APRESENTAÇÃO DE RESUMOS SELECIONADOS

19TH INTERNATIONAL PAPILLOMAVIRUS CONFERENCE
1 A 7 DE SETEMBRO, 2001, FLORIANÓPOLIS, SC – BRASIL

A infecção causada pelo Papilomavírus Humano é conhecida desde a antigüidade. No entanto, a sua relação com as lesões intra-epiteliais cervicais só foi devidamente estabelecida em 1976 através dos trabalhos de Meisels e colaboradores. Posteriormente, os estudos epidemiológicos demonstraram nítida relação do HPV com as lesões invasoras. Neste particular Bosch e colaboradores, evidenciaram que mais de 95% dos casos de câncer do colo do útero contém DNA-HPV.

Embora o avanço dos conhecimentos tenha sido muito significativo, a impossibilidade de cultivo do HPV limitou, de certa forma, o desenvolvimento da pesquisa clínica. A introdução na década de 70 dos métodos moleculares por Zur Hausen e colaboradores, o aprimoramento e a descomplicação da utilização desses métodos permitiu o avanço definitivo da pesquisa do HPV como fator necessário na carcinogênese do Trato Genital Inferior, em especial do colo do útero.

O crescente interesse do clínico sobre o conhecimento desses métodos e a sua aplicação na clínica com o devido senso crítico da utilização dos mesmos, permitiu estreitamento na relação entre pesquisadores da bancada e aquele.

A partir desse claro interesse tanto do pesquisador quanto do clínico ensejou a criação da *International Papillomavirus Society* (IPVS), atualmente presidida por Thomas Broker, motivando a realização dos vários eventos envolvendo, ao mesmo tempo, ginecologistas, dermatologistas, urologistas, citologistas, patologistas, imunologistas, virologistas, epidemiologistas dentre outros, com o intuito de juntos, discutirem os avanços epidemiológicos, clínicos e laboratoriais, pertinentes a infecção pelo HPV nos diferentes sítios humano.

De 1 a 7 de setembro de 2001, na cidade de Florianópolis, Brasil foi realizada a *19 th International Papillomavirus Conference* abordando todas as áreas anteriormente referidas. O evento foi organizados por *Luísa L. Villa (Brasil), Gustavo Amestoy (Argentina) e Eduardo L. Franco (Canadá) e patrocinado por Roche diagnostics, Merck, Sarp & Dohme vaccines, GlaxoSmithKline e 3M Pharmaceuticals, Children Vaccine Program at Path, Cytyc Corporation e Ludwig Institute for Câncer Research*. Contou com apresentação de 202 comunicações orais e de 312 pôsteres.

No entanto, com a realização de workshop clínico durante dois dias,foi dada especial atenção as patologias do trato genital inferior HPV- induzidas, onde foram discutidos os aspectos mais atuais da infecção ano-genital.

Com a finalidade difundir os tópicos ali debatidos sobretudo entre aqueles que embora interessados não puderam comparecer àquele evento, selecionamos os que julgamos serem de maior interesse para os clínicos para serem divulgados no DST - Jornal Brasileiro de Doenças Sexualmente Transmissíveis.

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EPIDEMIOLOGIC EVIDENCE

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STATE OF THE ART EVIDENCE ON THE ROLE OF CERTAIN HPV IN THE ETIOLOGY OF THE DISEASE.

The evidence relating HPV infections to cervical cancer includes a large and consistent body of studies indicating a strong and specific role of the viral infection in all countries where investigations have taken place. The association has been recognized as causal in nature by a number of international review parties since the early 90's (IARC 1992, IARC 1995,National Institutes of Health 1996).

Nucleic Acid Amplification Techniques (NAT) regularly identifies HPV DNA in 90-95% of the cervical cancer specimens both squamous cell and adenocarcinomas. Detailed investigations of the few cervical cancer specimens that appear as HPV DNA negatives in every series has been conducted and the results strongly suggest that these are largely false negatives. The reasons of non-detection are attributable to: a) poor quality of the specimen, poor preservation or absence of cancer tissue b) HPV DNA integration into cellular DNA with increased target fragmentation and c) NAT technology, notably length and target sequences of the b globin probes and of the HPV type specific probes. (Bosch et al. 1995, Walboomers et al. 1999).

HPV TYPES

Of the more than 35 HPV types found in the genital tract, some 10 (HPV types 16,18,31,33,35,45,51,52,58 and 59) have been adequately evaluated as high risk types in relation to invasive cervical cancer. For all of them, risk estimates were greater than 30 (range 35-350) strongly suggesting that these associations are causal in nature (Muñoz et al. HPV2000, abstract 053 reported in Bosch et al. 2001).

HPV 16 accounts for some 50% to 60% of the cervical cancer cases in most countries, followed by HPV 18 (10-12%) and HPV 31 and 45 (4-5 % each). Cervical adenocarcinomas showed a slightly different distribution and the most common types are HPV 16 (some 45%) HPV 18 (some 40%) and HPV 45 and 59 (4-5 % each).

In series of women without cervical lesions, (corresponding to controls in most case control studies or ad-hoc HPV prevalence surveys from the general population) the HPV type specific distribution embraces a much larger series of viral types. HPV 16 remains again the most common type (some 20 %) followed by HPV 18 (some 10%) HPV 45 (some 8%) HPV 59 (some 2 %) and smaller proportions of some 30 additional HPV types. Many of these rare types are occasionally found in controls and still convey a high risk for cervical cancer. (Muñoz et al., abstract 53 and Bosch et al. abstract 64 reported in Bosch et al. 2001).

It is of interest to notice that the geographical variation in type distribution has not been fully documented. Some recent studies from areas where little work has been done in the past suggest that some additional variability could be expected. For example, high rates of HPV 35 and 58 in the general population in Mozambique is now being reported (X Castellsague et al. submitted). New technical developments are also describing high frequencies of multiple HPV infections that were most probably undetected by previous testing systems (J Kornegay special contribution).

Finally, studies on HPV variants (variation within HPV types affecting down to one nucleotide of the viral genome) are beginning to unveil that the risk of some HPV 16 variants (non-european like) may differ from that of the HPV 16 European prototype (Xi et al. 1997, Hilldesheim et al. 2001). The geographical distribution of HPV variants is still being described and its relevance for HPV testing and for vaccine developments is still uncertain.

COHORT STUDIES

Cohort studies have consistently shown that HPV infections precede by some 10-15 years the development of cervical cancer. In addition to HPV DNA detection, additional markers of neoplastic progression includes HPV type, estimates of the viral load, persistency of the viral detection (as determined by repeated sampling) viral integration and possibly the presence of other environmental factors and some, less known, host factors. Most importantly, close follow up of cohorts of women investigated as to their HPV status have established that 1) the presence of HPV DNA is necessary for the development and persistency of cervical neoplasm and 2) that disappearance of the viral DNA predicts regression of the neoplastic cells, (Ho et al., 1995 & 1998, Koutsky et al. 1992, Nobbenhuis et al. 1999, Wallin et al. 1999). even at a stage of HGSIL (Meijer et al 2001 pc). The latter (i.e identification of HGSIL that is scheduled to spontaneous regression) may prove to be of substantial importance in the evaluation of screening methods and tests. Additional markers may be developed that provide information on morphologically identical lesions with opposite prognosis. To this respect, viral load, viral integration or markers of viral and cellular genetic interaction may prove to be of value.

Understanding of the time intervals of transient infections is likely to be of critical importance to define how best to use HPV testing in screening programs. From various sources it has been estimated that the mean duration of transient infections for most high risk viral types seems to follow a normal distribution with averages is in the order of 8 months and that HPV16 may perhaps double this interval. This seems to be consistent in both high risk and low risk countries (Ho 1998; Koutsky et al., 1992; Franco et al., 1999).

Cohort studies are also indicating that infection with one HPV type does not confer protection against novel infections with philogenetically related HPV types or with other HPV types (Thomas et al. 2000, Liaw et al. 2001).

CASE CONTROL STUDIES

The IARC research program on HPV organized a series of case control studies in different countries, mostly in areas at high risk for invasive cervical cancer. To date, this represents the largest data set on invasive cancer in high-risk countries and a major source of reference data.

Preliminary results on the pooled analyses of studies

in nine countries, including some 2288 invasive squamous cell carcinomas, 141 adenocarcinomas and 2513 matched controls were presented at the 18 th International Papillomavirus Conference (Bosch et al. 2001). The adjusted Odds Ratios (the factor by which the risk of cervical cancer of a given woman is multiplied if HPV DNA is detected) for HPV DNA detection was OR= 83.3 (95%CI: 54.9-105.3). Type specific risk estimates were as follows: HPV 16: OR= 182; HPV 18: 231; HPV 45 OR= 148; HPV 31 OR= 71.5; HPV 33 OR=77.6; HPV 35 OR=34.8; HPV51, OR= 42.7; HPV 52 OR= 145.7; HPV 58 OR= 78.9; HPV 59 OR= 347.3. The estimates of the attributable fraction AF %, (the proportion of disease that is related to HPV DNA) in most studies range from 90 to 98%.

The magnitude and the consistency of these estimates indicate that the association is one of the strongest identified for any human cancer making the case for claiming a necessary cause of the disease (i.e. that HPV negative cervical cancer cases are extremely rare). (Walboomers et al. 1999).

HPV AND PRE INVASIVE CERVICAL NEOPLASM

In developed countries and in areas where screening programs are operational, invasive cervical cancer is a relatively rare disease and most diagnosis are achieved at earlier stages (i.e. Carcinoma in Situ, HGSIL or CIN III). Several studies in both developed and developing countries have also shown that HPV is related to these precursor lesions with the same strength (as measured by the magnitude of the ORs) than the more advanced invasive cancers. (Schiffman et al. 1993, Bosch et al. 1993, Olsen et al. 1995, Moreno et al. 1995, Liaw et al. 1995, Kjaer et al. 1996, Herrero et al. 2000). As the HPV detection methods developed, the prevalence of HPV DNA in LGSIL / HGSIL increased steadily to levels of 80-90%. In fact, the very high prevalence observed in recent studies promoted the notion that HPV testing would not be suitable for triage of LGSIL (ALTS study group). A substantial part of the variability observed across studies is related to variability in the definition of the pre neoplastic lesions rather than variability in HPV testing.

OTHER RISK FACTORS FOR CERVICAL DYSPLASIA AND CERVICAL CANCER

Before HPV was investigated, epidemiological studies identified a series of factors as being more prevalent in cases of cervical cancer than in their control groups. This was the case for different sexual and reproductive behavioral traits, use Oral Contraceptives (OC), smoking or history of venereal infections, typically Herpes virus type 2. The ORs observed for such associations were in the range 1 to 3 and the results were inconsistent across studies. Having unveiled the very strong associations with HPV, all these putative additional factors require reevaluation.

Most investigators have attempted such reevaluation by restricting the comparison of the relevant exposures in cases of cervical cancer - most of which were shown to be HPV-positive- with their HPV-positive controls (women in the same age groups from the same underlying population, with HPV infection but without cancer). Preliminary results of the case control studies included in the IARC program were reported at the 18 th International Papillomavirus Conference and identified long term use of oral contraceptives as an environmental risk factor for HPV infected women. According to these results, users of Ocs for 5+ years and HPV DNA positive are at a 4-fold increased risk that equally HPV exposed women without Ocs exposure. The results are equally valid for cervical adenocarcinoma (Moreno et al. and Bosch et al. quoted in Bosch et al., 2001). Other factors that probably play a role are exposure to HIV infections and advanced immunosupression, (IARC, 1996) therapeutic immunosupression post transplant, exposure to Chlamydia Trachomatis (Anttila et al., 2001) and perhaps smoking .

ENVIRONMENTAL CO-FACTORS IN HPV CARCINOGENESIS

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Epidemiological and natural history studies have shown that despite the high prevalence of cervical HPV infections in sexually active women, only a small fraction of women infected with oncogenic HPV types will eventually progress to high-grade intraepithelial lesions (HSIL) and cervical cancer (CC). As infection by oncogenic genital HPVs is a necessary cause of CC, it has been assumed that there must be an important role of co-factors, which acting in conjunction with HPV, influence the risk of transition from cervical HPV infection to cervical malignancy.

Candidate co-factors may be classified into three groups: a) environmental co-factors, including parity, oral contraceptives (OC) use, tobacco smoking, co-infection with HIV or other STDs, and diet; b) HPV-related co-factors, such as genotype, co-infection with other types, HPV variants, viral load, and viral integration; and, c) host co-factors such as HLA, and immune response-related co-factors. The purpose of this presentation is to summarize and review the evidence of the role of environmental co-factors in HPV carcinogenesis.

Ideally, the study of environmental HPV co-factors for cervical cancer requires a study group known to be exposed to HPV. Thus, this review will mainly focus on the key studies that, using reliable HPV DNA detection methods, reported associations with co-factors within a well-defined HPV-positive group. These include: a case-control of CIN from the Portland (USA) cohort (Schiffman *et al.*, 1993), a US case-control study of CIN3 and CC (Lacey, Jr. *et al.*, 1999); a case-control study of ASCUS, LSIL and HSIL from the Copenhagen cohort (Kruger-Kjaer *et al.*, 1998); a case-control study of CIN3 from the Manchester cohort (Deacon *et al.*, 2000); a case-control study of HSIL and CC from the Costa Rica cohort (Hildesheim *et al.*, 2001); and a sero-epidemiologic case-control study within a cohort of Nordic women (Koskela *et al.*, 2000). Preliminary results are also presented from

the pooled analyses of the International Agency for Research on Cancer (IARC) case-control studies conducted in Spain, Colombia, Brazil, The Philippines, Thailand, Morocco, Peru, and Paraguay (IARC, in press).

High ***parity*** has consistently been found in most case-control studies to be associated with both CC and carcinoma in situ (CIS). Most of the major studies restricting the analy-

sis to HPV-positive women report na increased risk for CC or HSIL with increasing number of pregnancies. In the IARC study –which included 1853 cases and 255 controls, all positive for HPV DNA–, women with 7 or more full-term pregnancies had a 4-fold increase in the risk of developing CC as compared to nulliparous women (OR=3.8, 95% CI, 2.7-5.5). Risk of HSIL/CC significantly increased with increasing number of live births in the Costa Rica study, which included 146 HPV-positive cases and 843 HPV-positive controls. A similar trend was also found among HPV positive women in the Portland CIN study. A borderline association with CIN3 was found in the Manchester study. The Copenhagen case-control study, which included 71 HPV-positive cases and 155 HPV-positive controls, did not detect an effect of parity on HSIL, but this could be due to the low parity of the study population. Hormonal, traumatic and immunological hypotheses have been put forward as biologically plausible mechanisms to explain this association, but because of the concordance of effects with OC use, hormonal influences can be considered one of the most promising candidates in the search for HPV co-factors.

Use of ***oral contraceptives*** (OC) has also been found to be associated with CC in many, but not all, epidemiological studies. Among HPV positive subjects, the Manchester study, which compared OC use among 199 cases of CIN3 and 181 controls, found an OR of 1.5 (95% CI, 0.8-2.9) for 8-year or more of OC use. The two US studies did not find an increased risk for CIN or CC, but the study by Lacey found a moderate association with cervical adenocarcinoma in situ. The Copenhagen study found a RR of 3.8 (95% CI, 1.0-4.0) for women using OCs for 9 or more years. In contrast, the pooled data form the IARC study among HPV-positive women show that use of OCs for 5 or more years may be a cofactor that increases up to 4-fold the risk of CC. In the Costa Rica study, an increased risk was found only among women with less than 3 pregnancies. Hormonal-related mechanisms may influence the progression from pre-malignant to malignant cervical lesions by promoting integration of HPV DNA into the host genome. Alternatively, OCs might act by facilitating HPV infection or persistence.

The effects of ***smoking*** have been well studied in many case-control studies and show a moderate and statistically significant association with CIN and ICC, even after taking into account the strong effects of HPV. These findings are consistent with those found for ever smoking among HPV positive women in the IARC study (OR=2.2), the Costa Rica study (OR=2.3 for current vs. never), the Portland study (OR=2.7 for CIN 2-3), theCopenhagen study (OR=1.9 for current vs. never), and the Manchester study (OR=2.2). The fact that tobacco-specific carcinogen N-nitrosamines have been detected in the cervical mucus of smokers (Prokopczyk *et al.*, 1997) further strengthens the hypothesis of a synergistic action between cigarette smoking and high-risk types for the development of high-grade CIN and CC.

HPV Infection with other ***STDs*** has inconsistently been associated with CC. A case-control study provided sero-epidemiologic evidence that past infection with ***C. trachomatis*** conferred an increased risk for subsequent development of CC (OR=2.2, 95% CI, 1.3-3.5) after adjustment for, or stratification by HPV exposure (Koskela *et al.*, 2000). The IARC studies show that, among HPV positive women, the effects of *C. trachomatis* and ***HSV-2*** seropo sitivity are modestly associated with CC risk (OR=1.6, and OR=1.7, respectively). In contrast, ***HIV*** positive women have consistently been shown to be at an increased risk of developing cervical SIL when compared with their HIV-negative counterparts. Women infected with both HIV and HPV are at a much higher risk of SIL than women infected with either of the two viruses separately (La Ruche *et al.*, 1998). Consistent with these findings, evidence is also accumulating for a higher risk of anal squamous intraepithelial lesions among HIV-positive women (Holly *et al.* , 2001). As HIV infection is related to an immunocompromised state, these findings underscore the importance of host's immunological co-factors in HPV carcinogenesis. Concerning co-factors that may distinguish high-grade lesions from invasive CC, two studies provide strong evidence that, except for age, both CIN3 and CC share the same risk-factor profile (Moreno *et al.*, 1995; Thomas *et al.*, 2001).

Other putative, although less consistently identified co-factors include nutritional factors, socioeconomic-related variables, and genital hygiene.

In summary, based on the largest epidemiological studies that using sensitive HPV DNA detection methods allowed for the strong effect of HPV by means of restriction to HPV-positive women, high parity, long-term OC use, smoking, and HIV co-infection are the most consistently identified environmental co-factors likely to influence the risk of progression from cervical HPV infection or its benign lesions to CC.

THE ROLE OF HPV AND COFACTORS IN CERVICAL CANCER: THE IARC STUDIES

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Background: In the current scenario in which HPV is considered as a necessary but not a sufficient cause of cervical cancer, the role of cofactors should be envisioned either influencing the acquisition of HPV or the progression from HPV infection to cancer. We currently believe that a stratified analysis restricted to HPV DNA-positive women is the best strategy to identify cofactors influencing the rare progression of HPV infection to cancer. **Objective**: To assess the role of cofactors influencing the progression from HPV infection to cervical cancer. **Methods**: A pooled analysis of 10 IARC case-control studies on cervical cancer (eight studies on invasive cervical cancer and two on carcinoma *in situ*) was conducted. It included 1,853 cases (1,465 squamous-cell carcinomas and 135 adenocarcinomas/ adenosquamous carcinomas) and 255 control women, all positive for HPV DNA at PCR-based assays. Women were interviewed with a standardized questionnaire, and provided cervical specimens and blood samples. Associations with the follo-

wing cofactors were investigated: contraceptive use, parity and other reproductive factors, tobacco smoking, antibodies to herpes simplex virus type-2 (HSV-2) and to *Chlamydia trachomatis*. Pooled odds ratios (ORs) were computed by unconditional multiple logistic regression models and adjusted for sexual and non-sexual confounding factors. The 95% confidence intervals were estimated by treating the OR as floating absolute risks. **Results**: Use of oral contraceptives (OCs) for five or more years was associated with a four-fold increase in the risk of cervical carcinoma (OR = 4.0; 95% CI = 2.0-8.0) (Moreno *et al.*, submitted). Women with 7 or more full-term pregnancies (FTP) also had a 4-fold increase in risk compared to nulliparous women (OR = 3.8; 95% CI = 2.7-5.5) (Muñoz *et al.*, submitted). The proportion of women who have used OCs for more than 5 years and who have had more than 5 FTP was small in our study population (8% among cases and 3% among controls), but they showed a nearly 12-fold increased risk for cervical cancer. A two-fold increase in risk was observed in ever-smokers compared to non-smokers (OR = 2.2; 95% CI = 1.5- 3.2) (Plummer *et al.*, in preparation), as well as in women with antibodies to HSV-2 (OR = 1.7; 95% CI = 1.1- 2.6) and to *Chlamydia trachomatis* (OR = 1.6; 95%CI = 1.1-2.4) (Smith *et al.*, in preparation). Most of the above associations were found for both squamous-cell carcinoma as well as for the most rare types (adenocarcinoma and adenosquamous carcinoma). **Conclusions**: Long-term use of OCs and high parity increase the risk of progressing from HPV infection to cervical cancer. A modest increase in risk was also observed for smoking, HSV-2 and *C. trachomatis*. The role of host and viral factors (genetic susceptibility, immune response, HPV variants and viral load) remain to be identified.

CRITICAL VIEW ON MORPHOLOGICAL METHODS TO ASSESS HPV INFECTIONS

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Historical Perspective

Great progress has been made since Ayre's descriptions of HPV infection as "nearocarcinoma" in 1951 and Koss and Durfee's description of "koilocytosis" in 1956. Direct evidence linking the koilocyte to HPV accumulated over ensuing years using electron microscopy, immunohistochemistry and molecular techniques. However, the central role of HPV in cervical carcinogenesis remained unproven until the 1990's and consequently, koilocytotic atypia and dysplasia were viewed by many as representing separable infectious and neoplastic processes respectively until about a decade ago. Once HPV was established as the cause of cervical cancer, the pathologic distinction between viral changes and dysplasia (cervical intraepithelial neoplasia (CIN)) became moot. This trend was strengthened by widespread implementation of the Bethesda System in the US, which included the term "low-grade squamous intraepithelial lesion (LSIL)" to designate both koilocytotic atypia and mild dysplasia / CIN 1, thereby formally unifying these findings. Finally, natural history studies suggesting that most HPV infections in young women spontaneously regress (Ho GY et al N Engl J Med 1998) has led to increasing emphasis on recognizing and treating high-grade squamous intraepithelial lesions (HSILs) and identifying safe means for following women with LSIL.

Diagnostic Accuracy and Reliability of LSIL / CIN 1

The accuracy and reliability of cytologic interpretations of LSIL have improved in the last 5-10 years. In a review performed in the early 1990's, HPV DNA was detected in 94% of women with cytologic LSIL between 16 to 28 years of age compared to only 53% among those 40 years old and above (Schiffman MH et al J Natl Cancer Inst 1993;85:1868). The poor specificity of LSIL interpretations in older women suggested that morphologic mimics of HPV led to cytologic misclassification in parallel with the declining prevalence of infection. However, recent data from the ASCUS LSIL Triage Study (ALTS) indicate that the specificity of LSIL cytologic diagnoses may have improved. In ALTS, oncogenic types of HPV DNA were detected in 83% of women with community cytologic smears interpreted as LSIL, including detection of HPV 16 in nearly 25% of women. Notably, only 18% of these women were over age 30 (ALTS Group J Natl Cancer Inst 2001;92:397). In ALTS, 68% of thin-layer LSIL interpretations rendered by clinical center pathologists were confirmed in the first masked review performed by the pathology quality assurance group compared to only 43% of biopsy diagnoses of CIN 1 rendered by the clinical centers (Stoler MH et al JAMA 2001;285:1500). Thus, cytologic interpretations of productive HPV infection seem more reliable than histologic ones. In contrast, the QC panel agreed with 47% of thin-layer interpretations of HSIL and 77% of biopsy diagnoses of CIN 2 or worse, suggesting that histopathologic diagnoses of high-grade disease are more reproducible than cytologic ones.

Atypical Squamous Cells (ASC)

Management of equivocal cytology previously termed "atypical squamous cells of undetermined significance" and now provisionally re-named as "atypical squamous cells (ASC)" in the last revision of the Bethesda System remains central to cancer prevention. In some laboratories, ASC is the most common antecedent of biopsy confirmed CIN 2 or 3 (Kinney WK et al Obstet Gynecol) reflecting the extreme frequency of this interpretation. A comparison of two recent College of American Pathologists surveys has demonstrated an increase in "ASCUS" reporting from 2.8% in 1993 to 4.5% in 1996 (Arch Pathol Lab Med 2000). The histologic diagnoses at enrollment for 1,149 women with "ASCUS" triaged to immediate colposcopy in ALTS were CIN 1 in 14.5%, CIN 2 in 6.3% and CIN 3 in 5.1%. Approximately 55% of women with ASCUS tested positive for oncogenic types of HPV DNA, including about 96% of those with histologic CIN 3 (Solomon D et al J Natl Cancer Inst 2001;93:293). An additional finding from ALTS is that pathologists can identify a small subset of ASC (perhaps 5%) that reflects an extremely high risk for oncogenic HPV infection and a significant risk for underlying histologic CIN 2 or 3 (Sherman ME et al Am J Clin Pathol 2001). Based on ALTS and other studies, the draft of the revised Bethesda System proposes to dichotomously stratify ASC into cases mainly suggestive of LSIL (ASC of undetermined significance (ASC-US)) and ones suggestive of HSIL (ASC-H). ASC-US is a numerically largegroup associated with a low risk per patient for CIN 2 or 3, but most

women with ASC who have an underlying CIN 2/3 will have ASC-US. The risk per woman with ASC-H will be much higher, but this small group will likely detect a minority of CIN 2/3.

Endo cervical Adenocarcinoma

Many pathologists think that a definitive cytologic interpretation of AIS is possible in some cases. Nonetheless, even cytologically suspected cases of AIS are usually reported as atypical glandular cells of undetermined significance (AGUS). Paradoxically, most serious lesions associated with AGUS show squamous rather than glandular differentiation. Management of suspected glandular lesions is difficult because some gynecologists think that only a cone can safely exclude a lesion in the endocervical canal. Accordingly, better management strategies for AGUS are needed, but comparatively few biomarker studies have been performed in women with these findings. Furthermore, the relatively poor results of cytologic screening for cervical adenocarcinoma is concerning and knowledge of the pathogenesis of these tumors is limited. Milder degrees of endocervical abnormality than AIS are generally not recognizable microscopically and problems with both false positive and false negative interpretations are problematic, suggesting that improved diagnosis and management of glandular lesions is an important area for future research.

EVIDENCE FOR THE EFFICACY OF CYTOLOGIC SCREENING AND QUALITY ASSURANCE

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No randomised control trials have been undertaken for cervical screening. Nonetheless the effectiveness of cytologic screening programmes in reducing mortality from and incidence of cervical cancer was well established.

Wilson and Jungner set out the principles and practice for successful screening for any disease in 1968. For screening to be effective, the condition should pose an important health problem; the natural history should be well understood; there should be a recognisable latent or early stage; treatment of the early or latent stage should be more successful than treatment started later; there should be a suitable test; the test should be acceptable to the population; screening should be repeated at intervals determined by the natural history of the disease; there should be adequate facilities for the diagnosis and treatment of any abnormalities detected; the chance of physical or psychological harm should be less than the chance of benefit; the cost of case finding (including diagnosis and subsequent treatment) should be economically balanced against the benefit it provides.

Cervical cancer meets most of these criteria. However, it is important that cervical screening programmes have organised quality control of all parts of the programme including: compliance of women, smear taking, laboratory interpretation, colposcopy and follow-up, office and administrative systems; and that evaluation and monitoring of the whole programme is organised in terms of incidence and mortality rates at the level of the total target population among those attending and among those not attending.

To measure and improve the quality of a cervical screening programme, it is necessary to have: explicit standards; an information system which allows the necessary data to be collected; a quality assurance system which allows action to be taken should any programme fail to meet those standards.

The success of the NHS Cervical Screening Programme in the UK will be discussed with particular reference to these criteria. 83% of eligible women are screened regularly. Screening prevents 3,900 cases of cervical cancer each year and the invasive cancer rate has fallen from 16/100,000 women in 1986 to 8.9/100,000 in 1996. 1,300 lives are saved each year and the death rate continues to fall at 7% per annum. The aim of the current UK Pilots of liquid based cytology and reflex HPV testing to further improve the quality of the UK Cervical Screening Programme will also be discussed.

RELIABILITY OF MOLECULAR TECHNIQUES TO DIAGNOSE HPV INFECTION

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Direct detection of HPV genomes in biological specimens can be achieved by a series of hybridization procedures that include Southern and dot blots, in situ hybridization, Hybrid Capture (DIGENE Co., Gaithersburg, MD, USA), polymerase chain reaction (PCR) and DNA sequencing. A variety of signal detection procedures are also available which can further impact in the performance of these assays. Some of these procedures have the potential do be automated, facilitating result interpretation and allowing their use in large-scale, population based studies. The sensitivity and specificity of the several methods available vary largely, but have improved considerably in the last decade, due to improvements related to reagents quality and stability, and accessibility to once considered sophisticated equipments. The only procedure potentially capable of recognizing all HPV types and variants present in the biological specimen is DNA sequencing of an amplimer obtained by consensus primers-PCR, either after cloning into plasmids or by direct sequencing of the PCR fragment. This methodology, however, is presently labor-intensive and requires expensive equipment if using an automated sequencer. Moreover, direct sequencing dot not seems to be suitable for identification of specimens containing multiple HPVs, since it will detect preferentially the over-represented type.

At present, HPV DNA tests validated in large trials and epidemiological studies are PCR protocols employing consensus primers and Hybrid Capture II (HC2) HPV DNA assay. The third generation of the latter assay is expected to be available soon. This system has a lower background, increased sensitivity and reduced cross-reactivity between HPV types. Furthermore, a highly automated devise capable of running 700 samples per day based is under development. Several studies performed in the last decade employ consensus PCR that amplify a region of the highly conserved major viral capsid L1 gene, since it is potentially capable of detecting all mucosal HPV, with high sensitivity and specificity.

Analysis of the amplified products is generally performed by different hybridization protocols, but can also be done in an ELISA format, and ultimately can be coupled to cycle DNA sequencing. Another very sensitive and simpler approach consists of a reverse line blot hybridization of PCR products obtained with different consensus primers. These methods shall be available in the near future in automated, high-throughput, versions. HPV DNA quantification in the biological sample can be achieved by PCR-based methods, including Real-Time PCR.

The sensitivity and specificity of the several PCR-based methods available vary largely, depending mostly on the primers set, the size of the PCR product, reaction conditions and performance of the DNA polymerase used in the reaction, the spectrum of HPV types amplified and ability to detect multiple types, and accessibility of a type-specific assay. In most comparisons between HPV detection methods there is good to excellent agreement between tests performed with HC2, generic PCR employing MY09/11, PGMY09/11, GP5+/6+ and SPF systems (see references below). It is recognized, however, that any particular HPV detection method may undervalue the real prevalence of HPV in biological specimens, leading sometimes to type misclassification. Reference samples, validated reagents and standardized protocols are needed in order to increase reliability of HPV detection in both large epidemiological studies and vaccine trials.

EVIDENCE OF BENEFIT FOR CANCER SCREENING METHODS IN GENERAL: CERVIX VERSUS OTHER SITES

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Background: The Pap test is one of the first cancer screening tests and is undoubtedly the one with the best record of accomplishments in medical practice. Pap test screening targets mainly the detection and treatment of cervix cancer precursors, thus arresting neoplastic development within the cervical epithelium before it becomes invasive. The same rationale does not always apply to screening for other common cancer sites, many of which are not as accessible to cytomorphological probing as the uterine cervix. Many of the available cancer screening tests are able to detect cancer precursor lesions in the respective organs or sites, e.g., Pap test, mammography, sigmoidoscopy, oral exfoliative cytology, and skin examination, whereas the remainder are able to detect early invasive cancers. In this brief review I summarize the information on screening benefit for the available cancer tests. **Evidence-based public health**: The most persuasive arguments for the efficacy of screening come from randomized controlled trials (RCTs), investigations that take many years to complete, particularly if they focus on later endpoints in the natural history of cancer, such as invasive cancer or cancer-specific death. RCTs are not always feasible, however, because of ethical reasons — for example, when discovery of the intermediate endpoint is already an accepted basis for treatment, such as in high grade cervical lesions — or because they would have to be extremely large to the point of being impractical (e.g., rare cancers such as neuroblastoma). The new era of evidence-based medicine has spawned a number of consortia specialized in reviewing and ranking published clinical and epidemiological evidence on a systematic basis. As far as effectiveness of cancer screening is concerned, proof of mortality reduction in RCTs is typically assigned the highest level of evidence. Lower levels of evidence are obtained from case-control and cohort studies and other information such as the incidence of cancer before and after introduction of a screening intervention. Outcome measures for determining screening efficacy are ranked from the most persuasive to the least persuasive as follows: 1) decrease in cause-specific mortality; 2) reduction in incidence of advanced stage cancers; 3) increase in survival; and 4) shift in disease stage. **Cervical cancer:** The evidence for Pap screening efficacy is based on the following sources: (i) epidemiologic studies that indicate that the risk of invasive cervical cancer is 2-10 times greater in women who have not been screened and that risk increases with time since last normal smear or with lower frequency of screening; (ii) population surveillance, which indicates that cervical cancer incidence and mortality rates have decreased following the introduction of screening in Scandinavian countries, in Canada, and in the US; (iii) reductions in incidence and mortality seem to be proportional to the extent of population coverage; and (iv) multiple national and international consensus panels worldwide. The weight of the evidence in favor of Pap cytology obviates the need to have its screening efficacy scrutinized further in an RCT. In fact, such a proposition would be ethically untenable given that the Pap test is a widely accepted medical procedure.

In spite of its success, Pap cytology has important limitations, paramount among which is its low sensitivity. False-negative smears have important medical, financial, and legal implications; the latter being a particularly acute problem in North America where false-negative smears are among the most frequent reasons for medical malpractice litigation. Conversely, despite the test's relatively high specificity, false-positive results are particularly common in populations with low prevalence of cervical precursor lesions and cancer. False-positive results lead to unnecessary and frequently invasive procedures. The solution to minimizing errors in cytology is to improve the quality of smear taking, slide processing, and overall diagnostic performance of cervical cytology, which incur high costs for a screening program. This situation has elicited interest from the medical technology industry in developing new tests for detecting cervical cancer precursors. Prominent among them is human papillomavirus (HPV) testing, which has been considered both as a screening test per se or as part of a colposcopy triage strategy to manage women with abnormal referral Pap smears. HPV DNA testing for high-risk HPV types has higher sensitivity to detect high-grade disease than cytology but the specificity is somewhat lower than that of Pap cytology, as the prevalence of HPV DNA positivity in asymptomatic women varies markedly and tends to be high in young women. At present, the cost of HPV testing may be a barrier to wider use. However, HPV DNA testing is a promising tool as a primary screening method in women over the age of 35. Regarding HPV testing as a triage approach, there is mounting evidence that it is of value in helping to decide the need for a colposcopy among women with equivocal referral Pap smears.

Other cancer sites: RCTs have been conducted for only a few of the more common screening techniques — mammography in breast cancer screening being the one most thoroughly studied in several trials worldwide with unequivocal evidence of benefit among women aged 50-69 years. Sufficient evidence for screening effectiveness from RCTs has been obtained also for guaiac-based fecal occult blood testing in colorectal cancer, with a 15%-21% reduction in mortality. Removal of adenomas found on sigmoidoscopy, particularly the ones with severely dysplastic areas, decreases subsequent colorectal cancer risk. As yet there is no evidence of benefit for the remaining screening tests in reducing cancer mortality but many continue to be investigated in a variety of study designs in clinical and population-based settings. Conclusive evidence of no benefit has been obtained for Pap cytology in endometrial cancer, for chest x-ray and sputum cytology in lung cancer, and for urinary metabolite tests in neuroblastoma.

ROLE OF HPV TYPING IN LSIL MANAGEMENT

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Management of women with a cytological diagnosis of LSIL poses problems for the pathologist and gynaecologist. Firstly, the reproducibility of classification of this lesion is not more than 85%, and secondly, the clinical outcome for the individual woman is unknown. The gynaecologist circumvents these problems by following women with LSIL by repeating the smear within 6 months and when cytology remains abnormal women are referred for colposcopically directed biopsy. Histologically confirmed HSIL is only found in about 10% of women with cytologically LSIL. Therefore, follow up of women with LSIL seems unnecessary expensive and causes anxiety and stress in these women. Recent studies have shown that the persistent presence

of high-risk HPV (HR-HPV) is associated with persistence and progression of CIN lesions. Therefore, it has been advocated to use the presence of HR-HPV in the management of these lesions.

Next to identifying women with progressive LSIL on the basis of the presence of HR-HPV it is even more important to identify the women with regressive LSIL. Whether absence or clearing of HR-HPV in the latter women is associated with regression of the LSIL is unclear and asks for long term follow up studies.

In this presentation we will further restrict ourselves to women over 30 years of age since in Western countries women under this age mostly have a very low chance of developing CIN 3/cervical carcinoma. Moreover, the HPV prevalence is relatively high in women under the age of 30 years. The recent results of the ALTS trial already indicated that HPV detection for triage of younger women with LSIL is of limited potential since a very high percentage of them (i.e. 82% for women with a mean age of 25 years) had HR-HPV positive smears. Recently, we completed two studies in which triage by cytology and HPV assessment has been studied. The studies concern two cohorts of women from Amsterdam and Vlissingen, respectively. In the Amsterdam cohort women with an abnormal cervical smear (n=353) were followed for a median period of 36 months by cytology, colposcopy and HR-HPV testing using the GP5+/6+ PCR-EIA test every 3-4 months. Women with HR-HPV negative smears at baseline never showed progressive CIN disease, but more importantly, women who cleared the HR-HPV infection showed regression of their CIN lesion.

In the Vlissingen study women with borderline and mild dyskaryosis (BMD; n=278) underwent colposcopy and when lesions were found biopsies were taken. If histology appeared >CIN 2 lesions were treated by LLETZ. All these women, treated or not, were followed for up to 4 years (median 24 months) by cytology, colposcopy and HR-HPV testing using hybrid capture 2 (HC 2) every 6 months. All except one of the CIN2/CIN3 lesions found at study entrance were present in women with HR-HPV in their cervical smear. The one exception was a woman with a HR-HPV negative smear who had a CIN 2 lesion. All further CIN2/CIN3 lesions found during follow up involved women with HR-HPV positive baseline smears. Women who cleared the HR-HPV infection showed regression of their CIN lesion. These results do not only confirm earlier studies that HR-HPV infection is associated with the presence and/or the development of CIN 3 lesions, but also extend these observations. They indicate that HR-HPV detection identifies:

A group of women (HR-HPV+) with an increased risk of having and developing CIN 3.

A group of women who do not have CIN 3 and will not develop CIN 3 (HR-HPV-) within the follow up time (up to 4 years).

Based on these results we have developed a study protocol for the management of LSIL in women over 30 years of age. Instead of following these women for 6 months to see whether they show regression of their lesion as evidenced by a normal cytological smear or by colposcopy we now test these women for HR-HPV. Women who

are HR-HPV positive are directly referred for colposcopy, whereas HR-HPV negative women are referred to the national screening program, because the risk of developing CIN 3 in the interval to the next screening round is very low. Alternatively, it is possible to wait for 6 months before referring the HR-HPV positive women for colposcopy, since the risk of progression to cervical carcinoma within this time interval is non-existing and about 30% of these HR-HPV positive women will clear their HPV infection and subsequently regress their LSIL. Whether the costs of a recall of these HR-HPV positive women do outweight the profit of less referrals for colposcopically directed biopsy has still to be determined. Studies are now in progress to evaluate these guidelines in two cohorts of women with LSIL to reach a more definitive conclusion. This might help to prevent redundant follow up of women with regressive LSIL, to prevent overtreatment and to develop a direct see and treat protocol.

For women with ASCUS or women with BMD the re-

sults are more clear. Because of the much lower prevalence of HR-HPV in these women (varying from about 30% to 50%; 5,6) HR-HPV can be used for direct triage: HR-HPV negative women can be referred to the screening program whereas the HR-HPV positive women can be directly referred for colposcopy.

EVIDENCES OF BENEFITS FOR SYSTEMATIC TREATMENT OF LGSIL

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The management of LGSIL is an important medical and economic issue. In developed countries the main problem in this topic is to decide the best conduct after a cytologic diagnosis of low grade abnormalities. But in Latin-America and other regions, where colposcopy, followed by biopsy when necessary, is used as screening test in addition to cytology, making decisions about what to do with a patient with an histologic diagnosis of LGSIL is frequent.

In order to answer the question "does systematic treatment of LGSIL by any technique reduce the incidence or mortality of cervical cancer?" we conduct Medline search using a high sensitive approach. There are no randomized controlled trials (RCT) or observational studies evaluating this intervention with incidence and mortality as primary outcomes.

In order to contribute to this topic, we can ask several questions addressing some points: What are we treating or following up?, is LGSIL a precancerous lesion? There any benefit in treating it? So, the first question is "is LGSIL accurately diagnosed?": The high inter-observer variability of histologic diagnosis of LGSIL, as was stated by Stoler and Schiffmasn based on the data of the ALTS and published recently in JAMA, is an important problem specially when we must evaluate the effectiveness of an strategy based on it.

The second important question is "which is the risk of developing Cervical Cancer, or at least a HGSIL, in a patient with a LGSIL confirmed by biopsy?: At this point, there is some well designed cohort studies about the natural history of LGSIL but may be some doubts about the reproducibility of the diagnostic and follow up methodology. There is consistent evidence showing that only 10 to 20% of these lesions will progress. However, studies showed a higher risk of progression in High Risk HPV (HR-HPV) positive patients, specially in persistent infections.

Finally, the last question would be "is there any evidence on the efficacy of treatment of LGSIL histologically diagnosed on the subsequent development of new lesions?. At this point, considering the current evidence of HR-HPV infection as a necessary cause of Cervical Cancer, we conduct a search in Medline looking for RCT comparing the results of destructive or escisional treatment with negative results. However, a nonrandomized cost-effectiveness study conduced by Roland PY et al. shows that observation of mild dysplasia for regression provides the most cost-effective strategy compared with other strategies.

In conclusion, there is not available high quality evidence for or against the systematic treatment of LGSIL, but a lot of indirect evidence suggests a potential benefit. Since this decision carries on a high economic and social impact, I think that the design of a multicentric RCT should be considered.

NATURAL HISTORY OF HSIL

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Beginning in the late 1960's, the prevalent theory regarding the development of high-grade cervical disease was that it was the consequence of progression from CIN I to CIN 2 to CIN 3 (Richart 1968, Kiviat 1996). However, recent longitudinal studies have documented emergent CIN 3 without a detectable preceding low-grade lesion (Koutsky 1992, Woodman 2001). Although a majority of low-grade lesions are secondary to high-risk HPV types, only a small and unpredictable number of dysplastic lesions progress to invasive cancer and most low-grade lesions are transient. Present theory, therefore, is that low-grade SIL is a separate manifestation of HPV infection whereas CIN 3 is a monoclonal precancer secondary to high-risk HPV (Koutsky 1992, Kiviat 1996, Moscicki 1998) with cellular changes that promote the accumulation of genetic damage. Accumulation of genetic damage may eventually lead to invasive cancer if the lesion persists long enough. In the US CIN 2 is grouped with CIN 3 into HSIL, whereas in most of the rest of the world, CIN 2 is either considered a separate entity or grouped with CIN 1. The complex process that leads to cellular transformation from simple HPV infection with high-risk HPV to HSIL and finally to invasion is uncommon even though infection with these types appears to occur in the majority of sexually active individuals. In this presentation we will discuss what is presently known about the development of HSIL and what places it on the threshold of invasion.

Recent longitudinal studies on the natural history of CIN 3

Recent longitudinal studies have confirmed that persistent HPV infection, particularly HPV 16, is the most important risk-factor for the development and maintenance of CIN 3. Nobbenhuis et al followed 353 women referred with cytology read as mild, moderate or severe dyskaryosis with repeat cytology, HPV tests and colposcopy every 3-4 months for a median of 33 months (Nobbenhuis 1999). Ninety-five percent of women (98 of 103) developing CIN 3 during follow-up were persistently HPV positive. Woodman and colleagues evaluated 1075 initially cytologically and HPV negative young women (median age 18) every 6 months for a median of 29 months (Woodman 2001). Overall, 44% of these initially HPV negative women became HPV positive within 3 years and 60% within 5 years. The most common HPV type found in those who developed high-grade CIN was HPV 16, but it was also the most common type detected overall. Fourteen women developed CIN 2 and 14 CIN 3 during follow-up. The majority of HPV 16 positive high-grade lesions developed

within 6 to 12 months from first detection of HPV. The median time from first detection of all types of HPV until detection of high-grade CIN was 26 months. Only 10 of the 28 high-grade lesions had any evidence of LSIL prior to the detection of CIN 2 or 3. Quantitative viral loads fluctuated with the natural history of the lesion. Low HPV levels usually appeared at the beginning and at the end of the sequence. When cytologic abnormality occurred, it almost always appeared during the phase of detection of high viral load.

Koutsky et al followed a cohort of initially cytologically normal but HPV 16 or 18 positive women over a period of 2 years, detecting CIN 2 or 3 in 39% (Koutsky 1992). Women positive for HPV 31, 33, or 35 had na intermediate progression rate of 22%. What do these longitudinal studies tell us? First, high-grade CIN often develops in a very short time-span from first infection with HPV. Second, very high viral loads occur during the natural history of emergence of high-grade CIN, but also occur during transient expression of low-grade disease, with lower HPV DNA levels detected early and late in the coming and going of these lesions. Third, most high-grade lesions appear to develop *de novo* without a preceding low-grade HPV lesion.

Factors in Development of HSIL

Multiple factors are likely to come together in the multistep progression to HSIL and eventually to carcinogenesis. These factors include the necessity for infection with a high-risk HPV type, perhaps variants of certain HPV types, interaction with cofactors that may either promote the virus, diminish the immune response, or directly be responsible for the accumulation of genetic damage, and interaction with host immune factors. For example, specific variants of HPV 16 have been shown to be more successful at promoting persistent infection than the more common prototype HPV 16, which may partially explain why these specific variants are more prone to produce high-grade lesions (Zehbe I, 1997). Another variable that may separate lesions likely to progress from those that are likely to regress may be the identification of inactivated tumor-suppressor genes in one or more of four chromosomal regions (3p, 4p, 4q, and 11q) (Larson AA, 1997). Functional inactivation of these tumor-suppresser genes is assumed by detection of loss of hererozygosity (LOH) of the chromosomal region where they reside. LOH in putative tumor-suppresser loci has been documented in 0% of CIN 1 lesions, 22% of CIN 2 lesions, 41% of CIN 3 lesions, and 88% of invasive cancers. Additionally, LOH studies ofdifferent regions of large or multifocal high-grade lesions in individual patients indicates loss of the same allele at each locus without exception. This finding strongly suggests that high-grade cervical intraepithelial lesions arise from a single precursor lesion and that loss of specific tumor-suppresser genes is an early event in the development of high-grade lesions. Many other studies support the monoclonal nature of HSIL. For example, Park and colleagues noted that histologic specimens containing both CIN 1 and CIN 3 were more likely to have different HPV types in each lesion than specimens with only one grade change from CIN 1 to CIN 2 (Park 1998). Enomoto et la demonstrated monoclonality in 30/30 CIN 3 lesions (Enomoto 1997).

Other cellular events that appear to be associated with progression are aneuploidy and HPV integration. An increased DNA index and aneuploidy are noted in CIN 3 as compared with CIN 1 and CIN 2 lesions (Watanabe T, 1993). Evidence also exists that HPV integration increases with increasing grade of dysplasia and that genomic instability and, consequently, aneuploidy are likely mediated through disruption of p53 gene activity. The dramatic increase in the frequency of LOH seen in the transition from CIN 2 to CIN 3 may reflect a similarly dramatic increase in HPV integration, ultimately leading to inactivation of p53. Inactivation of p53 facilitates the subsequent accumulation of genetic damage, as evidenced by aneuploidy and LOH analyses. The long period of time required for progression to invasion most likely reflects the time necessary for random genotoxic events to occur.

Immune response to HSIL

Although HSIL may develop over a short time-span, it is likely that most HSIL persists for a long period of time before acquisition of cellular immortality. Evidence for this comes primarily from the median age for detection of CIS (age 29) and microinvasive cancer (age 42). Persistent HPV infection is necessary for the maintenance of an HSIL over such long periods of time. Persistence of HPV requires that host immunity is unable to detect or to clear the virus. T-cell mediated immune responses against high-risk HPVs are believed to play the primary role in the prevention of cervical carcinogenesis. It has been shown that HPV-16 E7-specific Th cell responses develop in women who are either demonstrating regression of CIN or in women with persistent HPV resulting eventually in development of CIN 3, but are less likely to be present in women with invasive cancer (de Gruijl 1998). The same authors noted that HPV 16 VLP-specific IgGs were consistently detected in all women persistently HPV-16 positive and whose disease ultimately progressed to CIN 3, but were less often detected in lesions that regressed. This suggests that HPV 16-specific antibodies are not important in clearance of virally induced CIN lesions, consistent with other evidence of the importance of the cell-mediated immune response (de Gruijl 1997).

Host Genetics

There may be some evidence that genetics play a part in the ability of the immune system to prevent development of CIN 3. Hildesheim et al determined in a case-controlled study of 24,000 women that those with HLA types B7 and DQB1*0302 were at increased relative risk for CIN 3 whereas DRB1*1501-DQB1*0602 and

DRB1*13 were at decreased risk (Hildesheim A 1998). These findings are consistent with several studies that show similar association of risk for these HLA types for cervical cancer and with evidence that human leukocyte antigens (HLAs) are important in the presentation of foreign antigens to the immune system.

CONE MARGINS AND ENDOCERVICAL CURETTAGE: ARE THEY IMPORTANT?

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Positive versus negative cone margins have practical importance in cases of micro-invasive squamous cell carcinoma of cervix and endocervical adenocarcinoma in-situ. Positive margins in these cases are associated with rates of residual lesions on the order of 50% and 60%, respectively. Thus, these necessitate further therapeutic interventions. Patients with negative margins are followed by non-surgical means as few, if any, residual lesions are found on follow-up.

The prognostic importance of cone margins in patients with squamous intraepithelial lesion (SIL) is less obvious and for all purposes, do not reliably predict presence or absence of residual/recurrent disease. Depending on the study, 20% to 80% of women with positive cone margins have no residual disease on follow-up and do not require further therapy. Conversely, negative cone margins do not guarantee cure for about 4% of women are found with residual disease on follow-up. In view of the data, all women regardless of status of cone margins have to be followed in the same manner, particularly during the first few years after conization. The most sensitive methods to detect residual/recurrent disease are a combination of cytology and colposcopy or cytology and HPV testing for high oncogenic risk HPV types.

The clinical value of routine use of endocervical curettage (ECC) has been controversial; some use it as na integral part of the initial work-up of women with abnormal Pap tests, others only if the endocervical limits of the disease are not colposcopically visualized; still, some never perform ECC because of patient discomfort, cost and

false positive and negative results. When ECC results are correlated with colposcopic localization of the endocervical margins of SIL, they are highly predictive of absence or presence of endocervical canal lesions. Also, ECC can detect endocervical adenocarcinoma in-situ and invasive disease. A well-performed ECC has better predictive value of endocervical canal disease than has colposcopy, and is of help in providing assistance in medico-legal cases involving "missed" invasive cancer. ECC, if positive immediately after conization, has better prognostic value for residual lesion than surgical margins. It is recommended particularly after electroconization (LLETZ / LEEP) in which surgical lines may be difficult to impossible to evaluate.

HPV INFECTIONS IN HIV POSITIVE WOMEN

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Infection with Human Immunodeficiency Virus (HIV) has been associated to an increased risk of Kaposi's sarcoma, lymphoid neoplasms, invasive cervical cancer (ICC), anogenital neoplasia, testicular seminoma, leiomyosarcoma in children and squamous conjuntival cancers. Many of these tumours are linked to an infectious origin other than HIV. The development of these neoplasms is likely to be the result of the immunsupression observed in HIV patients. In the recent years, some reports have been shade some doubts about the intervention of HIV in the development of some of these cancer types, in particular ICC. The purpose of this review is to summarise the epidemiological findings that explore the association of cervical cancer and HIV.

Cervical cancer is caused by the persistent infection of certain types of Human Papillomavirus (HPV). HPV characteristics such as type, viral load and time since infection together with the immune status of the subject have been advanced to influence the rate of progression from infection to cervical neoplasia. The natural history of HPV cervical infection is that women persistently infected with HPV are at high risk to develop high grade cervical lesions. Many women in the general population have at some point in time HPV infections but few will have it in a persistent manner. The addition of HIV infection to an already existent HPV infection may be a contributory factor to induce chronic infection and ICC development. This aspect is fundamented by the strong link between severely impaired cell-mediated immunity and HPV-induced carcinomas in non HIV- immune suppressed patients.

Although ICC has been included among an AIDS-defining condition since January 1993, the role of HIV in ICC development still remains controversial. It is well accepted that HIV positive women are more likely to be infected by HPV. The co-infection seems to be more common than what it would be expected by being both infections sexually transmitted. The effect of HIV is probably explained by the reactivation of latent HPV infections due to loss of immune competence. There has been, however, a lack of consistent changes in ICC incidence with HIV prevalence rates not only in high resource settings but also in African countries. Conversely, an association between HIV and ICC has also been described in several studies. Among women with AIDS reported to the Italian AIDS-Registry between 1993 and 1995, the frequency of ICC as AIDS-Defining disease was nearly 3 times higher among intravenous drugs users than those infected by heterosexual contact. In Italy the linkage of the National AIDS Registry and the populations cancer registries showed a RR of 15 for ICC for women with AIDS. The joint Italian-French follow up study of HIV positive women also showed a 13 fold increased rates of ICC for HIV positive women. In Spain, the Catalonian AIDS surveillance system detected 58 cases of ICC among 823 HIV positive women. This represented a 18-fold increased risk of ICC of AIDS patientsas compared to the general population. Frutcher et al 1998 reported that HIV positive women in New York had a 3 fold increase in invasive cervical cancer as compared to HIV negative women. Independent predictors were symptoms-duration and lack of Papanicolau smear. Similarly Chin et al in the USA reported an increased risk of ICC among black and hispanic women in the Sentinel Hospital Surveillance System. Recently, Sitas et al 2000 in South Africa have reported an increased risk of cervical and vulval cancer among HIV-1 infected patients as compared to hospital controls. A full evaluation of HIV as co-factor for ICC development requires data on several aspects related to the natural history of ICC. It is necessary to report on previous history

of Pap smears and also on cervical cancer treatment. This information is rarely expressed in the published reports. In high resource settings, women with low grade lesions that are detected to be HIV positive may undergo to drastic surgical treatment. Women with previous conization, history of previous cervical biopsies or previous cervical laser therapy may have their risk for ICC reduced. Survival in AIDS patients is strongly linked to treatment. It is likely that HIV positive women in poor countries with no access to medical care die before they have the time to develop cervical cancer or to have the medical attention necessary to be diagnosed. Studies on cancer incidence rates in HIV endemic countries should take into account HIV survival rates while evaluating ICC trends.

The controversy whether HIV promotes HPV infection and its adverse clinical consequences needs detailed information on therapeutic as well as preventive practices before it can be concluded that HIV does not affect the women's risk to develop ICC. Meanwhile, the scientific community should aim that HIV infected patients are attentively followed up for potential cervical damage and make all the possible efforts to guarantee the best available treatment for HIV irrespective of country of residency.

IS THERE ANY ROLE FOR SYSTEMATIC VULVOSCOPY AND PENISCOPY?

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Systematic (primary) screening for HPV-related lesions of the vulva, anus and penis with colposcopy is not cost-effective and is not recommended for the following reasons:

1. the vast majority of external anogenital lesions are detectable by naked eye examination or clinically palpable by both the patients and healthcare providers;
2. their discovery when symptoms and signs develop allows for sufficient time for adequate therapy;
3. the vast majority of external anogenital lesions are not life-threatening, i.e. condylomata, whereas precursor lesions have invasive potential of varying magnitudes and generally, long transit times to invasion;
4. colposcopy of the vulva, anus and penis does not seem to contribute to better histologic diagnosis of external anogenital diseases;
5. acetowhitening of the external anogenital epithelium is not specific for HPV infection; its discovery may lead to unnecessary diagnostic and often costly therapeutic procedures with inherent physical and psychosocial complications;
6. contact tracing of sex partners with or without colposcopy is not necessary for half of the current partners have no lesional tissue, and those with latent or subclinical infections have not proven to be the source of re-infection in stable sexual relationships; the so-called "male factor" in relation to risk of cervical cancer has been controversial.

Selective colposcopic examination of the vulva, anus and penis is clinically useful and recommended to more accurately determine the extent of disease, enhancing thereby complete removal of lesional tissues. Incomplete removal of disease is among the most frequent causes of treatment failures and "recurrences".

VULVAR, PENILE AND ANAL BIOPSIES: WHEN, HOW, WHERE AND WHY?

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The incidence of penile cancer is especially high in those countries with the highest incidence of cervical cancer. Vulvar cancer is less common than cervical cancer but occurs with increased frequency among immunosuppressed women, most commonly due to HIV or iatrogenic to prevent transplant rejection. The incidence of anal cancer has been steadily increasing in the general population in the last two decades and is especially common among men who have a history of receptive anal intercourse. Recent data also show that the incidence of anal cancer is increased among HIV-positive women compared with HIV-negative women. Anal-PV infection is nearly universal among HIV-positive men. Among both HIV-positive and high-risk HIV-negative women, anal HPV infection is more common than cervical HPV infection. Among both HIV-positive women and men, infection with multiple HPV types is common, with the mean number increasing with decreasing CD4+ level.

The primary reason to biopsy penile, vulvar or anal lesions is to rule out invasive cancer or high-grade dysplasia at these sites. If the clinical diagnosis is condyloma, biopsy may not be necessary, particularly if the lesion responds to standard therapy. A diagnosis of high-grade dysplasia should prompt a therapeutic approach designed to locally remove the lesion to prevent the development of cancer. Examination of the penis is optimally performed with 5% acetic acid and magnification. To apply the acetic acid the penis may be wrapped in acetic acid-soaked gauze or the acetic acid may be applied with a swab. After two to three minutes the surface of the penis and distal urethra can be examined under magnification such as a colposcope or magnifying glass. Indications for biopsy of penile lesions include signs suspicious for invasive penile cancer, including ulceration and rapid lesion growth. Penile lesions should be biopsied to rule out high-grade penile dysplasia since these lesions are potentially precancerous. Flat acetowhite, erythematous or hyperpigmented lesions should be considered for biopsy. Lesions that appear condylomatous but which do not respond to several courses of standard therapy should also be considered for biopsy.

Vulvar lesions should also be considered for biopsy if there is suspicion for anything other than condyloma acuminatum. Large lesions or lesions that are large, flat, hyperpigmented or ulcerated should be biopsied to rule out high-grade dysplasia and cancer. The threshold for biopsy of lesions should be lower in immunosuppressed individuals.

A very high proportion of both HIV-negative and HIV-positive MSM have anal intraepithelial neoplasia (AIN). The incidence of high-grade AIN is high among HIV-positive MSM. In one study, approximately half of HIV-positive men developed high-grade AIN over a four-year follow-up period. Among HIV-positive women, the incidence of high-grade AIN is higher than that of high-grade CIN and about 25% of HIV-positive women developed high-grade AIN over a 36-month follow-up period.

Indications for anal biopsy include the presence of peri-anal lesions that are flat, hyperpigmented or ulcerated. Among HIV-positive individuals, lesions that look clinically like condyloma often harbor foci of high-grade AIN and these should be considered for biopsy as well, particularly if they do not respond to standard therapy. Patients with peri-anal condyloma or AIN should be considered for high-resolution anoscopy (HRA) since presence of peri-anal disease is often accompanied by intra-anal disease. Similarly, individuals with na abnormal anal Pap smear should undergo HRA. To perform HRA an anoscope is inserted followed by insertion of a stick wrapped in gauze that is soaked in 3% acetic acid. The scope is removed leaving the gauze-wrapped stick in direct contact with anal mucosa for at least one minute. The stick is removed and the anoscope is reinserted. The entire intra-anal mucosa and the peri-anal region are visualized under magnification with the aid of a colposcope. Lesions within the anal canal should be biopsied if they are acetowhite, flat, and show vascular abnormalities such as mosaicism or punctation. Lesions that are particularly large should be removed surgically since these may harbor foci of invasion. As with peri-anal lesions, lesions that appear to be condylomatous may also be considered for biopsy, particularly in HIV-positive men and women.

Performance of penile, vulvar and peri-anal biopsies is simple and requires local lidocaine injection followed by lesion excision with a punch biopsy forceps, small Tischler or scissors. Biopsy of lesions in the office within the anal canal usually require no anesthetic unless they are close to the anal verge. Small instruments such as laryngeal forceps should be used to avoid complications. Complications are rare and include abscess and excessive bleeding. Contraindications to anal biopsy in the office include bleeding diathesis and anticoagulant use. Patients with these contraindications can be safely evaluated in an outpatient surgical setting where adequate hemostasis is possible. Patients with history of valvular heart disease should be considered for antibiotic prophylaxis prior to anal biopsy.

VULVA HPV-ASSOCIATED LESIONS: TREATMENT STRATEGIES

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The epidemic of genital human papillomavirus (HPV) infections is obvious to the medical community. Several therapeutic approaches are currently available for treatment of vulva-associated lesions, such as topical chemotherapy, local excisional or destructive procedures and immunotherapy.

Today, external genital warts (EGW) is the most frequent sexually transmitted disease. The link to anogenital malignancy has stimulated much interest in the treatment of HPV infections. To date, the most widely used therapies are ablative techniques, some of which have serious side effects and are also characterized by a high recurrence rate. More recently, patient applied therapies have become available. They offer several advantages over provider administrated therapies. Results obtained in clinical trials with Imiquimod 5% are described in the context of recommended treatment protocol for EGW.

Intraepithelial neoplasia of the vulva is encountered with increasing frequency.

Reports strongly suggest a relationship between HPV infection and VIN. The frequency at which this disease progresses to invasive carcinoma is presently unknown. Both the warty and basaloid types of vulvar intraepithelial neoplasia associated with HPV infection are often related to invasive squamous carcinoma of the vulva with similar morphological characteristics.

Both local excision and carbon dioxide laser are effective for treating vulvar intraepithelial neoplasia. The choice of the most appropriate therapeutical approach depends on the size and location of the lesions. Whichever technique is used, preservation of the vulva's normal anatomy and functions are of paramount importance.