. HPV vaccine types 6, 11, 16 or 18 were detected in 32.6% of positive women. Multiple infections were more frequent in women younger than 24 years and older than 60 years of age. Data regarding determinants of HPV infection will be presented.

These data correspond to the first population-based HPV prevalence study in Portugal and will contribute to a better understanding of the wide spectrum of HPV infection across Europe. This study will also provide a baseline for future assessment of the impact on HPV vaccination, in the country.

Epidemiologic Considerations for the Quadrivalent HPV Vaccine Anal Disease Study

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PhD, Chair, Department of Cancer Epidemiology and Genetics; Program Leader, Risk Assessment, Detection, and Intervention Program; H. Lee Moffitt Cancer Center and Research Institute, Tampa, Florida

Background: This analysis examined the appropriateness of population selection for a study investigating the impact of administration of a 3-dose regimen of quadrivalent HPV vaccine (qHPV) on the combined incidence of HPV 6/11/16/18-related anal intraepithelial neoplasia (AIN) or anal cancer in men having sex with men (MSM).

Methods: Utilizing data from the HPV in Men (HIM) Study and Merck protocol 020 (qHPV vaccine efficacy trial in men), we characterize anal canal HPV prevalence and risk factors in HIV-negative men. Both international studies recruited men free of disease. In the HIM Study, anal swabs were collected from 1,819 heterosexual men (HM) and MSM, and were analyzed for 37 HPV types via polymerase chain reaction. In Protocol 020 external genital swabs were collected from 3,463 HM and 602 MSM and anal swabs were collected from the MSM; swabs were tested for 14 HPV types.

Results: In both studies, HPV prevalence in the external genital area was higher in MSM than in HM. In the HIM Study, HPV prevalence in the anal canal was also higher in MSM vs. HM. In the same study, there was a significant association between presence of genital and anal canal HPV among HM, no association between any HPV and grouped HPV infections at these two anatomic sites was detected among MSM. Concordance of genital and anal HPV in MSM is only observed at specific HPV types - HPV 6 and HPV 16.

Conclusions: These epidemiologic data demonstrate the higher burden of anal infection among MSM, and support the selection of a population of MSM for the purposes of determining the efficacy of qHPV vaccine against HPV-related anal infection and disease. Although both HM and women (data from other studies in females) are at risk for HPV-related anal infection and/or disease, the higher risk of HPV infection in MSM allowed for a vaccine efficacy study feasible both in size and follow-up time.
LOW-RISK HPV INFECTION IN MORE THAN 40 000 DANISH WOMEN FROM THE GENERAL POPULATION

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2) Medical Virology, University Hospital of Tuebingen, Tuebingen, Germany
3) Sanofi Pasteur MSD, Lyon, France
4) Department of Pathology, Hvidovre Hospital, University of Copenhagen, Denmark
5) Juliane Marie Center, Gynecologic Clinic, Rigshospitalet, University of Copenhagen, Denmark

Objectives: The aim of the study was to estimate the overall prevalence and the age and type-specific HPV distribution of low-risk (LR) HPV types in more than 40,000 women from the general Danish female population. Furthermore, we wanted to examine the association between LR HPV types and cervical lesions in women positive for LR HPV types alone.

Methods: Consecutive liquid-based cytology samples (SurePath®) were collected over a 3 year period from Department of Pathology at Hvidovre Hospital, Copenhagen, which processes all cytology samples taken in Greater Copenhagen. After cytological examination, the rest of the respective samples were sent for HPV testing, which was done by Hybrid Capture II (both HR and LR probes) (HC2, Digene) and a PCR-based assay (INNO-LiPA, Innogenetics Inc.). We included 40,382 women (14-95 years of age) in the study. By linkage with the nationwide Pathology Data Bank the HPV test results were linked to cytological diagnoses made from the same samples and to subsequent histology results.

Conclusions: Overall, 2,790 women (6.9%) harbored LR HPV types, with HPV6, HPV70 and HPV44 being the most frequent LR HPV types detected. HPV11 was rare in the present study population. The highest prevalence was observed in the youngest age groups. The LR HPV prevalence was 6.3% in women with normal cytology, 20.9% in ASCUS/LSIL, and 12.7% in those with HSIL. However, the corresponding numbers in women exclusively positive for LR HPV types were 2.0%, 3.5% and 0.7%, respectively. In conclusion, cytological abnormalities are rarely caused by low-risk HPV types alone. Associations with subsequent histology and age will be presented.

HPV PREVALENCE AND RISK FACTORS IN A COHORT OF TUSCANY WOMEN AGED 18-24

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(2) Clinical and Descriptive Epidemiology Unit. ISPO, Florence, Italy
(3) Screening Unit. ISPO, Florence, Italy
(4) Obstetrics and Gynecology Unit. Santa Maria Annunziata Hospital, Florence Italy
(5) Departmental Section of Cytohistological for Oncological Screening- Montevarchi, Arezzo Italy
(6) Pathological Anatomy Department, Ospedale Unico della Versilia, Viareggio, Lucca, Italy
(7) Sanitary District of Valdarno, Montevarchi, Arezzo, Italy

Objectives: A prospective cohort study was performed in different area of Tuscany to evaluate the rate of prevalence, incidence, acquisition and clearance of HPV types in young women (18-24) and the risk factors correlated with such events.

Methods: We enrolled 1066 women from the regional archives. Each woman was requested to perform the HPV DNA testing (HCII) and to answer a self-administered questionnaire. The study is scheduled for three years and it is planned to repeat the HPV testing and the questionnaire every year. Only in case of previous positive results the HPV testing is anticipated to 6 months.

Conclusions: Oncogenic types were found in 19.32% of the whole female population. HPV 16 and HPV 18 were present in 10.41% of this population. The use of condom showed a protective effect in multivariate analysis but these data became not statistically significant in univariate analysis. Further we evidenced a significant statistically relationship between HPV infection, number of previous sexual partners (in particularly in the last 3 years) and the number of previous partner's intercourses. At six months follow-up 121 women, out of 164, confirmed the HR HPV positivity. The prevalence of HPV oncogenic types is high in young Tuscany women. However the dynamic of the process of clearance, acquisition and persistence of HPV infection and the correlated risk factors could be better understood by the results obtained in the three years follow up of this cohort.
**TIME TRENDS OF HPV TYPE DISTRIBUTION IN ITALIAN WOMEN WITH CIN**

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**Objectives:** It is assumed that the circulation of HPV types in a given population is stable over time although there are limited historical data to support this view. The existence of potential cohort effects in the circulation of HPV types has major implications for vaccination strategies and risk assessment in infected women. We investigated the distribution of HPV types among Italian women with CIN over the years 1985-2007 using DNA extracted from archival biopsy samples.

**Methods:** DNA from formalin-fixed paraffin-embedded cervical biopsies from the years 1985-1987 (n=67) and 1995-1997 (n=92) was HPV-typed by the SFP-10 Lipa assay. Cases were compared with 159 control biopsies from the years 2005-2007 matched by patient age and CIN grade.

Quantitative PCR was used to compare titres of HPV sequences in DNA extracted from samples of the three periods. Type-specific PCR was used to confirm HPV51 and HPV52 typing by SPF10 Lipa.

**Conclusions:** HPV51,52,53,56,58 and 66 were markedly under-represented or undetectable in samples from past periods whereas they represented 5.7-30.8% of present infections. Frequency of multiple HPV infections and high risk infections (p=0.000) also increased in recent years. The main changes occurred over the last decade. Infections by HPV16 and HPV18 were three times more frequent 20 years ago than today (p=0.012). Loss of amplifiable HPV sequences over prolonged storage was not observed. Type-specific PCR confirmed all HPV51 and HPV52 infections.

Secular trends in the distribution of HPV types among women with CIN may occur in specific populations, possibly reflecting the introduction of new HPV types and/or new pattern of exposure. These results should be confirmed in independent cohorts and considering general population samples of larger size and over longer time intervals.

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**DISTRIBUTION OF HPV TYPES ASSOCIATED WITH CERVICAL CANCERS IN SCOTLAND AND IMPLICATIONS FOR THE IMPACT OF HPV VACCINES.**

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**Objectives:** To undertake a sufficiently powered, systematic evaluation of cervical cancer cases from all regions of Scotland to determine HPV type specific prevalence in order to gauge the potential impact of the immunisation programme which so far has achieved high uptake (currently around 90% for girls aged 12-18 in school).

**Methods:** Cases of confirmed invasive cervical carcinoma (ICC; n=370) were collected from across Scotland, sequentially from 2004, working backwards. To assess how representative our sample was, demographic information was compared to national data at the Scottish Cancer Registry. HPV type specific prevalence was performed on formalin fixed paraffin embedded material using the INNO-LiPA assay. Additional testing included mRNA detection using HPV Proofer in cancers infected with multiple types. Analysis of the relationship between HPV 16/18, FIGO stage and disease grade was also made.

**Conclusions:** HPV DNA was detected in 325/370 (88%) cases. HPV 16 was detected in 207 cases, HPV 18 in 66 cases and HPV 16 & 18 in 8 cases. HPV 16 and/or 18 was detected in 72% of cancers overall and in 82% of the HPV positive cancers. Preliminary evidence suggested that in cancers with multiple infections (10%), HPV 45 was preferentially expressed. There was no evidence of a relationship between HPV16/18 and FIGO stage or grade of disease. These results suggest a significant reduction in ICC in Scotland will be achieved by the national HPV immunisation programme using the bivalent HPV vaccine.
**PREVALENCE OF HPV TYPES AMONG INDIVIDUALS ATTENDING A CLINICAL LABORATORY FOR HPV DIAGNOSIS IN PORTO ALEGRE, BRAZIL.**

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**Background:** Infection of the genital mucosa by certain types of human papillomavirus (HPV) often causes irregular cell growth and warts. Persistence of HPV infection, particularly by those belonging to the high-risk types (HR-HPV), is associated with an increased risk for cervical cancer development. Low-risk HPV (LR-HPV) types are more often associated with benign warts. Microarray technology has been introduced into the clinical laboratory for HPV detection. One such method is the PapilloCheck\(^\circ\) (Greiner Bio-One GmbH, Germany), which is able to detect and identify 24 HPV types. The aim of this study was to determine the prevalence of these HPV types, including coinfection rates and association of the most prevalent types with men and women, among the population that uses our clinical laboratory for HPV diagnosis.

**Methods:** Genital samples were collected from 1144 patients referred to our lab for HPV screening using PapilloCheck\(^\circ\). Viral DNA was extracted from collected samples using the QIAamp DNA Mini kit (Qiagen). Extracted DNA was subjected to PCR amplification prior to hybridization of the PCR products onto HPV type-specific DNA probes fixed on the PapilloCheck DNA array. After hybridization, the DNA arrays were automatically analyzed using the Check Scanner.

**Results and Conclusions:** In this study, men represented 10.3% and women comprised 89.7% of the patients, and, overall, the HPV prevalence in our study population was 42.4%. Among women, the most prevalent low-risk HPV types were: HPV 42 (9.9%), HPV44 (8.5%) e HPV6 (7.8%), and among men were: HPV42 (16.0%), HPV6 (14.0%), HPV40 (8.0%) e HPV44 (8.0%). For HR-HPV types, the following types were the most prevalent among women: HPV16, HPV56, HPV51 e HPV31 (19.5%, 16.8%, 11.5% e 9.7%, respectively), and the types HPV16 and HPV51 were found in 14.0% and 16% of men, respectively. Considering only the most prevalent types, there was no statistically significant differences between men and women, which may be explained by the low number of men involved in this study. Interestingly, coinfection was observed in 42.5% of the HPV-positive patients and among these, 21.6% were coinfected by 2 HPV types, and 20.8% had 3 or more types involved. The following HPV types were more likely to be found in association (coinfection) with other types: 6, 16, 35, 39, 42, 44, 51, 52, 53, 56, 59, 66, 68, 73 e 82 (p<0.001). The clinical significance of these coinfections still remains to be determined. Microarray technology is a powerful tool and, in this format, it can be easily adapted to the routine of clinical laboratories for the detection and genotyping of HPV.

**DISTRIBUTION OF HPV GENOTYPES IN WOMEN WITH CERVICAL CANCER IN SLOVENIA AND GENOMIC VARIANTS OF HPV 16, HPV 18 AND HPV 33**

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**Objectives:** The aim of the present study was to establish the distribution of human papillomavirus (HPV) genotypes in representative population of women with cervical cancer (CC) in Slovenia in order to contribute the lacking data on HPV in CC and to assess the potential local benefit of future prophylactic HPV vaccination. Furthermore, we wanted to determine genomic variants of three most common HPV genotypes.

**Methods:** Polymerase chain reaction with GP5+/GP6+ primers was performed in all 278 CC samples for HPV DNA detection and genotyping. Negative samples were additionally tested using CPI/CPIIg primers and INNO-LiPA HPV genotyping assay. Genomic variants of HPV 16, HPV 18 and HPV 33 were determined by sequencing of LCR, E6 and E7 genetic regions. A total of 262/278 CC samples (94.2 %) were HPV DNA positive. HPV genotypes in Slovenian women with CC, in decreasing order of frequency, were: 16, 18, 35, 45, 31, 51, 58, 59, 35, 52, 73 and 82. Detailed genomic analysis was carried out on 40/178 isolates of HPV 16, 20/34 isolates of HPV 18 and 11/13 isolates of HPV 33. A total of 26 genomic variants of HPV 16 were identified. Thirty-eight isolates (95 %) belonged to the European branch; one isolate (2.5 %) belonged to the Asian-American branch and one (2.5 %) to African branch. A total of 18 genomic variants of HPV 18 were identified. Nineteen isolates (95 %) belonged to the European branch and one isolate (5 %) belonged to the African branch. Seven genomic variants of HPV 33 were identified. Five isolates (45.5 %) belonged to prototypic variants and 6 (54.5 %) belonged to non-prototypic variants.

**Conclusions:** Distribution of HPV genotypes in Slovenian women with CC established in the present study represents baseline distribution before implementation of HPV vaccination. Prophylactic HPV vaccination with currently available vaccines could prevent up to 77.1 % of CC in Slovenia caused by HPV 16 or HPV 18. Almost all isolates of HPV 16 and HPV 18 belonged to European branches. Prototypic and non-prototypic HPV 33 variants were almost equally distributed among Slovenian patients with CC.
We investigated type-specific prevalence of high-risk HPV (HR-HPV) in Northern Japan and the association with progression of ASCUS and LSIL.

**Methods:** Seven types of HR-HPV (type 16, 18, 31, 33, 35, 52, 58) were investigated using the PCR-RFLP method in 1120 women with a cytological diagnosis of ASCUS or LSIL. Colposcopy and directed biopsy were performed when signs of progression were found in the follow-up period.

**Conclusions:** HPV 16 was the most common HR-HPV type, followed by HPV 52, 58, 31, 18, 33, and 35. Seventy-two women had ASCUS and 1048 were diagnosed by cytology with LSIL. A total of 12/72 (25.0%) ASCUS cases were positive for HR-HPV and similar results were obtained for LSIL, 260/1048 (24.8%). With a median follow-up period of 25.7 months (range: 1-264), 30 women had lesions that progressed to CIN3, while none had progressed to invasive cervical cancer. CIN3 occurred more frequently in HR-HPV positive women (10.4%, 29/278) compared to HR-HPV negative women (0.12%, 1/842). The difference was highly significant (p<0.0001). The median period of progression to CIN3 according to HR-HPV type was 28 months (range: 1-233) in HPV 16, 18, and 33 (Group A) and 82 months (10-154) in HPV 31, 35, 52, and 58 (Group B). Progression was shorter in Group A compared to Group B (p=0.012). The risk of CIN3 according to HR-HPV type was as follows: 13% (15/115) in HPV 16, 6.7% (1/15) in HPV 18, 8.6% (3/35) in HPV 31, 36.4% (4/11) in HPV 33, 0% (0/6) in HPV 35, 11.3% (9/80) in HPV 52, and 2.9% (3/65) in HPV 58. ASCUS/LSIL with a Group A HR-HPV type tended to progress more frequently than Group B HR-HPV types, although the difference was not statistically significant (p=0.07). In conclusion, a type-related progression risk was observed in cases of ASCUS and LSIL in Northern Japan and information on specific HR-HPV type is important for the triage follow-up schedule of ASCUS and LSIL detected by conventional cytology.

**EUROGIN**

**ABSTRACTS**

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**SS 12-12**

**HIGH-RISK HUMAN PAPILLOMA VIRUS INFECTION (HPV) AMONG WOMEN ATTENDING WOMEN’S HOSPITAL IN QATAR**

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**Objectives:** This study aimed to determine the prevalence and ideal detection method for high-risk Human papillomavirus (HPV) genotypes, in order to evaluate prevention strategies in cervical cancer and other HPV-related disease in Qatar. The study compared performance of cervical cytology and HPV DNA test to detect high-risk Human HPV genotype (16, 18, 31, 33, 39, 45, 51, 52, 56, 58, 59) infections in a sample of Qatar’s female population, using High Risk Screen Real-Time PCR test.

**Methods:** A series of 95 women attending the Gynae-oncology clinic at Hamad Medical Corporation between August 2007 and May 2008 were enrolled in this study. Cervical smears isolated from patients were subjected to High Risk Screen Real-Time test to confirm the presence of HPV DNA. The smears were characterised as ASCUS, LGSIL and HGSIL.

**Conclusions:** The overall prevalence of high-risk HPV in our study population (n=95) was 64%, with HPV 52, 56 and 16 being the commonest types detected. Of the 95 samples in the study, 93 were tested using Pap smear and RT-PCR. 11 samples found to be HPV DNA positive by Pap smear were confirmed by RT-PCR; 34 samples were found to be negative using both tests; and 48 samples which were shown to be negative using Pap smear were found to be positive using RT-PCR. Considering RT-PCR and Pap smear as stand-alone tests, the techniques did not show similar sensitivity. The RT-PCR showed better specificity and sensitivity than Pap smear.

The prevalence of HPV in the different types of lesions was compared in 65 women who had abnormal smears among the study population. HPV DNA was detection rate was 60.7%, 85.7% and 50% within ASCUS, LGSIL and HGSIL cytology, respectively.

The study also showed that molecular techniques are more sensitive than conventional methods for detection of HPV infection. The relatively high prevalence of HPV 52, 56 and 16 among the study group has important implications in vaccine prophylaxis in Qatar.
Background: HPV is now recognized as the absolute cause of cervical cancer and its prevalence in squamous lesions is high. Many studies describe the distribution of HPV infections in normal population, or in women with ASC-US, CIN 1 or CIN 2-3 lesions. The incidence of cervical cancer in Israel is low 5.0-5.7/100,000 women and there are a few articles that describe the prevalence of HPV infection in the Israeli population.

Objectives: To describe the distribution of HPV types in the Haifa district in women with ASC-US on Pap smear or CIN 1, CIN 2-3 lesions. To compare these finding with HPV incidence from other studies, and to access the clinical implications of these findings.

Methods: We conducted a multicentral prospective evaluation of HPV typing in all consecutive patients referred to the colposcopic clinics due to abnormal PAP smear or cervical complaints. DNA was extracted from brush samples, transported to the laboratory in viral transport medium, or from biopsy samples. HPV viral sequences were identified by nested PCR using L1 consensus primers that enable the amplification of the majority of HPV genotypes. Following amplification HPV positive samples were identified by electrophoresis and genotypes were determined by sequence analysis.

Results: 1285 samples were evaluated of these, 697 (55.5%) were HPV positive. HPV 16 was the commonest virus reaching 26.0% of the cases, multiple HPV types were found in 10.0%, HPV 66 in 8.3%, HPV 31 in 7.6% and, HPV 6 in 5.5% of the evaluated women. The incidence of HPV 18 and HPV 11 was low (4.2%, 1.4% respectively). In the non Jewish population (144 Arabs and Druses women) the HPV types were similar to the types in the Jewish population. 53.5% of the 400 ASC-US cases were found to be positive for HPV. 26.2% were positive for HPV 16. And 9%, 9%, 7.5% were positive for multiple HPV types, HPV 66, respectively.

In 116 women with CIN 2-3 HPV 16 was found in 48.4%, HPV 31 in 12.1%, HPV 18 in 3.4%.

43% of the 143 women with CIN 1 were negative for high risk HPV types.

Conclusions: In the Northern part of Israel HPV 16 is the most prevalent virus, but multiple HPV infections and HPV 66 is found in 10% and 8.3% respectively. HPV typing may have a role in the triage of women with ASC-US or CIN 1. Our report suggests that the HPV vaccine has a long term potential to prevent approximately 50% of CIN 2-3 lesions in the Israeli population.

GENITAL WARTS CONSTITUTE A SIGNIFICANT WORK LOAD AT SWEDISH YOUTH CLINICS AND HOSPITAL BASED STI CLINICS

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Objectives: There are good reasons to believe that genital warts are at least as common as Chlamydia. However, there is no mandatory reporting of genital warts in Sweden. It has previously been shown that genital warts give rise to a significant work load in STI clinics in large age groups (1). In Sweden, HPV-vaccine will be offered to girls born 1999 or later starting from 2010. Screening for cervical cancer begins at the age of 23; therefore it will take many years for vaccinated cohorts to reach an age where it will be possible to monitor the effect of the HPV vaccination using cervical screening samples. One of the quickest markers for the effect of vaccination should therefore be the reduction in the prevalence of genital warts. Such a tendency has already been seen in Australia (2). Due to this, knowledge about the number of genital warts cases and resource utilization before the start of mass HPV vaccination is of importance.

Methods: To define the prevalence of genital warts in patients visiting 9 selected Youth Clinics and two hospital based STI clinics in the County of Stockholm, Sweden through retrospective real world data collection for the years 2004-2008. Data was extracted from electronic patient records through a specific extraction and data management method. In total, approximately 75,000 patients records were screened. The study population consisted of patients with documented diagnosis of genital warts or registered prescription of podofyllotoxin/imiquimod or case confirmation by free text search.

Conclusions: Approximately 7% of visits at youth clinics (age 15-23 years) and 14% of visits at hospital based STI clinics (age 24 years and above), constitute consultation and treatment for genital warts. This represents a significant work load, particularly at Swedish youth clinics, since these mostly deal with contraception counselling and the majority of visits are taken care of by midwife nurses.

1) Dempsey et al, Sex Transm Dis. 2007;34(7):503-7
2) Fairley et al, Sex Transm Infect. 2009 (in press)
Objective: To describe the feasibility, utility, acceptance and effectiveness of various 'screen and treat' approaches in cervical cancer prevention in low- and medium resource countries.

Methods: 'Screen and treat' initiatives in the different countries in Asia, Africa and Latin America are reviewed and discussed.

Observations: Providing treatment for women with cervical cancer precursor lesions is a critically important step to ensure success of cervical cancer screening programs. Yet this is the one of the weakest links in many programs due to the insistence and the need for repeated visits before treatment is provided in conventional algorithms of managing screen positive women. Adherence to treatment is rather low if repeated visits are mandatory for treatment in low-resource settings. ‘Screen and treat’ approaches aim to link treatment or diagnosis and treatment in the same session as screening on the same day and thereby minimize loss to treatment. They are essentially single visit approaches (SVA). Such approaches require screening tests that can provide results in real time or quickly within few hours. Visual screening tests and new developments in HPV testing such as the 'careHPV test' facilitate screen and treat approaches. The most basic approach involves treatment of all screen positive women with cryotherapy or cold coagulation, after clinically excluding those with large lesions and invasive cancer. An alternative approach involves colposcopic triage of screen positive women, directing biopsies and providing treatment in the same session, which facilitates histological characterization of the lesion treated. Both the approaches have been used in study and programmatic settings in Asian, African and Latin American countries, and their feasibility, safety, acceptability and effectiveness will be discussed. The summary evidence indicates that both the screen test approaches are set to stay and will play a major role in extending successful cervical cancer prevention opportunities to disadvantaged populations.

Cervical cancer prevention in low-resource settings, where more than 80% of cervical cancer occurs, remains a vexing problem. Papanicolaou (Pap) smears/cervical cytology-based screening has poor sensitivity and reproducibility, especially in low-resource settings. Visual inspection with acetic acid (VIA) is a low-cost method of screening but probably only detects the most overt cancers and the largest of precancerous lesions unless it is conducted in a very non-specific manner. And HPV vaccines, while highly promising, remain unaffordable, require cold-chain delivery, and probably will require 2-3 doses to be effective. These barriers will limit access to current and future HPV vaccines by the underserved. In addition, current HPV vaccines only prevent HPV infections and do not treat them. To rely on HPV vaccination as a sole method of cervical cancer prevention is to doom approximately 10 million women to cervical cancer-related deaths over the next 30 years. Low-cost molecular testing for HPV may offer a solution. One low-cost test for carcinogenic HPV DNA has already been developed, and has been shown to be almost as clinically sensitive for cervical precancer and cancer as a well-established, U.S. FDA-approved test in one study. A second test targeting the E6 oncoprotein for 7-8 carcinogenic HPV genotypes is being developed. Early validation studies show that it is more specific for cervical precancer and cancer than HPV DNA detection for the same HPV genotypes. These tests could be employed in a screen-and-treat program or in the more traditional screen, diagnosis, and treatment algorithms. Both tests, plus novel triage strategies for risk stratification among carcinogenic HPV DNA-positive women, will be evaluated in a large clinical study in China in 2010. Despite the progress, more low-cost, robust molecular tests are needed and strategies for using these tests in low-resource settings must be developed and validated.
HUMAN PAPILLOMAVIRUS INFECTION IN A POPULATION- BASED SAMPLE OF WOMEN FROM TBILISI, GEORGIA.

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Although cervical cancer holds the second place among women with neoplasia in Georgia no data are available about the prevalence of high-risk human papillomavirus (HPV) infection in Georgia, which, in the absence of good-quality screening, is known to correlate with cervical cancer incidence. Therefore in Nov 2007 the Dept of Pathology of the Vrije Universiteit Amsterdam in close cooperation with the International Agency for the Research of Cancer /IARC/ (Lyon, France) and Institute of Morphology (Tbilisi, Georgia) has started an HPV prevalence study in Tbilisi.

Cervical cell specimens from 1,309 women aged 18-59 years from the general population in Tbilisi were obtained. DNA of 44 HPV types was detected using a GP5+/6+-based PCR assay. The prevalence of cervical abnormalities was 4.7%. HPV prevalence was 13.5%, being highest in women aged 15-24 (17.2%) and 25-34 years (18.7%). HPV prevalence then fell to between 8.6% and 9.5% for all age groups above 34 years. High-risk HPV prevalence was 8.6% overall, being 6.8% and 38.9% among women with normal and abnormal cytology, respectively. In conclusion, we report a relatively high burden of HPV infection in Tbilisi, Georgia. Since only 2.2% of women reported a previous PAP smear, improving cervical cancer prevention by screening and/or HPV vaccination, is an important public health issue for Georgia.

To this end we have set up a randomised controlled trial comparing cervical screening by HPV with cervical screening by conventional cytology, as is currently practised in some small areas of Georgia. The trial will involve 10,000 women with 5000 women in each arm and will start in the beginning of 2010. We expect that the experience with implementing HPV screening in Georgia can be used as a model for other low budget countries.

HPV PREVALENCE AND CYTOLOGY PATTERNS AMONG YOUNG WOMEN IN A NORTH INDIAN COMMUNITY

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Objective: Evaluation of HPV prevalence and cytological abnormalities in women aged 16-24 years and correlation with time since coitarche.

Methods: Married, non-pregnant women aged 16-24 years, residents of an urban slum in New Delhi gave exfoliated cervical cells for conventional cytology and HPV DNA testing by consensus PCR and Hybrid Capture 2 (HC2) Probe B. HPV genotyping was done on all positive samples by the Reverse Line Blot Assay (Roche®).

Results: Out of 1873 eligible women, 1300 women were enrolled from March 2006 to March 2008. All were of low socioeconomic status. The median age was 22 years. HPV positivity was seen in 91 (7%) and 110 (8.4%) women by PCR and HC2 respectively. On HPV genotyping 104 were positive, with single infections in 64 and multiple infections in 40 women. The five most common types were HPV16, 52, 51, 39, 59 and 18. HPV 16/18 accounted for 41.

The cohort was divided into two groups: Group 1 - women married for ≤3 years (n=356, 27.4%) and Group 2 - women married for >3 years (n=944, 72.6%). In Group 1, HPV positivity by HC2 and PCR was 38 (10.6%) and 30 (8.4%) respectively. In line blot positive (n=34) HPV 16/18 were seen in 35%, other high risk types in 41% and low risk HPV in 23.5%. Multiple infections were found in 29.4%. In Group 2, HPV positivity by HC2 and PCR was 68/944 (7.2%) and 60/944 (6.4%) respectively. In line blot positive (n=70) HPV 16/18 were seen in 44.3% other high risk types in 30% and low risk HPV in 25.7%. Multiple infections were found in 32.8%.

On cytology, inflammatory smears were found in 869 (66.9%) women. Pap abnormalities were seen in 29 women (ASCUS - 7, LSIL - 20, HSIL - 2). Both the subjects with HSIL were married for >3 years. Overall 19/29 (65.5%) abnormal Pap smears were HPV positive. There was no significant difference in the prevalence of cytological abnormalities with respect to time since coitarche (2.2% in Group 1 vs 1.9% in Group 2).

Conclusion: Inflammation is a common finding but there is a low prevalence of cytological abnormalities in the younger age group. High grade lesions were seen >3 years after coitarche. HPV 16/18 account for a substantial proportion of the infections but a wide spectrum of HPV genotypes is seen in this young population.
OBJECTIVES: Basic Health International (BHI) is a non-profit organization working to prevent cervical cancer in low-resource settings. Since 2005, BHI has worked with the Salvadoran Ministry of Health to integrate a single-visit cervical cancer screening and treatment program into an ongoing cytology program in order to increase screening coverage to the rural population.

METHODS: BHI worked with the Salvadoran Ministry of Health to establish visual inspection with acetic acid (VIA) as the primary screening tool for cervical cancer in the rural departments. Women over 25 presented for cervical cancer screening during BHI delegations. Women over 50 received VIA and Pap smear. VIA screening was considered adequate if the squamo-columnar junction could be fully visualized. Women of all ages who received cryotherapy were re-screened 6-24 months later with VIA, Pap smear, colposcopy, ECC, and 4-quadrant biopsy.

CONCLUSIONS: VIA can be successfully integrated into an ongoing cytology program without difficulty. Since January 2006, BHI has trained 64 nurses and generalist physicians in VIA and cryotherapy. During their training, BHI has screened 4482 women. Of these women, 432 were VIA+ (9.6%). Of the women who were VIA+ 133/432 (30.8%) had a biopsy confirmed lesion. Cryotherapy efficacy was evaluated in 45 biopsy-positive women. Treatment was successful in 42/45 (93.3%; 95% CI: 86-100%). 27/28 cases of CIN 1 were successfully treated (96.4%; 95% CI: 89.5-100%). 12/14 CIN 2/3 lesions were successfully treated (85.7%; 95% CI: 71-100%). Preliminary results for adequacy of VIA in post-menopausal women indicate that it could be an appropriate screening method in all ages. For respective age groups adequacy is the following: 20-29 = 98% (95% CI: 96-100%), 30-39 = 93% (95% CI: 88-97%), 40-49 = 85% (95% CI: 79-91%), 50-59 = 81% (95% CI: 77-86%), 60-69 = 72% (95% CI: 64-79%), and 70-79 = 64% (95% CI: 51-77%). Preliminary results for adequacy in women whom previously received cryotherapy indicate that VIA may be an appropriate tool for subsequent screening of patients following cryotherapy. Of 121 subjects previously treated with cryotherapy, 101 or 83.5% (95% CI: 86.9-91.1%) had adequate exams.

Objective: The authors present the results to Cervical Cancer Program of State of Paraná (CCSPP), southern region of Brazil, during the period ranging from October 1997 to June 2009.

Methods: The smears were take of general doctors, gynecologists and other health care professionals. The examination had no cost to the patient, and all the health centers were provided with Ayre spatulas, cervical brushes, glass slides and cell fixatives. The slides were screened by 62 pathologists and 24 cytotechnologists from 43 pathology laboratories linked the CCSPP. The nomenclature applied was modified Bethesda system. The quality control are performed in Hospital of Federal University of Paraná.

Results: Of the 6,412,840 cervical smears examined during the 11-year period. The adequacy of the smears examined was 98%. 115,819 (1.80%) cases were altered, LSIL was diagnosed in 34%, HSIL in 19%, squamous cell carcinoma in 1.53%, adenocarcinoma in situ in 0.16%, adenocarcinoma in 0.15%, ASC, in 40.5% and ACG 4.90%. In the Quality Control Unit 571,520 smears were examined (10.2%). The overall rate of diagnostic disagreement was 2,40% and the commonest causes of errors were in ASC category.

Conclusion: The methodology of CCSPP in Paraná state had subtly dropped our mortality index. The number of cervical cancer diagnosed in this 11 years reduced of 43.48/100,000 women in 1997 to 13, 23/100.00 women in 2009 and mortality is 4.44/100.000 women.
COMPARISON OF DNA-IMAGE CYTOMETRY, HC II HPV TESTING AND LIQUID-BASED CYTOLOGY AS A PRIMARY CERVICAL CANCER SCREENING TEST

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Objectives: Organized, quality assured, cytology-based screening programs have substantially reduced cervical cancer incidence in many developed countries. However, 80% of cervical cancers occur in low-resource settings, particularly in developing countries where the lack of qualified cytopathologists is the main barrier to cytology-based screening. An effective, inexpensive, alternative approach for cervical cancer screening is required. The goal of this study was to compare three different approaches to cervical cancer screening in the context of a family planning program in XiaMen, China.

Methods: A total of 4964 women were enrolled in the study. Samples were taken with a cervix brush and transported into a fixative solution. Two slides were made from each sample: one stained with Feulgen DNA specific stain and the other Pap stained. Part of each sample was tested for HPV by Hybrid Capture (HC) II. Patients with a Pap smear result above ASCUS, or positive by DNA image cytometry or positive for high-risk HPV infection were recommended for biopsy by fluorescence- and reflectance spectroscopy-directed digital colposcopy. We report the apparent sensitivities, specificities, positive and negative predictive values (PPV, NPV) of these 3 tests.

Results: Based on 60 patients for whom colposcopically directed biopsies were read by 3 intern pathologists and using CIN2+ histopathology as the positivity threshold and gold standard, the respective sensitivity and specificity were: 0.58 and 0.99 for conventional cytology; 0.79 and 0.99 for DNA ploidy; 0.84 and 0.91 for HCII. The PPV and NPV were respectively 0.23 and 0.99 for conventional cytology, 0.19 and 0.99 for DNA image cytometry, 0.04 and 0.99 for HCII.

Conclusions: Automated DNA image cytometry may be a useful tool for cervical cancer screening and has a competitive sensitivity, specificity, PPV and NPV compared with conventional cytology and HPV typing. The total training time for medical students to perform and interpret DNA measurements is about three weeks. The typical turnaround time for DNA image cytometry is 24-48 hours. Automated DNA cytometry may be an effective technology for cervical cancer screening in developing countries.

DIGITAL DOCUMENTATION OF COLPOSCOPY-BIOPSY AND THE NCI-BIOPSY STUDY

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Objectives: Visual signs of cervical precancer have low specificity and are not well reproduced. Therefore, colposcopic impression is frequently inaccurate and biopsy sites may not target the worst lesion on the cervix. Missing the worst lesion in colposcopically directed biopsy affects cervical cancer screening performance, evaluation of new screening assays, and tissue-based etiologic research. We launched a new study, the NCI Biopsy Study, to analyze cervical disease on the lesion level and to study the inaccuracy of colposcopy and the benefit of taking multiple biopsies.

Methods: The study population includes women referred to colposcopy for abnormal cervical screening results at the University of Oklahoma colposcopy clinic. We implemented an extended biopsy protocol with up to four biopsies of distinct acetowhite areas and a random biopsy if less than four biopsies are taken. Colposcopic impression, annotations of acetowhite lesions, and biopsy sites are recorded directly after colposcopy using a digital colposcopy system developed by NCI and the National Library of Medicine. All biopsies are processed and evaluated separately; corresponding LEEPs are analyzed in 12 segments to allow relating lesions with visual findings and biopsy sites.

Conclusions: In the Biopsy Study, we will be able to analyze the effect of collecting multiple biopsies on cervical disease ascertainment. Extensive digital documentation of colposcopic impression will allow better analyses of the relation between visual and histological findings and may improve current colposcopy-directed biopsy procedures. At the same time, we will be able to improve our gold standard and achieve better precision for etiologic and biomarker studies.
Three cases of cervical intraepithelial glandular neoplasia (CIGN) - a term encompassing adenocarcinoma in situ and glandular dysplasia of the uterine cervix - were studied clinically, colposcopically and histologically, histochemically for mucins (neutral mucins, sialomucins and sulfomucins), immunohistochemically for the affinity of four lectins (WGA, PNA, RCA, UEA). For comparison, six cases of cervical invasive adenocarcinoma and ten cases of cervixes without tumor were similarly studied. Criteria for histologic grading of CIGN into three degrees were proposed according to the hyperchromasia and the stratification of nuclei, number of mitoses, and amount of intracellular mucin. Two different types of CIGN were distinguished according to their histological aspect and their mucin pattern: CIGN type A, where the mucin pattern was qualitatively similar to that of normal endocervical mucosa, i.e., neutral mucins, sulfomucins and sialomucins; and CIGN type B, where the glandular cells resembled small intestinal goblet cells and the mucins consisted of neutral mucins and sialomucins with the absence of sulfomucins. Nine cases of CIGN were of type A, 2 of type B, and 12 of both type A and B. Differences in lectin binding existed between normal columnar cells, CIGN, and invasive adenocarcinomas, as well as between CIGN of type A and B. The intensity of the positive immunochemical reaction varied, as well as the type of bound lectin and its localization in the cell. There was a great heterogeneity in the same histologic group from one case to another, and even in the same case from one cell to another.

Objective: Analyze the differences in the brightness of the cervical epithelium on Optical Coherence Tomography (OCT) images as a distinguishing characteristic of normal, low-grade, high-grade, and cancer histologies.

Methods: 483 women participated in real-time studies of Niris OCT as a diagnostic adjunct to colposcopy (300) or VIA (unaided visual inspection) (183) to compare the relationship between the brightness of the OCT images to corresponding histology. All patients undergoing colposcopy or VIA were evaluated by cervical quadrant. Areas were likewise evaluated by OCT. All women had biopsies obtained from any abnormal areas in any quadrant. In normal quadrants biopsies matching the OCT sites were taken at 2, 4, 8, or 10 o'clock at the squamo-columnar junction depending on the quadrant. Brightness of the epithelium was measured at 3 and 12 o'clock and averaged together to create a normal brightness reading for each patient. Abnormal lesions were then measured for brightness; normal images were then subtracted from the abnormal image to create a difference from normal. All brightness measures were on a log scale. Mean difference from normal was used to compare brightness levels by histological grade. Two sample T-tests were used to look at differences in brightness between histological grades.

Results: Histologic diagnoses were 6 squamous metaplasia (SQMET), 52 CIN II, 64 CIN III, and 22 cancer. Mean brightness was 0.16, 1.56, 3.36, and 4.71 for SQMET, CIN II, CIN III, and cancer respectively. Mean brightness differed significantly between histologic grades for the comparisons of CIN I to CIN II (p<.000), CIN II to CIN III (p<.000), CIN II to cancer (p<.000), and SQMET to cancer. For the comparison of mean brightness for CIN III to cancer p=.018.

Conclusions: Epithelial brightness is a statistically significant distinguishing feature of OCT images of the uterine cervix.
**SS 14-4**

**LOOP CONE OR COIN TREATMENT IN CIN 1 - AN AGE TAILORED TREATMENT**

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**Introduction:** CIN 1 treatment is controversial and can be classified as destructive or excisional. Directed biopsy in CIN 1 cases can give a false negative result for CIN 3 in 30% of the women. The ideal treatment should be able to make a correct diagnosis without causing damage.

**Method:** Our aim was to examine the pathological results in women treated by LLETZ because of CIN1, to find if there are any differences for CIN 2-3 diagnosis according to the patients' age. We examined retrospectively our results for the years 2001-2003. From 2004-2007 young women up to 35 years old with CIN 1 on cervix biopsy after persistent lesion for 12 months were treated by LOOP Coin and older women were treated by the LOOP Cone. We documented the final pathological results, the volume of the cone, and the height of the conus and recorded the complications.

**Results:** 464 women underwent LOOP Cone excision due to CIN 1. In 2 women Carcinoma was diagnosed, in 19.2% the final diagnosis was CIN 2-3, in 44.4% CIN 1 was found and normal histology was found in 35.8% of the patients. In young women age 18-24 the average volume of the conus was 1.47 cubic cm. and the height was 0.59 cm. and it gradually grows in women 45-54 years to an average of 2.79 cubic cm. and height of 1.05 cm.

**Conclusion:** Tailored treatment by a thin LOOP Coin in young patients diagnosed with CIN 1 had a diagnostic advantage of correct diagnosis of CIN 2-3 in 19.2% of the patients, with a minimal potential risk for future complications.

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**SS 14-5**

**CERVICAL STENOSIS AND CONIZATION**

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**Objectives:** To identify the reasons of (CS) after conization.

**Methods:** This is a prospective study including 282 patients who underwent conization between 1999 and 2007. The different parameters studied were at the moment of the surgical procedure:
- patient's age
- menopausal status
- parity
- height of removed tissue
- diameter of the endocervical canal at the top of conization
- and the size of the external os, three months after conization

We have defined cervical stenosis as an external os diameter <2 mm

**Results:** We observed 34 stenosis on 282 cases (12,41%)

We used a logistic model for predicting stenosis
- Influence of patient's age: The percentage of CS in women aged of <45 years old was 9,5% versus 47,5% for women aged of >45 years old. p = 0.005 . RR:2.95. CI [1.37-6.34]
- Influence of menopausal status: The percentage of CS en pre-menopausal women was 10,85% versus 57,14% for post-menopausal women. P = 0.001. RR:5.6. CI [2-15.6]
- Influence of number of pregnancies: The average size of the external os in patients who were never pregnant was 5,87 mm, 5,36mm in women who had few pregnancies, and 7 mm in women who had numerous pregnancies but with too few cases to be significant.
- Influence of the height of the cervical cone: The average height of the cervical cone was 14,3mm. In patients with CS, it was 16,48 mm whereas 13,98mm in the non-CS patients. p = 0.04. RR: 1.08. CI [1.002-1.15]
- Influence of the diameter of the cervical cone: The percentage of CS was respectively 6.61, 13.7 and 22.72% for loop of diameters 25, 20 and 15mm.
- Influence of the endocervical canal diameter at the top of excision: The average endocervical canal diameter for the overall patient group was 5,54 mm. In patients with CS, the average was 4,08 mm whereas 5,72mm in the non-CS patients, p = 0.0001. RR: 0.66. CI [0.53-0.82]

The multivariate logistic analysis (AUC=0.78) highlights that the main factor is the endocervical diameter at the top of excision (p= 0.003) followed by diameter of the cone (p=0.02), before height of excision (p = 0.06).

**Conclusion:** In our study, the main risk factors for cervical stenosis are: a narrow endocervical canal diameter, diameter of the cone and age.
From 1987 to 2008, the diagnosis of 3158 pre invasive or early invasive cervical lesions was made in our colposcopy clinic after histological control of patients with abnormal Pap smear. 100 of these patients (3.2 %) had already been treated for high grade (54) or low grade (46) lesion from one to 20 years before. All interval Pap smears were normal after the first treatment. This situation suggests a true recurrence of the lesion after an effective treatment. The previous treatment had been performed in our hospital (66) or elsewhere (34). The grade of recurrence was LGSIL (30), HGSIL (59), ACIS (2), micro invasion (4), or early invasion (5).

The mean delay before recurrence was 6,4 years. There was no correlation between the grade of the previous lesion and the severity of the recurrence and with the delay before recurrence.

The previous treatment was conization (32) or ablative treatment (68): LASER vaporization(59), electro coagulation(4), 4 cryotherapy and one application of 5FU). There was a positive correlation between severity of recurrence and ablative treatment.

**FIRST TREATMENT**

<table>
<thead>
<tr>
<th>GRADE OF RECURRENCE</th>
<th>CONIZATION 32</th>
<th>ABLATIVE TREATMENT 68</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOW GRADE SIL</td>
<td>15</td>
<td>15</td>
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<tr>
<td>HIGH GRADE SIL</td>
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<tr>
<td>And ACIS</td>
<td>16</td>
<td>45</td>
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<tr>
<td>Micro invasion</td>
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<td>invasion</td>
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</table>

Most HG/invasive recurrences occur after ablative treatments mainly after LASER vaporization for low grade SIL. It is suggested that after a destructive treatment, the lesion has been hidden in the canal because of the healing process with more less cervical stenosis. Because of difficulties for diagnosis, the recurrent lesion was discovered later at an advanced stage. The greatest care must be taken to perform LASER vaporization on low grade lesions close to the canal. There is a risk of difficulties for further controls. Long term follow up by endocervical cytology is mandatory after any treatment even for low grade SIL.

**THE ACCURACY OF COLPOSCOPIC GRADING IN PREDICTING CIN2+ HISTOLOGY**

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**Objective:** The aim of the study is to analyze the accuracy of the colposcopic grading in predicting CIN2+ histology in comparison with the citology.

**Material and methods:** We analyzed the colposcopic description and grading of 93 patients with CIN2+ histological result after LLETZ/conisation. We classified the colposcopic lesions according to the International Colposcopic Classification 2002 and using Reid Colposcopic Index . We were looking for : the quality of colposcopy, the presence of acetowhiteness, the grading of the lesion, the prevalence of the main colposcopic aspects in relation with dysplasia and we calculated the sensibility of the colposcopy in comparison with the cytology.

**Results:** We included in the study 93 patients, with ages between 20 and 57, median 37, with histopathological results of CIN2+. The colposcopy was unsatisfactory in 2/93 cases (2,15%). The results of the grading score indicated:- benign lesion in 2/93 cases (2,15%), - G1(mild displasia) in 41/93 cases (44,08%), - G2 (severe displasia ) in 46/93 cases (49,47%),- high suspicion of cancer 2/93 (2,15%). The prevalence of the main colposcopic aspect correlated with displasia was: mosaic 53/93 cases (56,98%), punctuation 48/93 cases (51,61%), atypical vessels 14/93 cases (15,95%), ulceration (10,75%), leucoplasia ( 12,90%), acetowhite epithelium 62/93 cases ( 66,66%), cuffed glandular openings 17/93cases (18,27%), exophytic lesions 8/93cases (8,6%).In 90 cases there were associated aspects. The cytology was: HGSIL in 70/93 cases (75,26%), ASC-H in 3/93 cases (3,22%), persistent ASCUS in 4/93cases (4,30%), persistent LGSIL 13/93 cases( 13,97%), invasive carcinoma in 3 cases (3,22%), not correlated with clinical aspect .

**Discussions and conclusions:**
1. The colposcopy show acetowhiteness in all the cases when satisfactory, but the grading couldn't predict the high grade displasia or more.
2. The sensibility of colposcopy in detecting CIN2+ lesions were lower than for the cytology.
MOTIVATION TO PARTICIPATE IN COLPOSCOPY AND REASONS FOR NON-PARTICIPATION

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Objectives:
In a population-based cohort study on cervical cancer screening (MARZY) in Germany, conventional as well as liquid-based cytology and HPV-testing was performed. Women with positive test results were invited to undergo expert colposcopy at a University hospital. Women who did not attend colposcopy were contacted by telephone for further motivation or investigation of reasons for non-participation.

Methods:
All women who tested positive (ASCUS or worse, or HPV high risk positive) were invited to undergo expert colposcopy. Women who did not attend colposcopy by two months after invitation or missed an appointment were contacted by phone for motivation to receive colposcopy. If motivation was not successful, reasons for non-participation were investigated. Telephone interviews followed a standardized questionnaire.

A total of 108 women did not attend colposcopy after invitation and had to be contacted. Of those, 100 telephone interviews were completed (92.6%). On average four calls were necessary for a successful contact. After the telephone contact, 29 more women participated in colposcopy. The main reasons for non-participation were “lack of time” (28.1%), “organizational problems” (19.2%), “fear” (13.2%), “difficulties to get an appointment” (11.3%), “refusal to visit the University hospital” (9.6%) and “being advised against colposcopy by the office-based gynecologist” (9.0%).

Conclusions:
Our results show that motivation to participate in colposcopy is possible via telephone contact with study participants. Moreover, several of the reasons for non-participation could be addressed and alleviated in a study setting.

CONSERVATIVE APPROACH TO INVASIVE CERVICAL CANCER

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Summary: In modern medicine, treatment of cervical cancer has always been RADICAL: Radiation therapy to the pelvis with or without cervico-vaginal « boost », or radical hysterectomy with complete pelvic and/or para-aortic lymphadenectomy. The results of these treatments have been important on the cure rate of cervical cancer, but with major «side effects»: fibrosis, stenosis of the vaginal, enteritis, fistula etc. In younger patients, loss of fertility and often premature menopause were the rule.

The last 20 years have been fertile in advances in surgical procedures responsible for a more conservative approach in early-stage cancer of the cervix. Operative laparoscopy has opened the way to well accepted new techniques: pelvic and para-aortic lymphadenectomy, the technique of the sentinel node, radical hysterectomy and trachelectomy without opening the abdomen (vaginal or total laparoscopic technique). More recently, fertility-sparing cervical conisation or radical trachelectomy after neoadjuvant chemotherapy showed very good oncologic and reproductive results. Even less aggressive techniques are the subject of prospective trials: Laparoscopic lymphadenectomy followed by conisation or simple trachelectomy, without parametrectomy, and laparoscopic removal of the sentinel nodes only, followed by large conisation, simple trachelectomy or simple hysterectomy without parametrectomy.

Those advances are important since the SEER data shows that 30% of the 10,000 cases of cervical cancer in the USA affect women under the age of 40. It is speculated that half of them are candidates for a fertility-sparing procedure.

In higher stage disease, laparoscopic staging with or without ovarian transposition made possible better orientated radiotherapeutic approach.
MANAGEMENT OF ANNEXIAL MASSES
S. Dexeus, R. Carreras, G. Misson, G. Mancebo, D. Dexeus

The widespread use of ultrasonography has increased the number of hospitalizations for annexial masses (AM). In the USA, they account for almost 300,000 cases per year. This figure gives us the magnitude of the sanitary problem and the need for a proper diagnosis and treatment. Sometimes, ultrasonography is done by people with inadequate training and/or low-resolution apparatus.

The number of patients submitted to surgery is around 30%. The laparoscopic approach, has reached almost 86%. Laparotomy, as primary surgery, has been employed in less of 10% of the cases.

Benign pathology represents more than 90% of the cases and the multimodal preoperative study had a sensitivity of 91%, specificity of 46% and PPV of 37% and NPV 93%.

The so-called ovarian screening, gives rates of detection which are very low: 0.15%-0.21%.

IMPROVEMENTS IN TREATMENT AND OUTCOME OF CERVICAL CANCER IN THE NETHERLANDS, 1989-2006
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2 Erasmus Medical Centre, Dept of Public Health, Rotterdam
3 Maastricht University Medical Centre, Dept of Gynaecology, Maastricht
4 Medisch Spectrum Twente, Dept of Radiotherapy, Enschede
5 Comprehensive Cancer Centre South, Dept of Research, Eindhoven

Objectives. Did changes in detection and treatment contribute to better survival of cervical cancer in the Netherlands?

Methods. All newly diagnosed patients with squamous cell (SCC) or adenocarcinoma (AC) of the cervix diagnosed in the period 1989-2006 (n=11,547) in the Netherlands Cancer Registry.

Conclusions. The proportion of patients diagnosed in FIGO stages IB2 & IIB-IVA increased from 30 and 4% in 1989-93 to 35 and 7% in 2004-06. In FIGO IB1 & IIA, the proportion of patients receiving surgery increased from 80 to 95%, versus a decrease in the use of radiotherapy (RT) from 17 to 8%. The use of chemoradiation (chemRT) increased in these stages: both primarily (1.4 to 6.8%) and adjuvantly (0.2 to 6.4%). Treatment of patients diagnosed in FIGO IB2 & IIB-IVA changed from RT (35% in 2004-06) to chemRT (41% in 2004-06). Relative 5 year survival rates increased for AC from 65 to 74%, but remained the same (71%) for SCC. The rates for FIGO IB1 & IIA increased from 78 to 88% and for FIGO IB2 & IIB-IVA from 41 to 50%. In multivariable analyses, survival in FIGO IB1 & IIA and in FIGO IB2 & IIB-IVA increased with increasing time and decreasing age, being better for patients with negative lymph nodes. Treatment did not affect prognosis independently. The hazards in FIGO IB1 & IIA were 3 times higher for patients receiving RT compared to patients receiving surgery and in FIGO IB2, IIB-IVA hazards were lower for patients receiving chemRT compared to patients receiving RT. In conclusion, especially when taking into account that most slow developing tumours will be detected as premalignant lesions following cervical cancer screening, leaving the more aggressive tumours to be treated, treatment and survival of cervical cancer have improved during the last decades. This is due to the introduction of new diagnostic procedures as better staging leads to better treatment. However, also remarkable improvements in the treatment of cervical cancer have been achieved, for example the introduction of chemoradiation, which is accompanied by much better survival rates.
Human papillomavirus (HPV) infections have been well studied during the last decades although some key questions concerning the epidemiology of HPV remain unanswered. For example, very little is known about the probability of re-infection with HPV, especially with the same type. There is also a paucity of data concerning the source of HPV infection in older women. Some studies have shown that re-infection with the same HPV type is a common occurrence and that a woman continues to be at risk of being re-infected with the same type after clearance. Some studies also suggested that re-infection in older women can be a new incident infection contracted via sexual activity (rather than being explained via the reactivation of latent infection acquired at younger age). Moreover, the recent data obtained in vaccine trials have shown a benefit of the HPV vaccination among older women (protection against incident HPV infection) although little is known about the risk of lesions following re-infection that occur at older age. The objective of this presentation is to present the most recent data and new evidence concerning the epidemiology of HPV, more specifically re-infection with HPV.

Background: Quadrivalent HPV vaccine (GARDASIL®) is 90% (95% CI: 69, 98) efficacious against vaccine HPV type related external genital lesions in men. The purpose of this analysis was to examine the incidence of and HPV types identified in external genital warts (EGW) among heterosexual men (HM) and men having sex with men (MSM) enrolled in an efficacy trial of GARDASIL®.

Methods: Of 4,065 men aged 16-26 years with <5 lifetime sexual partners enrolled in a randomized, double-blind clinical trial, 2,030 received placebo. Subjects underwent genital exams and HPV sampling from the penis, scrotum, and perineal/perianal area at Day 1, month 7 and every 6 months afterwards. All lesions were biopsied for diagnosis and PCR testing. An incident case of EGW was defined as a subject who had no reported history or a diagnosis of EGW at Day 1, but developed a subsequent EGW as determined by the pathology panel. An HPV type-specific endpoint was an incident case of EGW with a specific HPV type (HPV 6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59) found in the lesion via PCR.

Results: Inclusive of 2.9 years of follow-up (median), 83 cases of EGW were identified with an incidence rate (per 100 person years) of 1.83. The incidence rate among HM (1.50) was lower than in MSM (4.70). The incidence rate was 1.12 per 100 person years (0.97-HM, 2.79-MSM) in subjects PCR and seronegative to all 4 vaccine HPV types at enrollment. 71 (86%) of all EGW was related to ≥ 1 of the 4 HPV types in the vaccine. HPV types 6 and 11 were the most common HPV types found in EGW lesions with 48 (58%) and 24 (29%) cases, respectively. Co-infections with other tested HPV types were detected in 20 (24%) of EGW.

Conclusions: These data demonstrate the high incidence of EGW amongst a population of young men with few prior sexual partners. A large proportion of EGW was associated with HPV 6 or 11. Considering the high efficacy of GARDASIL® against external genital lesions, these data suggest potential benefit of vaccination with GARDASIL® and prevention of EGW in men.
SS 16-5

GROWING EVIDENCE OF EPIDEMIOLOGIC LINK BETWEEN ORAL CANCERS AND HPV

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Approximately 570,000 new cases of head and neck squamous cell carcinoma (HNSCC) are diagnosed annually worldwide, for which smoking and alcohol drinking are the major risk factors. Early case studies suggested that Human papilloma virus (HPV) infection may play a causal role in a subgroup of predominantly oropharyngeal, and especially tonsillar HNSCC. These findings were further confirmed by larger epidemiological studies. About 95% of HPV-associated HNSCC harbor DNA of HPV type 16. Recently, an IARC monograph working group concluded that there was sufficient accumulated evidence for the carcinogenicity of HPV16 in the oral cavity, oropharynx and tonsil. Nevertheless, the attributable fraction of HPV to HNSCC remains unclear. The reported prevalence of HPV DNA in HNSCC ranges widely, with this disparity being related to differences in the anatomic site of the tumors as well as geographical region, with higher HPV DNA prevalence seen in recent studies from the U.S.. Furthermore, despite the firmly established association between HPV16 and certain sub-sites of HNSCC, the HPV detection in tumor specimens is not sufficient to indicate causality so there remain questions about the extent to which the HPV presence in these tumors is biologically relevant. This presentation will review the accumulating epidemiological evidence on this topic.

SS 17-1

TRENDS IN INCIDENCE AND MORTALITY OF CERVICAL CANCER IN THE NETHERLANDS: INCREASE OF INCIDENCE AFTER DECADES OF DECLINE?

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3) Department of Pathology, VU Medical Centre, Amsterdam, The Netherlands
4) Eindhoven Cancer Registry, Comprehensive Cancer Centre South, Eindhoven, The Netherlands

Objectives: To explore recent trends in incidence and mortality rates of cervical cancer in the Netherlands by different age groups, stages and morphology, and explain trends by screening activities. New guidelines regarding the screening programme were implemented in 1996. One of them was the increase of the screening interval from 3 to 5 years in a broader target age group.

Methods: Population based data was retrieved from the Netherlands Cancer Registry from January 1st 1989 till December 31st 2006 (incidence data) and from Statistics Netherlands from January 1st 1969 till December 31st 2007 (mortality data). Incidence rates and estimated annual percentages change (EAPC) were calculated by age, FIGO stage and morphology. Mortality rates and EAPC were calculated by age.

Results: Total age-adjusted incidence rate (European Standardised Rates (ESR)) declined from 9.1 in 1989 to 7.3 per 100,000 woman years in 2006. It decreased with 1.3% (95% CI -2.2%, -0.4%) annually during 1989-1998, then decreased with 6.3% (95% CI -16.0%, 4.5%) during 1998-2001, but seemed to increase during 2001-2006 by 1.7%, (95% CI -0.8%, 4.3%). The largest decrease in incidence rates during 1989-2006 was found for age group 60-74 years (-4.1%, 95% CI -5.2%, -3.0%). During the same period, incidence of FIGO IA tumours significantly decreased in all age groups <75 years. Incidence of FIGO 4B tumours significantly increased during 1989-2006. Incidence of squamous cell carcinomas decreased during 1989-2006 (-1.3%, 95% CI -2.5%, -0.2%), then decreased with 7.1% (95% CI -18.4, 5.9%) during 1998-2001 and increased during 2001-2006 (2.3%, -0.7%, 5.3%). Mortality ESR decreased dramatically from 8.1 in 1969 to 1.9 per 100,000 women years in 2007. It decreased with 4.1% (95% CI -4.6%, -3.7%) during 1969-1994, and with 2.6% (95% CI -3.8%, -1.5%) during 1994-2007.

Conclusions: After decades of decline, a possible increase in incidence rates was observed. Intensive screening during the implementation period of the revised screening programme after 1996 may have led to a temporarily more rapid decrease in incidence, followed by an increase in incidence due to less intensive screening.
**SS 17-2**

**HOW MANY WOMEN WERE SCREENED IN THE NETHERLANDS AND WHAT WAS THE ADVICE? RESULTS FROM MORE THAN 1000 CASES OF CARCINOMA.**

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**Objective:** What percentage of women with cervical carcinoma have actually been screened vs underscreened prior to the diagnosis and if so, what was the profile of results.

**Method & results:** We counted all cases of cervical carcinomas over 2006 and 2007 and controlled in the preceding years to diagnosis the presence or absence of smears. In addition, we measured whether these smears were programmed smears or not and what the results were of those smears. We utilized the National Pathology Database (PALGA) and restricted our analysis to all cases of squamous- and adenocarcinomas of the cervix uteri in invited age cohorts. and controlled 1) whether there was any smear history, 2) whether the smear was an invitational smear and 3) whether the smear diagnoses led to referral for further investigation.

We counted 1037 cases of squamouscell- or adenocarcinoma of the uterine cervix (age groups 30-67 year) over 2006 and 2007. In 349 (34%) cases, there was no screeninghistory, up to one screening round ago (under- or unscreened). In 688 (66%) cases, cervical cytology had been performed. In 493 of 688 cases, smears were invitational based while 195 of 688 were formally outside the programme. Of the 493 invitational based smears, 476 (97) had retrieveable information on the ‘advice’. We counted 178 (37%) times a default of regular advice (i.e. normal cytology) and 298 (63%) times a repeat advice (holding category) or referral advice for colposcopy. We observed no significant difference between adeno- and squamous-cell carcinoma. Total number of putative missed cases amounted to more than 100 over a 5 year period.

**Conclusion:** Fraction of unscreened or underscreened women prior to carcinomadiagnosis was lower than expected, ie 34% instead of 55% as shown earlier. In those cases screened, prior to carcinoma diagnosis, 63% had a referral of repeat advice, while 37% had a default advice. Although these results suggest improved cervical cytological programme-parameters compared to earlier studies of this type, there is still room for improvement.

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**SS 17-3**

**ATTENDANCE TO SCREENING AND RISK OF CERVICAL CANCER AMONG IMMIGRANTS IN SWEDEN, YEARS 1993 THROUGH 2002**

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**Objectives:** Cervical screening has effectively reduced the incidence of cervical cancer in high income countries, including Sweden. In this study we investigate the degree of participation and effect of adherence to the cervical screening program on the risk of developing invasive cervical cancer, among immigrant and Swedish-born women. We also investigated to what extent adherence to screening modifies differences in risk of cervical cancer between immigrant, compared to Swedish-born women.

**Methods:** Degree of participation to cervical screening was estimated for immigrant and Swedish-born women between 23 and 60 years from 1993 through 2002, stratified by age at migration. We also estimated incidence rates and rate ratios for women adhering or not to the cervical screening program. Finally, we assessed the effect of adherence to screening on the relative risk of cervical cancer for immigrant, compared to Swedish-born women.

**Conclusions:** The degree of participation was 56.7 % and 47.5% among the Swedish-born and immigrant women, respectively, with large variations among the immigrant groups. Degree of participation was lower with older age at migration and women who where non-adherent to the cervical screening program had a 5-fold risk increase of invasive cervical cancer, or more, compared to those who were adherent. However, screening adherence modified the relative risks of cervical cancer among immigrant, compared to Swedish-born women, only modestly. Although adherence to cervical screening drastically reduces risk of cervical cancer, it can not completely explain the differences in risk of cervical cancer observed between immigrant and Swedish born women. Immigration after age 40 to 50 is an important barrier for participation to cervical screening.
WOMEN’S SEXUAL BEHAVIOUR BEFORE THE INTRODUCTION OF NATIONWIDE HPV VACCINE PROGRAMS: A POPULATION-BASED STUDY IN FOUR NORDIC COUNTRIES

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Objectives: To describe patterns in sexual behaviour of women from the general population in Denmark, Iceland, Norway and Sweden prior to the introduction of HPV vaccination programmes.

Methods: We used an existing population-based cohort of 18-45 year-old women from Denmark, Iceland, Norway, and Sweden. Information on sexual behaviour e.g. age at first sexual intercourse and lifetime number of sexual partners was collected using a standardised questionnaire. The study population consisted of 65,623 women and the participation rate ranged from 81.3% in Denmark to 54.5% in Iceland. Descriptive statistics were used to illustrate inter- and intra-national patterns in sexual behaviour. Furthermore, survival analysis techniques were used to evaluate age at first sexual intercourse.

Conclusions: In Denmark, Iceland and Sweden the median age at first sexual intercourse was 16 years and in Norway the figure was 17 years. Median number of partners was 5 in Denmark and Norway and 6 in Iceland and Sweden. Focusing on the youngest women (18-25-year-old) approximately one third (one fourth in Norway) had experienced first sexual intercourse before age 16 years, and about one third (one fourth in Iceland) of all 18-21-year-old women had had no more than one sex partner. Among the young women who had experienced first sexual intercourse, the majority (93.6%) reported ever condom use and 28.6% reported regular condom use within the last 12 months. This information may be important in the decision of age limits for HPV catch-up vaccination.

CAUSES-OF-DEATH AND CANCER REGISTRIES SHOULD BE LINKED FOR CORRECT CERVICAL CANCER MORTALITY.

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Objectives: Monitoring the effectiveness of cervical cancer prevention is feasible using population based registers. Data reported must be validated to reflect the effects of changes in prevention strategies. All cervical carcinomas, but no other tumors should be included. Algorithms have been developed to account for death in cervical cancer, erroneously coded as unspecific uterine cancer. Globocan 2002 reports a cervical cancer mortality which is 50% (90 deaths) higher than the figures in the Swedish Causes-of-death register, due to an algorithm attributing half of the unspecified uterine cancer deaths (ICD10 C55) to cervical cancer (ICD10 C53).

We decided to investigate the true contribution of C55 to cervical cancer mortality in Sweden.

Methods: All 1660 deaths in C55 during the ten year period 1997-2006 were linked to the nationwide Swedish Cancer register to determine the preceding cancer diagnosis: 87% of all women with unspecific uterine cancer as reported cause of death already had a specific diagnosis (7% cervical carcinoma, 49% endometrial carcinoma, 8% carcinoma of non-uterine origin, 23% uterine sarcoma (SNOMED M88xx3/M89xx3)). Less than 13% had an undetermined or no previous cancer diagnosis, some of which might be reallocated to cervical cancer.

Reports to the Swedish Cancer registry require a specified diagnosis and ICD code by both pathologist and clinician. Death certificates, however, are issued in Swedish, often by general practitioners using simplified diagnoses, leading to C55 classification for both endometrial carcinoma and sarcoma. Swedish Cancer and Causes-of-death registers are not routinely linked for this follow-up.

Conclusions: The proportion of women with a death certificate of “unspecific uterine cancer” (C55) but who actually died from cervical carcinoma is closer to 10% than 50%. The Causes-of-death register and the Cancer register should therefore be linked before applying reallocation algorithms to obtain valid data suitable to evaluate cervical cancer prevention.
SS 17-6

SOCIOECONOMIC DISPARITIES IN CERVICAL CANCER SURVIVAL IN THE UNITED STATES

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Objective: Much research has been conducted into the socioeconomic disparities in cervical cancer screening; however the same is not true for cervical cancer survival. This study seeks to examine the relationship between cervical cancer survival and certain socioeconomic indicators within the United States.

Methods: Data from the Surveillance, Epidemiology, and End Results program (SEER), which was linked to US Census data, was used for the analysis. Logistic regression models were used to determine the effects of various socioeconomic indicators on five- and ten-year cancer survival. Education (above or below median rate of high school completion), poverty (above and below median poverty level) and unemployment (above and below median rate of unemployment) as county-level measures were used. Age of diagnosis and stage of cancer were controlled for where appropriate.

Conclusion: Relationships between county rate of high school completion, level of poverty, unemployment levels and cervical cancer survival were found. A level of education that fell under the median rate of high school completion was associated with an adjusted odds ratio (OR) of 1.39 (95%CI: 1.29-1.49) of less than five-year survival. The OR associated with lower than ten-year survival was 1.57 (1.42, 1.73). Falling below the median poverty level was associated with lower than 5-year (OR=1.29, 95%CI: 1.20-1.38) or lower than 10-year survival (OR=1.44, 95%CI: 1.34-1.55). Likewise, a level of unemployment under the median rate was associated with poorer survival for 5-year (OR=1.30, 95%CI: 1.25-1.35) and 10-year rates (OR=1.30, 95%CI: 1.25-1.37). An income analysis using a random effects model will also be shown to explore differences between county-level and individual-level data. In summary, there are important socioeconomic disparities among rates of cervical cancer survival in the United States.

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HUMAN PAPILLOMAVIRUS INFECTION AND RISK OF IN SITU AND INVASIVE CERVICAL ADENOCARCINOMA

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Objectives: Human papillomaviruses (HPV) are thought to cause adenocarcinoma of the cervix, but the evidence is based on retrospective studies. We investigated prospectively the association between specific HPV types and risk of in situ (AIS) and invasive adenocarcinoma (AC) of the cervix.

Methods: In two nested case-control studies among women who participated in cytological screening in Sweden, we collected 1434 smears in total from 118 women with AIS, 164 with AC, and their individually matched controls, during a median follow-up of 6 to 7 years. We analysed the smears for HPV using a sensitive PCR assay. Conditional logistic regression was used to estimate odds ratios (ORs).

Results: Being positive for HPV 16 in the first cytologically normal smear was associated with increased risks for both AIS (OR 11.0, 95% confidence interval (CI) 2.6-46.8) and AC (OR 16.0, 95% CI 3.8-66.7), compared to being negative for HPV 16. A first HPV 18 positive smear was associated with increased risks for AIS (OR 26.0, 95% CI 3.5-192) and AC (OR 28.0, 95% CI 3.8-206), compared to an HPV 18 negative smear. A first smear with either HPV 16 or 18 infection was associated with increased risks for AIS (OR 14.7, 95% CI 4.5-47.2), and for AC (OR 19.0, 95% CI 6.0-60.7), compared to being negative for HPV 16/18. Corresponding figures for the last smear (taken up to 12 months before date of diagnosis) was for AIS OR 59 (95% CI 8.2-426), and for AC OR 24.0 (95% CI 7.6-76.2). Non-16/18 HPV high risk types were not associated with statistically significant increased risk of AIS or AC. The HPV16/18 attributable risk proportion, estimated from first smear, was 36% for AIS and 35% for AC.

Conclusion: Infections with HPV 16 and 18 detected 15 years before diagnosis were strongly associated with increased risk of both AIS and AC. At least one-third of all incident cases in Sweden are attributable to these HPV types, and therefore potentially preventable through vaccination.
PROSPECTIVE STUDY ON HPV TYPES IN SQUAMOUS CELL CARCINOMA OF THE CERVIX


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Objectives: With a move into HPV-based screening, there is an imminent need to better understand and to quantify risks for cervical disease according to HPV status, not the least with regard to HPV types not covered in current vaccines.

Methods: In a case-control study, nested in the National Cervical Cancer Screening Register in Sweden, we identified 515 cases of in situ (CIS) and 320 cases of invasive squamous cell carcinoma (SCC). For each case patient one individually matched control woman was selected randomly using case-control pair sampling. Both cases and controls had an initial cytologically normal smear at entry. Validated and sensitive PCR-assays were used to detect the presence of seven low-risk and 16 high-risk HPV types in all available smears. The average follow-up time for both cases and controls was approximately seven years.

Conclusions: Being positive for HPV 16 and/or HPV 18 in the first (cytologically normal) smear was associated with an almost 9-fold (95% CI 5.3-13.7) increased risk of CIS, compared to being negative for HPV in the first smear. The corresponding figure for SCC was RR 19.1, 95% CI 9.2-39.8. The risk association increased from the first to last smear before diagnosis of the case, with a more pronounced increase for CIS (RR in last smear = 42.0, 95% CI 19.9-88.8) than for SCC (RR in last smear = 30.3, 95% CI 13.6-67.5). Being positive for any high-risk HPV other than 16 or 18 showed a significant risk increase at first smear (CIS: RR 4.4, 95% CI 2.8-6.8; SCC: RR 3.1, 95% CI 1.6-5.7), and a much stronger increase at last smear before diagnosis (CIS: RR 19.8, 95% CI 9.8-40.3; SCC: RR 15.7, 95% CI 6.4-38.7). Based on attributable risk proportions calculated from our relative risks, we conservatively estimate that between 30-50% of CIS and 40-50% of SCC in this cohort could have been removed through HPV 16/18 vaccination. An additional 20-30% of CIS, and 10-20% of SCC, could have been removed through non-16/18 HR HPV coverage. Our prospective study further elucidates the strong risk associations between HPV infection and subsequent development of in situ and invasive squamous cell carcinoma of the cervix.
HIGH RISK HPV PHYSICAL STATUS AND P16INK4A EXPRESSION IN CERVICAL INTRAEPITHELIAL LESION FROM THAI WOMEN

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Background: Overexpression of the viral oncogenes E6 and E7 in basal keratinocytes induces chromosomal instability and overexpression of the cyclin dependent kinase inhibitor p16INK4a that is therefore being used as marker for transforming HR-HPV infections. Integration of HR-HPV genomes in host cell chromosomes is hypothesized as potential mechanism resulting in overexpression of the E6-E7 oncogenes. In this study we investigated the physical status of HR-HPV genomes and their association with p16INK4a overexpression in cervical lesions of increasing severity.

Methods: 338 biopsy and cervical swab samples were collected that consisted of 36 cases of squamous cell carcinoma (SCC) and cervical intraepithelial neoplasia (CIN) (72 cases, CINII-III, 165, CINI) and 65 cases of no lesions. GP5+/6+ and reverse line blot hybridization were used for HPV DNA detection and genotyping. Amplification of papillomavirus oncogene transcripts (APOT) was used to investigate HR-HPV derived transcripts (HPV16, 18, 31, 33 and 45). p16INK4a immunostaining was performed in 90 formalin-fixed, paraffin-embedded tissue (FFPE).

Results: HPV infection rates were 27.7%, 41.8%, 75% and 80.5% in samples without lesion, CINI, CINII-III, and SCC. Integrate derived transcripts of HPV16, 18 and 45 were found 40%, 100% and 100% in SCC, respectively and only HPV 16 showed integrate derived transcripts in preneoplastic lesions (5%). HPV 18, 31 and 33 showed purely episomal derived transcripts in preneoplastic lesions. Unique integration sites were found in each clinical sample. The positive rates of p16INK4a were 0%, 16.2%, 89.4% and 100% in no lesion, CINI, CINII-III and SCC, respectively. Weakly staining was found in CINI (9.6%). Integrate derived transcripts were found 60% and 6.7% in p16INK4a positive SCC and CINII-III, respectively. Episomal derived transcripts only were found 100% in p16INK4a negative or positive lesions with either CINI or without detectable lesions.

Conclusions: Our observations suggest that p16INK4a overexpression substantially precedes HR-HPV integration in the course of the preneoplastic progression and samples with both p16INK4a overexpression and expression of integrated HR-HPV genome copies mark lesions with substantial risk for quick progression into invasive carcinomas.

PREVALENCE AND EPIDEMIOLOGY OF TRICHOMONAS VAGINALIS DETECTED BY REAL TIME PCR IN FLANDERS AND ITS RELATION TO HUMAN PAPILLOMAVIRUS INFECTION

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Objective: The goal of this cross-sectional laboratory based study is to assess the prevalence of Trichomonas vaginalis (TV) in Flanders, and to investigate the association between TV and human papillomavirus (HPV) infections in cervical samples.

Setting: Liquid based cervical cytology samples from unselected women, covering population of 14 to 97 years of age, resident of Flanders (North Belgium) and participating in cervical cancer screening were assessed for the presence of HPV and TV.

Methods: During 7 months in 2008, 62944 consecutive liquid based cytology cervical cancer screening samples were assessed for cytological abnormalities. All samples were tested by real time quantitative PCR for the presence of TV as well as for low-risk HPV (lrHPV) types 6, 11, 53, 66 and 67, and high-risk HPV (hrHPV) types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68. Correlations of TV and HPV infections with age, geographic area and occurrence of cytologic lesions were assessed.

Results: The overall prevalence of TV in the general population in Flanders was 0.37%, with the highest prevalence in women aged 41-45 years (0.53%). HPV was detected in 15.1% of cervical samples and peaked in younger women of ages 21-25 years (26.8%). The prevalence of TV was higher in women with HPV infections as compared to women without HPV (0.61% vs 0.33%, p < 0.0001). In women of suggestive foreign origin TV prevalence was 4 times higher than in the probably autochthonous population (1.16% vs 0.29%, p<0.0001). Working in the sex industry had an increased risk of both HPV and TV when compared to other women (OR 8.6, CL95% 4.4-16.9, p<0.0001) and a higher rate of TV was also observed in the city agglomerations, compared to the other municipalities (OR 1.7 (1.3-2.2), p=0.0002).

Conclusion: The prevalence of Trichomonas vaginalis in Flanders is ten times lower than what is published in the literature, whereas the prevalence of HPV infection is similar to other European countries. Both TV and HPV are sexually transmitted infections, but our prevalence data suggest that the epidemiology of HPV and TV are different in Flanders. Highest HPV prevalence is found in young women whereas TV is more frequent in older women. Although some epidemiological peculiarities of the society, such as promiscuity and import from overseas countries can possibly account for some of these differences, the exact reason for this difference remains to be elucidated in further studies.
ASSOCIATION OF TRICHOMONAS VAGINALIS AND CYTOLOGICAL ABNORMALITIES OF THE CERVIX IN LOW RISK WOMEN

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Background: Is Trichomonas vaginalis (TV) a provocative factor for the development of (pre-) cancerous lesions of the cervix?

Methods: We tested 63,251 of 63,487 consecutive liquid based cervical samples from Belgian women by real time quantitative PCR for the presence of TV as well as for 18 HPV types and for cytology.

Results: Overall prevalence of TV DNA was 0.37%, of low risk HPV 2%, of high risk HPV 13.2%, and 8.8% had cytologic abnormalities. Both LR-HPV and HR-HPV were significantly associated with all cytologic abnormalities, LR-HPV most strongly with LSIL and HR-HPV most strongly with HSIL. Presence of TV was associated with LR- and HR-HPV, ASCUS and HSIL, but not with other abnormalities. All women with TV and HSIL were also harboring HR-HPV, while this was only 59% of the women with TV and ASCUS. Amongst HPV negative women, TV was found in 1.3% of women with ASCUS, but only in 0.03% of women with normal cytology (OR 4.2, CL95% 2.1-8.6). In HR-HPV positive women, presence of TV increased the likelihood of cytological abnormalities somewhat (P=0.05), mainly due to an increase in ASCUS and LSIL, but not HSIL.

Conclusions: We conclude that TV infection is associated with both LR and HR-HPV infection of the cervix, as well as with ASCUS and HSIL. TV is a concomitant STI, but is no co-factor in the causation or promotion of HSIL and cervical cancer. However, TV this may lead to a significant number of false positive diagnoses of ASCUS.

EFFICACY OF GARDASIL® AGAINST EXTERNAL GENITAL LESIONS DUE TO 14 HPV TYPES IN MEN

Palefsky, J, for The Male Quadrivalent HPV Vaccine Efficacy Trial Team

Background: Efficacy of the quadrivalent HPV (types 6/11/16/18) L1 virus-like particle vaccine (GARDASIL®) against external genital lesions (EGL) (external genital warts, penile/perineal/perianal intraepithelial neoplasia, and penile/perineal/perianal cancer) related to HPV 6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 55, 58, and 59 studied in men.

Methods: Data are from 4,065 men aged 16-26 years enrolled in a double-blind, placebo-controlled trial. Subjects received GARDASIL® or placebo at Day 1, Months 2 and 6 and had genital exams and HPV sampling from penis, scrotum, and perineal/perianal area at Day 1, Month 7 and every 6 months. Subjects with visible or history of HPV related lesions were excluded at Day 1. All lesions were biopsied and PCR tested. Efficacy was calculated in a generally HPV naïve (GHN) population of subjects PCR negative to HPV 6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59 and who was studied in men.

Results: Among 1,275 vaccine and 1,270 placebo recipients, 42 developed EGL (6 vaccine and 36 placebo recipients). Vaccine efficacy (VE) was 83.8% (95% CI: 61.2, 94.4) against EGL related to any HPV type. 31 (86%) and 3 (50%) EGL cases in the placebo and vaccine recipients were PCR positive for HPV 6, 11, 16, or 18, respectively. VE against vaccine HPV types was 90.6% (95% CI: 69.8, 98.2). VE was 60.8% (95% CI: -139.1, 96.3) against EGL related to the 10 tested non-vaccine HPV types and 61.2% (95% CI: -137.0, 96.3) against EGL for which HPV type was not identified.

Conclusions: These data show the high efficacy of GARDASIL against vaccine-type EGL. Efficacy data for non-vaccine type EGL were not conclusive due to small sample size. As a high proportion of EGL is associated with vaccine-type HPV, vaccine impact on overall incidence of EGL in HPV-naïve populations is expected to be high.
TRANSMISSION OF MALE HPV INFECTION

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Objectives: To estimate male-to-female and female-to-male HPV transmission rates.

Methods: Recently-formed couples attending a university or junior college in Montreal, Canada, were recruited for the HITCH Cohort Study (HPV Infection and Transmission among Couples through Heterosexual activity). On average, couples had been in a sexual relationship for 3.9 months at enrolment. Self-collected vaginal swabs and clinician-collected swabs of epithelial cells from the penis and scrotum were tested for type-specific HPV-DNA using the Roche Linear Array. We analysed 4-month follow-up data from 100 couples for whom at enrolment the male partner had a type-specific HPV infection not detected in the female partner (F-M+), and from 62 couples for whom at enrolment the female had an HPV type not found in the male (F+M-). Each new HPV type observed at follow-up that was previously found in the only in the partner was interpreted as a transmission. Transmission rates were estimated using Poisson methods that accounted for multiple HPV types per person. Among the women who were initially F-M+ discordant, at follow-up 26% (26/100) had an HPV type(s) previously found only in the male. Among the men who were initially F+M- discordant, at follow-up 40% (25/62) had the HPV type(s) previously found only in the female. The male-to-female and female-to-male transmission rates were 38 (95%CI 28 - 52) and 50 (95%CI 35 - 72) per 100 person-years, respectively.

Conclusions: These data are consistent with a high rate of HPV transmission between sex partners. Male-to-female transmission was as equally likely as female-to-male HPV transmission.

SIGNIFICANCE OF FLAT PENILE LESIONS FOR HPV TRANSMISSION

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It has been widely accepted that genital HPV is sexually transmitted. Genital warts, reflecting mainly low-risk HPV infections, are highly contagious for sex partners. Limited insight is available about the clinical manifestations of high-risk HPV (hrHPV) infection in men and their contribution in the viral spread. Multiple studies of the last decade have been contributed to the insight that flat penile lesions (FPL) are likely to play an important role in the transmission of hrHPV. The positive relationship between hrHPV and the presence of FPL has been demonstrated by data that flat penile lesions have similar predilection sites as HPV, often contain hrHPV as identified by DNA in situ hybridisation in biopsy specimens and show a high association with hrHPV as identified by PCR in penile scrapes of lesional sites. Considering their contagious potential, an important finding is that the presence of FPL is associated with high viral copy numbers. In contrast, absence of FPL is generally associated with very low HPV copy numbers or absence of HPV. Therefore, we argue that FPL form the reservoir of hrHPV in men and contribute to the viral spread. Their bare visibility with the naked eye and their high degree of spontaneous healing explain why FPL have slipped the attention of the clinician. However, studying transmission of HPV in sex partners should not only focus on detection of HPV but also on the presence of flat penile lesions and viral load. Moreover, trials on HPV vaccinations in men should take into account the presence of flat penile lesions as an outcome measure for the efficacy of a vaccine.
PREVALENCE AND HISTORY OF SEXUALLY TRANSMITTED INFECTIONS AMONG MEN WHO HAVE SEX WITH MEN ENROLLED IN A QUADRIVALENT HPV VACCINE TRIAL

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

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Background: This analysis examined the history and prevalence of sexually transmitted infections (STI) among men who have sex with men (MSM) enrolled in a clinical trial of the quadrivalent HPV (types 6/11/16/18) virus-like particle vaccine (GARDASIL®).

Methods: 602 MSM aged 16-26 years with <5 lifetime sexual partners in worldwide randomized, double-blind, placebo-controlled trial received quadrivalent HPV vaccine or placebo at enrollment, month 2, and month 6. Those with a known history of or visible HPV-related lesion or HIV infection were excluded. A full medical history was obtained at enrollment. In addition, intraanal swabs were collected for chlamydia and gonorrhea culture. Anal cytology was performed, and blood for HIV and syphilis testing was collected.

Results: Among 602 MSM, 107 (17.8%) had reported a history of STI at enrolment. The most commonly reported STI's were anal Chlamydia trachomatis (8.8%, n=53), followed by syphilis (2.5%, n=15) and gonorrhea (2.2%, n=13). At enrollment, laboratory testing revealed that 54 (9.4%) subjects were positive for anal Chlamydia trachomatis, 15 (2.5%) were positive for syphilis, 11 (2.0%) were positive for HIV, and 5 (0.8%) were positive for gonorrhea. 583 subjects had intraanal swabs for cytologic examination, and 532 (91.3%) were satisfactory. 58 subjects (9.9%) had abnormal cytology at enrollment (23 [3.9%] atypical squamous cells of undetermined significance and 35 [6.0%] low-grade squamous intraepithelial lesion).

Conclusions: These data demonstrate the substantial risk of STI among young MSM with limited sexual experience enrolled in a large, worldwide clinical trial. The prevalence of these STI may be even higher in a general MSM population. Routine screening for the common STI in this high-risk population is crucial.

ANALYSIS OF THE EFFICACY OF GARDASIL® AGAINST EXTERNAL GENITAL LESIONS IN YOUNG MEN STRATIFIED BY BASELINE CHARACTERISTICS

Goldstone, S, for the Male Quadrivalent HPV Vaccine Efficacy Trial Team

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Methods: Data from this analysis are from 4,065 men aged 16-26 years without history of or visible lesions related to HPV enrolled in a double-blind, randomized, placebo-controlled (1:1) trial. Men received GARDASIL® or placebo at enrollment (Day 1), months 2 and 6. Subjects had genital exams and HPV sampling from penis, scrotum, and perineal/perianal area at Day 1, month 7 an every 6 months. Vaccine efficacy (VE) analysis was performed in a per-protocol efficacy (PPE) population including subjects who were seronegative and PCR negative to HPV 6, 11, 16 and 18 at Day 1 (PCR negative through month 7), and who received all 3 doses of study material. Case count started after Day 1, with median follow-up of 2.9 years.

Results: VE against EGL related to vaccine-type HPV was 90.4% (95% CI: 69.2, 98.1) in the PPE population. There were 3 cases of EGL among vaccinated subjects, 1 was among 16-20 year olds and 2 were among 21-26 year olds, resulting in VE of 94.3% (95% CI: 63.9, 99.9) and 85.1% (95% CI: 33.2, 98.4) in these age groups, respectively. VE among non-circumcised subjects was 91.4% (95% CI: 65.0, 99.0) and 87.6% (95% CI: 7.4, 99.7) among those circumcised. All 3 EGL cases were non-smokers, leading to VE of 84.1% (95% CI: 46.1, 97.0) among non-smokers and 100% (95% CI: 60.7, 100) among current smokers. No strong covariate predictors of efficacy related to race, geographic region, or number of sexual partners was identified.

Conclusions: GARDASIL® was found to be highly efficacious among young men. VE estimates were generally comparable for all subgroups within a particular baseline characteristic where there was sufficient data.
We also found that nearly 80% of the women were HPV-seropositive, HPV 6 being the most common serotype, followed by HPV16, 11, 18 infection could present a risk group for a pre-malignant disorder.

Oral HPV-infection was detected in 9%, while the infection cleared in 7% of the adults. One can speculate that a person with persistent HPV-infection of the other spouse. Among the parents, we identified different subgroups; half of the parents tested always HPV-negative in any sample, persistent infection to oral HPV infection, but a persistent oral HPV infection of the spouse increased the risk of persistent oral HPV infection 10-fold in the partners for oral and genital HPV every 6th month during three years. HPV detection rate varied from 15% to 27%. Oral sex had no association to HPV type-specific infection status (susceptible, infected, and immune) was developed. We identified multiple parameter sets that fitted age-specific sexual behaviour and epidemiological data. Strategies investigated included vaccination of: 1) girls, 2) girls + boys, 3) catch-up girls only, and 4) catch-up girls + boys. For each strategy, we varied: 1) coverage, 2) duration of protection and 3) efficacy.

Conclusions: Under baseline assumptions (vaccine efficacy = 95%, average duration of protection = lifelong), the model predicts that vaccinating 12-year-old girls will produce a rapid steep decrease in vaccine type specific prevalence and, at equilibrium, HPV-16/18 prevalence in females would be decreased by 64% (80% credibility interval (CrI): 54,82) and 81 (80%CrI: 68,98) with coverage of 70 and 90%, respectively. Vaccinating boys, in addition to girls, is predicted to produce a slightly faster decline in female vaccine type specific prevalence as well as a lower prevalence at equilibrium. Although at 70-90% coverage, vaccination of girls is not predicted to lead to elimination of HPV-16/18, a program that vaccinates 70% of 12-year-old boys and girls leads to elimination for 20% of simulations. The qualitative differences between the immunisation strategies are consistent for both HPV-6/11 and HPV-16/18 vaccination. The incremental benefit of vaccinating boys decreases significantly with improved vaccination characteristics (i.e. higher vaccine efficacy and coverage, and increases in the number of cohorts vaccinated). Countries decisions to vaccinate boys should depend, in part, on the coverage they can achieve in girls.

Both alpha- and beta-papillomaviruses can infect oral mucosa causing asymptomatic infection or warty lesions. Clinically detectable oral benign HPV lesions include papillomas, condylomas, warts and focal epithelial hyperplasia (FEH). FEH is familiar and caused by HPV infection with types 13 and 32. Recently, it was shown that population with HLA-DRB1*0404 allele is at risk for FEH.

Although the first evidence on HPV and oral cancer was presented already in 1983, the topic become an attractive research area only after the era of HPV vaccines. Numerous case-control studies have reported elevated odds ratios (from approximately 2 to 200-fold) of oral and oropharyngeal cancers among subjects with a detectable oral HPV infection. According to the IARC analysis, the overall prevalence of HPV in oral cancer was 16.0% in Europe and North America, but much higher (33%) in Asia. In oro-pharyngeal cancer, HPV detection rates were even higher in Europe (28%), North America (47%) and Asia (46%) due to the high detection rate of HPV in tonsillar carcinomas. HPV16 seems to be the single most prevalent type in all geographic areas followed by HPV6 and 18. HPV-positive cancers differ from the classical smoking- and alcohol-related cancers. No dose-related response for tobacco, alcohol or tooth loss are found in HPV16-positive oral cancers in contrast to HPV-negative cancers. Oral sex and use of marihuana are related to HPV-positive cancers, but not to HPV-negative cancers. The disease-specific survival are related to the expression of epidermal growth factor receptor (EGFR) and the presence of HPV DNA. The survival seems to be poorest for those patients who had high EGFR receptor activity and who were HPV negative.

Only few natural history studies on oral HPV infection exist. Our prospective Finnish Family HPV Study has tested pregnant women and their partners for oral and genital HPV every 6th month during three years. HPV detection rate varied from 15% to 27%. Oral sex had no association to oral HPV infection, but a persistent oral HPV infection of the spouse increased the risk of persistent oral HPV infection 10-fold in the other spouse. Among the parents, we identified different subgroups; half of the parents tested always HPV-negative in any sample, persistent oral HPV-infection was detected in 9%, while the infection cleared in 7% of the adults. One can speculate that a person with persistent HPV-infection could present a risk group for a pre-malignant disorder.

We also found that nearly 80% of the women were HPV-seropositive, HPV 6 being the most common serotype, followed by HPV16, 11, 18 and 45. There was no correlation between oral HPV-DNA detection and HPV-serology. The following HPV types were found in oral mucosa HPV 6,11,16,18,39,56,58 and 66.

Our data from the Finnish Family HPV Study also indicate that oral HPV infection can be acquired at early age. The mother might be the main transmitter of oral HPV-infection to her off-spring.
**SS 19-2**

**QUADRIVALENT HPV VACCINE EFFICACY AGAINST ANAL INTRAEPITHELIAL NEOPLASIA IN MEN HAVING SEX WITH MEN**

Palefsky, J. for The Male Quadrivalent HPV Vaccine Efficacy Trial Team

*Department of Medicine, University of California, San Francisco*

**Background:** Previous data have demonstrated the efficacy of the quadrivalent HPV vaccine (GARDASIL®) against external genital lesions (perianal/perineal/penile intraepithelial neoplasia and condyloma) in men aged 16 to 26. In this analysis we examined the efficacy of the vaccine specifically against HPV 6/11/16/18-related anal intraepithelial neoplasia (AIN) and anal cancer in men who have sex with men (MSM).

**Methods:** Data are from 598 MSM aged 16-26 who were randomized to receive vaccine or placebo at enrollment, month 2, and month 6. Serum was collected at enrollment and at months 7, 24, and 36 for analysis of anti-HPV antibodies. Subjects underwent detailed anogenital exams as well as sampling from the penis, scrotum, perineal/perianal and anal canal at enrollment, month 7 and at 6-month intervals afterwards. Efficacy analyses were performed in a per-protocol population (seronegative at day 1 and DNA-negative from day 1 through month 7 to the relevant vaccine HPV type). Median follow-up was 2.5 years (post-dose 3).

**Results:** Vaccine efficacy against HPV 6/11/16/18-related AIN and anal cancer in MSM was 77.5% (95% CI: 39.6, 93.3) (5 vaccine cases versus 24 placebo cases). Endpoints in vaccinated subjects were related to HPV 6 (n = 3) and HPV 16 (n = 2). Efficacy against high-grade AIN (AIN 2+) was 74.9% (95% CI: 8.8, 95.4). No anal cancer was seen in either treatment group.

**Conclusions:** These results demonstrate that the quadrivalent HPV vaccine is efficacious in preventing AIN and anal cancer related to HPV 6/11/16/18 in MSM subjects naïve to vaccine HPV types at enrollment.

**SS 19-3**

**ANALYSES OF THE ROLE OF HUMAN PAPILLOMAVIRUS (HPV) IN THE AETIOLOGY OF HEAD AND NECK CANCERS.**

Holger Sudhoff, David Winder, Jurg Ebmeier, Jurgen Franzer, Siolian Ball, Katie Vaughan, Margaret Stanley, Peter Goon.

**Background:** Head and neck cancer is the 5th most common cancer in the world, with a worldwide incidence of over 600,000 cases per year. 90% of these cancers are squamous cell carcinomas (HNSCC). The most important risk factors identified for developing HNSCC are tobacco use and alcohol consumption. Recently, the human papillomavirus (HPV) has also been implicated in approximately 40% of HNSCC. Strong epidemiological data has shown that tobacco and alcohol related HNSCC are decreasing in incidence (in concordance with falling rates of smoking and alcohol usage in the West) while HPV+ cancers are increasing in incidence. HPV positivity for HNSCC has assumed highly important prognostic and therapeutic significance as it has been demonstrated that HPV+ tumours have a much better prognosis compared to HPV- tumours, and the standard of treatment may need to be revised for these cancers as they have a much better response to chemo-radiation. It is therefore imperative to establish HPV status. We will test for HPV status, establish the prevalence of HPV-associated cancers in the cohort under study, and we will define the actively replicating HPV subtypes involved by first establishing HPV DNA viral load, and then testing for specific E6/E7 oncogene mRNA expression from tissue.

**Methods:** Our collaborators in Bielefeld, run the largest ENT unit in Germany, and currently operate on approximately 300 cases of head and neck cancer per annum. Only patients with fully informed written consent will be recruited into the study. We will analyse for HPV status via our sensitive nested PCR assay (established in our lab), and type for HPV subtypes via Linear Array™ (Roche) and direct sequencing of amplified products. Further analyses by qPCR to quantify the DNA viral loads of high-risk HPV subtypes 16, 18, 31, 33, 45 and low-risk subtypes 6, 11, 42 will be performed, and subsequent quantification of the mRNA expression of these specific E6,E7 oncogenes will follow. We will also test the hypotheses that DNA and/or mRNA loads from swabs and tissue will be useful in informing us of the presence and clinical activity of HPV in the adjacent tissue, and thus serve as useful biomarkers of HPV-associated dysplastic tissue and cancer. In this way, we seek to define the use of these parameters in prognosis and follow-up of patients with head and neck cancers and their presumed pre-cancerous lesions.

**Results:** We have shown that HPV infection of the oro-pharyngeal cavity is common. Data will be provided on the HPV status of head and neck cancers and pre-cancerous lesions in this cohort of patients, and we demonstrate that it is possible to obtain data on the replicative activity of these HPV subtypes. We will comment on the feasibility of using such data as diagnostic and prognostic biomarkers.
Background: Anal cancer has increased in incidence in all sections of society; but especially amongst HIV+ homosexual men (~70 - 128 per 100,000) (MSM - men who have sex with men). In the general population too, the trend has increased over the last few decades in both men (~ 1.1/100,000) and women (~2-3/100,000). The primary causative agent for anal cancer and its precursors is the human papillomavirus (HPV). Indeed, it is thought that the dominant high-risk HPV subtypes involved in 70% of cervical cancer (HPV 16, 18) are overwhelmingly dominant in anal cancer (over 90% of cases). The reason for this subtype prevalence dominance in cancers from different anogenital sites is unknown. We are analysing the natural history and prevalence of HPV in higher-risk groups such as HIV+ MSM, HIV- MSM, women with CIN disease and appropriate control groups.

Methods: Patients are recruited from the Departments of HIV/GUM Medicine and Gynaecological Oncology in the Chelsea and Westminster Hospital, London and Addenbrooke’s Hospital in Cambridge. The established and highly sensitive Linear Array system (Roche™) will be used to genotype for 37 different HPV types from anal and oral swabs and urines samples. We will determine HPV DNA viral loads for the most prevalent subtypes via RT-PCR. Further correlative analyses for mRNA expression of HPV E6/E7 oncogenes (to delineate the actively replicating HPV subtypes) will be performed, and thus obtain information on the subtypes causing disease.

Results: Multiple subtype infection is the norm in all anogenital sites in all groups but particularly high in HIV+ MSM. We now have data pertaining to the activity of different HPV subtypes in disease causation in the anal epithelium and this will have an impact on the vaccination programmes currently under way. The data will also inform future vaccination strategies.
VIROSTATIC AND IMMUNE MODULATORY LOCAL THERAPY OF HPV-ASSOCIATED HIGH GRADE VULVAR INTRAEPITHELIAL NEOPLASIA.

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Objectives: To test the efficacy of local therapy with imiquimod and cidofovir in the treatment of recurrent HPV-associated vulvar intraepithelial neoplasias (VIN) and to evaluate the mode of mechanism. Imiquimod is a low molecular derivate of imidazoquinolinamin with antiviral properties. It is registered for treatment of condylomata acuminata and acts via stimulation of the secretion of cytokines. Cidofovir is classified as a nucleotide analogue with anti-proliferative activity in virus-related human tumors.

Methods: 3 sexually active women (average age 33 years) presented with recurrent VIN. All 3 patients had their multiple recurrent VINs treated with surgical excisions and laser destructions. Despite the previous destructive therapies they developed new lesions of VIN. All biopsies revealed a high-grade VIN with demonstration of HPV-16 DNA by PCR analysis. All three patients received local treatment with 5% imiquimod for 2 weeks alternating with 1% cidofovir for 2 weeks with a total therapy length of 12 weeks. One and 5 months after completed local therapy all 3 patients had a clinical and histological remission without detectable HPV-16 DNA. One patient had a 5 year remission, during which she underwent a HPV vaccination. 6 months after completed vaccination, however, she developed a new lesion of VIN. The recurrent VIN was treated once again locally with imiquimod and cidofovir, which resulted again in a complete remission. The other two patients remained free of recurrences. Antigen-presenting dendritic cells were obtained from one patient and incubated in vitro with imiquimod for 12 hours. The expression of cell surface markers of the antigen-presenting dendritic cells was analyzed by flow cytometry. The treated dendritic cells revealed an increased expression of surface markers CD80/B7.1, CD86.

Conclusion: Virostatic / immune modulatory local therapy with imiquimod and cidofovir is an effective non-invasive treatment alternative for recurrent HPV-associated high grade VIN. The effect of imiquimod is based on an enhanced presentation of HPV-antigens to lymphocytes as documented by increased expression of co-stimulatory molecules and surface markers on the cell membrane of antigen presenting dendritic cells.

HUMAN PAPILLOMAVIRUS GENOTYPE DISTRIBUTION IN ANAL CANCER IN FRANCE: THE EDITH V STUDY

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Objectives: Anal cancer is a rare cancer but its incidence is increasing. Human papillomavirus (HPV) infection seems to be associated with the occurrence of most cases. The objective was to estimate the genotype-specific prevalence of HPV in anal cancer, unknown until now in France, to assess the potential benefit of HPV vaccination.

Methods: Randomly selected paraffin embedded anal cancer specimens (including adenocarcinoma and cloacogenic carcinoma) were retrospectively recruited in 2008 from 16 centres scattered in France. Medical records were examined for patient related data. Specimens were centrally tested for HPV genotyping using the INNO-LiPA assay allowing the detection of 27 genotypes (12 low-risk and 15 high-risk).

Results: Among the 366 anal cancer cases included in the analysis, 225 (62%) were females. HIV status was available for 96 cases (27%) and 50 cases (14%) were HIV+ cases. Majority of these HIV+ cases were male (45 cases, 90%). Mean age of all cases at diagnosis was 54.8 years in males and 66.4 years in females (p<0.001). HPV was found in 96.7% of samples, with 72% infected by a single HPV type. Presence of at least one high-risk genotype was observed in 91% (96% in females and 83% in males, p<0.001). Differences in mean age and HPV genotype distribution are observed HIV+ cases compared to HIV- cases. HPV16 was by far the most prevalent genotype (75%), followed by HPV18, HPV52, HPV33, and HPV51 (4-6%). HPV16/18 alone or in association were found in 78% of all cases and HPV16 or 18 alone (mono infection only) were found in 59% of all cases.

Conclusion: Our results indicate that HPV is associated with most of anal cancer and emphasize the predominant role of HPV16. The potential benefit of HPV vaccine on the occurrence of anal cancer should be further evaluated but HPV vaccination would be expected to significantly reduce the burden associated with the management and treatment of anal cancer in France.

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**Objectives:** To determine the prevalence of HPV-infection in 80 cases of penile carcinoma in Belgium.

**Methods:** All paraffin embedded specimens were submitted to a quantitative real-time PCR amplification for the detection of _β_-Globin. Extraction was done with the Qiagen DNA Mini KitÆ. For the HPV-detection and typing an in house real-time-PCR method was used.

**Results and Conclusions:** Fifty per cent of all samples (40/80) was HPV positive. Sixty-eight per cent of DNApos-samples (35/51) were HPV positive, versus 17% of DNAneg-samples (5/29). Top 3 of common types is HPV 16, followed by HPV types 56 and 58. HPV type 18 shares the fourth place with HPV type 11 (Table 1). Most samples were positive for one type only (31/40 or 77.5%) with HPV type 16 being most prevalent (60%). In 22.5% of cases, two or more types were found (Table 2). The samples were no DNA and no HPV was found, were all diagnosed as being Bowen's disease and erythroplasia of Querat.

**Table 1:** Fifty per cent of all samples (40/80) was HPV positive.

<table>
<thead>
<tr>
<th>HPV type</th>
<th>6</th>
<th>11</th>
<th>16</th>
<th>18</th>
<th>31</th>
<th>33</th>
<th>35</th>
<th>39</th>
<th>51</th>
<th>53</th>
<th>56</th>
<th>58</th>
<th>59</th>
<th>66</th>
<th>67</th>
</tr>
</thead>
<tbody>
<tr>
<td>%</td>
<td>3.3</td>
<td>5</td>
<td>51.7</td>
<td>5</td>
<td>3.3</td>
<td>3.3</td>
<td>1.7</td>
<td>3.3</td>
<td>1.7</td>
<td>3.3</td>
<td>6.7</td>
<td>6.7</td>
<td>1.7</td>
<td>1.7</td>
<td>1.7</td>
</tr>
</tbody>
</table>

**Table 2:** Percentage of mono- and multi-infection with HPV.

<table>
<thead>
<tr>
<th>Mono-infection (31/40 - 77.5%)</th>
<th>6 - 33, 56, 58 - 16</th>
<th>2.5 - 5, 5, 5 - 60</th>
</tr>
</thead>
<tbody>
<tr>
<td>11, 16</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td>11, 58</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td>16, 18</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td>16, 39</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td>16, 53</td>
<td>2.5</td>
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</tr>
<tr>
<td>16, 59</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td>16, 18, 56</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td>6, 16, 31, 51, 58</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td>16, 18, 31, 35, 39, 53, 56, 66, 67</td>
<td>2.5</td>
<td></td>
</tr>
</tbody>
</table>

With the introduction of the prophylactic HPV vaccine, it has become useful to know the HPV-prevalence in male malign lesions. This information is needed to predict the protective value of the vaccine, assuming it will be at least as effective in men as in women. In general and worldwide, HPV types 16, 18 and 45 (in Asia HPV types 16, 18 and 58) are most common. HPV types 16, 18 and 6 are most prevalent in penile cancer. As shown above, the prevalence of HPV types may differ according to lesion and to country.

**Objectives:** The aim of this study is to examine the potential indirect effect of vaccinating females against HPV-6/11/16/18 on prevalence of infection and genital warts in males.

**Methods:** A stochastic individual-based dynamic model of sequential partnership formation and dissolution, and HPV transmission (16, 18, 6, 11 and 14 other high risk HPV types) in a population stratified by age, gender, sexual activity and HPV type-specific infection status (susceptible, infected, and immune) was developed. We identified multiple parameter sets that fitted the following age-specific sexual behaviour and epidemiological data: % sexually active, number of partners in last year, % in stable partnership, type-specific HPV prevalence by level of sexual activity, genital warts incidence. Strategies investigated included vaccination of: 1) girls, 2) girls + boys, and 3) catch-up. For each strategy, we varied: 1) coverage, 2) duration of protection and 3) efficacy.

**Conclusions:** Under base case assumptions (vaccine coverage=70%, vaccine efficacy=95%, duration of protection=life-long), vaccinating 12-year old girls is predicted to reduce female HPV-6/11 and HPV-16/18 prevalence by 98% (80% credibility interval (CrI): 65,100) and 63% (80%CrI: 54,79) at 30 years post vaccination, respectively. In addition, vaccinating only 12-year old girls is predicted to reduce male HPV-6/11 and HPV-16/18 prevalence, at 30 years post vaccination, by 97% (80%CrI: 63,100) and by 56% (80%CrI: 47,76), respectively. The herd immunity impact of vaccinating females on HPV infection (and disease) in males increases with improved vaccination characteristics (i.e. higher vaccine efficacy and coverage, longer duration of protection, and number of age cohorts vaccinated). Given the important herd immunity impact of female vaccination on males, the incremental gains in vaccinating males may be limited.
COST-EFFECTIVENESS OF CERVICAL CANCER SCREENING IN THE NETHERLANDS: COMPARISON OF DIFFERENT SCREENING SCENARIOS AND POLICIES

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Objective: Since longitudinal data about the high-risk HPV DNA test have become available in the last two years, screening guidelines must be reconsidered to improve cervical cancer outcomes. This study determines the optimal screening program for cervical cancer in the Netherlands for the unvaccinated population, from a cost-effectiveness point of view.

Methods: We use the micro-simulation model MISCAN to compare a variety of screening programs. We consider seven different screening scenarios: a) cytological testing with repeat cytology for borderline/mildly abnormal smears; b) three scenarios of primary HPV testing with cytology or a combination of cytology and HPV triage for HPV positive tests; and c) four scenarios of primary cytological testing with HPV or a combination of HPV and cytology as triage for borderline/mildly abnormal smears. For all scenarios, we considered both conventional and thin-layer cytological testing and compared 171 screening policies. Screening policies varied by frequency, interval, and initiation age. As inputs for the analysis, we used estimated costs, utilities, and incidence levels based on the Dutch national screening program. We estimated the numbers of (quality-adjusted) life years gained and the costs of the different screening policies. We also determined the efficient screening programs and the optimal screening program, using a cost-effectiveness threshold of €20,000 per quality-adjusted life year. Finally, we investigated the sensitivity of the results for the background risk of cervical cancer in screening attenders, the costs of the HPV test, and the utility loss due to follow-up tests after a positive screen test.

Conclusions: In the base-case scenario, primary HPV DNA screening with two or three times cytology follow-up is the most cost-effective screening strategy. This holds as long as the costs of an HPV-test and the loss of quality of life due to being in triage after a positive HPV test are not higher than certain threshold values. The optimal screening intensity of four or five times per lifetime is sensitive for the extent to which current incidence and mortality are the result of false-negative cytology, or are due to non-attendance.

DECISION-ANALYTIC COST-EFFECTIVENESS OF HPV PRIMARY SCREENING FOR CERVICAL CANCER IN GERMANY

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Objectives: The objectives of this HTA commissioned by the German Federal Ministry of Health were to perform a decision analysis to systematically evaluate the long-term effectiveness and cost-effectiveness of different primary screening strategies including HPV testing alone or in combination with cytology for the German health care context.

Methods: A previously published and validated decision-analytic model(1) for the German health care context was extended and adapted to the natural history of HPV infection and cervical cancer to evaluate different screening strategies, including cytology alone, HPV testing alone or combined with cytology at the age of 30yrs, HPV testing at the age of 30yrs with cytology triage for HPV+, HPV testing at the age of 25yrs with cytology triage for HPV+, with screening intervals of 1, 2, 3 and 5 years. German clinical, epidemiological and economic data were used. In the absence of individual data, screening adherence was modeled independent from screening history. Test accuracy data were retrieved from international meta-analyses. Predicted outcomes were reduction in cervical cancer cases and deaths, life expectancy, lifetime costs, and discounted incremental cost-effectiveness ratios (ICER). A perspective of the healthcare system and 3% annual discount rate were adopted. Extensive sensitivity analyses were performed to evaluate robustness of results and to identify optimal screening strategies.

Conclusions: Based on our analyses, HPV-based cervical cancer screening should be more effective than cytology (depending on screening interval reduction of cervical cancer cases ranging from 71% to 97% for HPV vs. 53% to 80% for cytology) and could be cost-effective, when performed at intervals ≥ 2yrs depending on the willingness-to-pay threshold. Increasing screening start age to 25yrs may have no loss in effectiveness but save resources. For the German screening context (actual guideline: annual Pap from the age of 20yrs), an optimal screening strategy could be biennial HPV testing at age 30yrs with biennial cytology age 25-29yrs (ICER: 28,500 Euro/LY). An extension to a 3-yearly screening interval require substantial improved screening adherence. The implementation of an organized screening program for quality-controlled introduction of HPV-screening and -vaccination with continued systematic outcome evaluation is recommended.

COST-EFFECTIVENESS OF CATCH-UP PROGRAMMES IN HPV VACCINATION

de Peuter M. 1, Littlewood K. 1, Annemans L. 2, Quilici S 3.

1 Mapi Values, Netherlands; 2 University Gent, Belgium; 3 sanofi pasteur MSD

Objectives:
The cost-effectiveness of routine vaccination with the quadrivalent human papillomavirus (HPV) 6/11/16/18 vaccine has been reported in published reviews, but the impact and cost-effectiveness of catch-up programmes have not been addressed. We performed a search for publication of cost-effectiveness models that combine routine HPV vaccination with temporary catch-up programmes. Methods and underlying assumptions were reviewed to identify factors driving results.

Results: We reviewed eight cost-effectiveness papers published up to September 2009. With vaccination starting at 12 years old, temporary catch-up programmes for girls up to 24 years old was found to be cost-effective in five studies; two studies concluded that a catch-up programme would be cost-effective for a narrower age-group (12-15; 12-18), and one concluded that a catch-up was not cost-effective. Results were dependent on differences between the models used, their design and input data, although important modelling aspects and assumptions were not always sufficiently described, making comparison difficult. Despite this, several differences between models that are likely to impact results were identified. All models used dynamic transmission modelling techniques except for one (Markov model), which did not incorporate the effect of vaccine herd immunity. Catch-up strategies varied between models and the comparator strategies were not the same; the latter being screening only, or the ‘preceding non dominated strategy’. All models assumed life long duration of vaccine protection. Although HPV 16/18-related outcomes were considered in all base cases, those for HPV 6/11, when included, were only in sensitivity analyses. Thus, the impact of genital warts was not considered in all models leading to an underestimate for the real benefit of the quadrivalent vaccine. It is likely that ICERs values were underestimated in all models since the impact of HPV-related diseases on indirect costs was not considered.

Conclusion: Overall the cost-effectiveness evaluation of a catch-up programme is variable depending on the model’s underlying assumptions. With the exception of assuming a lifetime vaccine protection, all models seem to underestimate the full benefit of the vaccine and are therefore conservative.

MODELING COST CONSEQUENCES OF CERVICAL CANCER VACCINATION AT DIFFERENT AGES IN THE UK

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1 GlaxoSmithKline Biologicals, Belgium 2 GlaxoSmithKline Biologicals, UK

Introduction: Vaccination against cervical cancer has now been initiated in many countries worldwide especially in preadolescent girls. Meanwhile the cost consequence of vaccinating older women after sexual debut has been poorly reported especially in light of the more recent clinical trial results. We modelled the cost consequence of vaccinating girls beyond the age of 12 years old using the latest clinical trial results available including the vaccine effect of HPV cross-protection.

Method: A static population model was developed in MS Excel® to evaluate the annual impact of HPV vaccine on HPV related lesions (abnormal pap, CIN1, CIN2/3 and cervical cancer) and the costs in the UK. Based on the latest results from the PATRICIA clinical trial, vaccine efficacy includes some level of cross-protection against non-vaccine HPV-types. Overall vaccine effectiveness is based on HPV typing and vaccine efficacy on each lesion. We differentiate vaccine efficacy in girls regarding their pre/post -sexual debut status (<17 years of age - HPV naïve; TVC DNA negative irrespective of HPV serostatus). We also account for a time-delay between infection and the development of a lesion assuming 5 years for CIN1, 10 years for CIN2/3 and 15 years for cervical cancer. The age range of vaccination evaluated was from 12 to 40 years of age. Costing is analyzed from a healthcare perspective obtained from published sources and official tariff data in the UK. A 100% vaccination coverage was assumed. No discount is applied as results are evaluated over a one year period reaching vaccine steady state level.

Conclusion: Vaccinating 12 years old girls could reduce the number of cervical cancer cases by 1,943 per year among whom 268 cases would result from cross-protection and would offset a cost of £178 Million (£64 Millions from cross protection). Vaccinating 25 and 40 years old girls still would result in respectively 1,461 and 1,087 cancer cases prevented (154 and 114 from cross protection) associated with a cost offset of £93 and £37 millions. In conclusion, extending vaccination to girls post-sexual debut still could lead to a substantial reduction in cervical cancer clinical and cost burden. This reduction is strengthened by cross-protection against non-vaccine HPV types recently demonstrated in clinical trials of the bivalent vaccine.
CERVICAL CANCER SCREENING IN WHICH CONVENTIONAL CYTOLOGY IS REPLACED BY LIQUID-BASED CYTOLOGY: A COST-EFFECTIVE ALTERNATIVE?

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Objectives: Cervical cancer screening with liquid-based cytology (LBC) has been developed as alternative for conventional cytology. Based on a recent Dutch randomised controlled trial (RTC), we conducted a cost-effectiveness analysis comparing both technologies.

Methods: Data were used from a RCT (including 89,784 women aged 30 to 60 years from 246 family practices), and various national databases. We used the microsimulation model MISCAN to estimate the costs and (quality adjusted) life years (QALYs) gained of screening women aged 30-60 years every 5 years for 4 screening strategies: (i) conventional cytology both primary and in triage; (ii) LBC both primary and in triage; (iii) conventional cytology with human papillomavirus (HPV) testing in the triage, and (iv) LBC with HPV-testing in the triage. We performed sensitivity analysis to assess the effect of the sensitivity and specificity of LBC, the costs of LBC and the background risk of cervical cancer on the cost-effectiveness.

Conclusions: In a situation with a low rate of inadequate smears in case of conventional cytology testing like the Netherlands, LBC screening is not a cost-effective alternative to conventional cytology screening.

HOW TO SCREEN FOR CERVICAL CANCER AFTER HPV VACCINATION IN THE NETHERLANDS

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Objectives: 1. To use model-based cost-effectiveness analyses to identify optimal cervical cancer screening strategies for women vaccinated against HPV16/18 in The Netherlands. 2. To explore whether cervical cancer screening will remain cost-effective if HPV vaccines become available that protect against additional HPV types including HPV16/18.

Methods and Results: We developed a competing risk model that describes the relation between fourteen high-risk human papillomavirus (HPV) types and cervical disease. The model allows the occurrence of multiple type-specific infections, each giving an independent risk of developing cervical lesions and cancer. It therefore explicitly takes into account the masking of high-risk non HPV16/18 infections in HPV16/18 positive women.

We considered 20 different cervical cancer screening strategies, differing with respect to the primary screening instrument (cytology and the HPV DNA test), the number of screening rounds (7, 6, 5, and 4), and screening starting age (30 and 35 years). For vaccination, we assumed an attendance rate of 100%, 95% efficacy, and lifelong protection. When screening was added to HPV16/18 vaccination, our model predicted reductions in cervical cancer mortality between 60 and 81% (from 199 deaths to 37-79 for a cohort of 100,000 women). Screening 5 times with HPV DNA (€11,133/QALY) or 7 times with cytology (€17,627/QALY) were scenarios with comparable costs and effects and incremental cost-effectiveness ratios below the willingness-to-pay threshold in The Netherlands (€20,000 per QALY).

If a 5-valent (HV16/18/31/33/45) or 8-valent (HPV16/18/31/33/35/45/52/58) vaccine becomes available, screening for cervical cancer will still be cost-effective only if the number of screening rounds is reduced to one. The optimal strategy is a single HPV DNA test at the age of 40 (€5,385/QALY and 6,562/QALY for a 5- and 8-valent vaccine).

Conclusions: 1. Cervical screening is cost-effective after the introduction of HPV16/18 vaccination in The Netherlands. HPV DNA testing is attractive because less screening rounds are needed than in cytological screening. 2. Even if polyvalent vaccines become available in the future, screening for cervical cancer remains cost-effective, but the number of rounds needs to be reduced drastically.
Introduction: QIAensemble™ HC400 system is currently under development at QIAGEN Inc. The system is designed to run the digene eHC HPV DNA Test and two genotyping assays, HPV 16 and duplex HPV18/45. The eHC test is based on Hybrid Capture® technology with enhancements such as increased analytical specificity and reduced time to first results as compared to its predecessor, the digene HC2 High Risk HPV DNA Test. QIAensemble™ HC400 shares the same reagents and processing steps as QIAensemble™ HC2000. With a throughput of up to 400 specimens per 8 hr shift, the system is targeted for low to mid volume laboratories.

Objectives: The objective of this study is to integrate and optimize the assay on the HC400 to ensure a reproducibly high signal to noise ratio at a level similar to HC2000 and to achieve a processing throughput of at least 400 samples per 8 hr work shift.

Methods: Optimization of the eHC assay was performed on prototype HC400 instruments and achieved by investigating the impact on signal to noise ratio by systematically altering assay parameters including incubation temperature, reagent volumes and washing parameters. A plate layout of negative control and positive calibrator containing 1pg/ml HPV 16 DNA in alternating columns was applied. To determine inter plate uniformity a total of 9 plates were analysed on three different days using the optimized assay parameters.

Results and Conclusion: Our data demonstrate that a signal to noise ratio of ~ 5.0 and a plate CV% of less than 10 are consistently achieved when using optimized parameters. In addition, the HC-400 is able to process at least 400 samples in one eight hour work shift. In summary, the HC400 allows the seamless integration of the eHC assay into a fully automated system which would benefit laboratories with low to medium throughput requirements.

DETECTION OF HPV mRNA IN VARIOUS TRANSPORT SPECIMEN BUFFERS WITH THE APTIMA® HPV ASSAY

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Objectives: The objective is to evaluate the sensitivity of the APTIMA HPV Assay for detection of HPV mRNA in various specimen buffered solutions including Digene Specimen Transport Medium (DG-STM, Qiagen, Hilden, Germany), Universal Collection Media (UCM, Qiagen, Hilden, Germany), Abbott Multi-Collect Specimen Collection Kit medium (Abbott, Abbott Park, Illinois, USA) and PreTect HPV-Transport medium (NorChip, Klokkarstua, Norway), as compared to PreservCyt solution (Hologic Inc., Bedford, Massachusetts, USA) and APTIMA Cervical Specimen Collection and Transport Kit media (CSCT, Gen-Probe, San Diego, USA).

Methods: The APTIMA HPV Assay (AHPV) is a CE marked nucleic acid amplification test that detects the E6/E7 viral mRNA from 14 high-risk HPV types (16/18/31/33/35/39/45/51/52/56/58/59/66/68) in PreservCyt liquid Pap specimens and CSCT specimens. HPV infected SiHa cells or HPV 16 in vitro transcripts were spiked into PreservCyt solution and CSCT medium as well as DG-STM, UCM, Abbott and NorChip media. PreservCyt solution specimens, as well as DG-STM, UCM and NorChip media required dilution into APTIMA transfer solution prior to testing in the assay. Abbott and CSCT media were tested directly in the assay. Samples were tested and the analytical sensitivity of the AHPV assay in each solution was evaluated and compared. Stability of HPV mRNA (3 SiHa cells/reaction) in DG-STM, UCM, PreservCyt solution and CSCT medium was also evaluated at refrigerated and ambient temperatures.

Conclusions: The analytical sensitivity of the AHPV assay was similar for each of specimen media evaluated, with 1 cell or 100 copies of mRNA consistently detected. HPV mRNA stability varied between the sample types. For samples stored at 30°C, DG-STM samples were stable for 30 days, UCM samples for 60 days, PreservCyt solution samples for 30 days and CSCT samples for 90 days. DG-STM and PreservCyt solution samples once diluted into APTIMA transfer solution were stable for at least 90 days when stored at 30°C. Improved stability was observed for all sample types when samples were stored refrigerated. These results indicate that the APTIMA HPV Assay can be used with a variety of sample types.
FULLY AUTOMATED PERFORMANCE OF A NEXT GENERATION HYBRID CAPTURE® HIGH-RISK HPV DNA ASSAY ON QIAENSEMBLE™ HC2000

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Objectives: QIAensemble™ HC2000 is a next generation HPV diagnostic analytic system which is currently under development at QIAGEN. The digene eHC HPV DNA test (eHC) leverages Hybrid Capture® 2 technology with improved analytical performance and chemistry compatible with fully automated high volume throughput. The system is designed to analyze up to 2000 samples in one 8 hour shift.

Methods: QIAensemble™ HC2000 fully automates all steps from reagent and sample preparation to the final result reporting of the HPV test results. Patient samples in proprietary QIAGEN collection tubes are directly loaded into the system. Up to 15 plates or 1440 samples processed from liquid based cytology media can be simultaneously loaded into the system. The assay time to the 1st result was reduced by at least 40% from manual digene HC2® High-Risk HPV DNA Test. This high throughput HPV diagnostic system detects 15 different high risk HPV genotypes. The analytical sensitivity is 1875 copies (95% CI 1615-2290) of HPV 16 plasmid. Assay specificity was evaluated with 13 HPV low risk types. All HPV low risk types were tested at a high concentration of 2X10^8 copies/ml and there were no false positive results. The assay reproducibility on HC2000 was significantly improved over the manual assay using HPV 16 plasmids. The fully automated assay achieved consistent performance within plate, from plate to plate, day to day and instrument to instrument. No indication of target carryover was found when samples containing up to 10^9 copies/ml of HPV DNA type 16 were processed on HC2000 instruments.

Conclusion: QIAensemble™ HC2000 is a fully automated QIAGEN instrument capable of running the eHC assay. The system achieves an unprecedented throughput and significantly reduces the time to 1st result. The assay performance data show that the fully automated eHC assay on the HC2000 analytical system significantly improves assay specificity and assay reproducibility without compromising sensitivity of detection of high risk HPV types.

EVALUATION OF A MODIFIED RAPID CAPTURE SYSTEM WORKFLOW FOR USE WITH PRESERV CYT® SPECIMENS

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Objectives: The Rapid Capture System (RCS) is a semi-automated application that executes assays based on hybrid capture technology for high throughput testing. The workflow requires offline denaturation of samples followed by automated processing on the RCS. In the current study we describe the evaluation of a workflow modification in which denaturation is performed onboard the RCS, thereby eliminating the need for offline manual denaturation.

Methods: Workflows were compared for ease of use, hands-on time, and functional performance. Positive and negative calibrators from the HC2 High-Risk HPV DNA Test® kit (HC2) were tested using the improved RCS workflow and results were analyzed for functional performance. Further studies were conducted with 1537 residual PreservCyt® specimens. Specimens were processed with both the standard HC2 manual conversion method and the QIA symmetry® AXpH DNA kit® protocol. Crude lysates generated by manual conversion were tested on the RCS using standard 1-4C scripts. AXpH eluates were tested on the RCS using a modified denaturation reagent and the new workflow.

Conclusions: Though still in development, the modified RCS workflow described here is functional and the results are reproducible. Functional performance measured with plasmid models shows an acceptable percent CV. Workflow comparisons using clinical specimens resulted in excellent agreement (k=0.93). The new RCS workflow increases walk-away time by >2.5 hrs per 88-specimen plate. Further, undenatured extracts from cervical specimens, such as those collected in PreservCyt® and prepared with the QIA symmetry® AXpH DNA Kit®, can be used with this modified workflow. This increases process-control and reduces the number of repetitive motions associated with the manual workflow.

The applications presented here are for research use only. Not for use in diagnostic procedures.
**SS 21-5**

**MODIFICATION IN SAMPLE COLLECTION AND PREPARATION OF HPV IN FEMALE POPULATION SCREENING TESTS**

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**Objectives:** Cervical cancer screening applying Papanicolaou (Pap) smears or cytology has effectively reduced the incidence and mortality of cervical cancer where sufficient techniques have been instituted. However, arguably, cytology detection and hc2 test both have reached their limits in global effect on cervical cancer incidence and mortality.

**Methods:** Cytology is an insensitive test for the detection of precancerous lesions and is poorly reproducible. Its effectiveness in screening programs is the consequence of repeated screening to detect precancerous lesions during their slow progression to cervical cancer. The cervical cancer prevention program in the United States based on cytology screening, as successful as it is, comes at a significant price of $8 billion or more annually. The cost ineffectiveness of this program makes it unlikely for effective adoption in resource-limited regions. In the other hand, self Collection of HPV Samples promotes early screening for cervical cancer. In addition, Women who live in poor rural areas of the world can potentially benefit from the self-collection of vaginal samples to screen for humanpapilloma viruses (HPVs) the main cause of cervical cancer.

**Conclusions:** A safe and inexpensive media for the collection and preservation of self-collected specimens is available. This basic media is used to collect cervical and vaginal cells for screening tests and could be a potential preservative of cervical specimens for HPV DNA testing. By applying this media, even the most challenging specimen, those specimens with heavy mucus and large volumes of blood do not need extra processing. For collecting the Pap or HPV, a cervical swab has been designed. The advantage of this sampling device is the softness of the head that facilitate the cell collection without any harm to woman's body.

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**SS 22-1**

**ANALYTICAL PERFORMANCE OF A NOVEL ASSAY FOR GENOTYPING HPV16 AND HPV18/45 USING NEXT GENERATION HYBRID CAPTURE® TECHNOLOGY**


Qiagen Inc, Gaithersburg USA *Family Health Ministries, Durham USA

**Objectives:** Identifying the presence of specific HR HPV types that are strongly associated with the potential for progression to cervical cancer is useful for appropriate risk stratification and clinical management. QIAGEN has developed a next-generation diagnostic system, QIAensemble™ which uses re-engineered Hybrid Capture® chemistry to genotype HR HPV types 16, and 18/45. Analytical performance of this method has been well characterized with this test set providing the first clinical performance data for the novel genotyping assay.

**Methods:** Enhanced Hybrid Capture® technology is utilized in two separate reactions that use full length RNA probes complementary to HPV16, 18, and 45. The assay protocol contains the same basic steps as HC2™: denaturation, hybridization, capture, and detection, but with chemistry modifications that allow the assay to be performed in approximately three hours. Analytical performance of this assay with cervical specimens was assessed using the following testing strategy. Clinical samples that tested positive by the QIAensemble™ HR HPV DNA screening assay were re-tested in the QIAensemble™ HPV DNA 16/18/45 Genotyping Test. A subset of HR HPV negative samples was also tested to demonstrate specificity. A total of 510 clinical samples were tested using the HR screening assay followed by 115 samples with the novel genotyping assay. Discrepancies were tested by an in-house GP5+/6+ PCR/Luminex HR/LR genotyping assay and, where necessary, qPCR.

**Conclusions:** Of 510 samples screened, 68 were HR HPV positive by the NextGen HR HPV DNA screening assay (13.3%). A 2x2 agreement analysis (where the reference result was a composite of the HC2 result and a follow-up GP5+/6+ PCR Luminex genotyping— with concordance between the two reference tests scored as positive or negative and discordance was ruled indeterminate and not scored) showed 94.4% positive (86.4%, 97.8%) and 99.5% (98.3%, 99.9%) negative agreement and 98.8% (97.3%, 99.4%) total agreement. Of the 68 HR positive samples, 17 tested positive for HPV 16 and/or HPV18/45. Positive agreement of these results with those from the above genotyping assay was 100% (80.6%, 100%). Quantitative PCR demonstrated that some samples were detected with between 2000 and 4000 copies of HR HPV DNA.
A NOVEL COMBINED SCREENING AND GENOTYPING MOLECULAR HPV ASSAY


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Objectives: This study was undertaken to assess the performance of a new automated real-time Taqman® PCR assay which simultaneously screens for 14 high-risk HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68) and can provide genotyping information on 7 of these types (16, 18, 31, 45, 51, 52, 59) without the need to perform a reflex test. The assay design also includes amplification of an endogenous human gene, -globin, which serves as an internal sample processing and amplification control. It is designed to be run on instrument platforms that automate sample processing, nucleic acid extraction, reaction set-up, real-time PCR and results output.

Methods: A single harmonized sample processing procedure based on Ferric Oxide (FOX™) particle DNA binding and magnetic extraction (currently used in Chlamydia trachomatis (CT) and Neisseria gonorrhoeae (GC) assays run on the BD Viper™ System with XTR™ Technology) was developed for SurePath™ and ThinPrep® liquid based cytology specimens as well as an endocervical swab in BD ProbeTec™ Qx Swab Diluent. The assay was tested for cross-reactivity with non-targeted high- and low-risk HPV types and for inhibition with a range of potential interfering substances, including blood. Clean and clinical matrix limits of detection (LODs) were established for both ThinPrep® and SurePath™. Residual clinical cytology specimens were also tested and compared to results from the digene High-Risk HPV HC2 DNA Test (Qiagen).

Conclusions: The assay was found to have exquisite analytical sensitivity with a clinical matrix LOD of ≤ 100 copies for all 14 HPV targets in both SurePath™ and ThinPrep® media using an input volume of 0.5 mL of specimen. It exhibited zero cross-reactivity at 107 copies with non-targeted high- and low-risk HPV types and was not inhibited by any of the interfering substances tested. The results obtained were in close agreement with those from the HC2 assay. The high fidelity analytical performance and the ability to perform both screening and genotyping in the same assay, together with end-to-end automation, may offer considerable benefits for both laboratory and clinician.

A COMPARISON OF THE PAPILLOCHECK® HPV ASSAY AND HC2 IN LBC SAMPLES FROM THE ARTISTIC TRIAL

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Objectives: To present preliminary results of an ongoing trial examining the clinical utility of the Greiner PapilloCheck® HPV genotyping assay on archived cervical samples obtained from women enrolled into the ARTISTIC trial.

Methods: Stored archival samples of cervical cells obtained from approximately 4000 women during the course of the ARTISTIC trial are being examined during the course of this study. The majority of these samples fall into 2 groups, approximately 3000 samples are from women who had normal cytology but were found to be high risk HPV (HRHPV) positive by HC2 and 1000 samples are from women who were diagnosed with borderline or mild dyskaryosis by cytology. In addition a smaller number of women (approximately 100) diagnosed with severe dyskaryosis are to be tested. Results following testing 2000 of these samples will be presented. Nucleic acid was extracted using the Biomerieux EasyMag automated system after which HPV DNA amplified and detected using the Greiner PapilloCheck® assay. Cytology and HRHPV HC2 results were available on all samples. In addition, Roche prototype Reverse Line Blot (RLB) assay and Histology results were also available on a large number of these women.

Results: Initial results obtained from testing 1281 of the HRHC2 positive/cytology normal samples showed that 57% of these samples contained a HRHPV type by PapilloCheck® and 63.8% were found to contain a HRHPV type when tested using the Roche RLB assay. Of 547 samples from women with borderline dyskaryosis 93.2% concordance was obtained. In addition, Roche prototype Reverse Line Blot (RLB) assay and Histology results were also available on a large number of these women.

1. Kitchener HC et al, Lancet Oncol, 2009 Jul:10 (7); 672-82
DETERMINATION OF THE DIAGNOSTIC ACCURACY OF TESTING FOR HIGH-RISK (HR) HUMAN PAPILLOMAVIRUS (HPV) TYPES 16, 18 AND 45 IN PRECANCEROUS CERVICAL LESIONS: PRELIMINARY DATA

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Aims: In Germany, cervical cancer screening is regulated by the German Federal Ministry of Health and Social Security and is available for all women from the age of 20 on the basis of the Papanicolaou (PAP) smear. The purpose of this study was to determine the positive predictive value of HR-HPV testing for precancerous lesions of the cervix uteri. Therefore, this study especially focused on the diagnostic accuracy of testing for one or more of the HPV types 16, 18 and 45 for all HR-HPV positive women, since HR-HPV infections with subtypes 16, 18 and 45 have demonstrated a higher risk of developing cervical cancer [Bulk S, et al. Br J Cancer 2006;94:171-5].

Methods: Between 2007 and 2008 a total of 586 women were recruited: a group of 477 women with a history of known cervical lesions and/or HPV infections (eligibility criterion: HR-HPV DNA positive test result with HC2T) and a group of 109 women who were examined as part of their routine cervical cancer screening. Baseline HR-HPV status was measured at enrollment with the FDA-approved Hybrid Capture® 2 HPV DNA Test and the HR-HPV 16/18/45 Probe Set Test (HC2T, PST; QIAGEN, Hilden, Germany). Both tests use hybrid capture hybridization genotyping technology. Cervical smears were classified according to the Second Munich Nomenclature (1989). The results were converted to the nearest equivalent in the Bethesda system. In general, study subjects were followed up semiannually for a period of 11.2 years. The histopathological endpoint of CIN 2-3 lesion was used as a surrogate endpoint.

Results: Preliminary data for 194 women of the risk group (43.5%) and for the complete control group were available. To date, CIN 2-3 was confirmed in 77 HPV-HPV DNA positive women. 85.7% of these lesions were positive for one or more of the HR-HPV types 16, 18 and 45 (PST+). 88.2% (60/68) of the histologically confirmed CIN 3 lesions and six out of nine (66.6%) CIN 2 lesions were positive PST+. Furthermore, all women with a histologically confirmed squamous cell carcinoma (n = 4) were PST+. Besides, three (50%) out of six detected CIN 1 lesions were PST+. Nonetheless, histology confirmed no malignancy in three cases. Two of them were PST+.

Conclusion: These preliminary results demonstrate that starting cervical cancer screening at the age of 20 years remains important as seventeen (25%) of the 68 histologically verified CIN 3 lesions arose in women who were younger than 30 years. Furthermore, our data suggest that adding an HR-HPV test that detects one or more of the HR-HPV types 16, 18 and 45 in conjunction with cytology could help to identify women with an underlying cervical lesion who have an elevated risk of developing severe cervical lesions. This might offer the opportunity of a decrease in incidence and mortality rates that are related with invasive cervical cancer.

MULTIPLE INFECTION WITH HIGH RISK GENOTYPES IN MEXICAN WOMEN

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Objective: Identify the high-risk HPV genotypes present in a group of Mexican women.

Materials and Methods: 102 samples of cervical cells positive by Hybrid-Capture were included in the study. DNA extraction was performed with a column-based kit. Genotypes 16, 18, 31, 33, 39, 45, 51, 52, 56, 58, 59, 68 and 35 were identified using E6-E7 nested multiplex PCR (1), a Reverse Hybridization Kit and/or direct sequencing.

Conclusions: 25.5% of the studied samples were from women with normal cytology; 28.4% CIN I, 11.7% CIN II, 14.7% CIN III and 19.6% cervical cancer (CC). The high-risk HPV prevalence was 89.2%. Coinfections with multiple genotypes were found in 37.2% of the samples: 23 samples had double concurrent infections; 11 were triple, 3 were quadruple and 1 had five different genotypes. The frequency of coinfections according to cervical cytology was: 10.5% normal cytology, 21.0% CIN I, 23.6% CIN II, 15.8% CIN III y 28.9% CC.

The most frequently found genotypes were HPV16 (35.6%); HPV58 (18.8%), HPV52 (14.8%), HPV51 (13.8%) and HPV59 (9.8%). HPV type 18 was detected in 6.9% of all samples. X2 test was performed to determine association between the lesion grade and presence of multiple infections. The analysis showed association of multiple infection and high grade lesions and CC, p=0.000, OR 6.81 y IC 95% (1.81-27.78).

Genotypes different to those included in the approved vaccines are important within this group of Mexican women. Cervical coinfections with multiple high-risk genotypes are associated to the development of high risk lesions and cancer.

Objective: Measure the prevalence of high-risk and low-risk HPV genotypes in archived specimens of anal cancer.

Methods: 97 stored biopsies from 1995-2005 archived in a Montréal university hospital from that reference laboratory and 8 referring laboratories were processed. Extracted DNA was tested for the presence of HPV DNA and β-globin using AmpliTaq gold with PGMY and GH20/PC04 primers and the Linear array. β-globin-negative samples were retested with the primer pair PC03/PC04 amplifying a 110 bp fragment of β-globin DNA and the GP5+/GP6+ L1 consensus primer pair for HPV DNA detection. PCR-sequencing of HPV DNA amplicons generated with GP primers was then performed for HPV typing. PGMY then GP were done on negative specimens.

Conclusions: HPV was detected in 92% (89/97) of specimens. Overall HPV-16 was detected in 82% (80/97) of anal cancers and in 90% (80/89) of HPV+ specimens. Multiple infections were rare (6/97). Of those multiple infections, all involved HPV-16. Of those multiple infections, along with HPV-16, 5/6 were double infections (HPV-6 (1 case), HPV-11 (1 case), HPV-52 (1 case), and HPV-62 (2 cases) and one was a quadruple infections (HPV-6, HPV-53 and HPV-82). In the 9 cases of HPV+ cancers without HPV-16, the following HPV were found: HPV-33 in 3 cases, HPV-18 and HPV-58 in 2 cases and finally HPV-6 and HPV-56 in one case each. No HPV-97 case was found. Prophylactic vaccines have great potential to reduce the burden of anal cancer.

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Objectives: To evaluate the results of conventional anal cytology, cervical cytology and cervical HPV genotyping in a cohort of HIV positive women.

Methods: From October 2008 to July 2009, 109 HIV positive women were recruited at the Obstetric and Gynecology Unit of the University Hospital L.Sacco, Milan. Conventional anal and cervical cytology and cervical HPV genotyping for low and high risk types (Inno-Lipa, Immunogenetic, Belgium) were simultaneously performed in all patients.

Conclusions: Abnormal anal cytology (ASC positive) was found in 55% of adequate samples: ASC 4%, LSIL 48%, HSIL 3%. Abnormal cervical cytology was found in 25%: ASC 3%, LSIL 17%, HSIL 6%. Concomitant abnormal cervical and anal cytology was found in 20%. Positive HPV genotyping for high risk types was found in 64%, with 23% of multiple infections: HPV 52 was the most represented type. The rate of anal inadequate samples was 17%. In this cohort of HIV positive women the rate of abnormal anal cytology was double than the cervical one and was not related to cervical cytology and/or HPV genotyping. Anal cytology might be included as a screening test in the follow up of HIV positive women.

Liquid based cytology should be considered as an alternative to conventional cytology in order to reduce the rate of inadequate anal samples.
HPV GENOTYPING AND CONSEQUENCES FOR CERVICAL SCREENING IN 500 HEALTHY WOMEN ATTENDING A PRIVATE WOMAN’S CLINIC IN TOKYO

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Objectives: In Japan the HPV vaccine is not yet licensed and HPV testing is not covered by insurance. Many women are unaware of the connection between cervical cancer and HPV infection, and even those who can afford it feel reluctant to pay for HPV-DNA testing. Consequently, very little data is available on HPV genotypes in healthy Japanese women. This study aimed to investigate the relationship between HPV genotype, sexual activity, oral contraceptive use and lifestyle in 500 women attending a private woman’s clinic in Tokyo.

Methods: After written informed-consent, 500 women aged 20 to 60 underwent HPV-DNA Testing using the GeneSQUARE HPV Genotyping Microarray (KURABO Industries Ltd) between the period of August 1st and August 31st, 2009. Simultaneous Pap Smears were offered and the results compared to a self-completed questionnaire.

Conclusions: HPV 58 was the most common HPV type detected, followed by types 16, 52, 31 and 56, respectively. Only 4 women (3.8%) were HPV 18 positive and none were positive for HPV 6 or HPV 11. While HPV prevalence normally peaks in women aged 15-24, in this study, women aged 40-44 had the highest prevalence at 28.6%, followed by ages 30-34 (25.2%) and 50-54 (22.2%), respectively. Furthermore, 66.7% of women aged 50-54 were infected with a high-risk (HR) HPV. Other significant factors for HR-HPV infection were more than 5 sexual partners and sex within the previous 3 months (p<0.01). No significance was found for oral contraceptives or pregnancy. With regards to cervical cytology, women with a class IIb or IIIa Pap Smear were 100% and 67% HR-HPV positive, respectively. In conclusion, while HPV infection peaks between ages 15 and 24, a second smaller peak is seen around age 50. While some reports suggest hormonal changes due to menopause may be the cause, others have pointed out that since the age of the peak differs between countries it may also be due to social factors such as new partners after divorce or death of a spouse. Despite this, many countries recommend cessation of Pap Smears after age 60. Based on the result of this study, we believe sexually active women, but especially those with new partners, should continue to be screened, regardless of age.

HPV mRNA (E6/E7) VERSUS DNA ASSAYS FOR CERVICAL CANCER RISK EVALUATION

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Objectives: The major cause of cervical cancer is HPV infection and the development of malignancy requires continuous expression of E6 and E7 viral genes. It follows that E6 and E7 transcripts could be useful markers for disease progression. The aim of this study was to evaluate this possibility, using data from colposcopy and from cytological and histological tests to compare RNA assays for the E6/E7 genes against DNA testing.

Methods: The study was conducted on cervical brush samples from 180 women, admitted for secondary screening to the Colposcopy Outpatient Department, who underwent colposcopy, cytology, biopsy of suspected lesions (143 cases). Cervical samples were analyzed for HPV DNA by Hybrid Capture II HPV Test (HC2) (Digene), and for HPV E6/E7 mRNA by NuclISENS EasyQ® HPV assay (bioMérieux). The cytology was analysed according to the Bethesda System. Colposcopically direct cervical biopsy was performed by traditional punch or electrocautery loop.

Conclusions: HPV DNA was found in 57.8% (104/180) of the patients and HPV E6/E7 transcripts in 45%. Comparison with histological test, the rates of detection of HPV DNA and of E6/E7 mRNA were 33.3% and 25% for patients with normal findings; 51.4% and 31.9% for those with CIN1 and 61.1% and 44.2% for those with CIN2, respectively. All patients with CIN3 and 95.5% of those with SCC were positive by both assays. Comparison with cytological tests produced similar results. The lowest rates of concordance between DNA and RNA results were in patients with normal and low-grade lesions reflecting the transient nature of most HPV infections. In high-grade dysplasia and cancer, the concordance was high suggesting that the presence of E6 and E7 proteins is a specific marker for high-grade lesions. Overall, the RNA assay showed a higher specificity than the DNA test (72.7% versus 56.2%); given the positive impact on PPVs (59.3% versus 49.0%, respectively), it is likely that RNA assay will provide better risk prediction than DNA test. The E6/E7 mRNA test can provide sensitive, early-stage detection of persistent infections at risk of progression to malignancy representing a valuable diagnostic tool for triage and patient follow-up.
INTRODUCTION OF HPV E6/E7 mRNA IN CERVICAL CANCER SCREENING ALGORITHMS

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Background: Current cervical cancer screening algorithms are hampered by the low specificity of the HR-HPV DNA tests. Furthermore in the coming years, after the introduction of prophylactic HPV vaccines, it is expected a reduction in prevalence of cervical neoplasia and changes in the age-specific incidence and HPV type distribution of infection and of cervical neoplasia. It is therefore reasonable to expect that cytology and HPV DNA testing may be less predictive in the future. Thus, new triage strategies including new biomarkers in current and future cervical cancer screening programs become mandatory. HPV mRNA appears to improve the detection of women at greatest risk for developing cervical cancer. It is anticipated that the introduction of this biomarker in screening algorithms could be clinically useful for determining which women should be referred for immediate colposcopy and which could be followed-up.

Objectives: An ongoing regional study in Switzerland is assessing the usefulness of testing for E6/E7 mRNA in a low incidence of cervical cancer population.

Methods: The study population included women aged from 20 to 69 years who were undergoing routine screening, without history of cervical neoplasia. 7000 liquid based specimens are expected to be collected from 20 gynecologists. In order to investigate the cross-sectional predictive values of HR-HPV DNA test (consensus GP5+/6+/PCR), PAP cytology, and HPV mRNA test (NucliSENS® EasyQ HPV), women with abnormal cytology or positive for either HPV test will be followed by colposcopy and biopsy for the detection of lesions greater than or equal to CIN2.

Interim Results will be presented

Conclusion: This ongoing study will help to establish:
1. Whether detection of E6/E7 mRNA increases specificity and positive predictive value for high-grade lesions compared to HPV DNA detection and Cytology
2. To determine the clinical utility of adding E6/E7 mRNA testing to current cervical cancer screening algorithms.

INTRODUCTION OF HPV E6/E7 mRNA SCREENING IN ADDITION TO THE HYBRID CAPTURE 2 HIGH-RISK TEST AND HPV TYPING IN A ROUTINE DIAGNOSTIC LABORATORY: RESULTS AFTER 1 YEAR

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The screening for Human Papilloma Virus in women, in conjunction with cytology has been well established as an important tool in the evaluation of endocervical cancer risk. While the Hybrid Capture 2 (hc2) test (Digene) has proven to be a sensitive assay, it is known that it lacks in specificity. Recent assays that try to focus on a more direct detection of the cause of endocervical cancer have specialized on the screening of viral oncoproteins, such as the E6/E7 HPV proteins, in order to raise the specificity of laboratory assays. Here we have used in parallel the hc2 high-risk test (Digene) and the NucliSENS EasyQ E6/E7 HPV mRNA assay (biomérieux) on 648 endocervical scrapes of women requesting HPV screening in Luxembourg. Hc2 positive scrapes were also tested by a HPV typing PCR. 67% tested positive in both the hc2 and the E6/E7 mRNA assay while 13 % tested negative in both thus giving concordant results in 80 % of cases. All of these hc2 positive results were confirmed by PCR. 10 % of cases were hc2 positive, confirmed by PCR, but E6/E7 mRNA negative while 2 % were E6/E7 mRNA positive but hc2 negative. 8 % of hc2 positive cases could not be confirmed by PCR of which 2 % where E6/E7 mRNA positive and 6 % negative. No significant difference in age was observed for these groups. A follow-up of E6/E7 mRNA positive patients as well as a comparison with cytology is currently undertaken.

These results show that in at least a fifth of cases the E6/E7 mRNA screening gives additional results to the HPV detection alone thus providing potentially important information for the gynecologist in deciding the further cause of action to be taken.
**USE OF HPV mRNA TEST IN HPV TRIAGING - EXPERIENCE FROM THE UNIVERSITY HOSPITAL OF NORTH NORWAY**

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**Objectives:** Tests for detection of human papillomavirus (HPV) are used as confirmatory tests in the Norwegian Cervical Cancer Screening Programme in cases where cytology is uncertain (ASC-US) or show low grade changes (LSIL). HPV vaccine was implemented in the Norwegian children vaccine programme the autumn of 2009. The objective of this study is to evaluate the routine diagnostic practice at the University Hospital of North Norway (UNN) and suggest how the HPV vaccine may perform in our population in the future.

**Methods:** Our study comprises samples from 1,798 women. Cytology examinations were performed on these samples including HPV mRNA tests between 2006 and 2008. The tests were done according to the guidelines of the Norwegian Cancer Registry. The HPV mRNA test (PreTect HPV-Proofer) detects and types 5 high-risk genotypes (16, 18, 31, 33 and 45). The HPV mRNA findings were compared to cytology and later biopsy up to August 2009. HPV negative women with cytological ASC-US or LSIL were followed with a new PAP smear after 12 months. 327 women (18%) were HPV mRNA positive. 230 women (13%) had CIN2+ in biopsy and 141 women (7.8%) had CIN3+. Fifty-seven percent of the women with a positive HPV mRNA test had CIN2+ and 39% had CIN3+. The sensitivity of the HPV mRNA test to detect CIN2+ and CIN3+ was 81% and 84%. The specificity for CIN2+ and CIN3+ was 91% and 87%. The negative predictive value of the HPV mRNA test was 0.97 for CIN2+ and 0.99 for CIN3+. Of ten women with cervical cancer, nine were positive for HPV type 16 or 18.

**Conclusion:** Compared to existing literature, we find that due to its higher specificity, the HPV mRNA test is more suitable than HPV DNA tests as a confirmatory test in women with uncertain and low grade cytological changes. HPV vaccines against HPV type 16 and 18 may prevent a high number of cervical cancers in Norway.
Objective: Evaluate the sensitivity and specificity of the APTIMA HPV Assay (AHPV, Gen-Probe Inc) for detection of HPV mRNA in samples collected with the APTIMA Cervical Specimen Collection and Transport Kit media (CSCT, Gen-Probe) and compare to results from samples collected in PreservCyt solution (Hologic). CSCT samples are an alternative to liquid Pap samples for AHPV assay testing.

Methods: Paired CSCT and PreservCyt samples were collected from colposcopy referral (n=535) and routine cervical screening (n=1028) populations. PreservCyt samples were tested with the AHPV assay for mRNA and the Hybrid Capture 2 High-Risk HPV DNA test (HC2, Qiagen Inc.). The CSCT samples were tested with the AHPV assay for mRNA. Positivity was calculated for both tests stratified by cytology result for the two study populations. Sensitivity and specificity of both tests for the detection of histology-confirmed high grade lesion or worse (CIN2+) was determined for the referral population.

Conclusions: In the referral population, AHPV positivity in PreservCyt samples for normal, ASC-US, LSIL and HSIL was 60.5%, 64.3%, 87.7% and 100%, respectively. Similarly, AHPV positivity in CSCT samples was 62.8%, 67.1%, 87.7% and 100%. HC2 positivity for PreservCyt samples in this population was 60.4%, 71.3%, 93.3% and 100%, for normal, ASC-US, LSIL and HSIL cytology results. In the screening population, AHPV and HC2 positivity rates were 100% for HSIL samples and 44.4% for LSIL samples. In subjects with ASC-US and normal cytology results, HC2 positivity was higher than AHPV (21% vs 13% and 6% vs 4%, respectively). AHPV sensitivity for detection of CIN2+ was 100% in both PreservCyt and CSCT samples, whereas HC2 sensitivity was 97.7%. AHPV assay specificity was 40% in PreservCyt samples and 35% for CSCT samples, as compared with 34% for HC2 for PreservCyt samples. These results demonstrate that AHPV assay performance is similar with CSCT and PreservCyt samples, providing an alternative sample type for sites using conventional cytology.

Objective: Urine testing for oncogenic HPV may be an alternative cervical cancer screening tool for women who are reluctant to undergo pelvic examination or to self collect a vaginal sample. Our objective was to compare test performance of urine HPV testing to that of cervical and self collected vaginal samples.

Methods: Fifty women referred to colposcopy because of an abnormal Pap smear collected a first void urine (FVU) sample and vaginal samples (VS) with a dual flocked swab (Copan Italia). One VS was placed into Hybrid Capture 2 (HC2) STM (Qiagen) medium and another into a dry tube. The colposcopist obtained a cervical ThinPrep (Hologic) liquid-based Pap (L-Pap) sample, and a biopsy was taken when indicated. Gen-Probe APTIMA HPV assay (S/CO) was used to test urine (FVU-APTIMA). HC2 (RLU/CO) was also used to test urine (FVU-HC2), L-Pap (L-Pap-HC2) and VS. In both tests, values between 1.0 to 2.0 were repeated.

Conclusions: Sensitivity and specificity of FVU-APTIMA for detection of oncogenic HPV infection, defined as positive L-Pap-HC2, were 0.72 and 1.00, respectively. Sensitivity and specificity, respectively, for CIN2+ using FVU-APTIMA were 0.62 and 0.73, with FVU-HC2 0.23 and 0.76, with wet VS 0.77 and 0.49, with dry VS 0.77 and 0.49, and with L-Pap-HC2 0.87 and 0.63. FVU-APTIMA was positive in 8/13 women with CIN2+, in 2/5 with CIN1 and in 8/32 without detectable CIN. The median L-Pap-HC2 result in women with positive FVU-APTIMA was 470.60 RLU/CO (range 3.76-2328.60), compared to 10.57 RLU/CO (range 2.78-542.57) in women with negative FVU-APTIMA (p<0.05). In summary, urine specimen testing with APTIMA is more sensitive than urine specimen testing with HC2 for detection of CIN2+, with similar specificity. The cervical L-Pap-HC2 result in women with a negative FVU-APTIMA test result appears to be lower than in women who test positive with FVU-APTIMA. While FVU-APTIMA is less sensitive than either L-Pap-HC2 or VS for CIN2+, urine testing for oncogenic HPV may provide an opportunity for cervical cancer screening of women who refuse pelvic examination and who do not wish to perform vaginal self sampling.
**Performance of RNA isolated from BD SurePath liquid-based cytology specimens in the PreTect HPV-Proofer assay**

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**Objective:** To investigate the performance of RNA extracted from BD SurePath liquid-based cytology (LBC) specimens in the PreTect HPV-Proofer assay for the detection of HPV E6/E7 mRNA.

**Methods:** Residual anonymized BD SurePath cell pellets from women presenting for routine screening were collected. RNA was isolated using the RecoverAll Total Nucleic Acid Isolation kit (Invitrogen) and tested for the presence of HPV 16, 18, 31, 33 and 45 E6/E7 mRNA using the PreTect HPV-Proofer Assay (NorChip AS). A dual collection study was also performed to directly compare the performance of RNA isolated from cervical cells collected and stored in BD SurePath preservative fluid with storage in PreTect TM media (NorChip AS). For this study, two cervical samples were collected from each consenting patient with randomization of the order of collection into each preservative fluid.

**Conclusions:** A total of 628 residual BD SurePath LBC pellets were collected for analysis. The distribution of positivity for E6/E7 mRNA for the five HPV types across cytology grades was NILM 3.8%, ASCUS/AGUS 28.3%, LSIL 42.0%, HSIL/ASC-H 73.6%, consistent with published reports. A 92.5% first pass acceptance rate was demonstrated across all cytology grades. For the dual collection study, a total of 131 specimen sets (72 NILM cytology, 59 abnormal cytology) were available for analysis. The first pass positive rate was equivalent for both collection media, being 100% for normal and 96.6% for abnormal specimens, with two BD SurePath specimens having U1A values below the pass threshold. Analysis of a subset (n=31) of specimens where biopsies were available demonstrated 93.5% concordance between BD SurePath and PreTect TM media. One case with CIN3 biopsy tested positive for HPV 16 mRNA in the BD SurePath specimen, but negative in the PreTect TM sample. The other discordant sample with a reported atypical biopsy tested positive for HPV 18 mRNA in the PreTect TM sample, but negative in the BD SurePath specimen. In summary, these studies demonstrate equivalent results between RNA isolated from cervical cells stored in BD SurePath and PreTect TM preservative fluid. These findings support the compatibility of RNA isolated from BD SurePath LBC specimens with the PreTect HPV-Proofer assay.

**Intracellular HPV E6, E7 mRNA quantification (Oncotect) predicts CIN 2/3 in biopsies better than Pap screening for women regardless of age**

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**Objective:** Current recommendations for cervical cancer screening involve the use of the Pap smear and HPV testing. The combination of these tests is necessary since cervical cytology (Pap) has relatively low sensitivity but high specificity and conversely HPV DNA testing has high sensitivity but low specificity for dysplasia and cancer. We wanted to investigate avenues for improvement over the current screening algorithm.

**Methods:** We analyzed 3133 liquid based cytology specimens from women aged 19 to 75 using HPV Oncotect E6, E7 mRNA detection kit and compared the results to cytology and in 200 cases referred to biopsy. In addition, p16 immunohistochemistry was performed on a subset of equivocal biopsy specimens.

**Conclusion:** We report the use of an intracellular HPV E6, E7 mRNA quantification assay (HPV Oncotect), a single test that shows greater sensitivity and specificity than Pap for CIN 2/3 on biopsy. HPV Oncotect was positive in 69% of CIN 2 and 89% of CIN 3 cases in all women analyzed. The specificity of HPV Oncotect was 94.5%. In women <30 years old HPV Oncotect detected 90% of CIN 2/3 indicating this could be a valuable diagnostic for the under 30 age group. The mRNA detection method outperformed cytology in 60% of HSIL cases, was of equal or better predictive value than cytology in 88% of HSIL cases, and showed 50% correlation with p16 staining performed on biopsies. HPV Oncotect demonstrates similar sensitivity to HPV DNA and greater sensitivity than Pap for the detection of CIN 2/3 in cervical biopsies. HPV Oncotect shows much greater specificity than both HPV DNA and Pap for CIN 2/3. In cases where longitudinal biopsy data was available, the quantitative E6, E7 mRNA result was also able to predict CIN progression/regression. HPV Oncotect demonstrates a performance profile that might allow for use in primary cervical cancer screening for women regardless of age.
RNA TESTS FOR THE DETECTION OF CLINICALLY SIGNIFICANT HPV INFECTION.


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2 School of Cancer and Enabling Science, University of Manchester
3 Simpson Centre for Reproductive Health, Royal Infirmary of Edinburgh
4 Manchester Medical Microbiology Partnership, Manchester Royal Infirmary
5 Epidemiology and Statistics Core, Wellcome Trust Clinical Research Facility, Edinburgh
6 Academic Unit of Obstetrics and Gynaecology, University of Manchester, St Mary’s Hospital, Manchester

Objectives: Detection of oncogenic E6/E7 transcripts may denote clinically significant cervical HPV infection compared with DNA (L1) based assays. However more clinical data are required to substantiate this claim. To this end, we present an evaluation of the clinical sensitivity and specificity of the APTIMA® HPV RNA based assay (AHPV, Gen-Probe Incorporated) in comparison to the Hybrid Capture 2 DNA based assay (HC2, Qiagen Ltd) in a clinical setting.

Methods: Women attending two NHS colposcopy clinics in two city hospitals in the UK were invited to participate. Liquid based cytology (LBC) samples were collected and tested via the 2 HPV assays described. Biopsies were taken where clinically indicated and relative sensitivity and specificity of each assay for disease (defined as CIN2 or worse) were calculated.

Conclusions: A total of 1435 women have been recruited to the study so far. At time of abstract submission, 1032 LBC samples have been tested by both assays and have associated, confirmed pathology results. The following cross sectional analysis is based on this subset. Sensitivity and specificity of the AHPV for CIN2 or worse were 93% (226/243) and 66% (519/789) respectively. By comparison, sensitivity and specificity of the HC2 for CIN2 or worse were 93% (226/243) and 64.5% (509/789) respectively. These preliminary data suggest that APTIMA and HC2 assays show equivalent sensitivities and similar specificities for the detection of CIN2 or worse (in a population with a high prevalence of disease). Further data including performance of the assays within a post treatment subset will be presented (with associated 95% CI’s).

EVALUATION OF NUCLISENS™ EASYQ HPV IN ROUTINE DIAGNOSTIC SETTING IN UNIVERSITY HOSPITAL


1 Department of Clinical Chemistry, Microbiology and Immunology, 2 Pathology and 4 Gynecology, Gent University Hospital, Ghent, Belgium 3 GenoID Molecular Diagnostics Laboratory, Budapest, Hungary

Objective: Persistent expression of E6/E7 oncoproteins in women with HPV infection can serve as an indicator for progression to cervical intraepithelial neoplasia (CIN) and invasive cancer. Therefore detection of E6/E7 mRNA transcripts may have higher prognostic value as compared to DNA-based methods. The objective of this study was to evaluate diagnostic value and technical performance of the detection of HPV E6/E7 mRNA of 5 high-risk genotypes (16, 18, 31, 33, 45) by NucliSens™ EasyQ HPV (bioMérieux) in routine setting of university hospital.

Methods: After initial cytological screening of liquid-based Pap test, 1623 samples from 1465 patients were referred for HPV testing during the period of 17 months (13/02/08 - 13/07/09). HPV DNA detection was performed using HPV Full Spectrum PCR Amplification and Detection/Genotyping System by Lab2Lab Diagnostic Services, (GenoID) recognising 14 high-risk HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68. If one or more of high-risk HPV types 16, 18, 31, 33 or 45 were present according to HPV DNA result, HPV mRNA was analysed using NucliSens™ EasyQ HPV (bioMérieux).

Conclusions: Based on cytological result, 35.6% of samples were classified as ASC-R, 22.6% - CIN1, 11.3% - ASC-US, 7.8% - CIN2, 4.7% - CIN3 and 3.8% - ASC-H. The average prevalence of high-risk HPV types independent from cytological class was 55.7% ranging from 33.3% for ASC-US to 98.7% for CIN3. The average prevalence of high-risk HPV 16, 18, 31, 33 and 45 genotypes was 41.7% ranging from 34.8% for CIN1 to 68.9% for CIN3. High consensus for HPV DNA/mRNA data was observed (89.3-100% dependent on genotype) with an exception of high-risk HPV type 31 (62.1% DNA/mRNA consensus) which might be explained by technical issue confirmed by bioMérieux. Good technical performance for NucliSens™ EasyQ HPV was confirmed by low (6.4%) percentage of invalid results. Prognostic advantage of HPV mRNA as compared to HPV DNA on follow-up samples was observed for non-progressive cytology.
**THE ROLE OF HPV E6 AND E7 DETECTION IN CERVICAL CANCER SCREENING**

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**Objective:** Neoplastic transformation induced by HPV requires expression of the virally encoded oncoproteins, E6 and E7. To determine whether E6 mRNA expression correlates with cervical grade, we have analyzed the level of expression of the E6 mRNA for HPV16 in a panel of formalin-fixed and paraffin-embedded (FFPE) cervical tissues using real time RT-PCR.

**Methods:** DNA and RNA were extracted from 90 cervical biopsies using a modified MasterPure protocol from Epicentre. The tissues consisted of 17 normal, 44 CIN III, and 29 squamous cervical cancer (SCC) samples. HPV DNA genotyping was performed using real time PCR with primers/probes specific to genomic regions of HPV 16, 18, 31, 33, and 45. Following reverse transcription, HPV 16 E6 mRNA was analyzed with different and non-overlapping primers/probes using real time RT-PCR. Samples that had a GUSB Ct value of equal to or greater than 36 were eliminated from the analysis.

**Conclusions:** HPV genotyping results indicated that 58/90 specimens were HPV 16 DNA positive, 15 were positive for other high risk HPV types, and 17 were HPV DNA negative. Real time RT-PCR analysis demonstrated that all of the SCC samples that were HPV 16 DNA positive also expressed HPV 16 E6 mRNA. 97 percent of CIN III samples expressed HPV16 E6 mRNA with HPV 16 positive DNA. One CIN III sample was DNA positive but did not contain detectable E6 mRNA. Finally, none of the normal samples expressed any detectable E6 mRNA. Samples in which HPV16 E6 mRNA was not detected were either negative for HPV or were of another genotype. In this study, we have demonstrated that the majority of advanced cervical neoplasia samples that are HPV 16 DNA positive also express E6 mRNA.

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**SHENZHEN CERVICAL CANCER SCREENING STUDY I (SHENCCAST I)**


1. Peking University Shenzhen Hospital Department of OB/Gyn, Shenzhen, China; 2. Royal Ladies Gynecology Clinic, Shenzhen, China; 3. Preventive Oncology International Inc., Cleveland Heights, USA, 4. Cleveland Clinic Department of Quantitative Health Sciences, Cleveland, USA.

**Objective:** Determine the comparative sensitivity and specificity for ≥CIN2 for primary computer assisted liquid-based cytology with two primary HPV testing technologies

**Methods:** SHENCCAST I was conducted in Shenzhen, China. 2097 women ages 25-59, non-pregnant, no cervical cancer screening in ≥3 years, and no history of a hysterectomy or pelvic irradiation agreed to participate. All women had a cervical sample obtained for SurePath cytology read using the TriPath imager protocol. A second direct sample was placed in PreservCyt liquid (PC). 4cc of PC was used for Hybrid Capture (HC-II) and 1cc of PC was placed in Gen-Probe Transport Media for the Aptima HPV (AHPV). Participants positive on any test was recalled for colposcopy and biopsy using the POI directed and random biopsy protocol (>5 biopsies/patient). All 2097 subjects have complete HC-II, Cytology, and AHPV results. 36(1.7%) tested AHPV +, HC-II − and Cytology −. They were excluded from this analysis since their biopsy data were not yet available.

**Conclusions:** Mean age (SD) = 35.9(7.6); 2.9% were smokers; and 87.2% were married. Cytology >ASCUS 6.6 % (135/2061); >LGSIL 1.3 % (26/2061); and ≥ HGSIL 0.5 % (10/2061). Final biopsy ≥CIN2 = 1.2%(25/2061) and; ≥CIN3 = 0.7%(14/2061). HC-II and AHPV were positive in 19.2%(395/2061) and 10.4%(215/2061) respectively. The sensitivity of SurePath ≥ ASCUS , HC-II (at 1Pg), and AHPV for ≥CIN2 was 76%, 96%, and 100% respectively (p=.014); for ≥CIN3 it was 71.4%, 100% and 100% respectively (p=.046). The specificity of SurePath ≥ ASCUS , HC-II (at 1Pg), and AHPV for ≥CIN II was 94.3%, 81.8%, 90.7% respectively (p<.001); for ≥CIN3 was 93.9%, 81.4%, 90.2% respectively (p<.001) Kappa = 0.65 with a 95% CI (0.61, 0.70) between HC-II and AHPV.

<table>
<thead>
<tr>
<th>SCREENING TEST</th>
<th>SENSITIVITY FOR ≥ CIN 2 (%)</th>
<th>SPECIFICITY FOR ≥ CIN 2 (%)</th>
<th>POSITIVE PREDICTIVE VALUE (%)</th>
<th>NEGATIVE PREPRICTIVE VALUE (%)</th>
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<tr>
<td>AHPV</td>
<td>100</td>
<td>90.7</td>
<td>11.6</td>
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These data strongly support primary HPV screening. The lower false positive rate with high sensitivity is an advantage for the RNA based Aptima assay in this role.
Infection with high-risk human papillomavirus (HPV) is considered to play a central role in cervical carcinogenesis. Analyses of molecular markers seem to be important to increase HPV specificity and to predict the risk of disease progression.

**Objectives:** The aim of this study was to evaluate the clinical significance of viral load, integration status and E6/E7 mRNA expression of HPV 16 and 18.

**Methods:** Cervical smears from 219 HPV 16 and/or 18 positive women (69 with normal cytology; 46 with ASCUS; 59 with LSIL/CIN1; 40 with HSIL/CIN2+ and 5 with CC) were studied. E6/E7 mRNA transcripts was performed using NucliSENS™ EasyQ® HPV 1.0. Viral load and DNA physical status was determined by a quantitative in-house real time PCR, with TaqMan® and SYBR®Green, respectively. Viral load was expressed as HPV DNA copies/cell. All statistical analysis was performed using SPSS software version 16.0, with a p value of 0.05.

**Conclusions:** Viral load and integration status was assessed in 150 positive cases for HPV 16, 55 for HPV 18 and 14 positive for HPV 16+18. For E6/E7 mRNA transcripts 111 cases were studied (95 positive for HPV 16 and 21 positive for HPV 18). DNA viral load values ranged from 7.3 to 6.5x10^13 for HPV 16 and 1.7x10^2 to 9.5x10^11 copies/cell for HPV 18. Viral load increased with severity of cervical abnormality, ranging from 5.8x10^7 in normal cytology to 2.0x10^12 in HSIL/CIN2+ (p=0.000). These results show an increased risk for HPV persistence, which may be predictive for cervical cancer development. Regarding DNA physical status, the data obtained showed that episomal and integrated forms (concomitant) of HPV DNA were the most prevalent forms observed in HPV 16 and 18 positive cases. It was not observed a significantly association between the severity of cervical lesion and integration status (p=0.108). Detection of E6/E7 mRNA transcripts of HPV 16 and 18 increased gradually with the grade of lesions (p=0.164; α=0.01), suggesting that detection of E6/E7 mRNA transcripts could provide increased specificity of HPV testing.

In conclusion, the combination of HPV viral load, integration status and mRNA expression results seem to be important for patient management and cervical cancer prevention in women infected with HPV 16 and 18. Study is ongoing and further data will be presented to better evaluate the clinical significance of these results.

**REALTIME-PCR FOR mRNA TYPING OF 14 HPV TYPES**

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2 Department of Obstetrics and Gynecology, The Sahlgrenska Academy, Göteborg University, Sweden

**Objectives:** To evaluate the performance of a novel realtime-PCR based assay for mRNA genotyping in selected cervical samples with varying grades of neoplasia.

**Methods:** Liquid based cytology samples from 127 women (51 pregnant) attending gynecological screening or undergoing investigation of cervical neoplasias were included. Neoplasias were evaluated by biopsy and histological examination. DNA or total NA were extracted using a MagNA Pure LC instrument (Roche). In-house genotyping for HPV DNA and HPV mRNA were performed with TaqMan realtime-PCR targeting E6/E7. The HPV types included in the assays were HPV6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59. A DNase digesting step was performed before mRNA testing.

**Results:** In all mRNA-positive samples, DNA of the same HPV-type was also present. HPV16 and 18 were the most prevalent in histology-confirmed CIN3+ samples (20/32, 63%). Of all mRNA-positive samples, 46% (29/63) and 70% (44/63) were histologically confirmed CIN3+ or CIN2+, respectively. HPV mRNA of type 16, 18, 31, 33, 35 or 45 was detected in 84% (27/32) of histologically confirmed CIN3+ samples, and in three CIN3+ samples (9,4%), no mRNA was found. Out of 71 cytologically normal samples (some with abnormal histology), 17% (12) were mRNA-positive and 52% (37) DNA-positive.

**Conclusions:** In this small study, the sensitivity of detecting histologically confirmed CIN2+ or CIN3+ by mRNA realtime-PCR was 91%, and the specificity was 70% and 46%, respectively. Additional useful information from the test includes genotyping, semiquantitative data and occurrence of coinfection with several HPV types expressing oncogenic E6/E7. Overall, testing for mRNA with realtime-PCR might be a useful tool in HPV screening and investigation of neoplasias.
HUMAN PAPILLOMAVIRUS (HPV) DNA AND mRNA STATUS IN HUMAN IMMUNODEFICIENCY VIRUS (HIV) POSITIVE WOMEN

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Objectives: Infection with high-risk HPV is necessary for cervical cancer. HIV positive women suffer increased risk of oncogenic HPV infection, persistent HPV infection and as a result increased cervical disease. With the life expectancy of HIV positive patients increasing due to anti-retroviral therapy, the probability of developing cervical cancer is also increased. We examined a HIV positive cohort of women for HPV prevalence, HPV E6/7 oncogene expression and cytological status in relation to HIV risk factors (HIV viral load, CD4 counts, etc.). This study forms part of the CERVIVA consortium funded by the Health Research Board, Ireland.

Methods: PreservCyt™ cervical smears from 300 HIV positive women to date were recruited through the Genitourinary and Infectious Disease Clinic at St James’ Hospital Dublin. Smear samples were taken at time of recruitment and at 12-18 month follow-up periods. Clinical data (HIV viral load and CD4 count) was recorded for each patient at baseline. Cytological diagnoses were made according to BSCC guidelines. HPV DNA status was assessed using Hybrid Capture II (Qiagen Ltd., UK). HPV E6/7 mRNA expression was detected for 5 HPV types (16, 18, 31, 33 and 45) using PreTect™ HPV Proofer assay (NorChip AS, Norway).

Conclusions: At baseline the HPV DNA prevalence was 49%, the prevalence of HPV mRNA was 20% and the abnormal cytology rate was 25%. The HPV DNA prevalence in women with cytological abnormalities (88%) was over twice that of HPV mRNA (40%). HPV 45 was the predominant mRNA type followed by HPV 18, 16 or 33 and 31. To date HPV DNA and mRNA rates were 53% (26/49), and 21% (10/48) at baseline and 45% (22/49) and 23% (11/48) at follow-up. Correlation between low CD4 counts (<200 x 10⁶/L) and HPV DNA and mRNA positivity was detected, while no correlation was observed with HIV viral load.

Work supported by The Health Research Board, Ireland.

EVALUATION OF HPV E6/E7 mRNA EXPRESSION IN A COHORT OF HIV POSITIVE SUBJECTS (GISPAP*-COHORT)

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Objectives: Aim of our study was to evaluate the expression of HPV E67E7 mRNA in a cohort of male (M) and female (F) HIV positive subjects tested for HPV genotyping.

Methods: A total of 243 cervical (152) and anal (91) samples were collected respectively from F and M individuals, referred to STD and Gyn Unit University Hospital L.Sacco from March 2008 to May 2009. Samples were tested with INNO-LiPA HPV Genotyping (Innogenetic, Belgium) and with APTIMA HPV Assay (Gen-Probe, USA) for the detection of viral E6/E7 mRNA.

Conclusions: All cervical and anal samples were adequate for analysis and positive for HPV genotyping.
In F the rate of single type HPV infection was 49% while that of mixed infections was 48%; in M the rate of mixed HPV infections was 79% while a single type HPV infection was found in 19%. 25 different HPV genotypes were detected. HPV 16 (11%) was the most frequent genotype in F, followed by HPV 56 (10%), 52, 53 and 66 (8%), 6 (8%), 11 (5%); HPV 52 (12%) was the most frequent genotype in M, followed by HPV16 (10%), 51 (7%), 11 (11%), 6 (10%). In 3% of F and 2% of M respectively the HPV genotype was untypeable. E6/E7 mRNA expression was achieved in 77 F (51%) and 69 M (76%). In F the 16% of E6/E7 mRNA positive samples were infected with HPV 16.
In F the correlation between INNO-Lipa test and E6/E7 mRNA expression was confirmed in 95% of high risk genotypes (HR), in 4% of low risk (LR) and in 1% of untypeable. Similarly, in M the correlation confirmed in 86% of HR, in 12% of LR and in 3% untypeable.
In the present study we showed that mixed HPV infections were more represented in M (79%) than F (48%). HPV 16 and HPV 52 were the most frequent genotypes detected in F and M respectively. The HPV E6/E7 mRNA expression was more represented in M (76%) than F(51%).
More investigation about E6/E7 mRNA expression prognostic role are required.

Work supported by The Health Research Board, Ireland.
CLINICAL PERFORMANCE OF p16\textsuperscript{INK4a} CYTOLOGY AND HPV mRNA DETECTION FOR THE DIAGNOSIS OF CERVICAL NEOPLASIA

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Background: Cervical cancer screening is hampered by the low sensitivity of Pap cytology and limited specificity of HPV DNA detection. We evaluated two novel biomarkers that discriminate between transient and transforming HPV infections, HPV mRNA detection and p16 immunocytology in a cross-sectional clinical study for the detection of CIN2,3 and for the detection of p16 expression in immunohistochemistry (IHC) for which an improved diagnostic accuracy has been suggested.

Methods: 282 liquid based cytology specimens were collected from patients referred to colposcopy. The APTIMA HPV mRNA test (Gen-Probe), the CINtec p16 Cytology test (MTM Laboratories) and the Hybrid Capture 2 (HC2) HR-HPV DNA assay (Qiagen) was performed. p16 cytology was assessed according to a previously described nuclear scoring algorithm. 37.2% (105/282) of the patients had CIN3+ in a biopsy, 21.6% (60/282) had CIN2, and the remaining had CIN1 (28/282) or were disease negative (89/282).

Conclusions: APTIMA and CINtec yielded a comparable sensitivity for the detection of CIN3+ (95.2% for APTIMA and CINtec, 97.1% for HC2). Specificity for CIN3+ was 59.3% for CINtec, 54.2% for APTIMA and 46.9% for HC2. Sensitivity/specificity for the detection of CIN2+ was 87.9%/69.2% for APTIMA, 85.5%/73.5% for CINtec and 92.7%/63.2% for HC2. Twenty-three % (18/76) of CIN negative and CIN1 cases were p16 IHC positive. Of these, the majority was tested positive by APTIMA (15/18), CINtec (14/18) and/or HC2 (14/18). These results confirm the improved specificity/sensitivity profile of CINtec and APTIMA in comparison to HC2 and warrant future application of p16 IHC to refine disease endpoints, since previous studies have shown a higher risk of progression for p16 IHC positive vs. p16 IHC negative CIN1 cases and we demonstrated that test-positivity in low grade CIN was correlated with histological p16 expression.

VALIDATION OF SPF-10 LIPA HPV TYPING EXTRA ASSAY IN FORMALIN FIXED PARAFFIN-EMBDEDDED (FFPE) CERVICAL SAMPLES

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Objectives: To validate FFPE tissue for HPV typing with the SPF10 LiPA EXTRA assay, comparing cervical scrapings to punch and cone biopsy specimens.

Methods: We examined 88 paired cervical scrapings and FFPE punch biopsy samples, and 12 paired cervical scraping, FFPE punch and cone biopsy samples.

DNA from scrapings was extracted by lysis and digestion with proteinase K at 56°C for 1 hour; then proteinase K was inactivated for 20 minutes at 95 °C. For FFPE samples, three to five 10 \(\mu\)m-thick sections were incubated in 200 \(\mu\)l of a lysis solution with proteinase K for 24 hours at 58º C followed by heat inactivation of proteinase K.

HPV typing was performed using the SPF-10 LiPA EXTRA assay (Innogenetics). Kappa statistics were used to measure interrater agreement.

Conclusions: The overall agreement for HPV status was 100% in the 88 paired cervical scrapings and FFPE punch biopsy samples. 25 different HPV types were observed in the series. The mean number of HPV types detected per sample was 2.92 (range 1-11). In 73.9% of subjects, the same number of HPV types was detected in scraping and biopsy specimens (kappa = 0.678). The overall positive typing agreement was 92.4 (range, 90.8 to 94) for 191 out of 262 individual HPV type analyses.

Agreement was good for HPV6, 11, 31, 51, 53, 54, 56, 33, 35, 39, 43, 44, 26, 69/71 and 74 (kappa = 0.6358 to 0.7944), excellent for HPV16 and 52 (kappa = 0.8167 to 0.8287), absolute for HPV18,-45, 68 and 73. Punch biopsy specimens enabled the detection of more HPV types than scraping.

For the12 paired FFPE punch and cone biopsy samples, the overall agreement with respect to HPV status was 100%. Overall positive agreement for typing was 94.23% for 41 out of 59 individual HPV type analyses; 12 viral types were identified only in cone biopsy and 6 only in punch biopsy.

HPV typing by SPF-10 LiPA EXTRA performed equally well in cervical scraping specimens and standard pathological material.
**SS 25-2**

**A NEW APTIMA SPECIMEN COLLECTION AND TRANSPORTATION KIT TO DETECT HR-HPV mRNA BY APTIMA HPV**

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**Objectives:** The performance of APTIMA HPV (AHPV) on cervical and vaginal specimen and transportation (SCT) samples will be compared to cervical liquid-based ThinPrep and SurePath samples. Physician-collected vaginal SCT samples will be compared to self-collected SCT samples in the AHPV assay. The AHPV mRNA test will be compared to the HC2 DNA signal amplification assay and cytology on ThinPrep liquid-based Pap samples for the ability to predict CIN2+. Each patient will be asked to assess the ease and comfort of self-collection using the SCT kit.

**Methods:** Specimens are being collected from 500 women, signing consent and undergoing a routine examination; or as a follow-up to an abnormal Pap test, or a previously positive HR-HPV test. Each patient and the clinician is collecting a vaginal sample using the SCT kit before the cervical examination. After collecting the vaginal swab each patient is completing a questionnaire to determine the ease and comfort of self-collection. After insertion of a speculum a ThinPrep sample is collected for cytology, and tested for HR-HPV with HC2 and AHPV. Cervical samples are also collected using the SCT and the SurePath broom and both samples are tested in the AHPV assay. All HC2 and AHPV discordant samples are genotyped by Roche Linear Array. All APTIMA samples are processed on the TIGRIS instrument. Patients receive colposcopy and biopsy as required.

**Conclusions:** Patients have readily understood and expressed interest in participating in this study. Results comparing AHPV and HC2 on ThinPrep samples and AHPV on vaginal and cervical samples collected with the SCT or liquid-based Pap will be compared and presented.

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**SS 25-3**

**A MANUAL SAMPLE PREPARATION METHOD FOR LBC SAMPLES COMPATIBLE WITH THE NEXT GENERATION HYBRID-CAPTURE HPV DNA TEST**

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**Introduction:** A manual sample preparation method for PreservCyt® (PC) and SurePath® (SP) specimens is currently under development at QIAGEN, Inc. and DX Assays Pte Ltd. This method uses a novel DNA extraction chemistry that is compatible with the next generation hybrid capture technology, digene eHC HPV DNA Test (eHC, under development). The reagents and processing steps are the same as on the automated QIAensemble™ SP system.

**Objectives:** The objective of this study is to show the preliminary performance data of a manual sample protocol for processing sample from both PC media and SP media.

**Methods and Results:** The performance of the manual extraction protocol and eHC was compared with standard HC2 method using PC and SP clinical specimens. Studies were performed on HPV 16 plasmid DNA and SiHa cells in PC media. When using HPV positive SiHa cells at a concentration of 10^4 cells/ml spiked in PC media a mean RLU/CO value of 6.6 was achieved as compared to a mean value of 5.7 when cells where suspended in DCM and a mean value of 7.0 when cells where suspended in a negative pool of clinical material in PC media. Analysis was performed using the new sample processing protocol by three operators. With SP samples, 30 individual specimens were tested by manual extraction eHC and compared with HC2 SOP method. The agreement between the new method and HC2 was excellent. DNA extraction from 96 samples is achieved in 2 hours by one operator. Extraction and subsequent analysis of 96 samples on the QIAensemble HC400 low-to-mid volume instrument can be performed within one working shift by one operator.

Conclusion: Our data show a robust and reproducible manual sample preparation method for PC and SP samples which is compatible with the eHC assay. Processed samples can be directly analysed either manually or using the QIAensemble HC400 instrument. This method is especially suitable for low volume laboratories processing less than 96 specimens a day.
**SS 25-4**

**USING TURBIDITY LIGHT SCATTERING TECHNIQUES TO ENSURE ADEQUATE SAMPLE CELLULAR CONTROL OF CLINICAL CERVICAL SAMPLES IN QIAGEN HR HPV TESTING.**

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**Objectives:** The digene HC2 High-Risk HPV DNA Test® (HC2) has proven to be of extreme value as a component of cervical cancer screening programs and clinical management of ASC-US cytology patients. Currently HC2 HPV DNA testing yields a high negative predictive value of approximately 99.5% for prediction of cervical lesions of CIN3 or greater. Despite this data, an additional control is desired by many laboratories. We have approached this challenge of increasing confidence in the negative results obtained in QIAGEN’s QIAensembleTM HPV Assays by estimating the cell count in known fluid volumes using turbidity light scattering techniques.

**Methods:** Over 1000 clinical specimens in 1.5ml of PreservCyt® (Hologic, Inc.) medium were assessed for cellularity using commercially available turbidometer (Hach, Model 2100AN IS) and an in-house prototype turbidometer that will be integrated on our automated sample preparation platform. As a control we carried out cell counting of a small subset of this population using a hemocytomer (n=99). This comparative study of the turbidity and cell count data (R²=0.76) showed strong correlation and solidified the viability of this approach to determine cellularity. We are implementing this new approach to determine the minimum turbidity required to assure reliable results from QIAGEN’s QIAensembleTM HPV Assays, initial testing suggest that the rate of the indeterminate sample population will be less than 2%.

**Conclusions:** These results indicate that a light-scattering based sample-adequacy control implemented at the front-end of an automated sample prep process could improve the efficacy of results. This method is non-invasive and there is no consumption of sample. Further, by choosing not to test samples with low cellularity prior to the consumption of test reagents provides laboratories opportunities for cost and time savings.

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**SS 25-5**

**L-SHAPE CERVICAL FLOCKED SWAB FOR IMPROVED PAP TESTING.**

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**Objectives:** Sampling devices are important for proper Pap smear testing. It has been reported that the cytobrush+Ayre spatula combination produces better results than a spatula alone and inadequate sampling is responsible for false-negative results. A new L-shaped cervical flocked swab (Copan Italia) allows Pap specimen collection with a single device. The study objectives were to compare the performance of the L-shaped cervical flocked swab (LS) to the cytobrush+Ayre spatula (CS) for the collection of cervical specimens for liquid based Pap test smears and to assess patient comfort and staff ease of collection.

**Method:** During a routine visit for Pap testing 2 cervical specimens were collected from 108 patients who signed informed consent. A cervical swab was collected with the L-shaped cervical flocked swab and another with the cytobrush+Ayre spatula combination. A questionnaire, recording patient's comfort or bleeding and health care worker preference and suggestions was completed. The LS cervical specimen was always collected first in order to monitor bleeding during collection. Both collection devices were eluted in a vial of Thinprep medium and discarded. A liquid based Pap smear was prepared with each sample with a Thin Prep Processor. After staining all smears were read blindly by a cytologist and a pathologist. Each smear was assessed for quality, cell morphology, adequate number of endocervical and ectocervical cells, final test interpretation and comments. In the 108 samples collected with the LS, 12 Pap smears were positive for LSIL and 96 were negative. From the CS samples 7 were LSIL positive, 97 were negative. 3 were equivocal and one was inadequate.

**Conclusions:** The Copan L-Shaped cervical flocked swab was well accepted by the patients and induced less bleeding. The health staff found the L-Shaped cervical flocked swab easy to use and suggested modifications for patients with specific needs. Liquid based Pap smears collected with the L-Shaped cervical flocked swabs had better cells morphology and staining, were much easier to read and easily detected all patients with LSIL without any discordant results.
EVALUATION OF A NEW STRATEGY FOR CERVICAL CANCER SCREENING IN WOMEN WITHOUT ACCESS TO PAP SMEAR SCREENING IN WEST BRITTANY, USING A URINE TEST FOR HUMAN PAPILLOMAVIRUS (HPV) DETECTION (THE PAPU29 PHASE 1 STUDY)

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Objectives: In France, only 55% of women have had a Pap smear for cervical cancer screening due to the lack of organized screening; this rate decreases to 35% in west Brittany. The aim of our study is to evaluate a new strategy based on a rapid HPV-DNA assay in urine.

Methods: The HPV test was performed using 1 mL of urine with the NucliSENS EasyMAG extractor (bioMérieux) and real-time PCR Lightcycler (Roche) systems allowing automated testing of 48 samples per day (Payan JCM2007). Urine samples were obtained between December 2008 and July 2009, from women from 25 to 65 years old who were non-responder to a previous invitation to Pap smear, and living around Morlaix, north Brittany. Positive samples were genotyped using the LiPA HPV test (Innogenetics).

Results and conclusion: Among 5781 invited women, 913 were excluded (15.8%), 341 (5.9%) accepted the Pap smear test, showing abnormal cytology in 6 cases (1.9%); 4036 denied Pap test (70%) and among them 1170 (29%, mean age 47.2 +/-11.4) sent their urine sample to our lab (mean delay from invitation 54.7 +/-81.1 days); 304 (25%) were found HPV-positive and were invited to have a Pap smear by their general practitioners. Phase 2 study among 4151 other women is ongoing in West Brittany. With about a 5-fold increase in screening compared to Pap smear testing alone, our urine HPV test is adapted for screening in a large cohort of women from the general population with no Pap smear test over the last 3 years.

Grants from the French Ligue contre le Cancer.

HPV DETECTION IN URINE, A CHALLENGE OR A UTOPIA.

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Objective: The World Health Organization has recommended countries to introduce HPV vaccination in the (pre-)adolescent population, if programmatically feasible, affordable and cost-effective. Detection of HPV DNA in urine has been suggested as a tool to monitor the impact of HPV immunisation programmes. Several studies described the feasibility and applicability of this approach, but sometimes with contradictory outcomes. Our group has conducted a literature review to assess whether HPV detection in urine provides a promising concept to monitor the effect of future vaccination programmes.

Methods: For each study, study populations (eg. women with invasive cervical cancer, HIV+ patients, schoolgirls), type of urine samples (eg. first-voided, midstream, self-collected at the clinic, self-collected at home), volume of urine processed, storage condition (immediate processing, RT, 4°C, -20°C or -70°C), sample preparation (diluted or undiluted, centrifugation speed), extraction methods, and amplification methods were assessed.

Conclusion: Twenty-one studies on the detection of HPV DNA in urine have been analysed. The discrepancies in the settings and methods may explain the conflicting study results and hamper the interpretation of the data across different studies. Further research is needed to standardize the methodology and to determine the optimal approach for detection of HPV DNA in urine.
Completion of the productive papillomavirus life cycle requires the timely expression of different sets of viral genes as the infected cell moves towards the epithelial surface. This allows viral genome maintenance in the basal and parabasal layers, viral genome amplification in the mid epithelial layers, and assembly of infectious particles in the layers above this. The molecular events that regulate these timely changes provide a rational basis for the selection of biomarkers that can identify both the site of infection and disease severity. In productive papillomas caused by low-risk HPV types, the basal cells are not clearly marked by molecular changes, but in neoplasia caused by the high-risk papillomaviruses, the basal cells are driven into the cell cycle and can be visualized using cell cycle markers such as PCNA, Cyclins or the MCM proteins. The molecular pathways that participate in the up-regulation of these cellular markers involve the HPV E6 and E7 proteins and are well understood, with an additional marker (p16) providing additional information regarding viral de-regulation. The balance between sustained cell-cycle entry and differentiation varies according to disease severity, and is critically dependent on the infected cells ability to respond to cell contact inhibition and differentiation signals. Genome amplification will eventually begin in these cells as they move from being mitotically active, and progress for the final time into S-phase and then G2. Progression through these cell cycle states in the mid epithelial layers allows the accumulation of a second class of viral marker (E4) in the mid epithelial layers where genome amplification is occurring. E4 can be used as a marker of HPV type, and unlike MCM and p16 provides proof of active HPV infection. What regulates L1 expression in a subset of the E4 expressing cells is currently unclear. Marker combinations based on an understanding of the HPV life cycle, have potential in diagnosis and screening.

The productive program of human papillomaviruses depends on squamous differentiation of keratinocytes. Our lab has established a simple, efficient, and reproducible method to generate HPV-18 genomic plasmids in primary human keratinocytes (PHKs). Within a week of transfection with DNA vectors and liberation of the viral genome using Cre-loxP excision, the PHKs are used to develop organotypic raft cultures. The HPV-18 replicons amplify to high copy numbers in a large fraction of the differentiated keratinocytes and are then packaged into virions in the superficial cells, patterns identical to those observed in patient specimens. Transmission electron microscopy reveals a virion maturation process in cornified strata, resulting in paracrystalline arrays of icosahedral viral particles. The recovered viruses efficiently and productively infect naïve PHKs in raft cultures.

HPV E7 oncogene expression promotes S phase reentry by numerous spinous cells, resulting first in the induction of host chromosome replication, and then in a transition into G2 phase, which enables high level viral DNA replication. As viral DNA amplifies, the E7 activity is diminished and eventually extinguished. This in turn allows late gene expression of L1 and L2 capsid proteins to enable DNA packaging and virion morphogenesis.

Mutant HPV-18 genomes that carry site-directed mutations in the early genes E6 and E5 are being created and analyzed in the raft cultures to determine the functions of these viral proteins in their natural environment. This system obviates the need for immortalized cells and establishes the opportunity for genetic analyses of virus-host interactions leading to viral replication and virion production. The consequences of mutations will be presented.
The role of biomarker expression assessment in cervical cytological samples or histological specimens is proven, demonstrating that biomarker overexpression or downregulation correlates with the degree of cervical abnormality. However, several shortcomings of immunochemistry, such as the lack of standardized methodology, interobserver variation and the absence of systems for automatic interpretation, hamper the determination of its clinical role and the implementation in a screening setting. The most extensively studied markers to detect dysplastic cells are p16INK4A and cell cycle markers (Sahebali et al., 2004; Sahebali et al., 2006; Branca et al., 2006; Redman et al., 2008). In cytology, most markers are investigated as a locator or beacon, decreasing the time needed to detect suspicious cells. Some markers can be considered as surrogate markers for HPV infection. This has been proven for p16INK4A and recent data also show that overexpression of insulin-like growth factor I receptor is linked to high-risk HPV (Kuramoto et al., 2008). Molecular markers could also predict the outcome of CIN. The risk of progression of dysplasia to invasive carcinoma ranges from 1% to 12%. On biopsies, it has been proven that some markers, like p16INK4A (37% in carcinoma in situ versus 88% in invasive carcinoma) and survivin, show prognostic potential (Anschau et al., 2009; Branca et al., 2005). Combinations of markers are also useful and commercialized (e.g., ProExC). We recently studied the feasibility of quantitative PCR (qPCR)-based p16INK4a quantification in a LBC setting and concluded that qPCR analysis of biomarker expression is not appropriate for cervical screening purposes (accepted for publication in European Journal for Cancer Prevention). In typical LBC samples, the biomarker transcripts of the dysplastic cells are diluted by the RNA of the normal cells in such a manner that their overexpression cannot be detected by qPCR.

In conclusion, protein biomarkers could play an important role as locator or as triage tool for HPV positive patients. Further randomized trials are needed to detect their potential in a screening setting.

The viral vegetative cycle of human papillomavirus and the roles played by the viral proteins are not fully understood yet despite more than 20 years of investigation. An excellent example of this gap in our knowledge is our fragmented and incomplete understanding of the roles played by the viral E2 protein. The E2 transcription factor has been shown to regulate both transcription and replication of the viral genome and we and others have shown in vitro that it is a transcriptional repressor of the E6 and E7 viral oncogenes for the human papillomaviruses type 16 and 18, involved in cervical cancers. Accordingly, the E2 ORF is disrupted during integration of the viral genome in high grade lesions and cervical cancer. To gain more insight on the roles of the HPV16 E2 protein, we investigated its expression in clinical samples at various stages of cervical intraepithelial neoplasia (CIN).

We used new polyclonal antibodies raised against the HPV16 E2 protein to stain E2 by immunohistochemistry on paraffin embedded clinical samples and compared it with other markers of cell proliferation and differentiation.

E2 was found highly expressed in the nuclei and cytoplasmas of cells forming the intermediate and upper layers of CIN1 and CIN2. We could demonstrate that expression of E2 and p16INK4a, a surrogate marker for the oncopgenic E7 expression, were exclusive, thus implying that E2 was not expressed together with high levels of E7. Using markers of keratinocyte proliferation and differentiation, we could show that expression of E2 was topologically distinct from the proliferation markers while it coincided with squamous cell differentiation and episomal amplification of the viral genomes. These data indicated that E2 is an excellent marker of early stages of cervical lesions and validated previous assumptions of its crucial roles in HPV infection and cellular transformation.
Cervical cancer is a leading cause of cancer related deaths in women, with most cases in low resource settings. Implementation of appropriate cervical pre-cancer and cancer screening methods in low-resource settings is key to a global reduction in cervical cancer mortality.

Arbor Vita, in collaboration with PATH, has developed a rapid diagnostic test, the AV Avantage HPV E6 Test, for detection of cervical pre-cancer and cancer. Using a lateral flow based format (“Strip Test”), the test promises to be cost effective, it is simple and rapid to perform, and does not require complex machinery or a cold chain. The AV Avantage HPV E6 Test is designed to specifically detect E6 oncoprotein from the most prevalent HPV types (16, 18, 31, 33, 45, 52, 58), which are responsible for approximately 90% of cervical cancers. E6, in concert with E7, is necessary for oncogenic transformation of cervical epithelial cells. The AV Avantage HPV E6 Test, therefore, promises a high positive predictive value for those women at risk for progression to cervical cancer.

The AV Avantage HPV E6 Test has an analytical sensitivity of less than 5000 HPV-16, -18, or -45 transformed cervical cancer cells. A small clinical pilot study was performed to determine whether the analytical sensitivity and specificity would translate into acceptable clinical sensitivity and specificity. Cervical swab specimens from HPV DNA positive women with: negative pathology, histology confirmed CIN1, CIN3 or cervical cancer were run on the AV Avantage HPV E6 Test in a blinded fashion. E6 oncoprotein was detected in specimens from women with cervical cancer and in a majority of CIN3 specimens, but not in CIN1 or histology normal specimens. While the sample number in the clinical pilot study was small, the outcome is consistent with the premise that the AV Avantage HPV E6 Test provides a diagnostic tool of increased risk prediction value for cervical cancer.

Objectives: The human leukocyte antigen (HLA) plays a role in modulating innate and adaptive immune responses; the purpose of this study is to determine whether HLA-E or HLA-G polymorphisms play a role in human papillomavirus (HPV) infection susceptibility and persistence.

Methods: The McGill-Concordia Cohort is a prospective cohort study of the natural history of HPV infection and cervical neoplasia in female university students in Montreal, Canada. A total of 621 female university students were recruited between 1996 and 1999, and were followed for 24 months at 6 month intervals. At each visit, cervical specimens were collected for HPV DNA testing and typing using the MY09/11 PCR protocol. HLA polymorphisms were genotyped using purified DNA from cervical samples collected at enrollment. We used logistic regression to analyze the association between specific alleles or polymorphic regions and cumulative risk of HPV infection classified by HPV type, species, and group, and by whether infection was transient or persistent. We used dominant and recessive allelic models to gauge the associations. Persistence was defined as being HPV positive for the same species at two or more of the five visits, and contrasted with women who were negative for the same species on all five visits. All model-derived odds ratios were uniformly distributed around the null value and were non-significant.

Conclusions: We found no evidence of excess risk of HPV infection or infection persistence associated with any of the 12 HLA-E/G alleles investigated. This suggests that HLA-E or HLA-G polymorphisms may not influence susceptibility to HPV infection or to cervical carcinogenesis.
LEUKEMIA INHIBITORY FACTOR (LIF) SUPPRESSES TRANSCRIPTION OF HUMAN PAPILLOMAVIRUS AND INHIBITS THE GROWTH OF CERVICAL CANCER CELLS IN VITRO

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LIF (Leukemia Inhibitory Factor) is an endogenous human cytokine which is able to reduce transcription of the HPV oncoproteins E6 and E7 through repression of the viral LCR (long control region). Additionally, LIF has growth-inhibitory effects on human keratinocytes and cervical cancer cell lines.

LIF is a member of the IL-6 (interleukin-6) superfamily of cytokines, which also includes Oncostatin M, IL-11, and CNTF (ciliary neurotrophic factor). IL-6 is known to repress LCR-driven transcription through the activity of the transcription factor NF-IL6 (C/EBPβ), which may hinder the binding other factors such as AP-1 and NF-1.

LIF is a member of the IL-6 family of cytokines. Like IL-6, it negatively regulates transcription from the LCR of HPV, most likely through a C/EBP beta-dependent pathway. However, while IL-6 acts as a growth factor in cervical and other malignancies, LIF represses both LCR-driven transcription and cell growth.

LIF has previously been tested for clinical applications in fertility treatment and the prevention of chemotherapy-associated neuropathy. These studies have shown it to be well-tolerated and safe for human use.

HPV E5 PROTEIN UP-REGULATION OF THE MITOGEN-ACTIVATED PROTEIN KINASES CRUCIAL FOR VIRAL DNA AMPLIFICATION.

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Human papillomaviruses (HPVs) are prevalent pathogens that infect cutaneous or mucosal epithelia. The productive phase of HPV infection takes place only in differentiated keratinocytes of squamous epithelia. Our lab recently achieved robust HPV-18 production in organotypic raft cultures of primary human keratinocytes. Viral DNA replication is dependent on the ori and the virus-encoded ori binding protein E2 and the replicative helicase E1. All other replication machinery and substrates are supplied by the host cell. An 80-amino acid tract near the amino terminus of the HPV-11 E1 protein controls its cytoplasmic-nuclear shuttling and nuclear retention. This region contains a pair of basically charged sequences that are the bipartite nuclear localization sequence, a cyclin binding motif, and 3 consensus substrates for cyclin-dependent kinases (CDKs) or mitogen-activated kinases (MAPKs) located at Ser 89, 93, and 107, each followed by a proline residue. It also has a dominant CRM-1 dependent nuclear export sequence spanning Ser107. Cyclin/CDK mediated phosphorylation of S107 inactivates CRM1-mediated HPV-11 E1 nuclear export, whereas E1 S89A and S93A mutations significantly reduce nuclear import. Two MAPK docking domains located in the C-terminal helicase domain are highly conserved among many HPV genotypes, and alanine substitution mutations of the MAPK docking domains virtually abolishes E1 nuclear import. To determine whether MAPKs are also necessary for HPV-18 replicon amplification in PHK raft cultures, we examined wild type viral DNA replication in PHK raft cultures in the presence of inhibitors of the MAP kinases ERK and p38. These agents effectively inhibit viral DNA amplification without affecting host DNA replication or altering squamous differentiation. While E1 is a substrate of ERKs, it is not a direct substrate of p38. A systematic search for targets of p38-mediated phosphorylation revealed a potential substrate in the HPV E5 membrane protein. An HPV-18 E5 initiation-codon mutant has the same phenotype as inhibitor-treated raft cultures harboring the wild type genome, and several mutations of the candidate S-P motif verified that E5 itself is indeed phosphorylated by p38. This modification establishes a signal amplification circuit in which E5 activity increases after phosphorylation and then stimulates recycling of receptor tyrosine kinases to the infected cell surface, leading to augmentation of MAPK phosphorylation of E1 protein to facilitate the nuclear import crucial for viral DNA amplification. E1 nuclear translocation will be an effective platform to screen for small molecular weight chemicals that can inhibit DNA replication of most genotypes of HPV.
**SS 27/28-2**

**TRANSCRIPTION FACTOR: A NEW NOVEL BIOMARKER TO TRIAGE EQUIVOCAL AND LOW GRADE ABNORMAL CYTOLOGY**

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**Objectives:** We found a transcription factor (TF) Brn3a when binds to the Upper regulatory region of HPV can drive the expression of the oncogene E6 and E7 m-RNA1. We have reported previously that Brn3a is over expressed 300 folds in HSILs2. We believe, a proportion of women may have elevated Brn3a levels would therefore be particularly prone to cervical oncogenic transformation following HPV exposure. This novel biomarker can be successfully used to triage equivocal and LSILs.

**Methods:** It is a prospective study cohort study conducted from July 2008 to August 2009. Women with Liquid based cytology results suggesting ASCUS at least on two occasions or LSIL at least once were invited to participate. All women had colposcopy by accredited colposcopists. Two cervical scrape specimens were collected prior to colposcopy. One sample was utilized to measure Brm 3a, mRNA E6 and Brn3b. the other sample was used to detect presence of HR-HPV by hybrid capture (HC2) method. TF Brm3a, Brn3b and E6 level were measured by real time PCR. Colposcopy directed punch biopsies or excisional biopsies were taken if indicated.

Out of 300 women and 24 (8%) had CIN2/3 and 36 (12%) had CIN1. The results are as follows:

<table>
<thead>
<tr>
<th></th>
<th>Brn3a</th>
<th>95% CI</th>
<th>HPV DNA</th>
<th>95% CI</th>
<th>mRNA E6</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>100%</td>
<td>75.9 - 100</td>
<td>100%</td>
<td>75 - 100</td>
<td>93.7</td>
<td>67.7 - 99.6</td>
</tr>
<tr>
<td>Specificity</td>
<td>77.7%</td>
<td>70 - 83.3</td>
<td>42%</td>
<td>35.7 - 50.4</td>
<td>81.5</td>
<td>74.9 - 86.7</td>
</tr>
<tr>
<td>PPV</td>
<td>28.07 %</td>
<td>17.3 - 41.7</td>
<td>13</td>
<td>7.9 - 20.8</td>
<td>30.6</td>
<td>18.6 - 45.5</td>
</tr>
<tr>
<td>NPV</td>
<td>100</td>
<td>96.7 - 100</td>
<td>100</td>
<td>94.2 - 100</td>
<td>99.3</td>
<td>95.8 - 99.9</td>
</tr>
</tbody>
</table>

**Conclusion:** This study has shown Brn3a is as sensitive as HPV DNA but with much higher specificity. Hence it has achieved reasonably higher PPV for detecting HG CIN. Subgroup analysis showed that mRNA E6 has high predictive value to detect high grade. We have also looked at the role of another TF Brm3b in a subgroup of 100 patients. The final result will be available for presentation. Transcription factor can be a very useful biomarker in triaging ASCUS and LSIL.


**SS 27/28-3**

**CONTROL OF HPV 16 GENE EXPRESSION IN LATENT, PERMISSIVE AND TRANSFORMING INFECTIONS BY DIFFERENTIATION DEPENDENT METHYLATION OF THE URR.**

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**Objectives.** Replication and expression of HPV-genomes depends on the differentiation status of the epithelial host cells. Progression of acute to transforming HPV-infections is triggered by the expression of the E6 and E7 genes in basal cells. Methylation of the viral genome may affect regulatory features that control transcription of the viral genome. Here, we analyzed the methylation pattern of HPV16 URR during epithelial differentiation and neoplastic transformation and analyzed how changes in HPV URR methylation affect the viral oncogene expression.

**Methods.** HPV16 DNA isolated from laser-microdissected epithelial cells with different degrees of differentiation was analyzed by bisulfite genomic sequencing. Subsequently, we compared the methylation pattern of the HPV16 URR in LSIL and HSIL lesions with adjacent HPV-infected squamous cells not affected by pathological changes.

**Conclusions.** In epithelial areas without histopathological abnormalities all CpG dinucleotides were consistently methylated throughout the whole thickness of the epithelium. In low grade lesions, the promoter region in the basal or intermediate cell layers contained unmethylated CpGs. In contrast, in the superficial cells most of the CpG dinucleotides were methylated including the E2 and SP1 binding sites. The CpGs of the enhancer region including the ones within NFI and TEF-1 binding sites were heavily methylated in basal cells but showed less degree of methylation in more differentiated cells. In the 5'LCR region all CpG dinucleotides were unmethylated irrespective of differentiation stage. In the majority of high grade lesions (11/13, 85%), consistent methylation of E2BS1 was observed. Methylation of E2BS1 leads to 4-6 fold activation of the early p97 HPV16 promoter. These data underline the hypothesis that the methylation state of the viral genome is substantially changed depending on the degree of epithelial differentiation. Methylation of the E2BS1 allows for uncontrolled high level of viral gene expression.
Background: Coupling of therapeutics and diagnostics allows personalized medicine approaches to disease management in addition to accelerating drug discovery for certain conditions. Here, we report the novel inhibition of HPV-encoded oncoproteins E6 and E7 by the cytokine LIF (Enfilermin™), a compound found to be safe in humans for treatment of implantation defects in infertility.

Methods: Using intracellular quantification of E6, E7 mRNA expression by HPV Oncotect, we screened a library of compounds suspected of inhibiting pathways involved in E6, E7 expression in cells. In a microtiter format, we treated the human cervical cancer cell lines SiHa and CaSki and HPV 16 LCR luciferase-transfected human keratinocytes (HaCaT) with a matrix of compounds at serial concentrations. Results: At doses ranging from 100 pg/mL to 10 ng/mL of LIF in growth medium, SiHa and Caski cells exhibited a 40%-90% inhibition, respectively, of E6, E7 mRNA as determined by HPV Oncotect. LIF caused a similar inhibition of HPV 16-LCR expression in transfected keratinocytes as determined by luciferase activity. The downregulation of E6, E7 mRNA in SiHa and CaSki cells was accompanied by a 90% decrease in tumor cell proliferation in culture.

Conclusions: This is the first report of LIF activity in the downregulation of E6, E7 mRNA expression and the inhibition of cervical cancer cell growth in culture. In addition, we report the use of HPV Oncotect as a companion diagnostic for compounds such as LIF targeting expression of the HPV-encoded oncoproteins E6, E7.
IDENTIFICATION OF NOVEL PREDICTIVE BIOMARKERS FOR DEVELOPMENT OF CERVICAL HIGH GRADE LESIONS

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3 Gynecologic Clinic, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark
4 Genedata, Basel, Switzerland

Objectives: Persistent infections with carcinogenic HPV types 16 and 18 are a necessary precondition but not sufficient for cervical cancer. Using cervical swabs from a Danish cohort of younger women established in 1991-1993, persistently HPV16 or 18-infected women were identified by the LiPa genotyping assay (HPV16 or HPV18 present at two examinations with 2 years apart). Within a median follow-up time of 11.1 years, 45% of the HPV16-persistently infected women developed moderate dysplasia, severe dysplasia, CIS or cancer. To identify potential markers for persistent HPV16 and HPV18 infections and predictive markers for progression, RNA from cervical swabs of these women and controls was isolated and subjected to transcriptome analyses.

Methods: RNA was isolated by a modified Qiagen RNAeasy extraction protocol. The cellular transcriptome was determined with Affymetrix U133A 2.0 microarrays and then bioinformatically analysed. Differential expression of cellular and viral genes was confirmed by quantitative real-time PCR.

Conclusions: Transcriptome analyses revealed statistically highly significant differences between HPV-negative women and women persistently infected with HPV16 or HPV18. Also within the group of women with persistent HPV16 or HPV18 infection, significant differences were observed between non-progressors and women who developed ≥ CIN2 during follow-up. For validation by qRT-PCR differentially expressed genes were selected by statistical significance and by the extent of deregulation. Differences could be confirmed by qRT-PCR for 5 cellular genes between HPV-negative and HPV16-persisters and for 3 cellular genes between non-progressors and progressors to ≥CIN2 that have not been described before. We could identify putative novel persistence markers and novel markers predictive of subsequent progression to CIN2 or worse among women with persistent HPV16 or HPV18 infection.

DEREGULATED MICRORNAS IN THE PATHOGENESIS OF CERVICAL INTRAEPITHELIAL NEOPLASIA AND CERVICAL CARCINOMA

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Objectives: MicroRNAs (miRNAs) are a class of short non-coding RNA molecules that are involved in the post-transcriptional regulation of gene expression by binding to the target messenger RNAs (mRNAs). The precise mechanisms are not yet fully understood, however, emerging research has revealed that miRNAs play an important role in the control of basic cellular processes such as cell differentiation, proliferation, apoptosis and metabolism. Recent reports have shown that miRNAs may play an important role in carcinogenesis and tumor progression. This study aims at identifying miRNAs that are deregulated in cervical intraepithelial neoplasia (CIN) and cervical squamous cell carcinoma (SCC).

Methods: Thirty-three CIN, 51 SCC and 9 normal cervical tissue specimens were collected for this study. Microdissection was performed under a dissecting microscope to achieve a ≥90% purity of target cells. Extraction of total RNA was performed using TRIzol® reagent. Reverse transcription and TaqMan real-time quantitative polymerase chain reaction (qRT-PCR) were used for assessing each miRNA expression. hsa-let-7a was used as the endogenous control to normalize the expression levels of miRNA by correcting differences in the amount of cDNA loaded into PCR reactions.

Conclusions: Profiling of 204 miRNAs was completed in 24 CIN and 9 normal cervical epithelia. Using dChip software (http://www.hsp.harvard.edu/~cli/complab/dchip/), 12 miRNAs were found differentially expressed in CIN vs normal control with a lower bound fold change >2.0. These included 10 up-regulated and 2 down-regulated miRNAs. We further examined the expression of these 12 miRNAs in 51 micro-dissected SCCs. Seven miRNAs including miR-203, miR-20b, miR-338, miR-345, miR-512-5p, miR-518a and miR-9, were found significantly deregulated in SCC. Totally, 63 aberrantly regulated genes revealed in our previous global gene expression profiling of 29 SCCs were predicted as the target genes of the 7 miRNAs in SCC. This study has demonstrated deregulation of miRNA in CIN and might play an important role in the carcinogenesis of cervical squamous epithelium.
In conclusion, it appears that regimens which include an HIV protease inhibitor affect the regression of cervical dysplasia to a greater extent than HIV regimens which do not include a PI. There is emerging evidence in vitro to give a mechanistic explanation for these findings.

**The Association Between Use of HIV Protease Inhibitors and Regression of Cervical Dysplasia**

**Objective:** To obtain data with regards to the association between use of an HIV protease inhibitor (PI) and regression of cervical dysplasia in HIV seropositive women as compared to those on therapeutic regimens that do not include a PI.

**Methods:** Data on HIV seropositive women with a history of cervical dysplasia seen at our institution between years 2000 and 2007 was collected retrospectively. Only patients who were on highly active antiretroviral therapy (HAART) and had complete data were considered for analysis. 501 cases were on HAART and had complete data; 3,311 pap smears were collected on these women during that time. Cases were stratified by use of PI into three categories: 1) never used PI 2) used PI post initial pap smears 3) always used PI. Pap smear results were scored in order of increasing severity from 1 through 5, with benign coded as 1 and evidence of carcinoma as 5. Longitudinal analyses were assessed with a random trend model.

**Conclusion:** The mean pap score (in pap-years) was 2.89, 2.24/2.20, 2.22 for never used PI, pre/post PI, and always used PI respectively. Random trend model showed the strongest linear trend for regression of dysplasia among those who switched from non-PI to a PI containing regimen (p < .0001). On subgroup analysis, cases with a pre-PI score of 1-2 (benign/ASCUS) remained in this range over time and did not progress. Those with a pre-PI score of 3-5 (≥ LSILS) regressed over time post-PI (p < .001). In conclusion, it appears that regimens which include an HIV protease inhibitor affect the regression of cervical dysplasia to a greater extent than HIV regimens which do not include a PI. There is emerging evidence in vitro to give a mechanistic explanation for these findings.
In view of a better determination of the evolution potential of cervical lesions and therefore to perfectly and safely adapt the management and treatment, we suggest that the cervical lesion should be defined by the 5 following criteria:
- colposcopic characterization of lesions’s severity (grade: G)
- location (type: T)
- size of the lesion, (quadrants: Q)
- age of the woman,
- and concordance of cytological and histological results.

Each one of these 5 criteria should be subdivided into 3 groups depending on their severity. In addition, we propose the use of a basic colour code to make these grade of severity more striking: green = reassuring; orange = intermediary; red = worrying.

This classification may have two different advantages:
1- A simple and reproducible classification of colposcopic cervical lesions which may be useful for comparison of the lesions with 3 criterias GTQ: G for colposcopic grade; T for location of TZ and Q for area of the lesion

2- A simple and reproducible classification for treatment modalities and decision with 5 criterias: GTQ plus patient age and a cyto-histologic résumé.

The presence of at least one red code or two orange codes would contraindicate the choice of either surveillance or destructive therapy and therefore indicate the need for excisional technique with histological analysis and final diagnosis. More than a simple colposcopy classification, we here describe a way to take into account colposcopic features as well as parameters known to have significant influence on the risk of underestimation of a microinvasive disease. To avoid for further obstetrical complications, this nomenclature therefore safely allows for the selection of women who could benefit from destructive therapy or from a simple follow up.
METHODOLOGY FOR PREPARING EVIDENCE-BASED GUIDELINES ON CERVICAL CANCER PREVENTION IN THE EUROPEAN UNION USING HPV TESTING AND VACCINATION

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Objectives: To assist an European group of experts to prepare evidence-based guidelines for organised cervical cancer (CC) screening using HPV tests complemented or not by HPV vaccination.

Methods: A literature group composed of experts in CC prevention and critical appraisal of studies was constituted; it was responsible for retrieving, evaluating, and synthesizing data from relevant literature.

The methodology consisted in the following steps:

Clinical questions definition: For each topic, chapter authors formulated clinical questions containing the five PICOS elements.
- P: patient characteristics
- I: experimental intervention
- C: comparison interventions
- O: outcome measures
- S: study design

Bibliographic search: searches for literature are performed consulting several bibliographic databases. Articles suggested by experts are also considered. Inclusion criteria for each kind of question (efficacy, cost-effectiveness, diagnostic accuracy, acceptability), a hierarchy of the study designs and inclusion/exclusion criteria were produced.

Quality assessment: The quality of retrieved studies is assessed using validated checklists specific for each study design.

Grading: A grid integrating the level of evidence and the strength of recommendations was developed.

Evidence table and summary documents: For each question, evidence tables with information from single studies and a synthesis of the results are produced. Drawing up of guideline’s chapters: The chapter authors use the evidence tables and summary documents to draft texts for the guideline. Consensus: Drafts chapters will be circulated among authors, external reviewers and a multi-disciplinary editorial board to reach consensus on contents and recommendations.

Conclusions: Assessing effectiveness following well-established methods is a time-consuming but crucial activity which must precede the production of guidelines. This EU-sponsored process, , is currently taking place, which will result, in 2010, in two new supplements to the European guidelines for CC prevention.

EVIDENCE-BASED GUIDELINES ON CERVICAL CANCER PREVENTION IN THE EUROPEAN UNION USING HPV TESTING AND VACCINATION

De Vuyst H,1 Arbyn M,2 Franceschi S,1 Minozzi S,3 Armaroli P,3 Segnan N,3 Anttila A,4 von Karsa L1

1 International Agency for Research on Cancer, Lyon, France, 2 Scientific Institute of Public Health, Brussels, Belgium, 3 CPO and San Giovanni Battista University Hospital, Torino, Italy, 4 Mass Screening Registry/Finnish Cancer Registry, Helsinki, Finland

Objectives: Important developments in the field of HPV testing and vaccination since the publication of the 2nd edition of the European Guidelines for Quality Assurance in Cervical Cancer Screening (1) have prompted the European Union Health Programme to initiate the development of supplements with evidence-based quality assurance in HPV testing and HPV vaccination programmes (ECCG project GRANT 2006322).

Methods: Under the guidance of a multi-disciplinary editorial board, HPV experts from various European countries are reviewing and grading the available evidence from the literature. This information is then translated into recommendations by a panel of experts using an evidence grid.

Conclusions: Comprehensive background information is provided on the different HPV tests and the effectiveness and cost-effectiveness of HPV testing in primary cervical cancer screening, triage of minor cytological abnormalities or follow-up of treatment of cervical lesions, and the respective requirements for the tests in these different applications. Operational issues such as laboratory quality control guidelines and organisational aspects of HPV testing in primary screening programmes are discussed. The evidence on the efficacy of the available prophylactic HPV vaccines will be summarized and an overview will be provided of population trials and demonstration projects. An update of national vaccination policies and implementation in EU countries is given and recommendations will be formulated on delivery strategies, monitoring and quality assurance. The cost-effectiveness of potential HPV vaccination schedules in combination with HPV- and or cytology-based screening and triage options will be discussed. Since communication is an important aspect recommendations will be provided for addressing the public, health providers and stakeholders.

Evidence-based guidelines for quality assurance for HPV testing and vaccination will provide timely information for health policy development in Europe.

(1) http://bookshop.europa.eu/eubookshop/publicationDetails.action?pubuid=547021
NTCC is a RCT conducted within 9 organised cervical cancer screening programmes in Italy and comparing cytology with an experimental arm. The latter followed two phases: a first phase with HPV testing supplemented by liquid based cytology and a second phase with stand-alone HPV testing. Some 95,000 women were enrolled. HPV positive women were directly referred to colposcopy, except those aged 25-34 years enrolled in phase 1, who were referred immediately to colposcopy if cytology was also at least ASCUS and, otherwise, after 1 year if HPV testing persisted positive.

Data at recruitment showed that, among women age 35-60 years, using CIN2+ histology as endpoint, the cross-sectional sensitivity was increased by about 50% with HPV compared to cytology but the cross-sectional positive predictive value (PPV) decreased by almost 40%. Other RCTs (POBASCAM, Sweedscreen) that triaged HPV positive women for cytology/infection persistence observed similar (about 50%) gains in sensitivity but PPV was not reduced in comparison to cytology. In NTCC, triaging HPV-positive women for p16 over-expression (by immunohistochemistry) still led to an about 50% gain in cross sectional sensitivity vs. cytology but with no increase of referral to colposcopy. Other triage criteria based on HPV genotyping (or its combination with cytology) were dominated by p16 testing. Supplementing HPV with LBC led to negligible further increases in sensitivity vs. stand alone HPV but to a remarkable further loss in PPV (~60% vs. cytology).

At the second screening round the detection of CIN2 and CIN3 was reduced by about 50% in the experimental arm, suggesting that persistent lesions were detected in advance by HPV compared to cytology, therefore increasing the chance of treating them before invasion. A significantly lower number of invasive cancers were indeed observed in the experimental arm at round 2. The reduction was similar in both phases, again suggesting that stand-alone HPV is as protective as HPV + cytology.

Among women age 25-34 years a reduction of CIN3 at round 2 was observed only with direct referral to colposcopy of all HPV positive women but not with cytological triage of HPV positive women (phase 1). At this age, however, data suggest that, with both management strategies, HPV testing led to a relevant overdiagnosis of CIN2 that would have regressed spontaneously.

HPV testing has a higher sensitivity, a slightly lower specificity, but a significant higher negative predictive value (NPV) for high-grade CIN lesions (CIN2 or worse, CIN2+) compared to conventional or liquid based cytology. Randomised controlled trials comparing HPV testing alone or in combination with cytology versus sole cytology showed that 30-50% more CIN2+ lesions are detected by HPV testing. The higher NPV of HPV testing makes extension of screening interval possible, which makes the implementation of HPV testing as primary screening tool in cervical screening cost-effective. Nevertheless, HPV testing has a slightly lower specificity for CIN2+ compared to cytology. To increase the specificity additional triage of HPV positive women is therefore important. Based on two large trials with long term follow-up in The Netherlands (POBASCAM and VUSA-SCREEN) we evaluated more than 15 screening strategies including reflex cytology at baseline with repeat cytology at 12 and 24 months, reflex cytology with repeat cytology with HPV at 6 months, reflex cytology with repeat cytology with genotyping for HPV16,18 at 6 months and reflex cytology with HPV 16/18 genotyping at baseline with cytology at 6 months. To select the best strategy we calculated relative sensitivity for CIN2+, number of colposcopy referrals, and influence on cervical cancer incidence, taking into account that only one follow-up moment is preferable because of the loss of at least 20% of women in follow-up. We selected three strategies, which resulted in similar good results and these will be discussed.
LONGITUDINAL RESULTS FROM THE FINNISH RANDOMISED TRIAL ON HPV SCREENING

Ahti Anttila, Laura Kotaniemi-Talonen, Maarit Leinonen, Matti Hakama, Pekka Laurila, Jussi Tarkkanen, Nea Malila, Stefan Lönnberg and Pekka Nieminen

In Finland the conventional cytological screening programme for cervical cancer has markedly reduced the burden of cervical cancer. In the era before screening, the lifetime cumulative incidence of cervical cancer was about 2% and cumulative mortality 1% in the female population by age of 85 years; whereas nowadays they are 0.5% and 0.2%, respectively. In spite of high impact, there is a continuous need to assess developments in the diagnostic and clinical methods and study the potential for improved effectiveness and quality of life. From this background, primary screening using HPV-DNA with the Hybrid Capture 2 test was started within the Finnish programme in 2003, utilising a randomised screening design integrated into the programme. The main aim of the evaluation trial is to assess performance and effectiveness of HPV screening in comparison to cytological screening, by using subsequent cervical cancers as the ultimate outcome and screen- or otherwise detected pre-cancers as surrogate endpoints and markers. Also, future policies on targeting alternative age groups and screening intervals will be studied. For the time being, approximately 200,000 women have been invited to HPV screening or conventional cytology, with a 1:1 randomisation ratio. Information on the accrual invitation as well as follow-up data until the second invitation round has become available. Current results on cross-sectional and longitudinal data after the accrual invitation have shown an increase in the detection of CIN3+ in HPV screening compared to cytological screening. This indicates a potential to further decrease cervical cancer burden in the screened population. One problem is that detection of CIN1 and CIN2 is also higher in HPV-screening, compared with conventional cytology, indicating overtreatment. On the other hand, longitudinal information on impacts on cervical cancer will become available much later, from 2015 onwards, because cervical cancer incidence and mortality rates are extremely low (incidence at about 5/100,000 woman-years) after a negative cytological screening test in the programme and long duration of CIN, also of CIN3, if progressing to cancer. It is essential to include information on all screening and diagnostics services related to cervical smears within the health care into the evaluation. Meanwhile, in absence of sound observational information on cancer outcome, it is difficult to judge an optimal policy, i.e., how to maximise the impact and optimise quality of life. It is evident that one needs to consider fewer screening rounds in the future than the 7-9 invitations in a lifetime as done nowadays in the cytological screening programme, reducing potential adverse effects related to mild lesions or to borderline findings.

COMBINED HPV-CYTOLOGY SCREENING (CO-TESTING) VERSUS HUMAN PAPILLOMAVIRUS (HPV)
-ONLY BASED SCREENING

Castle, PE. U.S. National Cancer Institute, NIH, DHHS

Papanicolaou (Pap) smears/cervical cytology-based screening programs have been responsible for reducing cervical cancer incidence and mortality by 70% or more in countries that have effectively implemented it. Despite the success of cervical cytology, there is now overwhelming evidence that testing for carcinogenic human papillomavirus (HPV) DNA is more reliable and is 20%-40% more sensitive for the detection of cervical precancer and early cancer than any cytologic method. A randomized clinical trial demonstrated that a one-time screening using carcinogenic HPV DNA testing is more effective than Pap smears or VIA in reducing cervical cancer-related mortality. Given the evidence, it is now rational to switch to HPV-based screening. However, should the format be HPV-only based screening, with cytology or other methods for risk stratification among the HPV-positive women, or combined HPV and cytology screening (co-testing), which has been accepted as screening modality for women aged 30 and older in the U.S.? Because carcinogenic HPV DNA testing is approximately 95% sensitive for detection of any cervical precancer and cancer diagnosed within a few years of testing, inclusion of cytology to the primary screening adds only incrementally to the clinical sensitivity and reassurance of positive and negative HPV testing result, respectively. However, inclusion of cervical cytology will detect the relatively rare false-HPV negative cervical precancer and cancer, perhaps safely permitting even longer interval extensions among screen-negative women and providing greater reassurance among those who are rarely or irregularly screened. In addition, cervical cytology in the context of HPV testing may provide some added benefit of early detection of rare, non-HPV-related cervical cancers and some endometrial cancers (i.e., women with HPV-negative atypical glandular cells). A formal cost-effectiveness analysis is needed to assess under what scenarios (e.g., the length of the time interval between screenings among screen negatives) the inclusion of cytology with HPV testing could be cost-effective.

Reference List

Recently published randomized clinical trials, conducted in four European countries and Canada, consistently showed that HPV screening has a higher sensitivity for present or incipient high-grade cervical intra-epithelial neoplasia (CIN) or adenocarcinoma in situ than conventional cytology (pooled sensitivity ratio: 1.58 (95% CI: 1.36-1.83, p for inter-study heterogeneity not significant). However, in the ARTISTIC trial (UK), HPV screening was not more sensitive than screening with liquid-based cytology in detecting CIN2+ (pooled ratio 1.06; 95% CI: 0.87-1.69). The gain in sensitivity by adding cytology to HPV screening was small and statistically non-significant (pooled ratio: 1.04; 95% CI: 0.87 to 1.25).

The increased number of screen-positive women that results from HPV testing requires adequate triage strategies.

The most important aim of the trials was to demonstrate over time a reduced cumulative incidence of cervical intraepithelial neoplasia (CIN) of grade 3 or worse (CIN3+) among women who tested baseline HPV negative compared to those who had normal cytology (relative risk [RR] lower than 1). Lower cumulative incidence of CIN3+ can be considered as a proxy for decreased incidence of cancer.

This outcome was observed in all the trials that have published longitudinal outcomes of the second screening round, 3-6 years after the first round: RR = 0.53 in the Swedish and English trial, 0.45 in the Dutch trial, 0.29 in the first phase of the Italian trial, with confidence intervals always excluding unity.

These data indicate that HPV screening picks up more progressing cervical lesions than cytology and provides evidence for cervical cancer screening with an HPV assay followed by triage of HPV positive women. More in-depth meta-analyses and research should define best triage policies, target age groups and screening intervals, taking into account that certain future generations of women will have a lower background risk for developing cervical cancer (precursors) because of HPV vaccination.
REPORTING WOMEN’S ATTITUDES TO PREVENTIVE GYNAECOLOGICAL HEALTHCARE: SURVEY OF MORE THAN 6000 WOMEN IN THREE EUROPEAN COUNTRIES

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Objectives: A survey to assess how women, aged 18 to 50, are followed for gynaecological healthcare and their knowledge about causes and prevention of cervical cancer and HPV vaccination.

Methods: A total 6180 women from three European countries (Belgium: 2104; Germany: 2022; Spain: 2054) participated in an internet-based survey. Quota sampling was used to ensure that the women were representative on the basis of their age, region and education level. As the survey was long, the women could complete the questions in several sessions. The responses were analysed using descriptive statistics.

Conclusions: Eighty six percent of the women declared that they saw a doctor for gynaecological monitoring and 50% had started at 18 years old or younger. Forty percent of the women, irrespective of their age, said they found it difficult to talk with their physician about their sexual matters. Only 20% of women had talked about prevention of cervical cancer and 18% had talked about HPV vaccination for themselves with their gynaecologist. The women who saw their doctor more frequently were more likely to talk about these topics with them. 45% of the women said they did not know if they were at risk for HPV and 23% said they thought they were not. Fifty six percent said they thought women who have a relative who had cervical cancer should undergo HPV vaccination, and 42% said those with a genetic predisposition should be vaccinated, showing a misunderstanding of the cause of cervical cancer and the indications of HPV vaccination. The survey results imply that there are still many women who probably do not understand the significance of an abnormal Pap result, nor the link between HPV and cervical cancer. These results suggest that educational programmes on the causes and prevention of cervical cancer need to be intensified. Gynaecologists, who are mainly responsible for gynaecological monitoring, play a pivotal role in women’s health education. They are also essential for providing women with information about the role of HPV vaccination in preventing cervical cancer and the other benefits women in different age ranges could expect from vaccination.

QUALITY OF LIFE LOST FOLLOWING AN ABNORMAL CERVICAL CYTOLOGY RESULT: A PROSPECTIVE 3-MONTH STUDY

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Objectives: Vaccination against human papillomavirus is now recommended in many countries. Although valid quality-adjusted life-years (QALY) lost are needed to assess the cost-effectiveness of vaccination, very few data are available on QALYs lost because of an abnormal cervical Pap result. The aims of this study were to describe the psychosocial impact of having an abnormal cervical Pap result and prospectively estimate the QALY loss attributable to an abnormal result.

Methods: Between 08/2006 - 08/2008, 492 women with an abnormal Pap result and 471 women with a normal result, matched for age and clinic, were recruited across Canada. Health-related quality of life was measured at recruitment, and 4 and 12 weeks later with the following measures: EuroQol (EQ-5D and VAS), Short Form-12 (SF-12), short Spielberg State-Trait Anxiety Inventory (STAI-6), and HPV Impact Profile (HIP). EQ-5D and SF-12 scores were transformed into utility scores for QALY estimation. QALY loss during the 3-month period was estimated by aggregating differences over time between the utility scores of women with abnormal results and those of controls, adjusted for confounding factors.

Conclusions: At baseline, 46% of women with an abnormal result reported symptoms of anxiety/depression compared to 32% of the controls (p=0.0001). An abnormal Pap result was also associated with poorer mental health on all other mental health measures (SF-12 mental health, STAI-6 and HIP, all p-values < 0.0001). All between-group differences decreased over time. However, three months after getting their Pap results, women with an abnormal result still had significantly higher levels of EQ-5D anxiety/depression (p=0.0002), HIP scores (p<0.0001), SF-12 mental health (p=0.05) and higher STAI-6 anxiety (p=0.004), when compared to controls. The other EQ-5D domains (mobility, self-care, usual activities, pain/discomfort) were not or only slightly different between groups. As a result, QALY losses were small over the 3-month period (EQ-5D and SF-12 baseline utility weights both = -0.03; EQ-5D QALY loss = 0.006, SF-12 loss = 0.007). Receiving an abnormal cervical Pap result mainly affects domains of mental health and produces, over the first three months, QALY losses equivalent to 2.5 days of healthy life lost.
ES 1-3

PSYCHOSOCIAL IMPACT OF HPV-RELATED DISEASES IN WOMEN IN THE UK: THE PASQUAL STUDY

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Objectives: Data on health-related quality of life in cervical cancer patients are well reported, but data on other HPV-related diseases are scarce. Our objective was to assess the psychosocial impact of selected HPV-related diseases in the UK, using specific and generic instruments.

Methods: PasQuaL (Papillomavirus associated Quality of Life) was an observational cross-sectional study with a nested sub-population longitudinal follow-up. From May 2008 to March 2009, 1,264 subjects (men and women) aged 18-64 years, were recruited from 15 community and hospital healthcare clinics. They completed a series of self-administered instruments including the EQ-5D (generic) and the HIP (HPV Impact Profile), a specific tool capturing psychosocial aspects of HPV-related disease burden in women. Socio-demographic and clinical data were also collected.

Conclusions: Baseline analyses showed a mean HIP score [sem] of 21.67 [0.78] for normal cytology, compared with 39.43 [2.56] for borderline abnormalities and/or mild dyskaryosis; 41.88 [1.64] for CIN1; 44.71 [1.08] for CIN2/3; 43.81 [3.11] for VIN2/3; and 51.78 [2.06] for genital warts (p<0.05). For women with genital warts aged 18-25 years, the mean EQ-5D index score was 0.83, compared with 0.94 for the general UK female population (p<0.001). Women with VIN aged 18-64 years had a mean EQ-5D index score of 0.74, compared with 0.89 for UK population norms (p=0.001). Our data show that abnormal Pap smears, precancerous cervical and external genital lesions are associated with a significant decrease in quality of life. These results improve our knowledge and understanding of the qualitative aspect of HPV disease burden that could be prevented by quadrivalent HPV vaccination.

ES 1-4

SEXUAL PRACTICE AND HPV KNOWLEDGE AND RISK: WHAT CAN WE LEARN FROM THE AUSTRALIAN EXPERIENCE ABOUT WHO WILL BENEFIT?

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Objectives: This presentation draws on two nationally representative data sources to explore knowledge and behaviours relevant to HPV and the HPV vaccine. It examines these factors in the context of a vaccinated cohort of young women. For the past two years young women in Australia have been receiving the HPV vaccination mainly delivered via the schools vaccination program.

Methods: In late 2008 we asked 2,933 Year 10 and Year 12 students (both young men and young women) recruited from a representative sample of secondary schools throughout Australia, about their knowledge, attitudes and beliefs about HPV and cervical cancer, including their understandings of the vaccination. This information is set against their sexual practices and their trusted sources of information.

In the same year, drawing on the Australian Longitudinal Study of Health and Relationships (n= 9,542 men and women aged 16-64 years at recruitment), we examine current sexual practices and how they relate to age and gender of respondents. The specific sexual practices include age at first sex, serial monogamy, number of sexual partners and range of sexual behaviours, including oral sex. These data are interrogated with regard to HPV knowledge and risk.

Findings and Conclusions: Predictors of good HPV knowledge include gender (women have better knowledge), education level (higher education, better knowledge), age at first sex (lower age, poorer knowledge), relationship status those with a regular partner had poorer knowledge). There were no differences between states and territories. The implications of these changing sexual practices for vaccine acceptability and effectiveness will be discussed.
HPV INFECTION AND VACCINATION: LEVELS OF KNOWLEDGE AND ATTITUDES AMONG PRIMARY CARE PHYSICIANS

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Objectives: Primary care physicians have a key role in advising women about HPV tests and vaccination. Physicians' levels of knowledge about HPV infection and their attitudes towards vaccination will be an important determinant of the quality of the information women receive, thereby impacting on the likely success of cervical cancer prevention strategies. We undertook a national survey to determine HPV knowledge among primary care physicians in Ireland.

Methods: A questionnaire was mailed to a geographically-stratified random sample of two-thirds of primary care physicians in Ireland (n=1,995) during March-July 2007. This included a tool to assess HPV infection knowledge (13 factual questions), previously used in a US study, plus questions about knowledge, practices and attitudes towards HPV testing and vaccination.

Conclusions: The response rate was 44%. 16% of physicians answered ≥11 of the 13 questions on HPV infection correctly; 56% answered 8-10; 23% answered 5-7 and 4% answered 4 or fewer correctly. Male physicians, those who graduated longer ago, and those in solo practices were less likely to answer 8 of more questions correctly. 46% of physicians felt they knew enough about HPV infection to feel confident discussing it with patients. In general, levels of knowledge were lower in Ireland than in the USA. As regards HPV vaccination, 70% described their attitude as "positive" or "very positive". Only 29% felt they knew enough about HPV vaccination to feel confident discussing it with patients. One-third thought vaccination would give lifelong cervical cancer protection. Only 10% were aware that it could provide protection against other cancers. While 62% would be willing to vaccinate a sexually naïve girl under 16, more (72%) would administer the vaccine if she was sexually active. More than 50% would be willing to vaccinate sexually active women aged over 26. Knowledge of HPV infection was associated with willingness to administer vaccines to sexually naïve girls under 16. These findings highlight important gaps in primary care physicians' knowledge about HPV infection and vaccination. There is a need for further information or professional education initiatives among physicians to ensure that women have access to high quality information and advice.

KNOWLEDGE, ATTITUDES AND PRACTICES AMONG HEALTH CARE PROVIDERS REGARDING CERVICAL CANCER AND PREVENTION IN LAOS

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Background: Cervical cancer is the second most common cancer of females worldwide, but the commonest in low-income countries. Currently, little is known regarding cervical cancer incidence in Laos however it is anticipated to be high like in the neighbouring countries. To be able to develop cervical cancer prevention strategies it is essential to explore the knowledge and awareness of the disease not only among women but also among health care providers. The purpose of the current study was to evaluate the current level of knowledge regarding cervical cancer among staff working in facilities attended by adult women.

Methods: A descriptive quantitative study among health care providers, in two provinces in Laos, was conducted. Two-hundred and ninety-one medical workers, (doctors, medical assistants, midwives, nurses and nurses assistants), from provincial levels to health centers, were invited to participate. The study was a self-administered questionnaire containing both closed and open-ended questions about knowledge, attitudes and practice with respect to cervical cancer and screening.

Results: Two-hundred and sixty-one health care providers provided responses, 43% of them were nurses. Sixty-eight percent correctly identified cervix as the most common site for gynaecological cancer, but only 17% would be able to correctly identify risk factors associated with cervical cancer. Ninety-seven providers (37%) stated that they never discuss cervical cancer with their patients and 16% thought that Pap-smear was a test to detect sexually transmitted infections (STI). That early cervical cancer can be without any symptoms was known only by 116 providers (44%). It was more common that providers from Provincial Hospitals performed pelvic exams than providers from Health Care Centers.

Conclusion: The study indicated a lack of knowledge among health care providers regarding cervical cancer and its prevention. Providers need to be properly informed or educated about cervical cancer and prevention in order to motivate themselves and their patients to attend for examination. To be able to educate and introduce a future screening program an educational program for providers involved in women’s health is needed.

Keywords: Knowledge, attitudes, practice, cervical cancer, heath providers, Laos
ES 1-7

WHAT TEENS KNOW ABOUT HPV INFECTION AND VACCINATION

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Objectives: Evaluation of teens' knowledge on HPV infection and vaccination. to develop most effective educational tools.

Methods: During June, 2009, we’ve studied knowledge on HPV in 906 students (510 girls, 396 boys) of secondary school (mean age 15.7) by mean of an anonymous questionnaire, regarding HPV infection and related lesions, HPV transmission and vaccine, sexual/precautionary behaviours after vaccination. For statistical evaluation «2 test was performed.

Conclusions: 669/906 (73.8%) of students, [49.5% of boys and 92.7% of girls (p<0.001)] had heard about HPV (they form the study group-SG). 73% of SG reports sexual transmission of HPV, and 19% adds others wrong way. 88% knows HPV causes cervical cancer; only 19.6% retains HPV causes condylomata, but 23% believes HPV causes AIDS. Reported risk factor were: number of sexual partners (89% of SG), poor personal hygiene (55.8%), and smoking (5.4%). 71% considers condom the only preventive method, and other 21% adds wrong methods (personal soap, oral contraceptive-OC). 4.2% of SG reports only OC or personal soap as protective methods.

For diagnosis 62.8% reports medical examination and/or pap test, but 34.2% reports blood sample and ultrasound important for diagnosis.

645 students had heard about HPV vaccination; 91.3% of them knows that it protects against cervical cancer, 14% knows that one of the two available vaccine prevents condylomata. It is worrying that 10.6% thinks HPV vaccination protects against AIDS. 91% affirms it is important use condom during sexual intercourse out of a stable relationship after vaccination also, but only 70.8% affirms that vaccinated women will have pap test. No significant differences between boys and girls were observed in the SG.

Teen's knowledge about transmission and prevention of HPV infection is full of gaps, mainly: 1) to consider personal soap and OC preventing the transmission of HPV; 2) not identify risky behaviour; 3) to believe HPV causing AIDS. There is very poor knowledge on the relationship between HPV and condilomata, and about their prevention by one of the two available vaccine. The introduction of HPV vaccination may increase sexual risky behaviour among students believing it is protective against AIDS. To avoid dire consequences on the health of teen's it's important to increase and aim formative interventions at schools, taking advantage of the HPV vaccination campaign as a moment for sexual and health education.

ES 1-8

KNOWLEDGE OF HPV AMONG SWEDISH UPPER SECONDARY SCHOOL STUDENTS

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Objectives: To investigate knowledge of human papillomavirus (HPV) and attitudes to HPV vaccination and condom use among Swedish first year high school students.

Methods: In 2008, a classroom questionnaire filled in by 608 students from a strategic sample of seven high schools in Sweden.

Conclusions: The knowledge of HPV and of HPV vaccines was very low among first year high school students, despite marketing directed at potential vaccine consumers. Only 14% (n=82) of the students had heard about HPV and 6% (n=35) were aware of HPV vaccination. Girls and students in theoretical study programs generally had higher knowledge than boys and students in vocational programs. The students’ attitude towards vaccination was positive but most of them requested more information before considering vaccination (73%, n=443). Many students would like to receive such information from the school nurse (36%, n=220) or from the Youth clinic (30.6%, n=186). School nurses and nurse midwives thus have an important role to play in providing reliable information and counselling. The high cost of vaccination was the greatest obstacle (total group 37%, n=227); among girls the second major hindrance was the fear of needles (19%, n=65). Over 80% (n=512) of the students stated that they would be more inclined to be vaccinated if they knew that the vaccine also protected against genital warts. The students considered it less likely that they would use a condom when having intercourse with a new partner if they were vaccinated than if they were not (p<0.001). This risk should be taken seriously when planning information strategies about HPV.
CHANGE IN KNOWLEDGE OF WOMEN ABOUT CERVIX CANCER, HUMAN PAPILLOMA VIRUS (HPV) AND HPV VACCINATION DUE TO INTRODUCTION OF HPV VACCINES

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Objectives: Test knowledge of HPV, cervix cancer awareness and acceptance of HPV vaccination of women now and a year ago.

Methods: Questionnaire were filled out by 305 women visiting 4 gynaecologists of the Regional Hospital Heilig Hart, Tienen, Belgium during two subsequent weeks. Fisher T or Chi² were used as statistical methods to compare the data with the survey of 381 women exactly one year before.

Conclusions: Knowledge about HPV as a cause of cervix cancer and the presence of a vaccine rose from roughly 50% in 2007 to over 80% in 2008 (p<0.0001). Level of education and having daughters, boys or no children were no longer of influence in the level of knowledge or willingness to accept the vaccine. Most parents favour the age group 12-16 years as an ideal time for vaccination. In contrast with the 2007 survey, women below 26 had now acquired almost equivalent knowledge to older women about the virus, cervix cancer and the vaccine, but they were far less likely to accept the vaccine due to its cost price, unless it would be reimbursed (OR 4.2, CL95 1.6-11) p=0.0055). Therefore, one year after introduction of the first two prophylactic HPV vaccines, over 75% of women attending a ambulatory gynaecology clinic know HPV causes cervix cancer and that you can get vaccinated against it. Compared a year earlier, young and lower educated women had dramatically improved their knowledge. However, women below 26 are less prepared to pay the cost price for vaccination if it is not reimbursed.

KNOWLEDGE AND ATTITUDES TOWARD THE PROPHYLACTIC HPV VACCINE AMONG DOCTORS AND PATIENTS IN MEURTHE & MOSELLE (FRANCE)

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Objectives: Human papillomavirus (HPV) is the most common sexually transmitted infection and can lead to cervical and anogenital cancers and genital warts. Prophylactic HPV quadrivalent vaccine has been available in France since the end of 2006, and reimbursed since middle 2007. We assessed knowledge and attitudes of doctors and patients toward cervical cancer and its prevention, in Meurthe & Moselle (France).

Methods: Two-pages questionnaires were sent out to doctors in Meurthe & Moselle (general practitioners, gynecologists, and paediatricians) and to their over 14 year-old female patients, between January and March 2008. Response rates were: 11,1 % for practitioners, and between 4,1 and 36,7 % for patients.

Results: The knowledge rate about HPV vaccine was 97,4 % among doctors (better for over-40-year female general practitioners) and 78,8 % among women. Doctors got information from the health industry essentially while patients were mostly informed by the medias and their family doctors. Acceptability was high both among doctors (90 %) and patients (77 %, and 67 to 88 % of their parents). Reimbursement played a positive role multiplying by 10 prescriptions of the vaccine in our population. Reluctance to vaccin was scarce (3,5 % of our patients, 10 % of doctors) mostly due to lack of information. Regarding the cervical screening, it was significantly less known (67 %) and less available than HPV vaccine (80 %).

Conclusion: Levels of knowledge on the vaccine were quite satisfactory. Regarding the vaccine acceptability, we observed that family doctors and gynecologists played an important role, as well as the information level of the girls and of their mothers. These results highlight the need for additional education regarding HPV and cervical cancer prevention.
The Quality of Life of Patients with Genital Warts: A Qualitative Study

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Objectives: Genital warts (GW), which are caused by infection with human papillomavirus (HPV), are one of the most common sexually transmitted diseases in Europe. Although GW is commonly perceived as a non-serious condition, treatment is often long, of varying effectiveness and the recurrence rate is high. Very few studies have been performed on the personal consequences of GW. The aim of this qualitative study, set in Denmark, was to examine the ways in which GW may affect patients’ quality of life.

Methods: To obtain an in-depth understanding of patients’ perceptions of GW, we used qualitative focus-group interviews with five men and five women aged between 18 and 30 years who had GW. The interview guide was based on a literature review that identified important issues and questions. The data were analysed using a discourse theoretical approach.

Conclusions: The participants in this study considered their quality of life to be significantly lowered because of GW. Their experiences with GW were related to stigmatising cultural conceptions of venereal diseases and to the respective identities and sexuality of the sexes. The disease had negative psychological and social effects both for men and for women and it affected their sex and love lives, in particular. The psychological burden was increased by the uncertain timeline and the varying effectiveness of treatment. We identified a need for more patient information about the disease and its psycho-sexual aspects. The experiences described by the participants gave insights that may be valuable in treatment, counselling and prevention of GW. The quadrivalent HPV vaccine that has now been added to the childhood vaccination programme for girls in Denmark to prevent cervical cancer can also prevent 90% of cases of GW. Our results suggest that HPV vaccination could considerably reduce the largely unacknowledged psychological and social burden associated with GW. The results of a follow-up study of the long-term effects of GW will be included in the presentation if data are available at the time of the congress.

Civil Society Advocacy in the Introduction of HPV Vaccination in Europe: An Analysis

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Objectives: An analysis to assess the role of civil society, notably patients’ groups and women associations, in the introduction of HPV vaccination in Europe. The analysis also looks at future perspectives for civil society advocacy.

Methods: Analysis of processes and trends unfolding in Europe and literature research.

Conclusions: In the evolving environment of vaccinology, it is becoming clear that it is only through the concerted and active efforts of all stakeholders, including policymakers, health care professionals, and crucially civil society that vaccination programmes can be implemented. Civil society advocacy contributed to focus attention and increase market access of HPV vaccines both at EU and country level. However, some interest groups also posed a challenge to the introduction of vaccination. Despite its inherent limitations, we find that civil society advocacy is bound to play a greater role in vaccines’ introduction and implementation. Indeed, the future of health care is going from a disease-centered to a “patient-centered” model of care. Moreover, civil society groups bring health matters closer to the lay public and public support has never been more essential to the sustainability of vaccination programmes. Finally, lessons from the HPV experience will benefit the AIDS community. This experience actually provides an unprecedented opportunity for civil society groups to inform future access strategies and implementation mechanisms for an AIDS vaccine.
A POPULATON-BASED STUDY INVESTIGATING THE AWARENESS AND KNOWLEDGE OF HPV AMONG PARENTS OF CHILDREN AGED 12-15 YEARS.

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Objectives: We assessed HPV awareness and knowledge among parents to children aged 12-15 years of age to get a better understanding of how knowledge is influenced by various sociodemographic factors.

Methods: We invited 16 000 parents to girls and 4000 parents to boys, randomly selected from the Swedish population. Response rates were 11 187 (70%) and 2 759 (69%), respectively. Awareness of HPV was measured by asking ‘Have you heard of a virus called human papillomavirus (HPV) before taking part of this study?’. Binomial logistic regression models were applied to investigate correlates of HPV awareness.

Conclusions: In total 24% (3347) of the parents had heard about HPV (fathers: 17% and mothers: 29%). Knowledge was assessed by asking parents who had heard about HPV if they thought HPV can cause cervical cancer (79% answered yes), if HPV can cause other cancers (21%), if HPV can cause condyloma (52%), if HPV is sexually transmitted (86%), and if both men and women can be infected (72% and 92%, respectively). A logistic regression analysis of level of HPV knowledge was performed where five correct answers to six questions was considered high knowledge. Sex, country of origin and education were the most important factors associated with high levels of HPV knowledge. Mothers had better knowledge than fathers (OR: 2.49, 95% CI 2.18-2.83). Being born in other country than European was associated with lower knowledge compared to being born in Sweden (OR: 0.48, 95% CI 0.35-0.67) and low education levels were associated with little knowledge compared to having education equal to high school (OR: 2.03, 95% CI 1.49-2.76) or education levels above high school (OR: 6.13, 95% CI 4.51-8.31). Our results suggest that HPV information campaigns should particularly target parents with low education and born outside Sweden when promoting greater and more adequate knowledge of HPV in the population.

HPV VACCINE INTRODUCTION IN EUROPE: LESSONS LEARNED

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Objective: The 53 countries of the WHO European Region are diverse in income levels and health care systems. To date, 17 countries have introduced the HPV vaccine, with varying success and documentation of their immunization schedules. However, there has been scant documentation of these successes and the challenges encountered with HPV vaccine implementation in the WHO European Region in a consistent, standardized manner to allow for lessons learned. These experiences could benefit other countries in the Region considering HPV vaccine implementaton.

Methods: We will conduct a standardized phone interview with the 17 European countries that have introduced the HPV vaccine. Information regarding 15 items such as implementation strategies, target population, communication, funding, challenges, and coverage will be obtained using a standardized data collection questionnaire. Countries agreeing to participate will receive the questionnaire prior to the phone call. Data will be aggregated where similar strategies have been used to implement the vaccine.

Conclusions: In the WHO European Region, success of HPV vaccine introduction has varied widely, with some countries obtaining high rates of coverage and others very low. Financing mechanisms have also varied substantially in this economically diverse WHO region. Information from the interviews will be tabulated and presented, with specific examples offered. We will present strategies on the decision to use the vaccine and how it was implemented (mass catch-up campaigns or routine immunization programmes, and whether a comprehensive approach was used). We will collect and present samples of vaccine education offered to providers, patients, and media as well as problem solving strategies for handling adverse events and negative publicity. We will address finance mechanisms in this varied region with middle and high income countries. Disseminating the collective experiences of HPV vaccine implementation successes and challenges is vital to enable assistance to countries as they seek guidance towards introduction of the HPV vaccine.
Background: Since July 2007, primary prevention against Human Papilloma Virus (HPV)-related pre-neoplastic and neoplastic lesions through HPV vaccines is reimbursed by the social security (CPAM) in France. The vaccine is routinely recommended for 14 year-old girls and a catch-up vaccination should be offered to girls and women ages 15 to 23 before the first time they had sex or within one year of first having sex. We have little data on coverage and compliance while these aspects influence the effectiveness of a national immunization program.

Objective: To evaluate the coverage and compliance of HPV vaccine in Paris.

Methods: By selecting the female population living in Paris, aged 14 to 23 years (31/12/2008) and affiliated to the social security (n=77744), we analyzed data on reimbursement of HPV vaccines by the CPAM. We evaluated the dynamic of HPV vaccine dose reimbursement between July 2007 and April/May 2009 for this population and studied factors associated with coverage and compliance.

Results: The coverage rate in the study population was 17% (at least one dose). The compliance was not satisfactory since a complete vaccination scheme was observed in less than 43% of affiliates. Two doses have been reimbursed to 26% and only one dose to 31% of affiliates. The analysis by age and district showed that coverage and compliance depended on age and average family income (higher among 15-17 years, and districts with higher median income).

Perspectives and conclusion: The elasticity of the immunization schedule could allow prompt corrective actions to avoid losing the benefit of vaccination procedures initiated but not completed. To raise awareness on HPV and vaccine in terms of coverage but also compliance should be encouraged to improve effectiveness of primary prevention against cervical cancer in France.

ELIGIBILITY AND WILLINGNESS OF FIRST-YEAR STUDENTS ENTERING UNIVERSITY TO PARTICIPATE IN A HPV VACCINATION CATCH-UP PROGRAM.

Objectives: In France, Human papillomavirus (HPV) vaccine is routinely recommended for 14-year-old girls; a catch-up vaccination should be offered to girls and women 15-23 years of age before the first time they have sex or within the first year after sexual activity begins. The aim of the present study was to examine the eligibility and willingness of first-year college students of Toulouse University (France) to participate in a HPV vaccination catch-up program, and to estimate their knowledge of HPV vaccination and cervical cancer screening.

Methods: The study was conducted from January to April 2008 simultaneously at the three university medical centres (Science, Literature-Psychology, Law & Social Sciences). Female students entering the University were asked to complete an anonymous questionnaire at the time of their preventive medical visit. The questionnaire included questions on demographics, knowledge about HPV vaccination, sexual behaviour, and willingness to participate in the French vaccination program. In total, 606 women from the 3 colleges were included. The response rate of the questionnaire was 93%.

Conclusions: The median age of participants was 19 and 8.3% of them had already been vaccinated. Of the respondents, 67% were sexually experienced and 25% of them had their first intercourse less than one-year prior. Among respondents, 43% were eligible for catch-up vaccination according to French recommendations. 64% of eligible students were willing to be vaccinated. The reasons for refusing vaccination were mainly lack of knowledge on the vaccine (57%) and fear of adverse effects (22%). We did not observe significant differences among the three colleges. 74% of questioned students had already heard about HPV and HPV vaccine. However, knowledge of HPV infections, associated diseases, and prevention was limited. That finding indicates the need to pursue educational campaigns about HPV-related diseases and their prevention.
ACCEPTABILITY OF HPV VACCINATION AMONG WOMEN OF RHÔNE-ALPES. HPV-FEM STUDY - REMPAR PROJECT
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Objectives: To assess women's knowledge and acceptability of the papillomavirus (HPV) vaccination, recommended in France for all 14-years old girls and between 15 and 23, only before the end of the first year of sexual activity.

Methods: In July 2008, a self-administered questionnaire on cervical cancer (CC) prevention was proposed to 18-65 year-old women living in the Rhône-Alpes region by 39 general practitioners (GP) representative of Rhône-Alpes GP.

Conclusions: Of the 1,478 responders (mean age 40.5 years), 290 women (19.6%) (mean age 44.8 years) had a daughter aged 14-23 years (aged 14: 48 (16.5%); 15-18: 162 (55.9%); 19-23: 80 (27.6%)).

In this group, 162 (55.8%) had a gynaecological follow-up every year, 246 (84.8%) have had a pap smear (PS) in the 3 previous years, and 37 (12.8%) have had an abnormal PS at least once. A total of 196 women (67.5%) exactly knew the role of PS. Among the 136 (46.8%) women asserting that they knew the CC causal agent, 36.8% mentioned HPV (ie. 17.2% of the 290 women). Among the 249 women (85.9%) saying they knew the HPV vaccine, 16.4% cited the suitable ages of vaccination and 31.3% the modalities related to sexual activity.

A total of 131 women (45.2%) were favourable to HPV vaccine (21.4% had at least one daughter already vaccinated and 23.8% decided to vaccinate their daughter); and 42.1% preferred to wait or were opposed (39.3% and 2.8% respectively). Of these, 37.7% thought their daughter was not concerned and 15.5% feared possible side effects or a lack of backward path.

Characteristics associated with HPV vaccine acceptability were age < 50 years (OR=2.9 [1.1-8.0]), having a child previously vaccinated against pneumococcus (OR=2.7, [1.1-6.8]), knowing the target population of the vaccine (OR=4.6 [1.3-15.8]), and not knowing the role of PS (OR=3.3 [1.03-10.8]).

The limited knowledge on the HPV vaccine could be a barrier to vaccination because mothers believe that their daughters are not concerned. Main factors associated with HPV vaccine acceptability are knowledge of the target population of this vaccine, previous acceptance of new vaccines and, unexpectedly, the not knowledge of the role of PS; whereas compliance to PS screening had no effect. A better understanding of HPV vaccination could improve parental acceptance of vaccine for the girls. Further results in socially underprivileged women should specify the social and cultural predictors of acceptability.

WHY “IT’S NOT FOR US”: THE VIEWS OF PARENTS AND GIRLS IN THE UK WHO DECLINED THE HPV VACCINATION - A QUALITATIVE STUDY
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Objectives: The UK HPV vaccination programme for girls aged 12-13 years commenced in autumn 2008. At the time of implementation it was not known what the vaccination uptake would be, or on what basis decisions to accept or decline the vaccination would be made. As part of a larger study to develop and evaluate key HPV messages relevant to eligible girls, their parents and health professionals, a qualitative study was conducted to explore the reasons for non-uptake among girls and their parents. This is one of the first studies to examine and report on the behavioural responses to HPV vaccination invitation, and the underlying rationales. The findings from this study have the potential to assist in the development of interventions to ensure that vaccination decisions are made not on misunderstandings about HPV and the vaccine, but made on an informed basis.

Methods: Invitations to participate were sent via school nurses within one UK Primary Care Trust to all girls aged 12-13 years who had declined the offer of the HPV vaccination, and to their parents. In-depth, one-one interviews were carried out with all responding girls (14), and parents (20). Parents and girls were interviewed individually; a thematic analysis of verbatim transcripts was undertaken.

Findings: Reported reasons from the girls and parents for non-uptake of the HPV vaccination included: the age of the girls in terms of both the behavioural relevance of vaccination to them at that time, and their physical maturity; feeling pressured to make an immediate decision with insufficient information in a non life-threatening situation; the perception of the girls as ‘guinea pigs’ for an unproven vaccine; fear of unknown long term side-effects, and the impact of personal health histories and other experiences. This paper will focus specifically on the information needs which underpinned the reasons for non-acceptance of the vaccination, needs which were identified either by the participants, or apparent through their stated (mis)understandings. The implications of these findings to the ongoing implementation of the HPV vaccination programme will be considered and recommendations made.
Objective: To determine midadult women's knowledge, attitudes and perceptions about receiving prophylactic HPV vaccine.

Materials and Methods: Two hundred eighty-eight women 27-55 years who consulted at Philippine General Hospital Department of Obstetrics and Gynecology wards from May-June 2008 were made to complete a self-administered questionnaire that included demographic, reproductive and sexual history, knowledge, attitude and perception variables as potential correlates of vaccine acceptability. Chi square test was used to assess association of vaccine acceptability with these variables.

Results: Seventy two percent have heard of the HPV vaccine prior to the conduct of the study mainly from television followed by health centers. 50% have identified HPV as a specific risk factor for developing cervical cancer although 80% think that it is caused by an infection that is sexually transmitted. Overall acceptability rate is 79.2%. The main reason for wanting the vaccine is prevention of cervical cancer (86.40%) while the main reason for not wanting the vaccine is being in a monogamous relationship (65%). Majority of the respondents (93.86%) thought that men should also be vaccinated against HPV. There was significant association found between vaccine acceptability and age, employment status, gross family income, educational attainment, number of lifetime sexual partner, history of previous abortions and miscarriages, and perceptions of HPV and HPV vaccine.

Conclusion: There is high level of interest and acceptance of the prophylactic HPV vaccine by midadult women however its cost hinders vaccination to many.

ES 2-9

CERVICALSCREEN SINGAPORE - GEARING UP FOR THE NEXT LAP

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Objective: In Singapore, cervical cancer is the 6th most common female cancer and the 8th leading cause of cancer-related death in women. Each year, 172 women are diagnosed with cervical cancer and 77 women die from it. A population-based screening programme, CervicalScreen Singapore (CSS), introduced in 2004, encourages women aged 25 to 69 years to go for a Pap smear every 3 years. This paper 1) reports the knowledge and screening behaviour among women in Singapore, and the differences among the 3 main ethnic groups, 2) evaluates the effectiveness of the screening programme, and 3) highlights key gaps and opportunities to improve cervical cancer screening in Singapore.

Method: From the National Health Survey 2004, 80.8% of women were aware of the Pap smear test. The proportion of women who had ever had a Pap smear increased from 64.2% in 1998 to 70.1% in 2004. Women who had never had a Pap smear cited the following key reasons for their non-attendance: 1) “Not necessary as I am healthy” (28.6%), 2) “Never heard about Pap smear test” (22.4%) and 3) “Not sexually active” (13.2%). Between August 2004 to December 2008, 81,087 women were screened in the government clinics under CSS. Of the 1,340 women referred for assessment, 124 pre-invasive cancers and 26 invasive cancers were detected (0.33 per 1,000 women screened). Indian women had the highest lost-to-rescreen rate (75.4%) followed by Malay women (72.4%) and Chinese women (69.5%). Sensitivity for the test was 66.7% for first screens, while specificity was 88%. The proportion of pre-invasive cancer (CIN III) had also increased from 86% to 88% from 2004 to 2008.

Conclusion: While the findings indicate that the diagnostic accuracy and cancer detection rate for CSS are comparable to that of other more established programmes, one key challenge faced is bridging the knowledge-practice gap. To ramp up the coverage of cervical cancer screening, tailored marketing strategies are needed to reduce the barriers towards screening and to increase the rescreen rate, especially among the Indians and Malays. Greater accessibility of cervical cancer screening has also been offered to the community and workplaces through the Chronic Disease Management Programme-GP clinics and mobile screening bus.
IMPLEMENTING HPV TEST IN PUBLIC HEALTH HOSPITAL.

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Objectives: To analyse the performance of HPV test assessed with hybrid capture II (HC2) assay in women submitted to the gynaecological examination at Hospital das Clínicas of FMUSP in order to consider the introduction of molecular investigation of HPV as an alternative or complementary tool to Papanicolaou test (PapTest).

Methods: This analysis is part of a cross-sectional study carried out at a large public hospital attending predominantly low-resource population. The rationale of this study was to combine HC2-HPV test with the conventional Pap smear examination in women referred to gynecology examination for different reasons (previous abnormal PapTest, follow up of treated cervical lesion, ecc).

Conclusions: Seven hundred and four women were included in the analysis: 272 HPV positive (mean age of 36.3 years) and 432 HPV negative (mean age of 41.2 years). From HPV negative group, 3 cases were biopsy proven cervical high grade squamous intraepithelial lesion (HSIL), 2 high grade vaginal lesion (VAIN) and 1 vulvar high grade lesion (VIN); from the HPV+ group 18 were HSIL, 24 low SIL and 45 were cervicitis; also 2 VAIN 2 VIN and two vaginal and vulvar invasive carcinomas were identified. Papanicolaou test revealed 100 LSIL against 26 (26%) LSIL confirmed by biopsy; moreover, from 51 HSIL detected by cytology, only 13 were biopsy-proven. From HPV positive group, 132 biopsies were taken, and 70 of them (53%) have showed lesions. HPV positive test PapTest showed concomitant positive biopsy in 36 cases (53%), but in 13 (18%) cases PapTest showed categorization inferior to the biopsies, including 2 cases of invasive squamous cell carcinoma cytologically classified as HSIL. HPV test has proven to be more sensitive than cytology to recognize cervical alterations, including high grade lesions, in spite of slightly superior specificity of PapTest.

HPV VACCINE ACCEPTABILITY IN UNIVERSITY-AGED MEN

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Objectives: While several studies have explored factors related to HPV vaccine acceptability in women, few have studied vaccine acceptability in men. The present study examined HPV and HPV vaccine knowledge, attitudes, and beliefs and vaccine acceptability in university-aged men.

Methods: 45 male undergraduate university students (mean age = 20.4 years, SD = 1.7) participated. Participants completed a questionnaire assessing: (1) demographics, (2) knowledge about HPV and the HPV vaccine, (3) perceived susceptibility to and severity of HPV, (4) perceived advantages and disadvantages of the HPV vaccine, (5) physicians' and significant others' recommendations, and (6) sexual health and history.

Conclusions: Male undergraduate university students had limited knowledge about HPV and the HPV vaccine. While most had heard of HPV and the vaccine, knowledge was extremely low (M = 8.9 correct knowledge questions out of 22, SD = 4.9). Most men were not aware of the role of HPV in causing cervical cancer and genital warts, or the vaccine's role in preventing HPV infections that may lead to either of these diseases. Perceived knowledge (what the men thought they knew) about HPV and the HPV vaccine was low overall (M = 8.0 out of 21, SD = 4.3). Men not intending to receive the vaccine (60%) had significantly lower levels of perceived knowledge about HPV and the HPV vaccine, believed that the HPV vaccine was beneficial for women only, and believed that they were not susceptible to HPV. The most common reasons men indicated for receiving the vaccine included to protect themselves and/or their partner and to prevent the spread of HPV. Men who intended to receive the HPV vaccine also reported more favourable recommendations from physicians and significant others regarding the vaccine than those who did not intend to receive the vaccine (t(25.18) = -3.15, p < .01). Finally, frequency of condom use (r = 0.37, p < .05) was also, related to intentions to receive the HPV vaccine. It may be important to educate men about HPV and the HPV vaccine to increase their knowledge before the vaccine is approved for them. Once the vaccine becomes available for men, physician's recommendation may be a critical factor for vaccine uptake.
**Drivers and Barriers to Acceptance of Human-Papillomavirus Vaccination among Young Women**

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**Objectives:** Human papillomavirus (HPV) is a necessary cause of cervical dysplasia and cancer, and of genital warts. Few studies have examined attitudes to HPV vaccination since the introduction of HPV vaccines. We aimed to investigate the reasons for the acceptance or rejection of the quadrivalent HPV vaccine after its availability in Denmark.

**Methods:** A literature review assessed attitudes towards HPV vaccination and was used to identify relevant questions for telephone and focus-group interviews with women aged 16-26 who had decided to accept or reject HPV vaccination. 435 women across Denmark were interviewed by telephone. Qualitative focus-group interviews with a total of 33 women who had completed the telephone survey were undertaken. Four focus groups were set up according to age (16-20 and 21-25 years of age) and acceptance or rejection of the vaccine.

**Conclusions:** Of 839 women initially contacted, 94.6% had heard of HPV vaccination. 49% of the women said they accepted vaccination but only 24% had actually started or completed the vaccination series. 28.8% said they refused vaccination. Knowledge about HPV and its role in the development of cervical cancer and genital warts was poor. Prevention of cervical cancer was the main driver for acceptance of the vaccine, followed by parental encouragement and financial support, personal experience of someone with cancer and recommendation by health-care professionals. The greatest barrier to vaccination was its cost. A lack of information about the benefits of vaccination for sexually active women was also an important barrier and the older participants in particular considered that they were too old to be vaccinated. The difference between intention to be vaccinated and actually starting vaccination was considerable, and a large proportion of women aged 16-26 did not wish to be vaccinated. If the most important barriers to vaccination were addressed, it is likely that the uptake of vaccination in Denmark would increase substantially.
ORAL CONTRACEPTIVE USE AND RISK OF CYTOLOGICAL ATYPIA OF THE CERVIX

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Objectives: to assess by cytological method, the risk of cervical atypia associated with use of oral contraceptive (OC).

Methods: In this descriptive study, the risk factor for cytological atypia of the cervix among 100 women getting Papanicolaou smears at a referral gynecological Clinic, Khartoum, Sudan, was investigated. Of the 100 women, 50 were OC users ascertained as cases and 50 were non-OC users ascertained as controls. Relative risk (RR) and Odd Ratio (OR) were used for statistical analyses.

Two cytologists blindly categorized results from all women as normal (n=91) and atypia and low grade SIL (n=9). Out of the nine women with atypia and low grade SIL, seven (78%) were among cases and two (22%) among controls. Women with cytological atypia in each of the two groups were compared to women with normal cytological diagnosis. For the atypia among OC users, adjusted OR and the 95% CI were found to be 14.7 (13.07-16.32). Five women were detected as having candidasis, of whom four were cases (RR = 4.0). Furthermore, three of OC users were found with cytological evidences of viral infection.

Conclusion: OC use synergistically increased the risk of cervical cytological atypia and susceptibility for candidasis. Although, small numbers and inappropriate method prevented a reliable evaluation for viral infection, but the relation between OC use and susceptibility for viral infection require further assessment.
**P EP-2**

**HPV INFECTION AND MENOPAUSE. ESTIMATION OF EPIDEMIOLOGICAL DATA AND CLINICAL OUTCOME**


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**Objectives:** The epidemiological study of HPV infection in women in menopause and post-menopausal period. Evaluation of epidemiology in relation to other age groups and evaluation of clinical outcome of HPV infection in the post-menopausal period.

**Methods:** 168 asymptomatic women aged 48 to 60 years who attended the Gynecology Clinic of the “St. Savvas” Anticancer Oncology Hospital of Athens between May 2003 and September 2006 for cervical screening with Papanicolaou-test underwent cytological and HPV molecular analysis for HPV testing and HPV type distribution. The total prevalence of HPV infection regardless the HPV type and the related disease was 35% including oncogenic and non-oncogenic types. Regarding the HPV types distribution the most common oncogenic type was HPV 16 and the most common non-oncogenic type was HPV 42 followed by the HPV type 6. We must underline that in the negative cytological Pap smears, HPV prevalence was 26.4%.

**Conclusion:** This study provides very important information of the epidemiology in Greece due to the fact that the epidemiological literature data of HPV infection in this age group of women after menopause are limited. In addition we follow this age group in order to study factors that may be associated with increased HPV infection and factors influencing the evolution of HPV infection and developing cervical pathology in relation with the hormonal activity of women as shaped in the post-menopausal period.

**P EP-3**

**THE COLUMNAR EPITHELUM HYPOTHESIS OF CERVICAL CARCINOGENESIS**

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**Objective:** It is widely agreed that HPV infection of the cervix is initiated when minor trauma (e.g., sexual intercourse) exposes the reserve cells of metaplastic squamous epithelium of the cervical transformation zone to the virus. But does the decades-old concept of microtrauma of the overlaying layers of metaplastic squamous epithelium as the point of viral entry hold up to morphological and functional scrutiny? No one has seen such epithelial defects under the microscope or colposcope.

**Conclusion:** The following hypothesis takes issue with the dogma of microtraumata of metaplastic squamous epithelium. It is hypothesized that the major pathway of cervical carcinogenesis starts with HPV infection of a distinct number of sub-columnar reserve cells of the columnar epithelium with and without microtraumata (Fig. 1a), not with infection of the reserve cells of the metaplastic squamous epithelium. This hypothesis provides explanations for the following findings:

1. Why do glandular, squamous and mixed lesions occur? The target cells for HPV, the subcolumnar reserve cells, can differentiate in both directions.
2. Why is malignant transformation of the original squamous epithelium of the cervix uncommon? [3] The subcolumnar reserve cells are never located in the area of original squamous epithelium.
3. Why are recurrence rates lower after excisional or destructive treatments of SIL and AIS? The predominant concentration of subcolumnar reserve cells is near the external os of the cervix. [4] This area is removed or destroyed during treatment.
4. Why are early age at first intercourse and multiparity risk factors for cervical cancer? Early age shows a physiological eversion of columnar epithelium onto the ectocervix (ectopy) and a most active transformation. Also pregnancy shows repeated eversion of columnar epithelium onto the ectocervix. Anatomically, the subcolumnar reserve cells can now be easily reached by HPV.

Scientific theories have to be continuously verified and falsified. The present “Columnar Epithelium Hypothesis of Cervical Carcinogenesis” abstains from assuming traumatic epithelium defects of the metaplastic squamous epithelium to explain the HPV infection of reserve cells and thus is more compatible with objective observations.
EVALUATION OF CERVICAL, PHARYNGEAL AND ANAL HPV STATUS OF PROSTITUTES

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Objectives: HPV is the most common sexually transmitted infective agent known today. Approximately half of sexually active women are infected at least once during their lifetime. Chances of being infected and transmitting the virus correlates with the number of sexual partners. Sexual intercourse is not even mandatory in terms of development of HPV infection, genital contact (skin to skin) is fair enough. The use of condom decreases the risk, but doesn't give full protection. When talking about STDs we must focus our attention on prostitutes, who are mostly endangered. Every fifth switch goes hand in hand with an HPV infection, which means that prostitutes and their partners are in greater jeopardy every day.

In malignant transformations of chronic HPV infections high risk HPV types are involved. Latest data show, that HPV is the primary cause of more than 95% of cervical, 50% of genital (vagina, vulva, penis), 70% of anal and 20% of oro-pharyngeal cancers.

Methods: A survey based prospective study was carried out screening the sexual habits of women prostitutes and their knowledge of HPV. Thorough STD screening was supplemented with HPV testing using cervical, anal and pharyngeal samples along with Pap tests.

Conclusions: The questioned population had bare knowledge about HPV, the infection and its consequences. It can be stated that almost every one of them uses condoms on a regular basis as protection against STDs. We found HPV DNA positivity in 82.4% (14/17) of the cases. 11 HPV genotypes were identified, mostly HPV 16 and 33. 53% of HPV positive women (9/17) tested positive for high risk HPV, 23.5% (4/17) was positive for more than one type of HPV. High risk HPV positivity was proven as follows: 41.1% (7/17) of cervical, 17.7% of anal (3/17) and 11.8% (2/17) of pharyngeal samples. Screening, sampling, testing and evaluation are still under way.

HPV infection is in most cases symptomless and remains latent for months or even years, carrying the potential of malignant transformation. Since prostitutes are sources of infection and their partners are in danger of getting infected thus becoming a significant health hazard, routine STD screening was among prostitutes (Chlamydia, Hepatitis-B, HIV, Syphilis, Gonorrhea - once in every 3 months) should include HPV testing as well.

ROLE OF PLACENTAL INFECTION BY HUMAN PAPILLOMAVIRUS IN SPONTANEOUS PRETERM DELIVERY: PRELIMINARY REPORT

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Objectives: Human papillomavirus (HPV) infection is common among sexually active individuals and in some cases this infection could coincide with pregnancy in women (4). HPV infection of extravillous trophoblast cells induces cell death and may reduce placental cells invasion into the uterine wall. For this reason, it could be associated with adverse reproductive outcomes attributed to placental dysfunction, including spontaneous preterm delivery (9). The aim of our study was to determine if placental infection is associated with adverse reproductive outcomes attributed to placental dysfunction, in particular with preterm delivery.

Methods: We conducted a case-control study to detect HPV-DNA in the extravillous trophoblast region of placenta. The research included 84 pregnant women. The study group consisted of 42 cases of spontaneous preterm delivery. The control group consisted of 42 women who delivered at term. Samples of tissue from placenta were collected after delivery. Seventy-eigh percent (33/42) of cases and 43% (18/42) of controls were positive for the virus. Logistic regression analysis confirmed that HPV was detected more frequently in placentas from spontaneous preterm deliveries than in placentas from controls.

Conclusions: Our results support the hypothesis that HPV might be associated with some cases of adverse reproductive outcomes, such as the occurrence of spontaneous preterm delivery. However, further investigation should be made to determine a possible involvement of HPV in the development of some complications of pregnancy (4,13).

References
INCIDENCE RATES OF GENITAL WARTS IN GERMANY
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2) Sanofi-Pasteur MSD GmbH, Leimen, Germany

Objectives: Data on incidence rates of genital warts in Germany are only available from one cross-sectional study that was based on a survey of gynecologists, urologists and dermatologists. This study could not provide population-based data and was too small to estimate the incidence rates in male patients. Objective of our study is to estimate the incidence rate of genital warts in a large population-based sample of the German population.

Methods: A cohort study is being conducted in a large health insurance database including more than 14 million insurance members for the years 2004-2006 all over Germany. A case of genital warts is considered incident if a disease-free period of 12 months preceded the diagnosis of genital warts. Incidence rates will be stratified by sex, age, place of residence (federal state), and migrant status. All newly diagnosed cases of genital warts will be classified according to the type of physician consulted and treatment received.

Conclusion: This study will provide important data on the burden of disease of genital warts in Germany. Ultimately, with longer follow-up extending beyond 2006, it will allow to analyze the benefit of quadrivalent HPV vaccination on the reduction of the burden of genital warts.

1 Hillemanns P et al. Estimation of the incidence of genital warts and the cost of illness in Germany: a cross-sectional study. BMC Infectious Diseases 2008; 8: 76

ABSTRACTS

RATES OF HPV-16/18 PERSISTENT INFECTION AND ASSOCIATED CERVICAL LESIONS BY INITIAL SEROSTATUS IN THE CONTROL ARM OF THE PATRICIA TRIAL
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Objectives: The AS04-adjuvanted human papillomavirus (HPV)-16/18 vaccine (Cervarix®; GlaxoSmithKline Biologicals) showed high efficacy against HPV-16/18-associated cervical intraepithelial neoplasia (CIN)2+ in PATRICIA (NCT00122681). We present incidence rates (IR) of HPV-16/18 infections and associated CIN lesions in the control arm of the study stratified by initial serostatus.

Methods: Women (15-25 years) were randomised to receive HPV vaccine (N=9319) or control Hepatitis A vaccine (N=9325) at Months 0, 1, 6. Cervical samples were tested every 6 months by PCR for HPV DNA. Gynaecological/cytological exams were performed every 12 months. Baseline HPV serostatus was assessed by ELISA. IR (i.e. number of subjects reporting ≥ 1 event per 100 person-years of follow-up) are calculated for HPV-16/18 incident and persistent infections (6 and 12 months), CIN1+ and CIN2+ for the control group in the according-to-protocol cohort for efficacy (women who received all 3 doses, DNA-negative at baseline and Month 6 for the HPV type analysed, no protocol violations; N=8069).

Conclusions:

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<th>HPV-16</th>
<th>Incident</th>
<th>6 month PI</th>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Incident</td>
<td>6172</td>
<td>687</td>
<td>4.77 (4.41, 5.17)</td>
<td>1048</td>
<td>85</td>
</tr>
<tr>
<td>6 month PI</td>
<td>6018</td>
<td>345</td>
<td>2.38 (2.12, 2.66)</td>
<td>1007</td>
<td>38</td>
</tr>
<tr>
<td>12 month PI</td>
<td>5903</td>
<td>175</td>
<td>1.20 (1.02, 1.40)</td>
<td>980</td>
<td>20</td>
</tr>
<tr>
<td>CIN1+</td>
<td>6165</td>
<td>70</td>
<td>0.47 (0.36, 0.60)</td>
<td>1047</td>
<td>12</td>
</tr>
<tr>
<td>CIN2+</td>
<td>6165</td>
<td>46</td>
<td>0.31 (0.22, 0.42)</td>
<td>1047</td>
<td>6</td>
</tr>
<tr>
<td>HPV-18</td>
<td>Incident</td>
<td>6751</td>
<td>509</td>
<td>3.19 (2.90, 3.49)</td>
<td>793</td>
</tr>
<tr>
<td>6 month PI</td>
<td>6567</td>
<td>188</td>
<td>1.17 (1.00, 1.36)</td>
<td>767</td>
<td>10</td>
</tr>
<tr>
<td>12 month PI</td>
<td>6440</td>
<td>70</td>
<td>0.44 (0.34, 0.56)</td>
<td>750</td>
<td>4</td>
</tr>
<tr>
<td>CIN1+</td>
<td>6746</td>
<td>31</td>
<td>0.19 (0.13, 0.27)</td>
<td>790</td>
<td>0</td>
</tr>
<tr>
<td>CIN2+</td>
<td>6746</td>
<td>15</td>
<td>0.09 (0.05, 0.16)</td>
<td>790</td>
<td>0</td>
</tr>
</tbody>
</table>

N=evaluable subjects in each analysis n=subjects reporting ≥ 1 event

Despite having generally lower IR estimates, DNA-negative/seropositive women in the control arm remained susceptible to re-infection and lesion development, even though they had presumably cleared an HPV infection with the same type. While serology is not a perfect marker for prior exposure to HPV, these findings suggest that natural immunity as detected by ELISA does not reliably protect against re-infection and disease with the same HPV type.
The two vaccines directed against the oncogenic human papillomavirus (HPV) types 16 and 18 may impact the burden of anogenital cancers beyond cervical cancer alone. One of these vaccines (Gardasil) may also have an impact on cancers associated with HPV 6 and 11. Estimates of the prevalence of various HPV-types were extracted from recent systematic reviews addressing the distribution of HPV-types in cancers of the vulva, vagina, penis, and anus. HPV-type distributions of precursor lesions such as intraepithelial neoplasia (IN) or squamous intraepithelial lesions (SIL) were also extracted when available.

Table 1 shows the prevalence of different HPV types in non-cervical anogenital cancers. Anal cancer had the highest overall HPV prevalence (71.2%), followed by vaginal (65.5%), penile (47.9%), and vulvar (40.1%) invasive cancers. For HPV 16/18 combined, anal cancer again had the highest prevalence (72.2%), followed by vaginal (54.5%), penile (36.7%), and vulvar (30.6%) cancers.

HPV 16 was the most prevalent HPV-type for all cancers and precursor lesions, except for vaginal intraepithelial neoplasia-1 (VIN-1) and anal low-grade squamous intraepithelial lesions (LSIL): VIN-1 had HPV 6 as the most common type (23.8%), while anal LSIL had HPV 11 as the most common type (40.8%) followed by HPV 6 (28.6%). For the vulva, vagina, and anus, the lowest-grade precursor lesions (VIN-1, VAIN-1, and anal LSIL) all had far lower prevalences of HPV 16 than the higher-grade precursor lesions (VIN-2/3, VAIN-2/3, and anal HSIL).

These prevalence figures can be used to estimate the potential reduction in the burden of non-cervical anogenital cancers by the HPV vaccines (data not shown in abstract). While these estimates assume that a vaccine is both 100% administered and effective, a worldwide increase in HPV vaccination for cervical cancer may have a noteworthy influence on these other HPV-related cancers. Furthermore, the prevention of precursor lesions such as vulvar or anal intraepithelial neoplasias may broaden the impact of vaccination beyond invasive cancers.

Table 1: Prevalence of HPV-types in Non-Cervical Anogenital Cancers

<table>
<thead>
<tr>
<th>HPV Prevalence (%)</th>
<th>Overall HPV (%)</th>
<th>HPV 16/18 (%)</th>
<th>HPV 16 (%)</th>
<th>HPV 18 (%)</th>
<th>HPV 6 (%)</th>
<th>HPV 11 (%)</th>
<th>HPV 31 (%)</th>
<th>HPV 33 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vulvar Invasive Cancer</td>
<td>40.1</td>
<td>30.6</td>
<td>29.3</td>
<td>5.6</td>
<td>0.8</td>
<td>3.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VIN-2/3</td>
<td>80.4</td>
<td>65.7</td>
<td>71.2</td>
<td>5.5</td>
<td>1.3</td>
<td>7.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VIN-1</td>
<td>77.5</td>
<td>14.3</td>
<td>14.3</td>
<td>0</td>
<td>23.8</td>
<td>3.2</td>
<td>1.6</td>
<td></td>
</tr>
<tr>
<td>Vaginal Invasive Cancer</td>
<td>65.5</td>
<td>54.5</td>
<td>55.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>VAIN-2/3</td>
<td>92.6</td>
<td>60.5</td>
<td>65.8</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>VAIN-1</td>
<td>98.5</td>
<td>40.9</td>
<td>17.9</td>
<td>17.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Penile Invasive Cancer</td>
<td>47.9</td>
<td>36.7</td>
<td>30.8</td>
<td>6.6</td>
<td>6.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anal Invasive Cancer</td>
<td>71.2</td>
<td>72.2</td>
<td>68.5</td>
<td>5.1</td>
<td>5.1</td>
<td>1.0</td>
<td>1.7</td>
<td>3.3</td>
</tr>
<tr>
<td>Anal HSIL</td>
<td>90.7</td>
<td>68.7</td>
<td>62.9</td>
<td>7.0</td>
<td>5.8</td>
<td>3.5</td>
<td>4.5</td>
<td>4.9</td>
</tr>
<tr>
<td>Anal LSIL</td>
<td>88.1</td>
<td>27.4</td>
<td>6.1</td>
<td>-</td>
<td>28.6</td>
<td>40.8</td>
<td>8.2</td>
<td>-</td>
</tr>
</tbody>
</table>

P IM-1

BASELINE INCIDENCE RATES OF IMMUNE-MEDIATED AND NEUROLOGICAL CONDITIONS IN ADOLESCENT GIRLS IN SWEDEN 2005-2007

Janzon L and Young C
Sanofi Pasteur MSD, Stockholm, Sweden

Background: The introduction of a new vaccine, such as Gardasil (Quadivalent vaccine against HPV type 6, 11, 16 and 18), on the market and especially if introduced in the national vaccination program, always raise concerns about safety. Analysis of post-marketing surveillance data and identification of safety signals are often complicated by poor knowledge of incidence rates of medical conditions in the target population before and after introduction. In addition, when a specific safety issue is raised as a consequence of a cluster of adverse events, these kind of data are often requested at a very short notice by competent authorities.

Objectives: The objective was to establish baseline incidence rates of medical conditions that might appear as potential adverse events after introduction of HPV vaccination in the Swedish vaccination program, including immune-mediated and neurological conditions, in Swedish girls 10 to 17 years of age, in 2005 to 2007 before extensive use of HPV vaccine. This is the target population for reimbursed or national program for vaccination against HPV. We also wanted to establish a method for fast and reliable retrieval of baseline incidence rates of medical conditions for which a safety signal is detected.

Methods: Two nation wide registries, held by the National Board of Health and Welfare in Sweden, were used as source of data: Diagnoses in in-patient care and Diagnoses in out-patient specialist care. Both registries are based on personal identity number and allow for extraction of information on selected diagnoses, based on ICD-10 codes, in specific cohorts of girls. Data from both registries on all medical events during 2005 to 2007 recorded for girls 10-17 years of age at the time of medical care, were combined into one database. Medical conditions of special interest for safety surveillance of vaccination with Gardasil were analysed.

Conclusion: We present data on base line incidence rates on immune-mediated and neurological conditions in the target population recommended for HPV vaccination. Moreover by establishing a targeted database with individual diagnose data from two national registries, we have a platform for evaluation of adverse events following HPV immunisation in the Swedish vaccination program and reimbursed catch-up vaccination of adolescent girls.
CROSS PROTECTION IN HPV IMMUNIZATION: WHAT CAN WE EXPECT?

DENIS F. 1, LEDCMACH Y. 2, HAESEBAERT J. 2, JACQUARD AC. 2, SOUBEYRAND B. 2

1 Service de Bactériologie-Virologie-Hygiène, CHU Dupuytren, Limoges, France; 2 Sanofi Pasteur MSD, Lyon, France.

Objectives: Beyond the vaccine types specific prevention, a reduction in CIN 2/3 associated with other HPV types including HPV 31 (cross protection [xprot]) was reported. However, a significant number of CIN 2/3 are co-infected with HPV non vaccine types and vaccine types (i.e. 16 and 18). Therefore, the public health significance of these findings remains to be ascertained. Nevertheless, xprot may provide an additional number of cervical cancer (CC) prevented by immunization. We investigated here the potential theoretical additional impact on public health of such xprot.

Methods: We developed a model using French national incidence data from the InVS: 3068 CC cases in 2005. Type’s distribution of single and multiple HPV infections in CC was issued from the EDITH I French study. For CC with multiple infections, CC was attributed preferentially to 16 and 18, and then to 31, and then to 9 other types (33, 51, 45, 52, 58, 35, 39, 59). Efficacy of the quadrivalent vaccine against 16 and 18 used was 98.2%, the actual point estimate at end of study in the PPE population; efficacy for xprot was derived from the unrestricted susceptible population data: 55.6% on HPV 31, 7.9% on the 9 other types. A 100% vaccine coverage has been hypothesized for this model.

Conclusions: among the 3068 annual CC cases, the quadrivalent vaccine can potentially prevent 2557 cases, counting for 83.3% of annual incidence of CC in France, including 2470 cases attributed to HPV 16/18 (80.5%), 66 cases due to HPV 31 (2.1%) and 20 cases due to 9 other types (0.7%). Impact of HPV immunization in CC prevention is mainly driven by direct protection against HPV 16 and 18. Xprot is only associated with prevention of 2.8% of cases. Several methodological concerns and bias have been described in the measurement of cross protective efficacy: 1) analyses involving CIN 2/3 with multiple HPV lead to difficulties in HPV type assignment of cases; 2) due to the high efficacy of the vaccine on HPV 16/18 CIN2/3 in trials, the number of colposcopy performed in the placebo group may have artificially increased the number of CIN2/3 associated with non vaccine HPV types. Moreover unknown factors persist on biological reliability of cross-protective antibodies. Their weak specificity and avidity associated with a potential rapid decline raises the question of the durability of their protective efficacy.

HPV Xprot is an attractive concept but its public health impact remain questionable; HPV vaccines impact will be mainly driven by direct protection.

LONG-TERM PERSISTENCE OF IMMUNE RESPONSE TO HPV-16/18 AS04-ADJUVANTED CERVICAL CANCER VACCINE IN PRETEEN/ADOLESCENT GIRLS AND YOUNG WOMEN

Petaja T on behalf of the HPV-012 Study Group
University of Tampere, Tampere, Finland

Objectives: Vaccination against oncogenic human papillomavirus (HPV) types prior to sexual debut and long-term protection are important for the overall strategy of cervical cancer prevention. The HPV-16/18 AS04-adjuvanted vaccine (Cervarix®, GlaxoSmithKline Biologicals) has previously been shown to be highly immunogenic in preteen/adolescent girls aged 10-14 years. This follow-up study assessed persistence of immune response and safety profile through 48 months after administration of the first vaccine dose.

Methods: This was an open, multicentre, extension of a study (107481/NCT00337818) conducted in Denmark, Estonia and Finland. Subjects aged 10-14 and 15-25 years received 3 doses of the industrial scale HPV-16/18 AS04-adjuvanted vaccine at 0, 1 and 6 months and completed their Month 48 visit. Anti-HPV-16/18 antibody titers in serum and cervicovaginal secretions (CVS) were assessed by ELISA through 48 months post-first vaccine dose.

Conclusions: At Month 48, all subjects remained seropositive for both anti-HPV-16 and -18 antibodies with geometric mean titres (GMTs) in younger subjects (vaccinated at 10-14 years, seronegative at baseline (n=51) approximately 2-fold higher than older subjects (vaccinated at 15-25 years (n=169)). In both age groups, GMTs at Month 48 were substantially higher than natural infection levels and above the plateau level observed in subjects from another study in which sustained efficacy has been demonstrated (studies NCT00120848 and NCT00122681). At Month 48 antibodies in CVS were detected in 84.1% (n=69) and 69.7% (n=66) of the older subjects who volunteered and for whom samples were available for anti-HPV-16 and anti-HPV-18, respectively. A strong correlation was seen between antibody levels in serum and CVS (correlation coefficients: 0.88 for HPV-16 and 0.90 for HPV-18), suggesting sustained transudation of serum IgG antibodies to the cervical epithelium. The vaccine was generally well tolerated through 4 years post-vaccination. Results show that the HPV-16/18 AS04-adjuvanted vaccine induces a robust and sustained immune response in girls and women aged 10-25 years. The higher antibody response achieved in young girls at Month 7 was sustained through Month 48 and may predict longer-term protection.
COMPARATIVE IMMUNOGENICITY OF TWO PROPHYLACTIC HUMAN PAPILLOMAVIRUS CERVICAL CANCER VACCINES AGAINST HPV-31 AND -45

Einstein MH on behalf of the HPV-010 Study Group
Montefiore Medical Center, Albert Einstein College of Medicine, Bronx, NY, USA

Objectives: The superiority of HPV-16/18 AS04-adjuvanted bivalent vaccine (GlucoSmithKline Biologicals) vs HPV-6/11/16/18 quadrivalent vaccine (Merck) has been demonstrated in terms of HPV-16 and HPV-18 immune response in a comparative trial (NCT00423046). Additional study objectives included assessment of vaccine-induced immune responses against non-vaccine types HPV-31 and -45.

Methods: Women (n=1,106) were stratified by age (18-26, 27-35, 36-45 years) and randomised (1:1) in this blinded study to receive HPV-16/18 vaccine (Months 0, 1, 6) or HPV-6/11/16/18 vaccine (Months 0, 2, 6). Serum antibody responses were measured by ELISA and PBNA, CD4+ T-cell responses by cytokine flow cytometry and memory B-cell responses by ELISPOT.

Conclusions: Seropositivity rates for HPV-31 and -45 were comparable for both vaccines (Table), with higher GMTs (by ELISA) in women 18-26 years after HPV-16/18 vaccine. Antibody levels measured by PBNA were low and comparable between groups. HPV-16/18 vaccine induced a higher proportion of HPV-31/45 CD4+ T-cell responders. HPV-31/45 specific memory B-cell responses were comparable between groups. Clinical cross protection against HPV-31 and HPV-45 has been shown to differ between both vaccines. Although the mechanism of cross protection should be further investigated, T-cell responses may play an important role.

Results for ATP cohort (DNA/seronegative and T-cell or B-cell negative at baseline); *statistically significant; †across all age groups; #ELISA; ¶≥500 cells/million cells; ‡>0 cells/million cells

<table>
<thead>
<tr>
<th>HPV-31</th>
<th>Month 7</th>
<th>Month 18</th>
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<tbody>
<tr>
<td>HPV-16/18 vaccine</td>
<td>97-100</td>
<td>100-100</td>
</tr>
<tr>
<td>CD4+ T-cell responders</td>
<td>589-1246</td>
<td>603-866</td>
</tr>
<tr>
<td>CD4+ T-cell responders</td>
<td>1246 [1013-1532]</td>
<td>866 [735-1020]</td>
</tr>
<tr>
<td>% responders †</td>
<td>85</td>
<td>68</td>
</tr>
<tr>
<td>Gmean</td>
<td>978*</td>
<td>664</td>
</tr>
<tr>
<td>HPV-45</td>
<td>99-100</td>
<td>100-100</td>
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<tr>
<td>CD4+ T-cell responders</td>
<td>634-1248</td>
<td>829-1104</td>
</tr>
<tr>
<td>CD4+ T-cell responders</td>
<td>1248 [1041-1497]</td>
<td>1104 [939-1297]</td>
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<tr>
<td>% responders †</td>
<td>77*</td>
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<tr>
<td>Gmean</td>
<td>738*</td>
<td>402</td>
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COMPARATIVE EVALUATION OF THE IMMUNOGENICITY OF TWO PROPHYLACTIC HPV CERVICAL CANCER VACCINES BY MERCK’S COMPETITIVE LUMINEX IMMUNOASSAY (CLIA) AND GSK’S BINDING ELISA

F. Dessy, S. Poncelet, V. Xhenseval, D. Méric, G. Dubin, on behalf of the HPV-010 Study Group
GlaxoSmithKline Biologicals, Rixensart, Belgium

Objectives: Two prophylactic human papillomavirus (HPV) vaccines are currently on the market, the HPV-16/18 AS04-adjuvanted vaccine (Cervarix®, GlaxoSmithKline Biologicals) and the HPV-6/11/16/18 quadrivalent vaccine (Gardasil®, Merck). Cervarix was shown to induce higher HPV16/18 antibody responses compared to the quadrivalent vaccine [1]. The objective was to confirm that the use of the vaccine-specific virus-like particles (VLPs) in the respective assays did not introduce a bias in the serological evaluation of immune responses to the vaccines.

Methods: Women (n=1106) aged 18-45, were stratified by age and randomized (1:1) in this blinded study (NCT00423046) to receive Cervarix (months 0, 1, 6) or Gardasil (months 0, 2, 6). A subset of Month-7 sera (i.e., one month after the third vaccine dose) was evaluated using Merck’s competitive Luminex immunoassay (cLIA) (using Gardasil VLPs [2]) and GSK’s binding ELISA (using Cervarix VLPs [3]).

Conclusions: For both HPV-16 and HPV-18 antigens, Cervarix recipients showed higher antibody titers than Gardasil recipients independently of the assay (see table). A good correlation between the two assays was observed both with Cervarix and Gardasil recipients. No bias was introduced by using either assay when comparing sera from individuals receiving either vaccine.

Cervarix

<table>
<thead>
<tr>
<th>HPV-16</th>
<th>N</th>
<th>ELISA</th>
<th>GMT*</th>
<th>95% CI</th>
<th>cLIA</th>
<th>r</th>
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<tr>
<td>72</td>
<td>6456</td>
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<td>6778</td>
<td>5643-8141</td>
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<tr>
<td>72</td>
<td>2956</td>
<td>2435-3569</td>
<td>1466</td>
<td>1106-1708</td>
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Gardasil

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<th>N</th>
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<th>GMT*</th>
<th>95% CI</th>
<th>cLIA</th>
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<td>2502</td>
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<td>68</td>
<td>701</td>
<td>556-883</td>
<td>338</td>
<td>259-440</td>
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</tbody>
</table>

*ELISA GMT in ELU/ml; †cLIA GMT in mMerckU/ml

1. Einstein 2009, Hum Vaccin 5:705-19

Cervarix is a trademark of the GlaxoSmithKline group of companies, Gardasil is a trademark of Merck.
**IMMUNOGENICITY COMPARISON OF TWO PROPHYLACTIC HUMAN PAPILLOMAVIRUS (HPV) CERVICAL CANCER VACCINES AT MONTH 24**

Einstein MH, on behalf of the HPV-010 Study Group

Montefiore Medical Center, Albert Einstein College of Medicine, Bronx, NY, USA

**Objectives:** Vaccine-induced protection against HPV-16/18 has been demonstrated for HPV-16/18 AS04-adjuvanted vaccine (HPV-16/18 vaccine; GlaxoSmithKline Biologicals) and HPV-6/11/16/18 vaccine (HPV-6/11/16/18 vaccine; Merck). The immune response (serological and cell mediated) was assessed 18 months after third dose (Month 24) in this comparative trial.

**Methods:** In blinded study NCT00423046, women (n=1106) were stratified by age (18-26, 27-35, 36-45 years) and randomised (1:1) to receive HPV-16/18 vaccine (Months 0, 1, 6) or HPV-6/11/16/18 vaccine (Months 0, 2, 6). In the ATP cohort for immunogenicity, antibody responses in serum and cervicovaginal secretions (CVS) were measured by PBNA and ELISA, memory B-cells by ELISPOT and CD4+ T-cell responses by cytokine flow cytometry.

**Conclusions:** At Month 24, immune responses remained higher with HPV-16/18 vaccine than with HPV-6/11/16/18 vaccine. Across all ages, serum neutralising antibody (nAb) geometric mean titres were 2.4-5.8-fold higher (p<0.0001) for HPV-16 and 7.7-9.4-fold higher (p<0.0001) for HPV-18 with HPV-16/18 vaccine vs HPV-6/11/16/18 vaccine. In CVS, nAb positivity rates for HPV-16/18 vaccine vs HPV-6/11/16/18 vaccine were 24.4% vs 11.6% for HPV-16 and 2.2% vs 0.0% for HPV-18, with higher positivity rates using ELISA (77.8 vs 55.8 for HPV-16 and 68.9 vs 39.5% for HPV-18). Proportion of detectable antigen-specific memory B-cells with HPV-16/18 vaccine vs HPV-6/11/16/18 vaccine was 83.3% vs 66.7% (p=0.2122) for HPV-16 and 76.3% vs 52.9% (p=0.0489) for HPV-18, with significantly higher corresponding geometric mean ratios (GMRs) in responders with HPV-16/18 vaccine for HPV-18 (HPV-16: 1.34, p=0.4174; HPV-18: 2.54, p=0.0071). Proportion of antigen-specific CD4+ T-cell responders (subjects with ≥500 cells expressing ≥2 cytokines/million cells) was higher with HPV-16/18 vaccine than with HPV-6/11/16/18 vaccine (HPV-16: 90.9% vs 60.0%, p<0.0128; HPV-18: 74.3% vs 40.0%; p=0.0152), with significantly higher corresponding GMRs (HPV-16: 2.42, p<0.0001; HPV-18: 2.36, p=0.0025). Differences in immune response between these two vaccines may represent determinants of duration of protection. Long-term follow-up of immune profile endpoints is ongoing.

**HUMAN PAPILLOMAVIRUS (HPV)-16/18 AS04-ADJUVANTED VACCINE ADMINISTERED ACCORDING TO AN ALTERNATIVE DOSING SCHEDULE**

Esposito S1, Birilutiu V2, Jarcuska P3, Perino A4, Man S5, Vladareanu R6, Meric D7, Dobbetaere K7, Thomas F7 and Descamps D7

1 Fondazione IRCCS Ospedale Maggiore Policlinico, University Milano, Milano, Italy; 2 County Clinic Hospital, Sibiu, Romania; 3 Medical Faculty, University of P. J. Safarik, Kosice, Slovakia; 4 Dipartimento Materno Infantile, University of Palermo, Italy; 5 University of Medicine and Pharmacy “Iuliu Hatieganu”, Cluj-Napoca, Romania; 6 Carol Davila University of Medicine, Elias University Hospital, Bucharest, Romania; 7 GlaxoSmithKline Biologicals, Wavre, Belgium

**Objectives:** GlaxoSmithKline Biologicals has developed a prophylactic HPV-16/18 vaccine, which has been shown to be immunogenic and generally well tolerated. The recommended schedule for this vaccine includes three doses to be given at Months 0, 1 and 6. This study (NCT00552279) evaluated immunogenicity and safety of the vaccine when administered according to an alternative dosing schedule (0-1-12 months) compared with the standard dosing schedule.

**Methods:** This was a randomised, open, multicentre study conducted in Italy, Romania and Slovakia. Healthy young women aged 15-25 years were randomised (1:1) to receive HPV vaccine according to the standard schedule (HPV M0-1-6) (n=401) or an alternative schedule (HPV M0-1-12) (n=403). Anti-HPV-16 and -18 antibodies were measured by ELISA at Months 0, 2 and 7 or 13 (depending on group). Non-inferiority was tested sequentially for anti-HPV-16 and -18 seroconversion rates and geometric mean antibody titres (GMTs). The primary analysis of immunogenicity was based on the according-to-protocol cohort for immunogenicity. Vaccine safety and reactogenicity were also assessed.

**Conclusions:** The antibody response to HPV-16 and -18 induced by the alternative schedule (HPV M0-1-12) was non-inferior to the standard schedule (HPV M0-1-6) in terms of seroconversion rates for HPV-16 (100% vs 100%; difference: 0% [95%CI: -1.11, 1.13]) and HPV-18 (99.7% vs 100%; difference: 0.29% [-0.81, 1.62]) and in terms of GMTs for HPV-16 (11884.7 vs 10311.9 EU/mL; GM ratio [HPV M0-1-6/HPV M0-1-12]: 0.87 [0.75, 1.00]) and HPV-18 (4501.3 vs 3963.6 EU/mL; GM ratio: 0.88 [0.76, 1.01]). The HPV vaccine was generally well tolerated when administered according to either schedule. These data support administration of the third dose of HPV-16/18 AS04-adjuvanted vaccine any time between months 6 and 12 after the first dose.
EFFICACY OF THE AS04-ADJUVANTED HPV-16/18 VACCINE IN REDUCING ABNORMAL CYTOLOGY AND AGAINST LESIONS ASSOCIATED WITH VACCINE AND NON-VACCINE ONCOGENIC HPV TYPES: AN ANALYSIS OF WOMEN NEGATIVE FOR SPECIFIC HPV TYPES AT BASELINE

C Wheeler on behalf of the HPV PATRICIA Study Group

Univ. New Mexico, Health Sci. Ctr., Albuquerque, NM

Objectives: The AS04-adjuvanted human papillomavirus (HPV)-16/18 vaccine (Cervarix®; GlaxoSmithKline Biologicals) shows high prophylactic vaccine efficacy (VE) against cervical intraepithelial neoplasia (CIN)2+ associated with HPV-16/18. We present data on the reduction in abnormal cytology (atypical squamous cells of undetermined significance [ASCUS] or higher[+] and cervical lesions (CIN1+ and CIN2+) associated with vaccine and non-vaccine oncogenic HPV types.

Methods: In PATRICIA (NCT00122681), women 15-25 years were randomised (1:1) to receive HPV-16/18 vaccine (N=9,319) or control Hepatitis A vaccine (N=9,325) at Months 0, 1 and 6. Gynaecological and cytopathological examinations were performed every 12 months. Cervical samples were tested every 6 months for HPV DNA by PCR. VE (96.1% CI) is presented for the total vaccinated cohort (TVC) (women who received at least one vaccine dose) for women who were HPV DNA-negative for the corresponding HPV type at baseline, regardless of their serostatus.

Conclusions: VE against ASCUS+ associated with HPV-16/18 was 84.2% (80.3-87.5; p<0.0001). VE against ASCUS+ (irrespective of HPV DNA type and irrespective of baseline DNA and serostatus) was 10.7% (96.1% CI: 4.7, 16.3; p<0.0001). VE against CIN1+ and CIN2+ associated with HPV-16/18 was 89.1% (81.6, 94.0; p<0.0001) and 92.4% (84.0, 97.0; p<0.0001), respectively. VE against CIN1+ and CIN2+ associated with the 10 most prevalent non-vaccine HPV types (HPV-31/33/35/39/45/51/52/56/58/59) was 33.5% (19.0, 45.6; p<0.0001) and 47.3% (28.2, 61.6; p<0.0001), respectively. The HPV-16/18 AS04-adjuvanted vaccine provided protection against lesions associated with HPV-16/18 and with non-vaccine oncogenic types, suggesting it has the potential to reduce the overall incidence of cervical pre-cancer and cancer. These data reflect the vaccine efficacy in women who were DNA-negative for the corresponding type at baseline.

HPV-16/18 BASELINE DNA AND SEROLOGICAL PREVALENCE IN ADOLESCENT AND YOUNG WOMEN FROM A PHASE III TRIAL OF THE AS04-ADJUVANTED HUMAN PAPILLOMAVIRUS (HPV)-16/18 VACCINE (PATRICIA)

 Dishon Apter on behalf of the HPV PATRICIA Study Group

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Objectives: The AS04-adjuvanted human papillomavirus (HPV)-16/18 vaccine, Cervarix® (GlaxoSmithKline Biologicals), showed high prophylactic efficacy against cervical intraepithelial neoplasia (CIN)2+ associated with HPV-16/18. We report baseline HPV-16/18 DNA and serological prevalence by age in PATRICIA.

Methods: This study (NCT00122681) was conducted in 18,644 women aged 15-25 yrs, enrolled in the study irrespective of their HPV DNA status, HPV serostatus, or cytology at baseline. Study entry was restricted to women who had had no more than 6 lifetime sexual partners. We present baseline DNA prevalence (by PCR) and seroprevalence (by ELISA) of HPV-16/18 in all women who received at least 1 dose with data available, stratified by age (15-17 yrs and 18-25 yrs).

Conclusions: At baseline, 80.4% and 70.7% of women aged 15-17 and 18-25 yrs, respectively, were DNA-negative and seronegative for both HPV-16 and HPV-18. A higher proportion of women were DNA-negative and seropositive for either HPV-16 or HPV-18 in the age group 18-25 yrs (16.2% and 12.4%, respectively) compared to women aged 15-17 yrs (9.0% and 6.6%, respectively). The percentage of women who were DNA-positive for either HPV-16 or HPV-18 was similar in both age cohorts (15-17 yrs: 5.1% and 2.5%, respectively; 18-25 yrs: 5.5 and 2.3%, respectively). Less than 1% of women were DNA-positive for both HPV-16 and HPV-18, in either age group. Although serology is not a perfect marker for prior exposure to HPV, data suggest that the majority of women in either age group showed no evidence of current infection (by PCR) or previous infection (by ELISA) with vaccine HPV types. Of those with an indication of current or previous infection, most were seropositive only, or DNA positive for one vaccine type. Trial eligibility criteria regarding lifetime number of sexual partners would be expected to homogenise risk differences including HPV infection exposures in the older age group (18-25), and type-specific prevalence reported here may not be generalisable for this group.
DETECTION OF HIGH-RISK HPV mRNA IN LIQUID BASED CYTOLOGY (LBC) SPECIMENS WITH THE APTIMA® HPV ASSAY

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Objectives: The objective of this study was to evaluate the ability to detect high-risk HPV (hrHPV) mRNA with the APTIMA HPV Assay (AHPV, Gen-Probe Incorporated) and DNA with the Hybrid Capture 2 HPV DNA Test (hc2, Qiagen Incorporated) in disease positive LBC specimens (CIN2+ and CIN3+). The sensitivity and specificity for each method was compared to the sensitivity and specificity achieved with conventional cytology.

Methods: Almost 600 clinical specimens were collected from patients with abnormal cytology. Samples were stored in LBC vials at room temperature for up to 3 years and tested for hrHPV mRNA in the AHPV Assay, a qualitative nucleic acid test designed to detect the E6/E7 mRNA of 14 hrHPV types in LBC specimens. Detection of hrHPV DNA was also determined for a subset of n=425 samples with the hc2 test. AHPV results for all 425 samples were compared to conventional cytology, histology and hc2 results.

Conclusions: The AHPV assay yielded a positive result in 148 out of 150 CIN 3 and 10 out of 11 cervical carcinoma specimens (sensitivity 98.1% for CIN 3+). The one cervical carcinoma specimen that was missed by the AHPV assay contained HPV53 as the only HPV type, a type that has limited evidence for cervical cancer and is not detected by the AHPV assay. The hc2 test yielded a positive result in 146 out of 150 CIN 3 and 9 out of 11 cervical carcinoma specimens (sensitivity 96.3% for CIN 3+). One of the two cervical carcinoma specimens missed by the hc2 test was the high-risk type HPV18, and in the other specimen no HPV DNA was detected. However, both specimens were positive in the AHPV assay. Conventional cytology yielded a positive result in 142 out of 150 CIN 3 and 10 out of 11 cervical carcinoma specimens (sensitivity 94.4% for CIN 3+). The AHPV assay had the highest specificity (74.7%) of all three methods in disease positive specimen (CIN 2+). The specificity of the hc2 assay was 60.9% and the specificity for conventional cytology was 66.7%. These results indicate that the AHPV Assay is able to detect high-risk HPV mRNA in retrospective LBC specimens stored at room temperature for up to three years with strong correlation to disease. The AHPV assay showed an equal sensitivity but a higher specificity than the hc2 assay. The AHPV assay was not only more sensitive than conventional cytology but also more specific.

IMPROVED RECOVERY OF HPV mRNA FROM BD SUREPATH™ PRESERVATIVE FLUID AND DETECTION WITH THE APTIMA® HPV ASSAY

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Objectives: Evaluate detection of HPV messenger RNA (mRNA) from cervical cells (SiHa) stored in SurePath Preservative Fluid ((BD Diagnostics - TriPath; Burlington, North Carolina USA) with the APTIMA HPV Assay.

Methods: The APTIMA HPV Assay is a CE-marked target amplification nucleic acid probe test for the in vitro qualitative detection of E6/E7 viral mRNA from 14 high-risk HPV types (16/18/31/33/35/39/45/51/52/56/58/59/66/68). Messenger RNA purification and amplification can be very challenging out of liquid-based cytology media that contains formalin, such as SurePath preservative, due to extensive cross-linking, fragmentation and chemical modification that progresses over time and at elevated room temperatures. Several recent publications reported improved mRNA recovery with the incorporation of a proteinase K (pK) digest step prior to extraction. In this study, SiHa cells (10 to 100,000 cells/reaction) were stored in SurePath (BD Diagnostics - TriPath; Burlington, North Carolina USA) or PreservCyt (Hologic Inc., Bedford, Massachusetts,USA) media at various temperatures and the analytical sensitivity of the APTIMA HPV Assay was determined with and without a pK digest of the SurePath specimen.

Conclusions: Whereas the positivity of the APTIMA HPV Assay (tested at 100 SiHa cells/reaction) was 100% when cells were stored in PreservCyt solution for 10 days at 30°C, the positivity dropped to 0% when cells were stored in SurePath preservative at 30°C for more than 4 days. The incorporation of a pK digest restored positivity for SurePath preservative samples to 100% after storage for up to 10 days at 30°C. These data show that significantly more mRNA can be recovered from samples stored in SurePath preservative at elevated room temperatures that is suitable for amplification in the APTIMA HPV Assay when a pK digest is performed prior to testing.
The aim of this study was to evaluate the performance of the NucliSENS easyQ HPV on the Light Cycler 2.0 (Roche, Rothkreuz, Switzerland). Results were compared with the NucliSENS® easyQ (bioMérieux, France).

**Material & Methods**

90 specimen were tested in parallel on both systems NucliSENS easyQ and Light Cycler.

**RNA extraction:** ThinPrep (liquid based transport medium, Hologic) samples have been processed using the easyMAG extraction system (bioMérieux, France).

**Mix preparation:** The mix preparation has been done according to the NucliSENS easyQ kit procedure. The primer mix and sample was added to the capillary, centrifuged, then added with the enzymes and closed. First incubation on the Light Cycler 2.0 was 2 min at 65°C followed by 2 min at 41°C. Then centrifuge again to add the enzymes to the mix, and start the run.

**Run configuration:** 99 cycles / Target temperature: 41°C / Hold: 1min / Ramp rate 2°C/sec / Acquisition mode: single.

**Data analysis:** Channel 530 (HPV 16, HPV 31, HPV 33) and 610 (U1A, HPV 18, HPV 45) are used for analysis. Choose the “qualitative detection”. For the channel 610, deduce the channel 530 to discriminate between the signals that are overlapping from channel 530.

**Results**

80 of 90 specimen (89%) had concordant results in detecting equivalent subtypes when 10 (11%) were discordant. 10 discordant were positive on NucliSENS easyQ and negative on LightCycler.

**Conclusion**

The Light Cycler 2.0 shows to be a good alternative to NucliSENS easyQ system to perform the NucliSENS easyQ HPV test from bioMérieux.

Further improvements of adaptation could be useful to increase the concordant results rate.

**P HT-4**

**EVALUATION OF THE NucliSENS EasyQ® HPV KIT ON SELECTED DNA POSITIVE SAMPLES**

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**Christine Armbruster, Carmelo Martinez / bioMérieux (Suisse) SA, 1202 Geneva**

The aim of this study was to evaluate 83 selected DNA positive and determined cytology specimens with the NucliSENS EasyQ® HPV test.

Infection with high-risk (HR) HPV is the main cause of cervical intraepithelial and invasive neoplasias and HPV DNA has been detected in >90% of cervical carcinomas with the most common HPV types identified as HPV 16, 18, 31, 33, and 45 (Molden T, CEB 2005).

Recent studies demonstrated that HPV mRNA E6 and E7 oncoproteins may be more specific biomarkers of cervical neoplasia than HR HPV DNA. Detection of oncocenes E6 and E7 mRNA indicates neoplastic cell transformation as opposed to HPV DNA tests detecting the presence of the virus but not how it is behaving. The NucliSENS EasyQ HPV test from bioMérieux detects the presence of 2 oncogenic E6 and E7 transcripts. The test is based on the NASBA technology.

**Material & Methods:** ThinPrep (liquid based transport medium, Hologic) samples have been processed using the easyMAG extraction system (bioMérieux, France). NucliSENS EasyQ HPV kit, NASBA based amplification kit was used according to the supplier guidelines used on NucliSENS EasyQ instrument. (Specimen were also processed on LightCycler)

**Results:**

1. DNA positive specimen: n=83 (15 specimen with multiple subtypes). We found: 62.5% (n=56) of HPV16 were mRNA positive, 50% (n=14) of HPV18, 18.2% (n=22) of HPV31, 50% (n=6) of HPV33 and 100% (n=8) of HPV45.

2. Cytology: 83 specimens were included (16 normal cytology, 48 ASCUS, 19 LSIL). We found that: 43.8% of normal cytology specimen, 60.4% ASCUS and 57.9% of the LSIL were mRNA E6/E7 positive.

**Conclusion:** In almost 60% of the cytology cases (ASCUS and LSIL) an oncogenic activity is found. In the case of a normal cytology with positive DNA, an oncogenic activity is found in almost 44%, suggesting a lack of sensitivity of the cytological method.

Considering that all specimens were HPV DNA positive, we found a lower percentage of oncogenic E6/E7 activity. Thus the RNA method shows a higher specificity than the DNA one.

It is known that the prevalence of HPV DNA is higher than the prevalence of E6/E7 mRNA. These data suggest that the NucliSENS EasyQ HPV test has a higher triage effect and is more cost effective, reducing the number of positive cases referred to colposcopy.

These conclusions should be more investigated in a wider clinical study.
CERVICAL HPV DNA DETECTION IN RELATION TO MENSTRUAL PHASE

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Objectives: There is considerable interest in the use of HPV DNA testing for cervical cancer screening. As such, clinical management of patients would depend on single point detection of HPV. It is important to identify factors that affect HPV test accuracy. Hormonal fluctuations during the menstrual cycle are believed to affect the immune response in the female genital tract. Studies evaluating differences in HPV detection with respect to a woman's last menstrual period (LMP) have come up with conflicting results. There is a need to address these inconsistencies. We evaluated the effect of menstrual phase in relation to HPV detection using data collected in the Brazilian Ludwig-McGill cohort study.

Methods: During the first year of follow-up, subjects were interviewed every 4 months. At each clinic visit, individuals were asked to complete a questionnaire and to provide a cervical sample for HPV testing. We excluded LMP and associated HPV test information for women who reported LMPs greater than 31 days, or less than 5 days. After applying these exclusion criteria, data were available for 6100 patient visits. To account for possible auto-correlation due to repeated measures on the same women, general estimating equations were used to calculate odds ratios (OR) and associated 95% confidence intervals (CI). LMP data were separated into three categories: (1) days 5-10 (early phase); (2) days 11-21 (middle phase); and (3) days 22-30 (late phase). We examined the effect of relevant covariates (age, smoking, parity, oral contraceptive use, and number of sexual partners), by including these variables in the model. None were found to be informative and so they were left out.

Conclusions: Compared with early phase (referred group), HPV detection did not differ according to reported LMP for middle phase (OR = 1.06, 95% CI 0.93-1.22) or late phase (OR = 0.98, 95% CI 0.83-1.15). Similarly, no difference was observed in detection of oncogenic types (IARC classification), or individual types 16 and 18. For HPV positive samples, we evaluated the effect of LMP on total viral load and found that there was no effect. These preliminary results indicate HPV detection is not associated with menstrual phase.

PERFORMANCE OF NOVEL PREANALYTICAL EXTRACTION CHEMISTRY WITH ULTRA-HIGH-THROUGHPUT SAMPLE PREPARATION FOR HIGH-RISK HPV DNA TESTING


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Objectives: Cervical cancer is the second most prevalent cancer among women and human papillomavirus (HPV) has been established to be its most important etiological agent. Multiple studies have unequivocally established the cost effectiveness and clinical value of HPV triage in the management of patients with equivocal cytological abnormality. There is a high demand for reliable DNA extraction method from Liquid based cytology (LBC) media combined with high throughput automation. To meet these needs Qiagen developed an instrument (QIAssemble™ SP) with novel chemistry capable to process up to 1000 LBC specimens under 6 hours. The objective of this study was to evaluate the analytical performance of the new extraction chemistry combined with next generation Hybrid Capture technology digene® eHC HPV DNA Test (under development).

Methods: In this study, the assessment of a new NextGen protocol exploiting proprietary DNA-extraction chemistry was based on a pair-wise comparison of signal of the residual Clinical specimens from PreservCyt® (PC) media processed by a sample-prep instrument to the standard HC2 detection. The extracted DNA in 96-well micro-plate was then processed by the NextGen analytic assay method. The functional performance of the new NextGen protocol was evaluated by an agreement rate between 2 methods using the JMP statistical software. Any discordant samples were then adjudicated with PCR based genotyping method.

Conclusions: A set of 160 individual PC specimens was used in the study with 70 being positive and 90 being negative by HC2. Among the HC2 positive samples the new NextGen protocol detected 59 as positive and 11 as negative. Among the HC2 negative samples the new NextGen protocol detected 84 negative and 6 positive. The discrepant specimens were subjected to a 2_2 agreement analysis where the reference result was a composite of the HC2 result and a follow-up GP5+/6+ PCR Luminex genotyping The analysis showed 95.2% positive, 95.5% negative and 95.3% total agreement. In conclusion, we demonstrate an ultra-high-throughput automation solution for processing the residual PC samples with an excellent analytical performance.
COMPARISON OF SELF-COLLECTED FLOCKED VAGINAL SWABS AND THINPREP CERVICAL SAMPLES TESTED FOR RNA BY APTIMA HPV AND DNA BY HC2 ASSAYS


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Objectives: Women are capable of self collecting vaginal swabs (VS) for the diagnosis of sexually transmitted infections. This approach to testing vaginal samples for HR HPV may be a strategy to enable more routine testing of women who are reluctant to undergo pelvic examinations.

Methods: Women (n=100) referred to colposcopy because of an abnormal Pap test collected a dual flocked swab (Copan, Italia) vaginal sample. The colposcopist collected a cervical ThinPrep (Hologic) liquid based Pap (L-Pap) sample and a biopsy was taken when indicated. One of the swabs was transported to the laboratory in a dry state and the other in a tube containing specimen transport media (STM) (Qiagen). Both of the swabs and the L-Pap sample were tested for DNA by HC2 (Qiagen) and for RNA using APTIMA (AHPV) (Gen-Probe). The L-Pap samples were also processed for cytology.

Conclusions: Patients were determined to be infected with HR HPV DNA or RNA if either nucleic acid was found in the L-Pap sample or if both VS contained the same nucleic acid, when L-Pap was negative. Both assays identified 59 positive patients. Concordance of RNA and DNA positive women was 81.0%. Of 9 discordant positive patients, 6 were without DNA and 3 without RNA. Insufficient volume for testing all 3 samples was recorded in 11 patients with DNA and in 6 with RNA. The sensitivity of HC2 to detect a DNA infection was 0.98 for L-Pap and 0.87 for VS. Sensitivity of AHPV to detect an RNA infection was 0.91 for L-Pap and 0.93 for VS. The concordance of positive dry and wet VS was 90.9% for DNA and 89.4% for RNA, with no advantage for dry or wet transportation. Histology determined 24 CIN2+, 11 CIN1, 29 NEG and 36 patients did not receive a biopsy. For CIN2+ the sensitivities of HC2 were 0.95 for L-Pap and 0.71 for VS, compared to AHPV 0.87 for L-Pap and 0.90 for VS. L-Pap HSIL sensitivity for CIN2+ was 0.52. Testing VS by AHPV identified most CIN2+ cases, approximating the sensitivity of HC2 testing of ThinPrep L-Pap samples.

DEVELOPMENT OF NOVEL HPV GENOTYPING CHIP SYSTEM “CLINICIP HPV” USING ELECTROCHEMICAL DETECTION TECHNOLOGY

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Background and aims: Human papillomavirus (HPV) infection is the main cause of cervical cancer, and the risk of carcinogenesis on each HPV strains has been investigated. The HPV genotype has been analyzed using PCR-RFLP and/or PCR-sequencing. Our purpose is to develop a novel HPV genotyping DNA chip system that can detect and determine 13 types of high-risk HPV strains using electrochemical detection technology and LAMP (Loop-Mediated Isothermal Amplification) method.

Methods: 247 of cervical smear samples were collected from patients with abnormal cervical cytology and control subjects at the Hospitals. HPV DNA was extracted with a QIAamp then after target DNA was amplified of six tubes using LAMP amplification method with specific primer for 13 types. Amplified products were applied to Clinichip HPV for HPV genotyping. Clinichip HPV identifies genes, using an original current detection method, by electrical signal indicating specific bind of the target DNA to immobilized probes with the complementary sequence on the gold electrode of chip surface. The protocol was approved by the IRB of the Hospitals.

Results: HPV genotypes determined by Clinichip HPV were compared with PCR-sequencing using specific primer for 13 types. HPV positive rate was 63.6% (157/247) in the Clinichip HPV and 59.9% (148/247) in the PCR method. The genotyping results in HPV positive samples were 99.3% (147/148) concordant with the results of PCR-sequencing. In the negative samples, the results were 89.9% (89/99) concordant with the results of PCR sequencing.

Conclusions: The Clinichip HPV can rapidly determine 13 types of high-risk HPV in clinical samples with high sensitivity and great accuracy.
**P GT-2**

**COMPARISON OF TWO PCR PRIMER SETS FOR HUMAN PAPILLOMAVIRUS DNA DETECTION IN PCR-BASED HPV GENOTYPING**

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**Objective:** To compare the sensitivity of two PCR primer sets (SPF1/GP6+ and MY11/GP6+) in PCR-based assay for HPV DNA genotyping in cervical swab samples.

**Methods:** Eight cervical DNA swab samples with known HPV infection (HPV 16, 18, 31, 33, 39, 45, 52, and 58) were retrieved from our DNA bank. Type-specific PCR was used to confirm the HPV types. Viral load of each reference sample was quantified by real-time quantitative PCR. Then, serial dilutions to viral copy number of 20, 50, 10^2, and 10^3 copies were performed. Two PCR primer sets (SPF1/GP6+ and MY11/GP6+) were used to amplify the test sample of the defined HPV copy numbers followed by genotyping with Easychip HPV blot (King Car, I-Lan, Taiwan).

**Conclusions:** HPV infection at 20 copies in HPV 16, 18, 31, 33, and 39 was detected in both primer sets while HPV 45 was at 50 copies. In HPV 52, the threshold copy number for SPF1/GP6+ and MY11/GP6+ primer sets were 50 and 10^3 copies, respectively. On the contrary, in HPV 58, the detection threshold for MY11/GP6+ and SPF1/GP6+ primer sets were at 10^2 and 10^3 copies, respectively. In conclusion, the two primer sets were sensitive in most of the prevalent high-risk types, and SPF1/GP6+ primer set seemed more sensitive in HPV 52 and MY11/GP6+ more sensitive in HPV 58.

**P GT-3**

**HUMAN PAPILLOMAVIRUS GENOTYPE IN HIGH-GRADE CERVICAL INTRAEPITHELIAL NEOPLASIA BY LASER CAPTURE MICRODISSECTION**

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**Objective:** To determine the human papillomavirus (HPV) genotype of high-grade cervical intraepithelial neoplasia (CIN) formalin-fixed paraffin-embedded (FFPE) samples using laser capture microdissection (LCM).

**Methods:** FFPE of CIN 2/3 paraffin block from conization or hysterectomy specimens which were HPV negative in previous study were retrieved. Specimens were sliced in 5 µm. Four slices for each sample were required to obtain adequate amount of DNA by LCM. Genomic DNA was extracted according to laser-microdissected tissue protocol. The SPF1/GP6+ consensus primers were used to amplify a fragment of 184 bp in the L1 open reading frame. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) PCR was used as external control. 15 µl of each sample from the SPF1/GP6+ PCR product (in 25 µl) were then hybridized with an HPV Blot (King Car, I-Lan, Taiwan) membrane for HPV genotyping. HPV 16, 18, 33, 52 and 58 type-specific (E6 and L1) PCRs were performed if the results were HPV negative.

**Conclusions:** A total of 76 FFPE specimens were included. Twenty-two cases FFPE specimens contained no residual tumors. Fifty specimens containing tumor with GAPDH-positive were analyzed. HPV DNA sequences were detected in 30 (62%) of the 50 evaluable specimens, among which 23 (46%) contained single types, eight (16%) with multiple types. HPV 16 was detected in 10 (24.4%), HPV 33 in 7 (17.1%), HPV 52 in 6 (14.6%), HPV 45 and 58 in four (9.8%) each and HPV 18 in 3 (7.3%) of the samples. There are high-grade CINs that remained unresolved of their HPV status after LCM procedures.
**P GT-4**

**THE digene HPV GENOTYPING PROBESET TEST (PS) IS SHOWN TO BE COMPATIBLE WITH BOTH THE digene CERVICAL SAMPLER AND LIQUID CYTOLOGY SPECIMENS**

Gay T., Chen W., McLeod S., Pfister D., Thai H., Nazarenko I., Loeffert D.

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**Objectives:** The digene HPV Genotyping PS™ Test developed for the specific detection of HPV 16, 18, and 45 is intended to be utilized as a reflex genotyping test for the digene HC2 High-Risk HPV DNA Test (HC2). The test has been demonstrated to be compatible with both the digene Cervical Sampler which uses STM media and the commonly used liquid based cytology (LBC) media, PreservCyt® (PC). A study was performed to demonstrate that the PS test is also compatible with another frequently used LBC media, SurePath®. The study was to compare and demonstrate equivalent performance of the genotyping PS test regardless of the cervical specimen media tested. In addition, the study describes and details the procedure and protocol required for the specific specimen media.

**Methods:** The HPV 16, 18, and 45 reflex genotyping PS test is a non-target amplification platform leveraging the HC2 and the Hybrid Capture® technology. Sample input for the PS test is identical to the sample input volume required for the HC2 screening test for STM, PC, and SurePath media. Sample preparation for the LBC media are also identical to the preparation required for performing the HC2 test. To demonstrate compatibility of SurePath media with the Genotyping PS test, SurePath cervical specimens was used. In addition, to demonstrate equivalence between STM and SurePath solution specimens, recovery of HPV target in each of the media was examined and compared.

**Conclusions:** It was demonstrated with SurePath clinical specimens that the digene HPV Genotyping PS test is not only compatible with STM and PC but also with SurePath media. The PS test detected HPV 16, 18, and/or 45 infections at 5000 copies per assay or greater in SurePath clinical specimens with the results being confirmed by qPCR. In addition, the results demonstrated that recovery of HPV DNA is equivalent for STM and SurePath media. Each specimen type was processed according to its respective processing/denaturation procedures and tested with the PS test.

* This test is For Research Use Only. Not for use in diagnostic procedures.

* This test is not commercially available in the United States

**P GT-5**

**THE digene HPV GENOTYPING PROBESET TEST (PS) IS CAPABLE OF DETECTING HPV 16, 18, AND 45 SEPARATELY OR TOGETHER**

McLeod S., Chen W., Gay T., Pfister D., Thai H., Nazarenko I., Loeffert D.

Qiagen Gaithersburg Inc., Gaithersburg, MD, USA

**Objectives:** The digene HPV Genotyping PS Test, (PS™) a reflex test for the digene HC2 High-Risk HPV DNA Test (HC2), was developed for the specific detection of HPV 16, 18, and 45. The intended use of the test is to detect each of the three HPV genotypes separately. This requires three separate tests and three aliquots of the patient specimen. In this study, we wanted to demonstrate the ability to use the PS test to detect for the presence of HPV 16, 18, and 45 or any combination of the three from a single specimen aliquot. The capability to detect more than a single HPV genotype from one test will reduce the number of tests required and ultimately the volume of patient specimen required for the PS test. In addition, the protocol and procedure changes required to detect more than one genotype per test will also be described in detail.

**Methods:** The PS test is based on Hybrid Capture technology. The assay uses similar reagents and follows a similar protocol as the HC2 test. PreservCyt® (PC) specimens both negative and positive for HPV 16, 18, and/or 45 were utilized in the study. An assessment and comparison of the PS test was performed for detecting HPV 16, 18, and 45 targets individually versus detecting for two or more of the HPV genotypes together. When detecting for HPV genotypes separately, an individual probe mix is made for each genotype and when detecting for two or more of the genotypes together the specific target probes are combined into a single probe mix and used as a cocktail. In addition, analytical sensitivity and performance was also evaluated with HPV plasmid DNA as the target.

**Conclusions:** We used HPV plasmids to demonstrate that the PS test had the capability to detect two or more HPV targets in a single test. It was shown that the sensitivity of the assay was equivalent with a multi-probe mix as it is with a single-probe mix. With HC2 positive PC specimen additional results were generated to demonstrate that the PS test could be used to detect two or more targets with equal effectiveness. When detecting PC specimens both positive and negative for HPV 16, 18, and/or 45, the results were identical no matter if a single-probe mix or a multi-probe mix was used. In addition, the study also defines the changes to the assay flow of the PS test when detecting for more than a single HPV genotype per test.

* This test is For Research Use Only. Not for use in diagnostic procedures.

* This test is not commercially available in the United States
DISTRIBUTION OF HIGH RISK HPV GENOTYPES AMONG BULGARIAN WOMEN WITH CERVICAL INTRAEPITHELIAL NEOPLASIA AND INVASCIVE CARCINOMA

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Objectives: Human papillomaviruses (HPV) are associated ethiologically with premalignant cervical lesions and cervical carcinoma. Our objective was to perform a retrospective study concerning the prevalence and distribution of high risk HPV genotypes in Bulgaria and to analyze the obtained data in order to elucidate their significance for accurate diagnosis and therapeutic management.

Methods: In this study, by means of multiplex and real-time PCR systems we analyzed the genotype distribution of high risk HPVs in Bulgarian females aged between 16 and 70 with cervical intraepithelial neoplasia and invasive carcinoma. During a six year period (2002-2008) 1953 specimens were collected and tested for 12 high risk HPV types.

Conclusions: Out of the tested samples, 1611 (82.5%) contained one or more HPV types (44 samples). The most frequently observed HPVs were high-risk HPV types, especially type 16 (n=841/1611, 52.2%), while HPV types 18 (n=295/1611, 18.3%), 31 (n=192/1611, 11.9%), 33 (n=234/1611, 14.5%), and 56 (28/1611, 1.7%) were less frequent, whereas other HPV types (35, 39, 45, 52, 58, 59, 66) altogether accounted for 4.1% of the positive samples. The presence of HPV DNA significantly increased from 73.2% to 82.5% along with the severity of the cervical lesions from CIN I to CIN III. The highest prevalence of HPV showed the group of women between 21 and 30 year old. There was also a tendency HPV positivity rate to decline with age. These findings might contribute to the knowledge of HPV molecular epidemiology and may be useful in the implementation of vaccine strategies.

COMPARISON OF HPV-DNA DETECTION AND GENOTYPING IN URINE AND CERVICAL SAMPLES

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Objectives: Human Papillomavirus (HPV) represents the etiologic agent of cervical cancer. Pap test screening induced a significant decline in incidence and mortality for this cause but a consistent number of high risk persons (young women, HIV infected persons, immigrants or women living in developing countries) are, for several reasons, poorly reached from screening programs or prevalence studies. In the last years high sensitive molecular methods for the detection of HPV DNA and high (HR) and low (LR) oncogenic genotypes in cytological samples have been proposed as adjunctive to, or substitutes of cytological screening.

In this study we want to assess if urine samples, easier to collect and more acceptable, could substitute cytological samples in screening programs directed to hard-to-reach populations.

Methods: Paired cervical and urine samples collected in the same day from 107 women (median age 42 yrs, range 22-70 yrs) referred to the STD Unit of the Sacco Hospital, Milan (Italy) have been analysed for HPV-infection and genotyped. DNA was obtained through NucliSENS®miniMAG® (bioMérieux bv, The Netherlands). Viral genome was analysed with a multiplex-PCR on the HPV-L1 gene. Genotyping was performed with RFLP (Restriction Fragment Lenght Polymorphism) technique using 3 restriction enzymes (RsaI, HaellI, Ddel, Recombinant Enzyme, BioLabs inc, New England).

Conclusions: A high concordance (94.4%) of HPV-DNA detection was observed in cervical and urine samples (66.4% and 62.6% respectively). Preliminary genotyping results on the 65.7% of the positive paired cervical and urine samples showed the same genotypes as single (63.6%) or multiple (36.4%) infection. In particular, 60.7% of single infection were due to a HR-HPV genotype; in 75% of multiple HPV infections one or more HR-HPV types were involved.

These preliminary data suggest that the use of urine samples for HPV DNA detection and genotyping could be a valid tool which can widen the target population for screening programs.
VIRAL MARKERS IN LGSIL LESIONS

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Aims: To investigate viral markers (viral load and E6/E7mRNA of hrHPV) in low grade squamous intraepithelial lesions (LSILs) as a means of determining the persistence and lesions progression

Methods: From a cohort of 670 women (17-57 years old), eighty five women with cytological interpretation LSIL were followed for one year. HPV typing (Roche Linear Array), viral mRNAs presence (PreTect HPV Proofer) and viral load (Pathogen Detection Advanced Kit, Primer Design using 7300 Applied Biosystems Real-Time PCR) were determined in cervical-brush specimens at base line and in samples obtained at 12 months interval.

Results: At base line, 59/85 samples were HPV positive, type 16 being prevalent in single or co-infection. E6/E7mRNAs were present in 37 cases (HPV16,18, 31). HPV viral load was significantly higher for genotype 16 (Standard curve: Slope-3.644965, Intercept- 48.311424, R2-0.994560; 37.67-17x105 median 123.56) and genotype 18 (Standard curve: Slope-3.831552, Intercept- 45.947674, R2-0.979529; 2.34- 5639.83, median 6.89). HPV viral load values for both genotypes were lower in co-infections as compared with single infection. During follow-up, 5/85 patients underwent biopsy and 4/85 were subjected to LLETZ conisation (all tested positive for viral mRNAs) but 5 cases still tested HPV positive (with the same type or acquired new genotypes). From E6/E7mRNA positive cases at enrollment, 3 developed HGSIL lesions and 11 maintained LGSIL cytology, but in two cases no HPVDNA was detected. Patients who maintained HPV 16 genotype retested with high viral load (2, 81068 x103) while patients with low viral load values and no mRNAs became negative for HPV 16 DNA test.

Conclusions: Persistence was significantly higher for PreTect HPV-Proofer than for PCR for genotyping and for viral load.

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CLAUDIN 1 AS A BIOMARKER OF CERVICAL CYTOLOGY AND HISTOLOGY

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The claudin 1 (CLDN1) is major component of the tight junction structure and plays an important role in cell-cell adhesion. The role of CLDN1 in cervical cancer pathology was revealed by our previous work (Sobel G, Páska C, Szabó I, Kiss A, Kádár A, Schaff Z. Hum Pathol. 2005 Feb;36(2):162-9.).

In this study 360 cervical cytological, histological samples were collected from a colposcopic referral population. The histotological and cytological samples were immunostained using CLDN1, and as reference, CDK2A antibodies and were evaluated by experienced pathologist.

The results indicate high concordance between CLDN1 and CDKN2A immunostaining, which were in agreement with histology immune status, as well. CLDN1 might have a similar potential, than CDKN2A, to be used as histological/cytological biomarker to improve the clinical performance of cervical cytology/histology.
HPV SCREENING USING careHPV™ ON A HIGH-RISK HIV POPULATION IN KIGALI TOWNSHIP, RWANDA

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Introduction: WE-ACTx (Women's Equity in Access to Care and Treatment) is dedicated to improving access to health care, including specific HIV and cancer services to reduce the incidence, morbidity, and mortality in Rwandan women, where cervical cancer is the leading cause of cancer death. The careHPV™ test, currently in late-stage development, is the product of a partnership between QIAGEN, Inc., and PATH (Seattle, USA) to design and develop a high-risk-HPV-DNA screening test that is accurate, affordable, and acceptable in resource-constrained regions of the world. The foundation of the test is the Hybrid Capture® signal amplification chemistry, simplified and fortified to deliver robust, rapid, batched results that facilitate same-day community screening with clinical follow-up. Here we describe the preliminary performance of careHPV using final R&D reagent formulations and pilot instrumentation in an ongoing clinical study in Kigali, Rwanda.

Methods: More than 1700 HIV-negative and nearly 600 HIV-positive women were recruited to participate in a screening program over a several-month period. Screening methods included visual inspection with acetic acid (VIA), conventional Pap, and HPV screening using the careHPV test. Pap smears were sent to and reviewed at Montefiore Medical Center, USA, to produce cytology results. Women testing positive by either VIA or careHPV or cytology received follow-up clinical management.

Conclusions: From 2300 women screened, 215 (9.4%) were VIA+; 347 (15.1%) were HPV+; 593 (25.7%) were HIV+. Among HIV+ women, 193 (32.6%) were also HPV+; among HIV- women, 154 (9.3%) were HPV+. HPV prevalence was more than three times higher in the HIV+ group than in the HIV- group. Cytology results and correlation with other tests were compiled separately and are reported as part of the presentation. This was the first study to use final careHPV reagent formulations and pilot instrumentation in a pre-clinical-performance study. This study was funded, in part, by a Fogarty Foundation grant from the U.S. National Institutes of Health.

CERVICAL CANCER SCREENING BY HPV TESTING AND CYTOLOGY DURING PREGNANCY IN WOMEN WITH INADEQUATE GYNECOLOGICAL FOLLOW-UP.

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Objective: The objective of this study was to take advantage of the follow-up proposed during pregnancy to determine the prevalence of human papillomavirus (HPV) infection, the genotype distribution, the prevalence of cytologic abnormalities and HPV persistence in a population of young women who do not participate regularly to cervical screening programs.

Methods: 234 pregnant women aged 16 to 42 years who attended either a Health Center dedicated to women and children follow-up (n = 142) or the obstetrical department of our Hospital (n = 92) were included in a cervical screening program. Among inclusion criteria were the absence of previous cervical pathology, no cervical screening the year preceding the pregnancy, and a signed informed consent. Two cervical samples were performed: one for cytology analysis after Pap staining and the other, collected into a PreservCyt Solution (Cytyc), for HPV testing and viral genotyping. High risk HPV (HR-HPV) testing was performed using Hybrid Capture 2 (Qiagen, France). HPV genotyping was performed using the PapilloCheck®, assay (Greiner Bio-one). In case of abnormal cytology and/or positive HPV testing during pregnancy, women were treated according to the national (ANAES) and international (Eurogin 2008) recommendations.

Results: HR-HPV was detected in 19.6 % of women. Genotyping, performed on 187 samples, identified 22 HPV types including HPV16 (16.4%), HPV51 (12.7%), HPV39 (7.3%) and HPV53 (7.3%). Prevalence of multiple HPV infection was 16.4%. Abnormal cytology was detected in 9 / 234 cases (3 ASCUS, 5 LSIL, 1 HSIL). Follow-up was performed for 23 / 46 women with either HR-HPV positivity and/or cytology abnormalities. 14 / 23 women presented with transient HR-HPV infection with normal cytology and 9 / 23 women with persistent HR-HPV with or without cytological abnormalities. Colpocopy was performed for 6 women with a diagnosis of CIN2 for 2 of them leading to appropriate treatment. No adverse effect of cervical screening during pregnancy was recorded.

Conclusion: cervical screening combining HPV testing and cytology during pregnancy should be considered in young women with inadequate gynecological follow-up.
A UNIVERSAL SAMPLE PROCESSING WORKFLOW FOR SAMPLES FROM BOTH SUREPATH® MEDIA AND PRESERVCYT® MEDIA WITH THE QIAensemble™ SP INSTRUMENT

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Introduction: The HC2 High-Risk HPV DNA Test® (HC2), approved by the FDA for testing samples collected in PreservCyt® medium (PC) is routinely employed for adjunctive screening from residual sample after cytology. Despite several attempts, a standard and reproducible HPV DNA test method that uses residual samples from SurePath® (SP) medium is not yet available. Most methods use cumbersome procedures and longer lysis incubation time, which is not suitable for high volume lab workflow. In this study, we evaluated a novel extraction protocol that is compatible with various collection media and amenable to the ultra-high throughput next generation Hybrid Capture® system, digene eHC HPV DNA Test (currently under development). Our main objective was to develop a universal extraction chemistry protocol for both LBC media, SP and PC. The extraction chemistry should take no more than 30 minutes for processing 96 samples and be compatible with automation.

Methods: In this study, the novel sample processing chemistry was evaluated against the industry standard HC2 method. Negative and positive PC and SP clinical specimen pools were split and processed using standard HC2 manual conversion method and by the new extraction method. Samples processed by both methods then were tested side-by-side by the HC2 assay. The new chemistry protocol was integrated with prototype instruments and performance was evaluated by testing individual clinical specimens.

Conclusions: The results with PC positive and negative pools show similar performance from both sample processing protocols. In SP samples, the optimized new extraction method resulted in 3.5 fold higher signal than that from HC2. This method also showed low background with negative samples and good reproducibility with both SP and PC samples. The individual SP clinical specimens (n=160) processed by QIAensemble™ SP instrument followed by digene eHC HPV DNA Test compared with HC2 manual process showed 94.87%(90.2% - 97.4%) total agreement. In conclusion, we have established a novel universal protocol and automation solution for HPV DNA test with residual liquid-based cytology (LBC) specimens.

QIASYMPHONY® AXpH DNA KIT® AS AN ALTERNATIVE TO MANUAL CONVERSION OF PRESERVCYT® SPECIMENS FOR USE IN HYBRID CAPTURE® 2


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Objectives: Cervical specimens collected in PreservCyt® (PC) media are routinely used for cervical cancer screening and are acceptable for use with the digene HC2 High-Risk HPV DNA Test® (HC2). The current HC2 PC sample conversion protocol requires manual processing of 4 ml of PC specimen. In this research study, we compared eluates generated with an automated protocol for DNA purification from cervical PC specimens for use in HC2 with the current “manual conversion” method.

Methods: 2104 residual, de-identified cervical PreservCyt samples retained after cytology screening were tested at three different sites. 4 ml aliquots of cervical PC samples were processed using the standard HC2 manual conversion method. 4ml of the same specimens were likewise extracted with the automated the QIASympohony® AXpH DNA kit®. Crude lysates and denatured DNA eluates were tested with HC2. HC2 RLU/co values were plotted by scatter-plot analysis and results were compared in concordance tables.

Conclusions: 520 samples were tested at QIAGEN R&D (Hilden, Germany); positive agreement was 96%, negative agreement was 87%, total agreement was 94%, and K=0.84. 1056 specimens were tested at Molecular Pathology Laboratory Network (MPLN, Maryville, TN); positive agreement was 96%, negative agreement was 99%, total agreement was 97%, and K=0.94. 176 samples were tested at Shiel Medical Laboratory (Brooklyn, NY); positive agreement was 88%, negative agreement was 97%, total agreement was 96%, and K=0.82. Overall, positive agreement was 96%, negative agreement was 96%, total agreement was 96%, and K=0.92. Our data show that the automated QIASymphony® AXpH DNA kit® protocol gives equivalent results to the standard HC2 manual conversion methods with the additional benefit of minimizing valuable technologist hands-on time by approximately 2.5 hours per full plate.

The applications presented here are for research use only. Not for use in diagnostic procedures.
HPV-HSIL LESIONS, DIAGNOSIS TREATMENT AND RECURRENCE

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Objectives: To assess the correlation between colposcopy, Pap smears, cone biopsy viral identification of the HPV types and immunohistochemical staining.

Material and Method: We carried out a study over 382 patients with HSIL attending our health center. Pap-smear were classified according to the Bethesda system; colposcopy, cone biopsy, viral identification of the HPV types and immunohistochemical staining were performed.

were abnormal, representing 44% overall. Our study has focused on the 382 (7.6 %) patients with HSIL. All patients with HSIL were followed by colposcopy, 256 having major lesions. HPV determination was performed in all 382 cases, 234 (61%) were HR -HPV, in 27 cases more types of HR-HPV were associate.

All 382 patients had undergone LEETZ as a tool for diagnostic and therapy.

CIN 1 occurred in 26 cases, CIN 2-304 cases and CIN 3- 47 cases, microinvasive carcinoma 5 cases.

Diagnosis protocol at CIN 3 and microinvasive carcinoma included immunohistochemical staining for p16, Ki 67, p63, laminina, collagen IV, citokeratina.

Our study showed that the most accurate markers in assessing the risk of the relapse according to the aggressiveness of the lesions are Ki 67 si p16.

Patients were followed up through colposcopy and citology at 6, 12, 18 24 36 months and HPV typing at 12 months.

The most frequent HPV type was HPV 16 followed by HPV 31, 53 and 18.

52 patients that had undergone LEETZ, needed another surgical intervention (LEETZ or cone biopsy) because of the relapse.

Conclusions: Our study shows that patients diagnosed with HSIL-HPV, particularly those with HPV 16, 31, 53 and 18 or those with HR HPV associate have a more serious degree of dysplasia, certified by cervical conization, and immunohistochemical staining for p16 and Ki 67 show a higher rate of recureence.

HPV TYPES 16/18 INFECTION IN ATYPICAL SQUAMOUS CELLS OF UNDERMINED SIGNIFICANCE AND LOW GRADE SQUAMOUS INTRAEPITHELIAL LESIONS

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Objective: Atypical squamous cells of undermined significance (ASC-US) and low-grade squamous intraepithelial lesions (LSILs) with the infection of -risk human Papilloma virus 16/18 (HR-HPV) indicate risk for high grade cervical intraepithelial neoplastic (CIN) lesions. This hospital based study is proposed to analyze whether the presence of HR-HPV identifies the presence of CIN lesions in ASC-US/LSIL diagnosed women on Pap smear.

Methods: A total of 100 women with ASC-US (n=47) and LSIL (n=53) cervical cytology were studied by screening a total of 3500 women in a regional hospital. These 100 cases were enrolled for the detection of oncogenic HPV types 16 and 18 using DNA polymerase chain reaction (PCR). All Patients underwent colposcopy and biopsy wherever required. The HPV positivity was correlated with histopathologically proven CIN.

Results: The frequency of HR-HPV type 16/18 infection in ASC-US and LSIL smears was 21.3% and 28.3% respectively. All HPV positive cases were having HPV 16/18 DNA sequences. The sensitivity and specificity of 16/18 HPV DNA testing was 80.0% and 81.1% in ASCUS group and 80.0% and 86.2% in LSIL group. HPV infection (16/18) had odds ratio of 17.1(95% CI = 2.5-152.7) for detection of CIN in ASC-US group and an OR of 25.0 (95% CI = 3.91-197.9) for CIN in LSIL group. ASC-US and LSIL women with HR-HPV 16/18 infection had 17-25 folds high risk of having CIN lesions.

Conclusions: Use of High risk HPV DNA testing in the triage of patients with ASC-US and LSIL on Pap reduces the number of follow-ups and unnecessary biopsies in low economic countries with high prevalence of HPV. Further population based prospective studies are needed to eliminate the drawbacks of our study and to determine non-hospital based HPV prevalence in Indian Women.
NEW APPROACH TO CERVICAL CANCER DIAGNOSING USING OCT-COLPOSCOPY

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Similar colposcopic abnormalities may be observed for a variety of cervical conditions, benign and malignant reducing specificity and positive predictive value [1,2]. So, elaboration of new protocols for management of Pap and HPV positive women are important for ensuring adequate diagnosis and, at the same time, for avoiding over-referral to colposcopy, overtreatment and to maintain sustainable costs.

Material of clinical studies of 100 female patients with cervical pathology has been analyzed. Indications for colposcopy: history of abnormal colposcopic findings, abnormal results of Pap smear (ASCUS, LSIL; HSIL), positive HPV test for oncogenic viral type. The study was approved by the Ethical Committee for scientific studies with human subjects. A conventional time-domain OCT-device (spatial resolution 10-20 µm at the depth of approximately 2 mm) with a forward-looking OCT probe (2.7 mm OD) was used in a routine colposcopic procedure: Each OCT image 200x200 pixels was acquired in approximately 2 seconds. Histology studies of biopsy material were made by standard protocol.

We have compared data of colposcopy and OCT-colposcopy for taking a decision about cervical biopsy. According to our data on abnormal colposcopic findings, 72% of cases had indications for biopsy and only in 36% of cases malignant and suspicious types of images were detected by OCT. Histology studies of biopsy material confirmed the presence of malignancy in 26% of all the cases. In the benign types of OCT images only 1 case was estimated as neoplasia by histology. Our study demonstrates that inclusion of OCT into the protocol of cervical examination allows more accurate assessing of the state of the object under study and reduces substantially the number of invasive procedures (biopsies), which will have an important medical and economic impact. When no biopsy is taken in the case of benign types of images, OCT may be an objective follow-up technique.

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OUR EXPERIENCES WITH THE FISHER CONE BIOPSY EXCISOR.


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Objectives: Conisation of the cervix is widely used for the diagnosis and conservative treatment of cervical intraepitelial neoplasia (CIN) and is an effective method that preserves reproductive function. LEEP have some disadvantages, induces electrocautery artifacts and usually excises the cervical tissue as a fragment pieces, so that evaluation of margins are sometimes not possible. The surgical method using Fisher cone biopsy excisor is characterised by easy reconstruction of specimens at the time of pathological diagnosis.

Methods: 160 patients between age 18-59 (average age 34,2) were assigned to treatment with the Fisher cone biopsy excisor. Eligibility criteria included: 1/ CIN 2 or 3, 2/ cytologic - colposcopy (or biopsy) discrepancy, 3/ for a long time persistent CIN 1.

A pathologist analysed degree of neoplasia, specimens for margin interpretability and adequacy of excision.

Results: Specimens with interpretable margins were in 92% of cases, fully excised cervical lesions were in 88% of cases. In pregnant women there were no problems in course of pregnancy. In 5% of cases we found out recurrence of CIN and in one cause CIS of the cervix.

Conclusions: The Fisher cone biopsy excisor is a reliable method for conservative treatment of cervical neoplasia and is save for preserve of next pregnancy possibility. This method is also highly reliable for margin interpretability and is ease for use.
Today, at least 1 million new cases of genital warts are diagnosed every year and, it seems that human papilloma virus and its related conditions such as genital warts show an increasing trend. Sensitive detection tests for HPV DNA indicate that as many as 30% of sexually active adults may be infected; a similar rate is seen in pregnancy. Genital wart is a clinical manifestation of low risk HPV types 6 and 11 and often increases in size and number during pregnancy. This situation can pose some problems in the therapeutic management that may affect fetus. Occasionally, condyloma in the pregnant which becomes large and macerated would require surgical excision after the first trim.

Case: we are reporting a 24 years old pregnant woman in 21 weeks of gestation who was referred due to large cauliflower mass protruding from vagina. She underwent surgical excision. The post operative period was uneventful. She delivered at term and her baby was normal in appearance.

Conclusion: Surgical excision is a good treatment for giant vaginal condyloma in pregnancy.

DEVELOPMENT OF A MULTIPLEXED ISOthermal AMPLIFICATION ASSAY FOR THE DETECTION OF C. TRACHOMATIS AND N. GONORRHOEAE

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Objective: To create a highly specific, ultrasensitive multiplexed assay for the detection of C. trachomatis and N. gonorrhoeae utilizing isothermal helicase dependent amplification (tHDA). This assay would be compatible with high throughput automation currently under development in Qiagen for HPV testing.

Methods: tHDA is an isothermal amplification technology which utilizes helicase to unwind double-stranded DNA, thus removing the need for thermocycling. We have combined Qiagen’s proprietary Hybrid Capture® (HC) technology for sample processing with tHDA and endpoint fluorescence detection to develop a multiplex assay for the simultaneous detection of CT and NG in clinical samples. Hybrid capture sample preparation for this assay involved hybridization of short, sequence-specific synthetic RNA probes to target DNA. These RNA:DNA hybrids were captured by specific HC antibodies conjugated to magnetic beads. DNA was then amplified by tHDA without prior elution from the HC beads. HC method was used for the processing of 1ml samples in different media including PreservCyt® and urine. Oligonucleotide primers for the simultaneous amplification of two CT targets (genomic and cryptic plasmid) and one multi-copy NG opa gene were optimized for use in combination with amplification inhibition control in the same reaction. To detect tHDA products in a homogenous, closed-tube format we employed endpoint fluorescence detection with dual-labeled probes. Total time of this prototype assay did not exceed 3 hours.

Conclusions: The developed CT/NG multiplex tHDA assay delivers high analytical sensitivity and specificity. As little as two CT elementary bodies and less than ten NG cells per mL of sample could be amplified and detected in the presence of the internal control. This assay is amenable to automation for high-throughput C. trachomatis and N. gonorrhoeae screening.
**P EC-1**

**INCREMENTAL COST EFFECTIVENESS EVALUATION OF VACCINATING GIRLS AGAINST CERVICAL CANCER PRE- AND POST-SEXUAL DEBUT IN THE UK**

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**Introduction:** HPV vaccines against cervical cancer (CC) have now been implemented in many countries worldwide especially in preadolescent girls. The cost consequence of vaccinating adult women has been poorly studied. We investigated the cost effectiveness of vaccinating girls at the age of 12 years old until 40 years using the latest clinical trial results of the bi-valent HPV vaccine including its effect on HPV cross-protection.

**Method:** A published static lifetime Markov cohort model was adapted to the UK setting. The model replicates the natural history of HPV infection leading to CC and it includes the effect of screening reducing progression of CIN lesions with treatment and the effect of vaccination reducing infection of HPV through immunization. Based on the latest results of the PATRICIA clinical trial, vaccine efficacy includes cross-protection against non-vaccine HPV types. The vaccine efficacy in girls pre- and post-sexual debut (<17 years of age - HPV naïve; total vaccinated cohort DNA negative irrespective of HPV serostatus) is differentiated. Lifetime protection is assumed. Input data are extracted from literature review, national databases further validated by experts. The costing is analyzed from a healthcare perspective obtained from published sources and official tariff data in the UK. A 3.5% discount rate is applied on cost and effect. The incremental cost effectiveness ratio (ICER) of vaccination at an age ranging from 12 to 40 years of age is evaluated. Univariate sensitivity analysis is performed on key variables.

**Conclusion:** The model indicates that vaccinating a cohort of 100,000 girls aged 12 years old would prevent 510 CC cases over a lifetime with an ICER of £22,184 per QALY gained. Vaccinating at the age of 25 years would prevent 269 CC cases with an ICER of £37,819 per QALY gained and at the age of 40 years 117 CC cases would be prevented with an ICER of £98,334 per QALY gained. The ICER remains under the very cost effective threshold defined by the WHO (1 GDP/capita - £23,517) until the age of 17 years and under the cost effective threshold (3xGDP/capita) until the age of 40 years. Extending vaccination to girls and women post sexual debut could lead to a substantial reduction in cervical cancer and cost burden and remains cost effective in the UK compared with no vaccination.

**P EC-2**

**COST EVALUATION OF THE BIVALENT AND QUADRIVALENT HPV VACCINE AGAINST CERVICAL CANCER IN SWEDEN**

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**Objectives:** Sweden will introduce vaccination against human papilloma virus (HPV) in the national immunization programme for girls 10-12 years of age in January 2010 to prevent cervical cancer (cc). Two vaccines are available: a bivalent HPV-16/18 and a quadrivalent HPV-6/11/16/18 vaccine. The quadrivalent vaccine has an additional effect against genital warts, while the other offers broader protection against oncogenic non-vaccine types (cross-protection). The annual cost consequences of both vaccines on HPV-related morbidity (i.e. abnormal pap smears, CIN1, CIN2/3 lesions, CC and genital warts) is unknown and was evaluated for Sweden.

**Methods:** A static population model was developed in Excel® to assess the effect of the vaccines on yearly HPV related lesion incidences and associated costs in Sweden. The two vaccines differ in their cross-protection level based on the latest results from clinical trials using, for both, the HPV naïve study population (without current or past HPV infection) and WHO data for Northern Europe on HPV-type distribution in each related lesion. Incidences were determined from national statistics. In the base case analysis, lifelong protection was assumed for both vaccines. Costs were calculated from a healthcare perspective and based on a report from the National Board of Health and Welfare. Vaccine prices were assumed equal. No discounting was applied as results are reported over a one year period, after reaching steady state.

**Conclusions:** The quadrivalent vaccine results in 7 352 genital wart cases prevented per year, while the additional cross-protection observed with the bivalent vaccine leads to an additional reduction of 888 abnormal pap smears, 969 CIN1, 816 CIN2/3, and 29 CC cases compared with the quadrivalent vaccine. Comparing the benefits, the quadrivalent vaccine offers 3 558 977 SEK (330 146 €) additional costs averted while the bivalent vaccine gives an additional 236 life years saved. Within the Swedish setting, the additional level of cross-protection of the bivalent vaccine allows for more CC cases prevented and life-years saved at a low extra cost compared with the quadrivalent vaccine.
HOSPITALISATIONS FOR HEAD AND NECK CANCERS IN FRANCE

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Objective: With 16,005 new cases and 5,406 related deaths in 2005, France is particularly concerned by Head and Neck (H&N) cancers. In addition to tobacco and alcohol, Human Papillomavirus (HPV) has been reported as a risk factor for H&N cancers. The literature on the burden of these cancers in Europe is scarce. This study was performed to assess the medical and economical burden of hospitalisations for H&N cancers in France.

Methods: The French national hospital database (PMSI), in which admissions to public and private hospitals are recorded, was retrospectively analysed to assess the annual number of patients hospitalised for H&N cancers and associated hospital costs from the healthcare payer perspective. ICD-10 codes (16 codes classified as oral cavity, oropharynx, pharynx, salivary glands, larynx) were used to extract admissions for these cancers. Hospital stays, chemotherapy and radiotherapy sessions were extracted to assess patients’ management. Costs of admissions were obtained from French official tariffs.

Results: In 2007, there were 35,069 patients hospitalised for H&N cancers, of whom 81% were men, corresponding to 60,200 hospital stays and 242,935 sessions of chemo- or radio-therapy. Oropharynx cancer was the most frequent (28% of patients), followed by oral cavity cancer (25% of patients). The peak of frequency was observed in the 55-59 years age group. Patients were mainly treated in medicine (47%) and surgery (23%) units. Mean annual cost per patient ranged from €3,285 to 8,924, leading to a total hospital cost of €275 millions in 2007. Considering that 26% of H&N cancers could be associated with HPV, it would lead to 9,118 patients hospitalized annually for HPV-related H&N cancers with an associated cost of €71,5 millions.

Conclusion: The hospital burden of H&N cancers in France is considerable. Furthermore, these costs are underestimated since radiotherapy sessions performed in the private sector as well as expensive drugs were not available from the PMSI. Further research is needed to assess outpatient and indirect costs linked to these cancers.

PROPOSED ALGORITHM FOR BULGARIAN ARMY MILITARY FEMALE PERSONNEL VACCINATION AGAINST HPV

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Objective: Propose an algorithm for HPV vaccination of adult sexually active women, military female personnel. Compare the efficacy and immunogenicity of the commercially available bivalent HPV vaccine and quadrivalent vaccine.

Proposed algorithm: From the beginning of March 2009 by initiative of Ministry of defense officials and under Military medical Academy expert guidance a program for human papiloma virus (HPV) infection prevention amongst female military personnel within the Bulgarian Army was started. The program covers on voluntary basis approximately 4000 military female personnel aged 18 to 39.

The program includes five major steps: 1) Preparation, 2) Information sharing, 3) Personnel screening, 4) Immunization, and 5) Vaccinated personnel control with comparison between the vaccination groups.

After proper information sessions with the women and obtained written informed consent from the volunteers, a gynecological examination is performed to all participants. A PAP smear and RT-PCR testing for HPV serotypes 16 and 18 are obtained and processed. The women with normal PAP smears and negative HPV DNA results will be vaccinated at this step. Those with abnormal PAP smear will undergo corresponding treatment. Those with normal PAP smears and positive HPV DNA results are requested to come back to the office for a repeat HPV test and if the infection has cleared, they will be vaccinated at this step. Vaccination will be performed to all eligible subjects (informed consent obtained and negative HPV test, normal PAP smear results) with either bivalent or quadrivalent HPV vaccine. The randomization to a vaccine type (bivalent, GSK or quadrivalent, MSD) will be on a regional principle, 50:50.

Follow up of all women will be done every 6 months with PAP smear and HPV tests for up to 5 years. Anti HPV 16 and 18 antibodies will be tested at months 7, years 3 and 5 with the method (ELISA) and compared between the different vaccine groups as well as to efficacy results.

Discussion: With this program we propose an algorithm for vaccination of adult sexually active women. We suggest that only women with normal PAP smears and negative for the vaccines types HPV 16 and 18 should be vaccinated.
**Estimation of Sexual Education Level in a Hellenic Vaccinated Population Against HPV Infection From a Nationwide Recording Vaccination Network**

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**Objectives:** The assessment of the level of sexual education of young Hellenic adolescents studying a sample population vaccinated against HPV, which is recorded and monitored on a nationwide recording network of HPV vaccination.

**Methods:** 910 vaccinated girls and young women were recorded at University Gynecology Clinics, Public and Private Maternity Hospitals during the year 2008. We assessed the level of sexual education in the vaccinated population in the majority of women (55%), sexual debut was found to be between age 16 and 18 years. 13% of the sample population initiated sexual contact before the age of 16. The average number of sexual partners by the age of 26 years was 2.7. Friends and family were the main source (60%) of information on sexual health issues. 40% were using condoms regularly, 6% were using the combined oral contraceptive pill and 1.9% had had an IUD fitted. 73% of girls and adolescents did not smoke, and of those, 2% were previous smokers.

**Conclusions:** The age of coitarche in our sample was low. Although percent of the girls were sexually active, a large proportion of them were not using effective methods of contraception. More needs to be done to inform and educate young women in Greece in order to improve their sexual health. Vaccination against human papillomavirus acts as an incentive to approach health services and is likely to promote sexual education in addition to increasing the level of prevention from HPV.

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**EVALUATION OF IMPLEMENTATION OF HPV VACCINATION PROGRAM FROM A NATIONWIDE HELLENIC VACCINATION RECORDING NETWORK**

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**Objectives:** To establish a data recording network of a vaccinated female population against human papilloma virus to assess the implementation of HPV vaccination program in a Hellenic population.

**Methods:** 910 adolescent girls and young women were vaccinated at University Gynecology clinics, Public and Private Maternity Hospitals during the year 2008. An analysis of the sample showed that almost the entire sample was vaccinated with the quadrivalent vaccine against human papilloma virus (types 6,11,16,18). Regarding the compliance level of vaccination, 90% of vaccinated population completed the vaccination regimen. 25% of vaccinees delayed the 2nd and 3rd dose of vaccination. The majority of people in this group were young female students aged 18 to 23 years, which indicates the need for flexibility in dosing regimen when it comes to vaccinating teenage girls and young women after puberty. The majority of women (35%) were between 23 and 26 years of age. Finally regarding the safety of HPV vaccine there were no serious side effects reported.

**Conclusions:** Analysis of the sample population during the first year of the National HPV recommendations shows that the implementation of HPV vaccination has followed the Greek recommendations, leading to high compliance, high safety and good monitoring. The recording of HPV vaccination continues to study other parameters for evaluating the implementation of HPV vaccination in Hellas.
PARENTS AND YOUNG PEOPLE’S ATTITUDES TOWARDS HPV VACCINATION: A QUALITATIVE HEALTH TECHNOLOGY ASSESSMENT (HTA)

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Objectives: Human papillomavirus (HPV) infections can cause a range of burdensome conditions, one of the most severe being cervical cancer. How to implement HPV vaccination in national health care systems is currently being considered in several countries. In order for a national vaccination programme to be effective, public attitudes towards the vaccine is of paramount importance. The present study was part of the Danish Health Technology Assessment (HTA) of parents’ and young people’s attitudes towards HPV vaccination.

Methods: The use of qualitative social science research methods can contribute to a deeper understanding of perceptions of health and medical decision making processes. This qualitative study was based on four focus groups with Danish parents and young people of both sexes. The three focus groups with parents were divided according to the children’s age: 9-10 years, 11-12 years and 13-17 years. The fourth focus group comprised young people aged 18-22 years.

Conclusions: The participants mostly held positive views on the possibility of vaccinating against cervical cancer, though some parents of the younger children were concerned about unknown side effects. Attitudes toward the vaccine were not negatively affected by it being against a sexually transmitted virus. In light of the mode of virus transmission, the participants did feel that both sexes should be vaccinated and that vaccination should occur before the onset of sexual activity. Still, parents of teenage children also wished for their children to be vaccinated in case they had not yet been exposed to HPV. Participants aged 18-22 years thought they might already be infected with HPV, thereby making prevention irrelevant for them. The cost of vaccination was however of great importance. If the price was high (then nearly €500), the participants believed that HPV vaccination would be downgraded despite its perceived importance. They suggested that HPV vaccination be included in the childhood immunization programme.

PSYCHOSOCIAL IMPACT OF HPV-RELATED DISEASES IN MEN IN THE UK: THE PASQUAL STUDY

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Objectives: Data on health-related quality of life in women with cervical cancer is well reported, but data on other HPV-related diseases, particularly in men, are scarce. Our objective was to assess the psychosocial impact of genital warts in UK males, using specific and generic instruments.

Methods: PasQuaL (Papillomavirus associated Quality of Life) was an observational cross-sectional study with a nested sub-population longitudinal follow-up. From May 2008 to March 2009, 1,264 subjects (men and women) aged 18-64 years were recruited from 15 community and hospital healthcare clinics. They completed a series of self-administered instruments including the EQ-5D (generic) and the CECA (Cuestionario Especifico en Condilomas Acuminados), a specific tool capturing the emotional and sexual burden of genital warts. Socio-demographic and clinical data were also collected.

Conclusions: Baseline analyses showed a mean EQ-5D index score of 0.89 in men with genital warts aged 18-25 years, compared with 0.94 in the general UK population (p= 0.053); the mean EQ-5D visual analogue scale score was 79.27, compared with 87.15 in the general UK population (p=0.022). Data from the CECA questionnaire will be presented. Our data show that genital warts are associated with a significant decrease in quality of life in men. These results improve our knowledge and understanding of the qualitative aspect of the burden of disease for genital warts that could be prevented by quadrivalent HPV vaccination.
SELF COLLECTED HPV TESTING ACCEPTABILITY: COMPARISON OF TWO SELF SAMPLING MODALITIES

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Objectives: Strong evidences shows that HPV testing can be used as primary test in cervical cancer screening. HPV self sampling has the potential to substitute physician/nurse collected sampling and could be useful for patients that do not respond to screening or where organized screening system is still not fully implemented. Our objective was to compare the acceptability of 2 different self sampling methods.

Methods: 205 women undergoing an excisional procedure for CIN agreed to participate. 111 patients received a Hybrid Capture (HC) Cervical Sampler™ (Qiagen) and 94 received a self lavaging device, the Pantarhei® screener (Pantarhei Devices), both with written instructions. Self sampling was performed in the restroom with the HC sampler and on a gynecological bed with the Pantarhei screener, just before the clinician collected HPV test and the excisional procedure. Women were given afterward a questionnaire including 5 numeric scales to analyze the general and the physical acceptability of the self sampling and the embarrassment, pain and difficulty experienced using each device. Patients were also asked if they preferred the self or the clinician sampling method.

Results and Conclusions: Both self sampling methods were generally accepted with a high score, but the acceptability was statistically higher in the group using the Pantarhei screener (Cochran-Armitage test for trend, p-trend = 0.005). Embarrassment was generally low and it was significantly lower in the group using the Pantarhei screener (Cochran-Armitage test for trend, p-trend = 0.042). Both self sampling methods were physically well accepted, not painful and quite easy to be performed. More than half of the patients (68%) preferred the self to the clinician sampled test, specially the group using the Pantarhei screener (77.6%) compared to the group using the HC Cervical Sampler (60.4%). This difference, between the 2 groups considered, was statistically significant (Two-sample two-sided binomial test of proportions; p-trend = 0.005). In conclusion, the present study shows that self sampling is an HPV testing modality which is very favourably accepted by the women. A sampling device specifically developed for self sampling, like the Pantarhei screener, shows the highest degree of women satisfaction.
In addition to the evaluation of HPV vaccines in terms of efficacy and safety, HPV vaccine trials are sophisticated and detailed studies on the natural history of HPV and cervical neoplasia.

Some of the strengths of Phase III studies are: a careful follow up of large numbers of women with frequent visits and highly controlled assessment of the cytology results, the virology results and the diagnostic procedures.

Information gathered in such trials has identified amongst other that:

1) CIN 2+ often includes multiple HPV types. Causality allocation remains an issue that might impact the quantitative estimates of vaccine efficacy. More specific analyses of these cases confirms the notion that lesions are clonally in origin and that only one HPV type is responsible of any given lesion.

2) Sampling from the cervix using either spatula or biopsies generates a mix of DNA from different parts of the cervix, and has the potential to generate unclear result in the presence of multiple lesions and multiple infections.

3) HPV 16 and 18 results including consistent findings on histology and virology endpoints have validated 6 and 12 month persistent (type specific) HPV infections as endpoints for other HPV types and in future vaccine trials. These endpoints also avoid the etiological uncertainty of histological lesions with multiple HPV types.

4) the incidence rate of CIN 2+ in a protocol including a very close follow up and an aggressive scheme of diagnostic procedures seems consistent with the notion that the time lag between HPV exposure and CIN 2+ might be rather short in some instances. A limitation of current trials is that they were designed and powered to evaluate HPV 16 and 18 with the identified size and time to follow up. Interpretation of results for other HPV types remains a difficulty and has to rely on compound results from groups of HPV's (analyses irrespective of HPV types) and persistent infection endpoints.

The intensity and quality of the follow up requires careful translation and comparisons of the trial's results to the conventional results generated by clinics or screening programs.
HISTOIRE NATURELLE DE L'INFECTION À HPV
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Le cancer du col de l'utérus représente le deuxième cancer féminin à l'échelle mondiale. La majorité de ces cancers surviennent dans les pays en voie de développement. Dans les pays développés, l'incidence des carcinomes épidermoides du col utérin diminue alors que celle des adénocarcinomes est en pleine croissance. Les HPV sont également responsables d'un nombre important de cancers du pénis, de l'anus, de la vulve et du vagin ainsi que de cancers des voies oro laryngées. L'identification de HPV spécifiques considérés à haut risque comme cause de cancers génitaux offre l'opportunité d'améliorer l'efficacité des programmes de dépistage. Néanmoins cette perspective nécessite une meilleure compréhension de l'histoire naturelle de l'infection par les HPV et du développement des lésions génitales.

L'âge des individus, le génotype de HPV mis en jeu ainsi que l'immunité acquise lors de l'infection constituent des éléments importants de l'histoire naturelle des maladies associées aux HPV, en particulier dans le risque de progression ou de régression des lésions ou de ré infection. Au cours de cet exposé seront également abordés divers points susceptibles d'influer sur les stratégies de prévention des lésions du col, notamment la signification de la persistance de l'infection, de la charge virale, de l'intégration de séquences d'ADN viral au génome cellulaire, et des infections multiples. Enfin, une attention particulière sera apportée aux phénomènes épigénétiques du cancer du col utérin.

IMPACT D'UN RÉSULTAT ANormalAu TEST DE PAPANICOLAOU SUR LA QUALITÉ DE VIE.
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Objectifs : La vaccination contre le papillomavirus humain est maintenant recommandée dans plusieurs pays. Bien que des estimations valides des années-personnes sans invalidité (quality-adjusted life-years, QALY) soient nécessaires pour les modèles de coût-efficacité, très peu de données sont disponibles quant à l'impact d'un résultat anormal au test de Papanicolaou (Pap) sur la qualité de vie. L'objectif est de décrire l'impact psycho-social d'un résultat anormal au test de Pap et d'estimer prospectivement les années-personnes sans invalidité perdues à la suite d'un résultat anormal.

Méthodologie : Entre 08/2006 et 08/2008, 492 femmes avec un résultat anormal et 471 femmes avec un résultat normal, de même âge et provenant de la même clinique ont été recrutées à travers le Canada. La qualité de vie liée à la santé a été mesurée de plusieurs manières. Bien que des estimations valides des années-personnes sans invalidité (quality-adjusted life-years, QALY) soient nécessaires pour les modèles de coût-efficacité, très peu de données sont disponibles quant à l'impact d'un résultat anormal au test de Papanicolaou (Pap) sur la qualité de vie. L'objectif est de décrire l'impact psycho-social d'un résultat anormal au test de Pap et d'estimer prospectivement les années-personnes sans invalidité perdues à la suite d'un résultat anormal.

Conclusion : Au recrutement, 46% des femmes avec un résultat anormal et 47% des femmes avec un résultat normal, de même âge et provenant de la même clinique ont été recrutées à travers le Canada. La qualité de vie liée à la santé a été mesurée de plusieurs manières. Bien que des estimations valides des années-personnes sans invalidité (quality-adjusted life-years, QALY) soient nécessaires pour les modèles de coût-efficacité, très peu de données sont disponibles quant à l'impact d'un résultat anormal au test de Papanicolaou (Pap) sur la qualité de vie. L'objectif est de décrire l'impact psycho-social d'un résultat anormal au test de Pap et d'estimer prospectivement les années-personnes sans invalidité perdues à la suite d'un résultat anormal. Les années-personnes sans invalidité perdues à la suite d'un résultat anormal au test de Pap affecte principalement la santé mentale et occasionnelle, durant les 3 mois suivants l'annonce, une perte équivalente à 2,5 jours de vie en santé.
Dépistage du cancer du col, cancers et pré-cancers, données actuelles en France

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Le dépistage du cancer du col utérin en France est un dépistage individuel dont les modalités font l'objet de recommandations de la HAS. Celles-ci prévoient la réalisation d'un frottis cervical tous les 3 ans après deux frottis normaux espacés d'un an, chez les femmes entre 25 et 65 ans, à l'exception des femmes vierges et hystérectomisées.


Quatre expérimentations de dépistage organisé ont été lancées au début des années 1990 et trois sont encore en fonctionnement, en Alsace, Isère et Martinique.

La publication par l'INVS en 2007 de l'analyse de ces 3 sites a montré que l'invitation systématique des femmes non suivies, selon le cahier de charges national, conduisait à une progression de la couverture de la population de l'ordre de 10 à 15%. Les programmes départementaux permettent également de progresser en matière de qualité grâce à l'instauration d'actions d'assurance qualité et au suivi des femmes ayant présenté un frottis anormal.

En juin 2009, suite à un appel à candidature lancé par la Direction Générale de la Santé et l'INCa, dix nouveaux départements ont été retenus pour mettre en place des programmes de lutte intégrée contre le cancer du col utérin. Tous appliquent en matière de dépistage, le cahier des charges national, conduisant à une progression de la couverture de la population de l'ordre de 10 à 15%. Les programmes départementaux permettent également de progresser en matière de qualité grâce à l'instauration d'actions d'assurance qualité et au suivi des femmes ayant présenté un frottis anormal.

Le Plan cancer 2, associé à cette action pour la lutte contre le cancer du col, un programme d'expérimentations visant à définir la faisabilité sur le terrain, en France, d'une utilisation du test HPV en dépistage primaire, et qui feront l'objet de prochains appels à projet lancés par l'INCa.

DÉPISTAGE DES CANCERS DU COL : QUELS TESTS UTILISÉS ?

Hélène SANCHO-GARNIER

Le dépistage organisé du cancer du col par le frottis cervico-vaginal réalisé tous les 3-5 ans chez les femmes, à partir de 20-30 ans et jusqu'à 60-65 ans est l'exemple type de dépistage coût-éfficace (OMS 2002, IARC 2005), dans les pays où la qualité des tests et les taux de couverture sont adéquates. L'actualisation en 2007 des recommandations Européennes conforte l'utilisation de la cytologie en dépistage initial et est complétée par la recherche des virus HPV oncogènes en cas de diagnostique cytologique de type ASC-US (triage).

Parmi les facteurs intervenant dans l'efficacité du dépistage, les taux de couverture et la qualité du test utilisé sont des critères majeurs, ce qui pour le frottis cervical pose parfois problème. En effet, outre un manque de sensibilité intrinsèque, une qualité insuffisante du prélèvement, de la préparation et/ou de la lecture peuvent en plus fortement diminuer cette sensibilité. De plus l'acceptabilité du test varie avec l'âge et les différentes cultures, et dans les pays où les infrastructures sanitaires sont défectueuses, le faible nombre de cytologiste et l'absence de cyto-technicien sont des obstacles majeurs à l'utilisation du frottis.

Dans le but d'améliorer la sensibilité des frottis et la couverture des populations féminines, divers tests ont été développés, en particulier le test « VIA » d'inspection visuelle et le test de recherche des HPV oncogènes « HPV-DNA ».

Le test VIA consiste à examiner le col à l'œil nu, avec un bon éclairage après l'avoir badigeonné (ou pulvérisé) avec une solution d'acide acétique à 3 ou 5 %, (l'utilisation de lugol ne semble pas apporter d'efficacité supplémentaire). Les lésions observées peuvent être prélévéées, voir traitées d'emblée. Ce test a une sensibilité de l'ordre de 60 à 80 % et une spécificité de 75 à 85 %. Évalué dans le cadre d'un essai randomisé en cluster en Inde, ce test (VIA + traitement d'emblée) paraît diminuer l'incidence (IHR = 0,75 ; CI : 0,55 - 0,95) et la mortalité (MHR = 0,65 ; CI : 0,47 - 0,89) par cancer du col. Ce test est une solution temporaire pour les pays à incidence élevée et revenus limités.

Le test HPV-DNA détecte la présence d'un virus oncogène au niveau du col utérin. Il est plus sensible que le frottis mais moins spécifique, tout particulièrement chez les femmes jeunes (< 35ans) en raison des infections transitoires sans conséquences ultérieures. Le manque de spécificité entraîne des colposcopies inutiles, des sur-traitements, le tout accompagné d'augmentation de l'angoisse liée au diagnostic de cancer.

La vaccination contre les virus HPV 16 et 18 change les conditions d'efficacité du dépistage, en diminuant la prévalence des lésions détectables, ce qui diminuera encore la valeur prédictive négative du frottis. C'est pourquoi l'utilisation du test HPV-DNA en première intention suivi d'un frottis en cas de positivité semble être une solution d'avenir, en particulier chez les femmes après 35 ans dans les pays où une telle technologie peut être suffisamment développée.

Un auto test HPV-DNA a également été développé ; avec une sensibilité de l'ordre de 74 % et une spécificité de 84 %, il permet de contourner le problème culturel de refus du frottis. Il restera cependant à convaincre les femmes ayant un test HPV positif à réaliser un frottis et/ou une coloscopie. La stratégie de dépistage depuis la sensibilisation des populations, jusqu'à la prise en charge des femmes dont les tests sont positifs, repose dans tous les cas sur l'organisation quel que soit le test utilisé.
1. Tous les cancers du col utérin sont associés aux HPV
2. Le dépistage basé sur le frottis est imparfait
3. Le dépistage HPV est validé dans le triage des ASC-US. Cette option est privilégiée lorsque le frottis a été réalisé en milieu liquide.
4. Le dépistage basé sur le risque (frottis + HPV)
   - Permet d’espacer l’intervalle du dépistage à 3 ans avec une plus grande sécurité que n’offre le seul frottis (NP1), l’absence d’HPV rassure instantanément et durablement.
   - La présence d’HPV invite à la vigilance sans signifier pour autant une lésion sous-jacente
   - Permet de détecter les CIN HG plus fréquemment et plus précocement que le seul frottis (NP1)
   - Les tests ADN cocktails (HC2, Amplicor) et le test ARNm (Aptima) qui ciblent 13 et 14 types viraux à risque respectivement, sont les plus sensibles (NP2).
   - Lorsque le frottis est négatif et le test HPV positif, les tests ARNm et le génotypage augmentent la spécificité et la prédiction lésionnelle (en cours d’évaluation).
   - Le dépistage des vaccinées basé sur le test viral est en cours d’analyse
5. Le dépistage basé sur l’HPV en première intention suivie doit encore être évalué
   - Le triage des HPV+ avec le frottis améliore la spécificité.

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Worldwide data on age-specific human papillomavirus (HPV) prevalence among female adolescents would be useful, in conjunction with data on age at first intercourse, to inform future policies to maximize the potential benefits of prophylactic HPV vaccination.

Genital HPV infection in women is predominantly acquired during adolescence, relatively quickly following initial onset of sexual activity given the high probability of HPV transmission between sexual partners. Among female university students (aged 18-20 years) in Seattle, the 2-year cumulative incidence of incident HPV infection was 32.3% (95% confidence interval (CI): 28.0, 37.1). Among sexually active participants at enrollment, the 2-year cumulative incidence of HPV infection: 38.8% (95% CI: 33.3, 45.0), was similar to that among virgins initiating sexual activity: 38.9% (95% CI: 29.4, 50.3).

Age trends of HPV prevalence differed across surveyed countries, but generally decreased with increasing age from a peak prevalence in younger women (≤ 25 years of age). In several regions, the highest HPV prevalence occurred among adolescent women, including in rural Mozambique (55%), Honduras (71%), Argentina (64%), and among attendees of a sexually transmitted infection (STI) clinic (91%) in Maryland. In the United States, HPV prevalence generally increased sharply among girls 14-19 years (24%), peaking at 20-24 years (45%). HPV prevalence among female adolescents and young adults in Australia ranged from 6% (19 years olds) to 41% (18-20 years). Worldwide variations in HPV prevalence across age appear to largely reflect differences in sexual behavior and sexual onset across geographical regions.

Further data on HPV DNA prevalence are needed among adolescent populations worldwide, given that many countries, especially in Asia, currently have limited available data. These data on the lifetime risk of acquiring an HPV infection may help in informing vaccination policies and assessment of the potential long-term impact of the HPV vaccines.
Les papillomavirus humains (HPV) sont à l’origine de l’infection sexuellement transmissible la plus fréquente dans le monde. Plus de 100 génotypes différents d’HPV ont été définis, dont environ 40 infectent les muqueuses génitales. Des études épidémiologiques ont montré que l’infection à HPV était acquise au début de l’activité sexuelle, le risque d’infection étant corrélé au nombre de partenaires sexuels. On estime que 80 % des femmes sexuellement actives auront été infectées à l’âge de 50 ans. Les HPV 16 et 18 sont les HPV à potentiel oncogène les plus fréquemment retrouvés dans les prélèvements cervicaux. Ce sont les agents étiologiques d’environ 70 % des cancers du col. La plupart des infections cervicales par les HPV ne sont pas associées à des anomalies cytologiques et représentent donc des infections latentes, inaparentes, asymptomatiques et le plus souvent transitoires. L’infection persistante peut entraîner la formation de néoplasies intraépithéliales cervicales (CIN) dont l’incidence et la prévalence sont déterminées à partir des programmes de dépistage des lésions cervicales par le frottis. On estime que la prévalence des frottis anormaux varie de 2,4 % à 5,5 % selon la technique utilisée (frottis conventionnel ou frottis en couche mince). La progression des CIN de haut grade (CIN3) vers le cancer est rare et lente, elle survient en 10 à 20 ans chez la femme immunocompétente. Il est maintenant reconnu que la prévalence des infections par les HPV et de la co-morbidité associée est élevée chez les personnes immunodéprimées, qu’il s’agisse d’une immunodépression iatrogène dans le cas d’une greffe d’organe ou d’une immunodépression liée à l’infection par le VIH. Les femmes séropositives pour le VIH ont un risque deux à trois fois plus élevé que les autres d’avoir des tâches d’HPV décelables dans les sécrétions cervico-vaginales et cinq fois plus élevé d’avoir des lésions cervicales, des condylomes vulvo-vaginaux ou des lésions intra-épithéliales anales. Les adolescentes séropositives pour le VIH sont également très souvent infectées par des HPV et porteuses de lésions cervicales. Plusieurs études récentes ont montré que les hommes infectés par le VIH avaient un fort taux d’infection anale par les HPV. L’incidence et la prévalence des cancers du col de l’utérus et du canal anal sont élevés chez les personnes infectées par le VIH.

Il n’a pas été démontré que les femmes transplantées rénales d’augmentation de la prévalence de l’infection par HPV, ni de hausse de celle des CIN de grade 3 depuis l’introduction de la CsA. Pour le cancer invasif du col utérin, presque toutes les données publiées plaident en faveur d’une augmentation significative du taux d’incidence standardisé, mais son amplitude est très variable, allant de 3,3 dans une étude australienne importante (mais déjà ancienne) à 25,3 dans une étude française récente. L’interprétation des données épidémiologiques doit tenir compte d’une augmentation du risque démontrée dès le stade de l’insuffisance rénale chronique et de la dialyse par rapport aux femmes issues de la population générale.

Dans ce contexte, les personnes immunodéprimées pourraient bénéficier d’une vaccination mais l’immunogénicité, l’efficacité et la tolérance n’ont pas été évaluées dans cette population. En France, dans l’avis du 9 mars 2007, le Comité Technique des Vaccinations et le Conseil Supérieur d’Hygiène Publique de France demandent que des études soient menées spécifiquement sur la vaccination chez les jeunes filles et jeunes femmes immunodéprimées. Un avis complémentaire relatif à l’âge de la vaccination contre les HPV des jeunes filles devant bénéficier d’une greffe a également été rendu par le Haut Conseil de la Santé Publique, recommandant que la vaccination contre les HPV puisse être proposée aux jeunes filles devant bénéficier d’une greffe avant l’âge de 14 ans et selon les données de l’AMM.

**Objectifs :** Revoir le fardeau des pathologies causé par les HPV 6, 11, 16 et 18 chez l’homme et les coûts engendrés par un programme de vaccination « neutres aux sexes ».

**Méthode :** Revue de la littérature pertinente aux sujets ci-haut mentionnés.

**Résultats :** Les souches HPV 6 et 11 causent mondialement et annuellement chez l’homme approximativement 15 millions de nouveaux cas de verrues génitales, 2000 à 3000 cas de papillomatoses laryngées juvéniles et adultes, alors que les souches 16 et 18 sont responsables à 90% de 40,000 mille cas de cancer de l’anus, 5000 cas de cancer du pénis et 50,000 cas des cancers oropharyngés. Le fardeau économique de ces lésions est dans le milliard de dollars US annuellement. L’efficacité du vaccin prophylactique quadrivalent 6, 11, 16 et 18/Gardasil™ contre les condylomes et les lésions génitales précancéreuses a été prouvée chez les hommes hétérosexuels et homosexuels : 90% de 40,000 mille cas de cancer de l’anus, 5000 cas de cancer du pénis et 50,000 cas des cancers oropharyngés. Le fardeau des pathologies-causé par les HPV 6, 11, 16 et 18/Gardasil™ contre les condylomes et les lésions génitales précancéreuses a été prouvée chez les hommes hétérosexuels et homosexuels, âgés de 16 à 26 ans naïfs aux souches HPV 16, 11, 16 et 18 à l’ordre de 90% et 79%, respectivement. Le vaccin quadrivalent est recommandant que la vaccination contre les HPV puisse être proposée aux jeunes filles devant bénéficier d’une greffe avant l’âge de 14 ans et selon les données de l’AMM.

**Conclusions :** Le fardeau des pathologies dues à HPV 6, 11, 16 et 18 chez l’homme est cliniquement et financièrement important. L’effet protecteur du vaccin quadrivalent est établi chez les garçons de 16 à 26 ans naïfs aux HPV 6, 11, 16 et 18. La vaccination universelle, « neutre aux sexes » résulterait en une immunité de groupe plus rapidement que si elle n’était pas limitée qu’aux jeunes filles. Exclure les garçons du programme de vaccination sous entend que seules les filles devraient porter le fardeau des considérations économiques et la responsabilité de la santé sexuelle des garçons.
Comme au niveau du col utérin chez les femmes, bon nombre d'hommes et de femmes sont contaminées au niveau de l'anus lors des premières expériences sexuelles. Les rapports anaux sont logiquement un facteur de risque majeur, particulièrement pour les lésions endo-canalières. Une centaine de génotypes peuvent infecter l'anus. L'histoire naturelle n'a pas été bien décrite jusqu'à présent mais nous observons que certains patients ne développeront jamais de lésion alors que d'autres auront des condylomes et/ou des dysplasies plus ou moins sévère voir un carcinoma épidermoïde. Cette évolution vers la dysplasie et le cancer est largement favorisé par une infection à un génotype oncogène. L'HPV 16 est le principal puisqu'il est retrouvé dans 75% des cancers anaux sur une cohorte récente de 366 cancers. L'autre principale facteur influençant cette évolution pérjorative est l'immunodépression et particulièrement l'infection VIH. Ce facteur multipliant le risque de cancer de l'anus par 40 à 80 selon les séries et les autres co-facteurs associés. A tel point qu'il y a actuellement 2 grands types de populations révélant un cancer anale : les femmes d'âges mûres et surtout récemment les homosexuels VIH +. Devant la forte incidence des cancers de l'anus et d'une prévalence de 23% de condylomes (36% chez les homosexuels, 15% chez les femmes et 11% chez les hétérosexuels masculins) chez les patients infectés par le VIH, des recommandations ont été rédigées pour réaliser un dépistage systématique dans cette population. Tous les VIH + et particulièrement les homosexuels masculins et les patients avec antécédents de lésion à HPV (anale ou génitale) doivent bénéficier d'un examen proctologique avec anuscopie. Elle peut être idéalement avec haute résolution et on pourra s'aider de la coloration à l'acide acétique et/ou au lugol. Les condylomes, les dysplasies de bas grade et de haut grade sont électrocoagulées sous anesthésie locale ou générale selon leurs étendues. Les récidives sont fréquentes et imposent un suivit très régulier. Les cancers ainsi dépistés sont traités par radio-chimiothérapie et/ou chirurgie avec une guérison dans 80% des cas.

**PR3-5**

**HPV AND CANCER OF THE ORAL CAVITY AND PHARYNX**

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**Objectives:** Ninety percent of oral cancer worldwide is attributable to tobacco smoking and chewing, and alcohol drinking. Poor eating habits and oral hygiene also play a role that has been, however, difficult to quantify.

**Methods:** The International Agency for Research on Cancer (IARC) Multi-centre Oral Cancer Study (1) is the largest case-control study published on the topic so far (1,670 cases and 1,732 controls from 9 different countries). It has provided insight into the broadest range of different molecular and serological markers of HPV infection.

**Conclusions:** Conclusions were largely based on the prevalence of antibodies against HPV16 early (E) protein 6 or 7, that were associated with risk for cancer of the oral cavity [odds ratio (OR)=2.9; 95% confidence interval (CI): 1.7-4.8] and of the oropharynx (OR=9.2; 95% CI: 4.8-17.7). The IARC study showed that the effects of HPV and smoking were additive, rather than multiplicative. Risk additivity would suggest that HPV and smoking operate, at least in part, at the same step or steps of multi-stage carcinogenesis in the oral cavity and oropharynx. It has recently been shown that one such step is the inactivation of the tumour supressor gene TP53 and hence HPV E6-induced p53 degradation and chemically-induced TP53 mutation can be seen as alternative mechanisms leading to a phenotype of p53 pathway inactivation. For the relationship between TP53 mutation and HPV16 to be mutually exclusive, it is not sufficient, however, to find HPV DNA in tumour biopsies; HPV E6 DNA must also be transcriptionally active (2). In addition, some studies based on in situ hybridisation, have pointed to a specific interaction between HPV and the tonsillar epithelium (3).

**References**


La stratégie choisie par les industriels pour le développement de vaccins prophylactiques anti-papillomavirus et qui découle des données acquises lors d'expériences de protection dans des modèles animaux a été d'utiliser des particules virales vides recombinantes recréant une structure tridimensionnelle analogue à celle de la capsule virale. Ces vaccins sont constitués de la protéine majeure de capsule des papillomavirus (L1) obtenue par surexpression en système baculovirus/cellules d'insecte ou par des levures recombinantes. Les protéines L1 sont assemblées sous forme de pseudocapsides virales, structures antigéniques qui portent les épitopes conformationnels responsables de la production d'anticorps neutralisants protecteurs. Le but des vaccins est d'induire des anticorps qui empêcheraient le virus d'atteindre sa cible, c'est-à-dire les kératinocytes de la couche basale de l'épithélium, évitant ainsi l'infection virale. Le vaccin doit être injecté par voie intramusculaire avec un protocole de 3 doses de 0,5mL en une période de 6 mois.

L'immunogénicité des vaccins anti-HPV est très bonne puisque plus de 99% des sujets vaccinés contre les types 16 et 18 développent des anticorps après la troisième injection. Les titres d'anticorps anti-HPV16 observés sont 66 à 148 fois plus élevés après vaccination qu'après une infection naturelle, et 19 à 129 fois pour le type 18. Les titres d'anticorps anti-HPV sont à un taux maximum un mois après la troisième injection de vaccin, déclinent rapidement pendant les 24 mois suivants, puis plus lentement. À trois ans, les titres sont toujours 2 à 20 fois supérieurs à ceux qui sont observés à la suite d'une infection naturelle, surtout vis à vis de la VLP-16. Ces anticorps persistent pendant au moins 5 ans à un titre supérieur à celui qui est observé lors d'une infection naturelle.

La protection est assurée par les IgG présentes dans le sang, anticorps neutralisants capables de transsuder dans les sécrétions cervicales. Il existe une corrélation entre le titre des anticorps détectés par ELISA et le titre des anticorps neutralisants. Du fait du nombre limité d'études réalisées et du faible nombre de sujets infectés après vaccination, il n'a pas pour l'instant été possible de déterminer s'il existait un titre d'anticorps neutralisants protecteur, ni son niveau.

Chez les sujets vaccinés, il existe aussi une réponse immune de type cellulaire, caractérisée par une augmentation de la prolifération des cellules T et par la production de cytokines versus les sujets témoins. L'incubation des cellules mononucléées du sang (PBMCs) de sujets avec des VLP conduit en effet à la production de cytokines spécifiques des profils Th1 et Th2 et de l'IL-10. Les réponses cellulaires sont les plus élevées chez les sujets présentant les plus forts titres d'anticorps. Cette réponse cellulaire est importante pour l'induction de la réponse immune et sa persistance, et elle pourrait jouer un rôle dans l'efficacité vaccinale et la durée de la protection.
Les cancers de la vulve surviennent le plus souvent (> 90%) dans les suites d’une néoplasie vulvaire intra-épithéliale ou VIN. Les VIN représentent 57% des néoplasies vulvaires et sont en réalité plus fréquentes que les carcinomes invasifs. Les VIN correspondent à 2 entités anatomo-pathologiques : il peut s’agir soit de VIN liées au papillomavirus humain (HPV) ou VIN classiques (aussi appelée VIN indifférenciée), soit de VIN survenant dans un contexte d’hyperplasie épithéliale ou de lichen et qui ne sont pas liés à l’HPV (VIN différenciée).

On suspecte une VIN différenciée devant l’apparition d’une leucoplasie dans un contexte de lichen (peau pâle, mince avec une fusion des petites lèvres, un rétrécissement de l’introït et un encapuchonnement du clitoris). Toute leucoplasie doit a priori être biopsiée. Les anomalies histologiques des VIN différenciées siègent dans les couches basales uniquement. Elles sont localisées au tiers inférieur de l’épithélium (kératinocytes éosinophiles de grande taille, cellules basales nucléolées, chromatin vésiculaire).

Les lésions de VIN classiques sont dues au papillomavirus humain (essentiellement l’HPV16) et se partagent en 3 groupes selon les manifestations cliniques et dont les risques de dégénérescence sont différents :
- la papulose bowénoïde qui survient chez la femme jeune et qui dégénère rarement.
- la VIN extensive qui survient chez la femme jeune immunodéprimée et qui dégénère souvent.
- la maladie de Bowen survenant chez la femme âgée et qui dégénère dans 20% des cas.

Sur le plan histologique les lésions observées sont identiques. Ce sont des dysplasies sévères étagées associant une désorganisation architecturale cellulaire avec un déséquilibre nucléocytoplasmique (gros noyaux), de nombreuses atypies nucléaires et parfois une activité mitotique intense. Des cellules dyskératosiques sont présentes sur toute la hauteur de l’épithélium.

Le traitement de choix du lichen scléreux ano-génital est l’utilisation d’un dermocorticoïde extrêmement puissant tel le Dermoval® en pommade (clobétasol propionate à 0,05 p. 100, topique corticostéroïde de classe I). Il est recommandé d’appliquer et de faire pénétrer sur la vulve une quantité de la grosseur. Le 1er bilan se fait au terme de 4 à 6 semaines car toute leucoplasie doit avoir complètement disparu. Toute lésion persistante, toute ulceration persistante, si petite soit-elle, doit alors être biopsiée si elle ne l’a pas été initialement.

Le traitement des VIN classique dépend en réalité de la forme clinique et de l’extension des lésions. Des études récentes ont montré l’efficacité du traitement des VIN classiques par Imiquimod (Aldara®) en application locale. On observe une régression des lésions histologiques avec une bonne tolérance. L’Imiquimod est devenu la thérapeutique de référence pour les VIN classiques, au moins pour les formes multicentriques. Le principal problème conceptuel du traitement médical des VIN est la possibilité de méconnaître une invasion débutante (10%). Ainsi l’exérèse avec 5 mm de marge reste le traitement de référence des lésions unifocales et donne l’assurance de ne pas méconnaître un invasif débutant aux prix d’une morbidité et d’une morbidité psycho-sexuelle non négligeable.

Les cancers du col utérin touchent souvent des femmes souhaitant conserver leurs possibilités de concevoir. D. Dargent a conceptualisé il y a 20 ans, une intervention radicale conservatrice : la trachélectomie élargie. L’intérêt de cette intervention est la possibilité d’avoir des grossesses et des enfants en bonne santé chez des patientes présentant un petit cancer du col utérin. La technique opératoire associe une recherche du ganglion sentinelle puis une lymphadénectomie pelvienne per-coelioscopique, et finalement le geste de trachélectomie élargie par voie vaginale. Le but de l’opération est de réaliser une exérèse du col utérin avec une collarote vaginale et la partie proximale des paramètres (équivalent à une hystérectomie élargie de type Piver II). De 1986 à 2009, 160 trachélectomies élargies ont été réalisées dans notre service. Huit (5%) rechutes ont été observées aboutissant au décès dans 6 cas. Le seul facteur pronostique des rechutes statistiquement significatif est la taille tumoral (en cas de tumeur > à 2cm, 6 rechutes (21%) ont été observées contre 2 rechutes (2%) lorsque la taille est < à 2cm). L’actualisation des grossesses obtenues après trachélectomie élargie a été réalisée en 2004. A cette date, nous avions noté la naissance de 49 enfants vivants et en bonne santé après trachélectomie élargie pratiquée dans notre service. Nos données ont été confirmées par d’autres équipes à travers le monde. La trachélectomie élargie est une intervention efficace et non dangereuse qui permet de préserver la fertilité chez des femmes jeunes souhaitant des enfants. Après réalisation de cette intervention le risque de rechute est de moins de 5% et les chances d’avoir un enfant vivant en bonne santé sont de l’ordre de 65%.
Current methods for HPV screening rely on the detection of L1 DNA from high risk genotypes (HRHPV). These assays have very high negative predictive values (~99%) and as such have been used to triage women to longer screening intervals. The literature has shown that the positive predictive value for pre-cancerous and cancerous lesions (CIN 2+) is less than 50% for HPV DNA screening. The purpose of this study was to compare HPV DNA screening with intracellular HPV E6, E7 mRNA quantification in an effort to improve the overall performance of cervical cancer screening while potentially reducing the number of women requiring colposcopy. Liquid based cervical cytology specimens collected in either PreservCyt (Hologic, Marlborough MA) or SurePath (Tripath Imaging, Burlington NC) were submitted for routing cytology, HPV HRDNA detection by Hybrid Capture 2 (Qiagen, Gaithersberg, MD) and HPV E6, E7 mRNA quantification in cells using HPV OncoTect (incellDx, Menlo Park, CA). We analyzed a total of 743 samples including 73 with CIN 2, CIN 3, or squamous cell carcinoma and 670 samples from women with normal cytology. Biopsy was performed based on current standards of care.

The positive predictive value of HPV E6, E7 mRNA quantification in cells for CIN2+ was 83% which was greater than HPV DNA alone (29%). The specificity was 96% based on 670 samples with normal cytology. There was a statistically significant difference in the percent of ectocervical cells expressing E6, E7 mRNA in women with CIN2, CIN3, or cancer (mean 10.7%) compared to women with normal cytology (mean 1.1%) with a P<0.001. With similar sensitivity and greater specificity, HPV E6, E7 mRNA quantification in cells is an improvement over HPV DNA for cervical cancer screening especially in the developing world.

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

References