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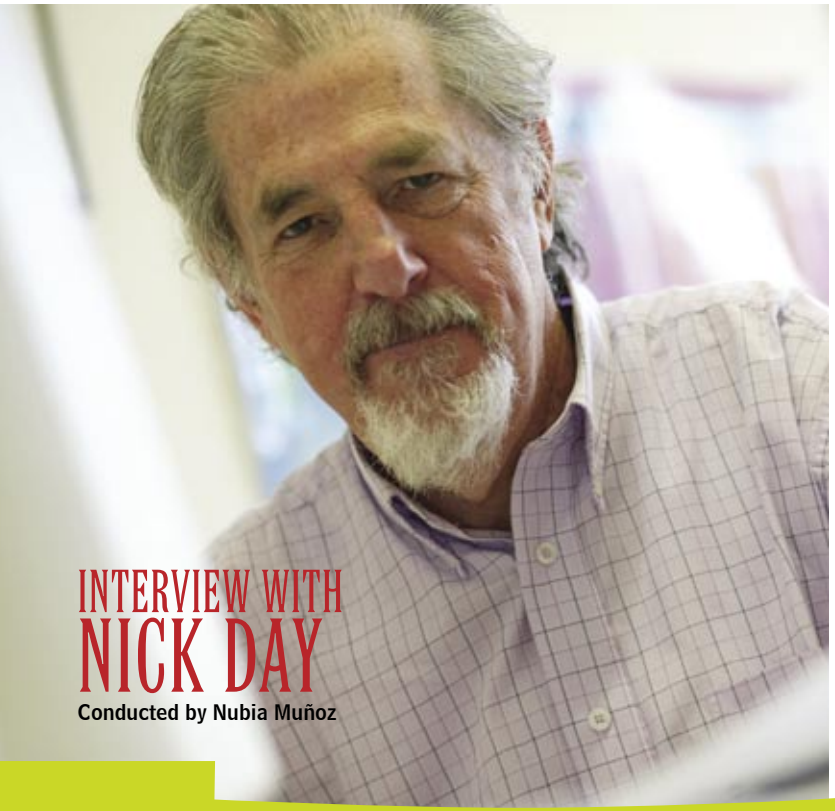
DEVELOPMENT OF SCREENING PROGRAMS IN THE LAST 20 YEARS



INTERVIEW WITH NICK DAY

Conducted by Nubia Muñoz

The International Agency for Research on Cancer (IARC) has recently evaluated and prepared a monograph on new technology for cervical cancer screening. What have been the main advances in the development of screening programs in the past 20 years?

There have been two major developments in the public-health approach to cervical cancer screening over the past 20 years. The first development relates to the traditional cytological methods, and the second to the identification of HPV as the fundamental cause of cervical cancer. Twenty years ago there was widespread, although not universal, acceptance that cervical cytological screening should reduce both the incidence of, and mortality from, cervical cancer. There was little understanding, however, of the real quantitative benefits that could result from high-quality screening, nor of the factors that determine its effectiveness. There was little information on the relative effectiveness of different screening frequencies, and serious misconceptions of the most effective age groups to target. The result of this lack of understanding was well exemplified by the experience in the UK. Millions of smears were being taken each year, with no discernible impact on cervical cancer incidence or mortality. The first IARC Workshop in 1985, and the work that subsequently flowed from it, established the impact on cervical cancer morbidity that could be achievable by cervical cytology screening, and the organization and monitoring of the screening programme that is required to ensure that this potential was realised. In the UK the effect was rapid. Reorganisation of the cervical cytology programme in the late 1980's, with the introduction of targeted monitoring, led to a steady decline in both the incidence and the mortality of cervical cancer throughout the 1990's.

(continues on page 3)

Nº 06 April 2005

EDITORIAL

THE CHANGING SCENARIO OF CERVICAL CANCER PREVENTION: BETTER SCREENING TOOLS AND THE UPCOMING HPV VACCINES

The International Agency for Research on Cancer (IARC) convened a workshop in April 2004 to review evidence on new technologies for cervical cancer screening. The party concluded that, in addition to conventional cervical cytology, there is now sufficient evidence to consider that each of the three novel methods — liquid-based cytology (LBC), computer-read cytology, and HPV testing — are adequate to reduce the incidence of, and mortality from, cervical cancer. The implication for HPV tests of this conclusion is that it is now scientifically acceptable to propose large, population-based demonstration projects that directly compare cytology and HPV testing in a randomized manner. Since women allocated to an HPV screening arm would not be at any increased risk (by not receiving cytology as well) these projects should also be ethically justifiable. Prof. Nick Day, chairman of the IARC monograph, elaborates in this issue on the context and importance of the conclusions of the working party.

Dr. J. Thomas Cox summarizes the interim guidance for the management of women undergoing screening with both HPV tests and cytology in the US. The protocols are consistent with the recommendations issued by several European-based studies. These guidelines, when applied to large numbers of patients, should generate a wealth of information in the near future and clarify unambiguously the contribution of HPV tests to screening programs under different scenarios.

Dr. John T. Schiller discusses some of the relevant issues on second-generation HPV vaccines. As the promising results of the Phase II trials continue to arrive, it is an increasing concern to ensure that HPV vaccines amenable to widespread use in developing countries will be developed and made available. While in affluent countries HPV vaccines may impact primarily on the incidence of pre-invasive cervical neoplasm, in extensive parts of developing countries cervical cancer is consistently the leading cause of cancer mortality among women.

HPV infections in children are introduced by Prof. Stina Syrjanen. The role of pediatricians in the management of HPV is of growing interest. The clinical responsibilities of this professional group linked to HPV are currently related to conditions such as laryngeal papillomatosis or warts. Once HPV vaccination reaches maturity, there is the potential for pediatricians to become a focal point for HPV vaccine delivery and thus for the prevention of cervical cancer.

The European Research Organization on Genital Infection and Neoplasia (EUROGIN) organized its expert meeting in Nice in October with a number of novel topics and new scientists arriving to the field. The work that is currently being done on the social and psychological impact of HPV is remarkable. Interesting issues concerning the acceptability of HPV vaccines by health professionals, administrators, and citizens were also presented.

F. Xavier Bosch
HPV Today

Website: www.hpvtoday.com

Correspondence and collaborations:
E-mail: box@hpvtoday.com

Published by:
BYPASS Ediciones
C/ Los Álamos, 16.
28813 Torres de la Alameda.
Madrid. España.

Legal Deposit: M-40590-2002

NICK DAY, PhD

Professor of Epidemiology. Strangeways Research Laboratory. University of Cambridge. UK

(from page 1) Total deaths from cervical cancer are now 60% lower than in the late 1980's, in the face of increasing rates, in younger women, of pre-invasive neoplasia. Cytology, however, has its limitations. In particular, the sensitivity of a single test for detecting early pre-invasive lesions is not high, and the resources required for a high-quality programme deter its implementation in many developing countries. Some programmes mitigate the lack of high sensitivity by repeating the test every three to five years, which, with the extended natural history of pre-invasive lesions, can provide good programme sensitivity. The demonstration, however, that almost all cervical cancers are caused by HPV is in the process of transforming the outlook for cervical screening. Modern HPV testing is highly sensitive for cervical pre-invasive lesions. The interval between initial HPV infection and the development of an invasive lesion is typically long. On the evidence now available from short-term studies, the IARC 2004 monograph concluded that HPV testing should be at least as effective as cytology as a screening test, with the advantage that for those women found to be HPV negative a considerably longer inter-screening interval than is recommended for cytological screening may be appropriate.[1]

How can the efficacy and effectiveness of HPV testing as a screening modality be evaluated?

In developed countries that already have effective cytology-based programmes, what is required is a large public-health demonstration programme in which, within a particular health-care system serving a specified population, two well defined subpopulations are compared, one of which receives usual cytology screening whilst the other receives screening by the chosen combination of HPV testing and cytology. Given the results summarised in the 2004 IARC monograph, this combination is likely to consist of HPV testing as the primary screening test, with later triage by cytology of those found HPV positive. I use the phrase "demonstration programme" rather than trial deliberately, since the operation should not be viewed as a trial in the normal sense where individual randomisation is considered the gold standard, and full individual consent required. Rather, the process could be viewed as the slow replacement across the total population of one screening modality, cytology, by another for which there is clear evidence to assume superiority. Short-term endpoints need to be monitored, in terms of sensitivity, specificity, cost, psychosocial parameters etc., but the design of the process needs to be focused on the comparison between the two subpopulations of subsequent cervical cancer incidence, preferably by stage, and mortality. With the rates of advanced cancer and mortality as the endpoints, the demonstration programme clearly has to be large. Provided the expected effectiveness of the combined screening modality relative to cytology screening is achieved, and cost-effectiveness demonstrated, then one would envisage that combined HPV-cytology screening would become established across the population.

Should cytology-based programmes continue? If so, why and where?

Well-organised screening programmes based on high-quality cytology should clearly continue until a more attractive alternative is available. Not all countries, or populations, will necessarily want to be in the first wave of countries launching demonstration programmes for primary HPV testing. Unforeseen problems with the implementation of primary HPV screening may come to light in the programmes that are launched. Health services which continue with cytological cervical screening should be aware that the recommendations on screening interval and target age group in the 2004 IARC monograph have been reformulated in the light of new epidemiological evidence. More frequent screening in the younger age groups appears indicated, and in women over 60 years of age with a history of negative cytology, continued screening may not be warranted. Which countries or populations decide to opt for the staged introduction of HPV testing as the primary cervical screening test will depend on a range of local circumstances. It is likely, however, that its introduction will snowball once a few populations have moved ahead and demonstrated how it can be delivered.

Age standardized incidence of invasive cervical cancer and coverage of screening, England, 1971-95*

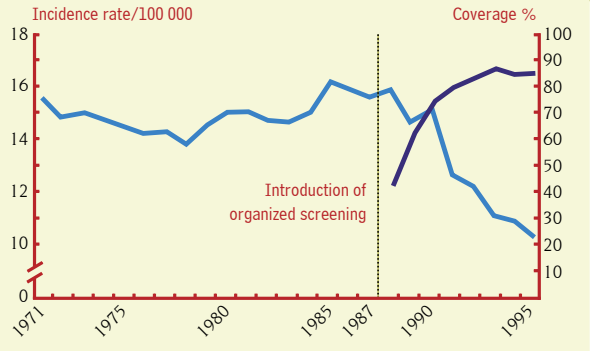


*Figure reproduced from: Quinn M, Babb P, Jones J, Allen E. Effect of screening on incidence of and mortality from cancer of cervix in England: evaluation based on routinely collected statistics. BMJ 1999; 318: 904. Reproduced with permission from the BMJ Publishing Group.

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HPV IN SCREENING AND TRIAGE

INTERIM GUIDANCE ON THE USE OF

In 2002 the American Cancer Society (ACS) issued new primary-cervical-screening guidelines for the US that included the option of screening with HPV testing combined with cytology for women age 30 and above.[1] This option was given in anticipation of the likely Food and Drug Administration (FDA) approval of the use of Hybrid Capture 2 (HC2) for such screening, which occurred the following year. In 2003 the American College of Obstetricians and Gynecologist (ACOG) released its 2003 Practice Bulletin on cervical screening that mirrored the American Cancer Society (ACS) option of combined HPV DNA and cytology screening.[2] The existence of this new screening option prompted the convening of an expert panel of representatives from the National Cancer Institute (NCI), the American Society for Colposcopy and Cervical Pathology (ASCCP), ACS, ACOG, and the Center for Disease Control and Prevention (CDC).[3] The recommendations from that panel now guide US clinicians as they begin to adopt combined screening with HPV testing and the Pap test. These management recommendations have been termed "interim guidance" in recognition that they may change as new data continues to evolve.

Primary Screening Sensitivity: CIN 2/3+				
Study	**No.**	**Age**	**Pap**	**HPV**
Cuzick J	11,085	30-60	76.6%	97.1%
Clavel C	4076	30-76	57.7% CP	100%
			84.4% LBP	

Table 1

The data from the HART[4] *and Rheims*[5] *studies mirror the difference in sensitivity of cytology and* HPV *testing for* CIN 2/3+ *that was presented in a number of studies for* FDA*-approval of the use of* HC2 *as an adjunct to the Pap in the primary screening of women age* 30 *and over.* CP=*Conventional Pap.* LBP=*Liquid-based Pap.*

What was the basis for such dramatic changes in screening options?

In a number of primary screening studies from several countries HPV testing has been found to be, on average, 25–40% more sensitive than a single conventional Pap test for the detection of cervical intraepithelial neoplasia, CIN 3+ and 10–25% more sensitive than a single liquid-based Pap test. Recent data from the HPV in Addition to Routine Testing (HART) study,[4] reported by Dr. Jack Cuzick in the October issue of HPV Today, and data from the study of Dr. Christine Clavel and colleagues in France,[5] support and augment the eight studies originally presented to the FDA for the approval of combined HC2/Pap screening.

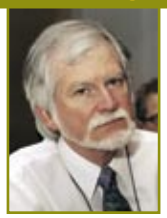
In all these studies the negative predictive value of the combined test was 99–100%, indicating that women negative on both are at exceedingly low risk for CIN 2,3 or cancer. This very high protective value of the combined test is valid not only for present disease, but also predicts low risk for a number of years in the future, thus providing the rationale for screening no more frequently than every three years. The advantage of this approach over the other option given by ACS and ACOG of screening every two to three years for women having three consecutive negative/normal Paps is that the reassurance provided is not subject to the significant variability in test sensitivity noted with the Pap, which requires serial screening to achieve high sensitivity. This is particularly important because the women at highest risk are those who are screened infrequently and are therefore less likely to obtain the three negative/normal tests required for extended screening. The guidelines from the ACS, ACOG, and the "Interim Guidance" Committee all strongly recommend that combined screening be done no more frequently than every three years for women negative on both tests. This should reduce overscreening that would likely increase cost without benefit and potentially result in the overtreatment of many women having only transient HPV infections.

When should the HPV testing/Pap combination not be used in primary screening?

There are several areas in which HPV testing should not be used as an adjunct to the Pap in primary cervical screening. First, the HPV testing/Pap combination is not recommended for women under the age of 30 because of the high prevalence (15–46%) of mostly transient HPV infection at this age. This contrasts to the approximately 5% prevalence of high-risk HPV in women aged 40 and above. Even women in this age range who are HPV positive/Pap negative have a relatively low risk for detection of CIN 2,3. In the Portland NCI prospective study[6] the risk for detection of CIN 3+ over the 3–5 year period following a positive HPV test was 4.4% but only 0.24% for women testing HPV negative. It is this nearly 20-fold increased risk with a positive HPV test that confirms

HPV TESTING COMBINED WITH CYTOLOGY IN PRIMARY CERVICAL SCREENING

J. Thomas Cox, MD Director of the Gynecology and Colposcopy Clinic. University of California Santa Bárbara. Past-Executive Medical Director of the National HPV and Cervical Cancer Prevention Resource Center (American Social Health Association). USA.



the need for increased surveillance and the very low risk over 3–5 years for detection of a significant lesion in HPV-negative women that verifies the safety of three year extended screening intervals. Immunosuppressed women should also not be screened in this manner, as the prevalence of HPV in these women is too high (up to 60% in some studies), and high-grade lesions may progress more rapidly and be more common. Women having had a hysterectomy for benign disease, with no evidence of CIN 2,3 or cervical cancer at the time of the hysterectomy, do not need any cervical screening as per the ACS and ACOG guidelines.

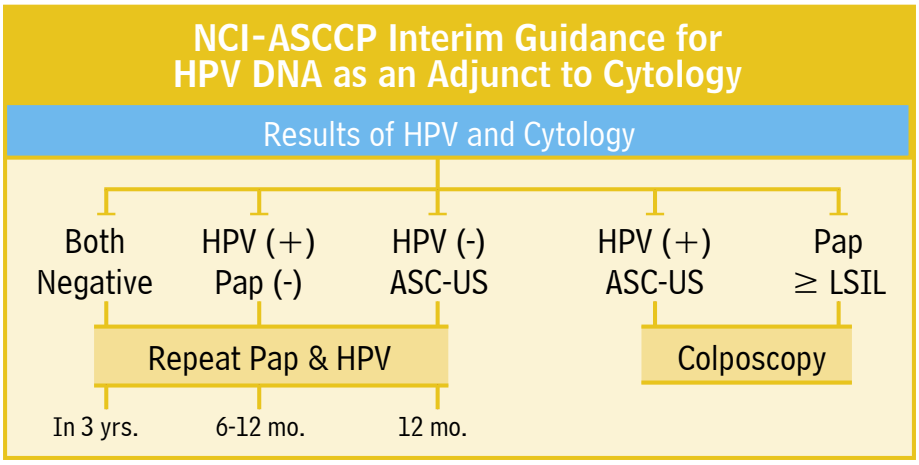


Figure 1

Interim Guidance for the management of women using the combination of cervical cytology and HPV testing in the primary screening of women ≥30. Women positive of either the HPV test or having an abnormal Pap at the 6-12 month evaluation are to be referred to colposcopy. Women with ≥LSIL are to be referred to colposcopy irrespective of their HPV result. Source: Wright TC et al. Interim guidance on the use of HPV DNA testing as an adjunct to cervical cytology. Obstet Gynecol 2004;103:304-9. Reproduced with permission.

"Interim Guidance" on managing women with various combined screening results

The "Interim Guidance" recommendation for women with a single positive HPV test/negative Pap, is to test for persistence of HPV six to twelve months from the initial screening result and to not refer to colposcopy unless the repeat Pap test is abnormal or the repeat HPV test is positive. This recommendation is based upon the necessity for HPV to be persistent if high-grade precancer is to develop and risk of cervical cancer to occur. Testing for persistence prior to referral to colposcopy should capture almost all significant lesions within a reasonable timeframe, while allowing nonpersistent HPV (45–60% will become HPV negative within 6–12 months)[4,7] to resolve. In contrast, until further data suggest otherwise, women negative on the HPV test but having cytology interpreted as low squamous intraepithelial lesion (LSIL), AGC, or high squamous intraepithelial lesion (HSIL) should be referred to colposcopy, whereas HPV negative atypical squamous cells of undeterminated significance (ASC-US) can be managed by repeat Pap and HPV test in 12 months as per the 2002 American Society of Colposcopy and Cervical Pathology (ASCCP) Guidelines.

Further "Guidance"

Educating women about the usually benign nature of a positive HPV test is imperative if women are to not be made exceedingly anxious about testing positive for "high-risk" HPV. The term "high-risk HPV" must be clarified such that women understand that, despite the association of certain HPV types with high-grade CIN and cervical cancer, the risk of development of either of these lesions is relatively low and that only persistent HPV infections indicate enough potential for a treatable lesion to warrant colposcopy. Patients should be reassured that most women have high-risk HPV at some time in their lives, and that even if it is not possible to know whether HPV is completely cleared, or just suppressed, once the HPV test becomes negative the individual is not likely to continue to be infective to a new partner. It is also imperative to reassure the patient that detectable HPV probably indicates that she shares this virus with her partner and that successful clearance or suppression of HPV by either partner is only dependent upon one's own immunity and is not affected by possible re-exposure through continued sexual activity with their partner. These messages are complex and take time to discuss, but the importance of providing such education cannot be underestimated.

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SECOND-GENERATION HPV VACCINES

John T. Schiller, PhD
National Institutes of Health (NIH),
National Cancer Institute (NCI).
Bethesda, MD. USA

Prophylactic HPV vaccines based on noninfectious L1 virus-like particles (VLPs) are undergoing intense clinical evaluation. In proof-of-concept efficacy trials, these vaccines demonstrated 100% efficacy at preventing type-specific persistent cervical HPV infection and the development of type-specific cervical intraepithelial neoplasia (CIN).[1,2] There is widespread optimism that a VLP vaccine will become commercially available within the next few years. The prospects for the development of alternative HPV vaccines must therefore be considered in light of the likelihood that a safe and effective prophylactic HPV vaccine will soon be available. Three questions need to be addressed:

Is there sufficient need for a second-generation vaccine?

The current VLP-based vaccines might reasonably well meet the needs of developed countries. However, they have fundamental weaknesses for widespread distribution in developing countries. First, VLP-based vaccines are expensive to manufacture, since they are produced in eukaryotic cell culture and must be extensively purified. Second, they are expensive to distribute, as they involve three intramuscular injections of a vaccine that requires a cold chain for storage. Third, protection will almost certainly be type specific, and so the current vaccines aren't expected to protect against the 25% of cervical cancers that are HPV-16 and -18 independent. Incomplete type coverage is especially problematic for developing countries because most do not have effective screening programs as an alternative to reduce cervical cancer risk from minor oncogenic types. Fourth, the L1 VLP vaccines are not expected to induce regression of established HPV-induced neoplasia. Because it generally takes more than a decade for incident HPV infection to develop into cervical cancer, the public-health benefit of VLP vaccines will be substantially delayed. It might therefore prove easier to convince public-health officials to invest in a vaccine with therapeutic potential, since it could produce benefits earlier.

Are there sufficiently attractive candidates to invest in at the present time?

An ideal HPV vaccine would be inexpensive to manufacture and distribute, should protect against all oncogenic types, and act both therapeutically and prophylactically. None of the second-generation vaccines currently under development meets this ideal. Either new candidates will need to be developed or we will need to decide which goals are most important for reducing cervical cancer deaths and to what extent they are likely to be achieved. Obtaining prophylactic protection against HPV-16 and -18 in as many women as soon as possible would seem a top priority. Table provides a partial list of second-generation-vaccine candidates that are currently undergoing preclinical development and lists their potential strengths and weaknesses.

Is there a realistic development/commercialization path?

There are many hurdles to the successful development of a second-generation HPV vaccine. First, it may prove difficult to attract sufficient funding once an effective HPV vaccine is commercially available. Second, it will

2ND GENERATION HPV VACCINES

VACCINE	POTENTIAL ADVANTAGES	POTENTIAL LIMITATIONS
Additional VLP Types[3] (HPV31, 45, 33, 52, etc)	Established technology	Increased cost, modest increase in protection from cervical cancer
Heat Stabilization of VLPs[4]	Decreased implementation costs	Unproven technology for HPV VLPs
Slow Release Formulation[5]	Lower cost of administration, if fewer injections required	Unproven technology for HPV VLPs
Upper Respiratory Tract Delivery of Purified VLPs[6]	Needle free delivery; Induction of sIgA; Lower cost of implementation?	Consistency of immune response? Safety?
Oral Delivery of VLP in Crude Plant or Yeast Extract[7]	Low cost production and administration; Induction of sIgA	Very low level expression in plants; Low immunogenicity in animal models
L1 DNA[8]	Lower cost of production	Less immunogenic than VLPs? Unknown oncogenic potential of injected vectors
L1 Pentameric Subunits[9]	Lower cost of production (made in bacteria)	Less immunogenic than VLPs?
L1 Recombinant Bacteria[10]	Low cost of production and administration (i.e. mucosal)	Regulatory issues with genetically modified (GM) organisms; Safety/ immunogenticity uncertain
L1 Recombinant Virus[11]	Lower cost of administration if mucosal; Lower cost of production?	Regulatory issues with GM organisms; Safety/immunogenticity uncertain
Chimeric VLPs[12]	Combined prophylactic/therapeutic efficacy; Earlier benefits	Modest therapeutic effect in early trial
VLPs combined with a therapeutic HPV vaccines[13]	Combined prophyactic/therapeutic efficacy; Earlier benefits	Efficacy of current therapeutic vaccines limited; Interaction with VLPs uncertain
L2 protein or peptide[14]	Induction of type cross-neutralizing antibodies; Lower production costs	Lower titers of neutralizing antibodies than VLPs

be difficult to conduct placebo-controlled trials once there is a commercial vaccine. Third, it may be difficult to obtain regulatory approval for candidates based on more novel technologies, especially those involving live microbial vectors. Fourth, intellectual property issues will likely be impediments if sales in developed countries are contemplated. If a substantially different second-generation vaccine is to become a reality, it will likely require continued public sector support of preclinical and early-phase clinical development to identify the most attractive candidate(s) for efficacy trials. Determination of an immune correlation of protection in the current efficacy trials could help enormously in this endeavor. Funding for expensive efficacy trials may require combined public/corporate sponsorship. However, if protection from infection proves to be a strong surrogate marker for protection against high-grade CIN in the current trials, smaller trials using a virological end point could be considered. In my opinion, it would be a mistake to look only to the large vaccine manufacturers in developed countries for production of a second-generation vaccine. Many safe and effective vaccines are manufactured in countries of intermediate development. Because they would likely be less constrained by intellectual property issues, the vaccine manufacturers is these countries may more readily become interested in manufacturing a second-generation HPV vaccine, especially if it were less expensive to mass produce and deliver than the current VLP-based vaccines.

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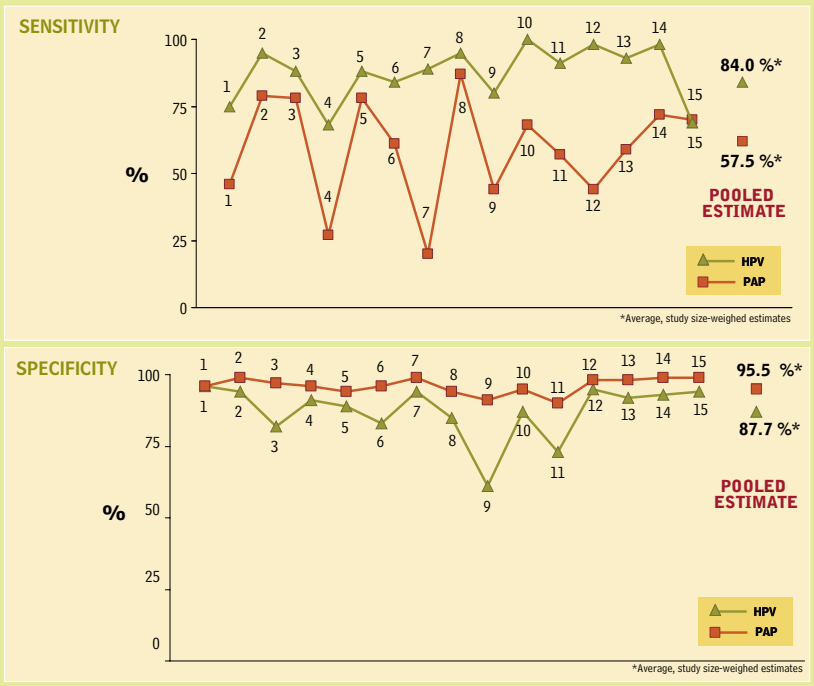
HPV IN 100 SLIDES

STUDIES THAT COMPARED HPV TESTING WITH CYTOLOGY IN THE CONTEXT OF POPULATION SCREENING



Results of the 15 best studies that compared HPV DNA testing with cytology in screening for HSIL / cervical cancer. The studies were conducted between 1995 and 2004 in developed and developing countries. Cytology includes conventional or liquid-based methods.
It is noteworthy that the variation in sensitivity across studies for an HPV DNA test is smaller – range of sensitivity 68% to 100% – than cytology, which showed greater inter-study variability – range of sensitivity 20% to 87%. In contrast, the specificity is consistently very high across studies for both HPV tests and cytology. The range of specificity for an HPV test is 61% to 96% and for cytology 90% to 99%.

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The Pooled estimate excluded study number 2.

HPV INFECTIONS IN CHILDREN

Stina Syrjänen, PhD, DDS

Professor and Chairman of Oral Pathology.
Institute of Dentistry, Faculty of Medicine, University of Turku.
Director of Turku Immunology Centre, University of Turku.
Finland.

The Finnish HPV Family Study Group

What lesions are associated with HPV in children?

The disease pattern, diagnosis and management of HPV infections in children were recently reviewed by this author[1]; these data are briefly summarized here. Skin warts and laryngeal papillomas are the best known pediatric HPV-associated diseases, while oral papillomas associated with HPV types 6 or 11 are the most common epithelial tumors of the oral mucosa in children. Until recently, genital HPV infections were considered to be a sexually transmitted disease (STD) that affects sexually active age groups only. More recently, however, a number of reports on genital HPV infections in children have been published (reviewed in references 1 and 2).

Skin warts are by far the most characteristic HPV-associated lesions in children. These are rare in children below the age of 5 years; their peak incidence appears at between 10 and 14 years of age, followed by a rapid decline until 20.[3,4] Skin warts are classified according to their anatomic distribution, clinical appearance and histology. Specific HPV types are associated with these distinct morphological types of skin warts. According to a recent Cochrane review, the best available evidence substantiates the use of simple topical treatment with salicylic acid.[5]

Epidermodysplasia verruciformis (EV) is a rare disease associated with a high risk of skin cancer.[3,6] In EV-susceptible patients, HPV infections cause disseminated flat wart lesions at a young age. Recently, evidence was found for a non-allelic heterogeneity of EV with two susceptibility loci mapped to chromosome regions 2p21-24 and 17q25.[7]

Oral HPV infections in children have not been systematically studied, and thus no reliable data on their prevalence are available.[1–3] Oral mucosa is particular in that several distinct morphological entities associated with HPV exist, including the following: 1) squamous cell papilloma, 2) skin wart, 3) condyloma, 4) focal epithelial hyperplasia (FEH).[3]

Squamous cell papilloma is estimated to represent about 7–8% of all oral tumors in children. FEH is caused by HPV types 13 or 32 and the viruses are nearly always present at high copy numbers in these lesions. Clinically, FEH appears as multiple, soft, flat or rounded, slightly elevated nodules. The lesions are asymptomatic and may persist for several years, but tend to regress spontaneously and may also recur. The highest prevalences (7–36%) have been found among Indian children in Venezuela, Guatemala and Colombia, and in Eskimos from Alaska and Greenland. For FEH, a family history has been strongly suggested, as seen with EV. Recently, an association of HLA-DR4 (DRB1*0404) with HPV infection in patients with FEH was described.[8]

HPV DNA is also detectable in normal oral mucosa, with the prevalence varying from 0–47% among children at age of 4 months to12 years.[1,2,9–12] The most prevalent types

are HPV 6/11 and 16. The significance of these latent, clinically unapparent oral HPV infections of newborn babies and in early childhood is being currently assessed in a prospective cohort study entitled: *the Finnish HPV Family Study*, with this author as the principal investigator[13] run in collaboration with the Department of Obstetrics and Gynecology, Turku University Central Hospital (Dr. S. Grenman).

Juvenile laryngeal papillomas is another well-established pediatric HPV-associated disease, currently known as recurrent respiratory papillomatosis (RPP).[1,14] The incidence and prevalence of juvenile-onset recurrent respiratory papillomatosis is approximately 0.3–0.6/100 000. Sometimes, extensive papillomas may cause respiratory obstruction and lead to death in early childhood. Up to the end of 1998, 27 studies had been published on HPV DNA detection in juvenile laryngeal papillomas. In total, 78% of the 375 samples examined contained HPV 6 or HPV 11; HPV 16 or 18 was detected in only 1.3% of the cases.[3] Vertical transmission has also been implicated as the mode of virus spread in this disease from an infected mother to the newborn.[1,2] Other risk factors associated with juvenile onset (JO)-RRP include the age of the mother (<20 years) and first-order births.[14] The susceptibility to RRP was recently associated with DRB1*0301, while HLA-DRB1*14 might be associated with juvenile-onset disease.[15]

An anogenital condyloma acuminatum in an infant was first reported in the English literature by Smith in 1903. The prevalence of anogenital warts in prepubertal children seems to be rising. This might be both a) due to an increased awareness of the disease and b) a true increase in the incidence of these infections in children.[1,2]

The literature has expanded rapidly, with at least 700 reported cases.[1] External anogenital warts in pre-pubertal girls affect the vulva, urethra and perianal area, while most of the lesions in boys are confined to the perianal region, and more rarely in the penis.[1–3] Only a few reports are available on vaginal HPV infections in children, each concluding that sexual contact is the major transmission mode of vaginal HPV infections in children.[16,17] By the year 2001, the presence of HPV DNA had been analyzed in 390 anogenital warts of children, of which 285 (73%) were HPV DNA-positive.[1] The mucosal HPV types 6 and 11 clearly predominate (57%), but HPV 16 and 18 were identified in 4% of these warts.[1] The implications of the HPV typing data as markers of disease origin is controversial, because little is known, for example, about latent and subclinical HPV in the genital mucosa of children. Also, studies on HPV infection during the neonatal period have yielded highly variable results [1,2,10–12,18].

Are HPV antibodies detectable in sera of children?

Few studies are currently available where serum HPV antibodies have been analysed in children.[1,19,20] When HPV 16 peptides are used, the detection rate of serum HPV antibodies is much higher (24–33%) than with virus-like particles (VLPs) of HPV 16 (3–15%). The seroprevalence is also related to the number of VLPs tested. The preliminary results from our Finnish HPV Family Study indicate, however, that seroconversion to both low-risk and high-risk HPV types can be detected already at the age of 6 to 12 months. The significance of HPV antibodies acquired during the early childhood is poorly understood.

How is HPV transmitted in children?

The potential modes of transmission for the pediatric HPV infections include perinatal transmission, auto- and hetero-inoculation (through hands, baths, beds, underwear etc.), sexual abuse and possibly indirect transmission via fomites.[1,2] Previously it was thought that genital warts in children are all contagious, although it was subsequently proposed that sexual abuse during childhood is the most common mode of HPV transmission, HPV 6 and 11 being the two most prevalent viral types detected [1]. The epidemiology and social significance of anogenital warts in pre-pubertal children are controversial. Debate continues as to the frequency with which these lesions have developed as a result of sexual abuse or transmission by non-sexual routes [1,16,17]. More recent studies suggest that perinatal infections (i.e., vertical transmission) and auto- and/or hetero-inoculation are much more common than originally thought [10,11,13]. Recent evidence indicates that, although HPV infection can be acquired during the passage through an infected birth canal, transmission in utero or acquisition postnatally seem to be possible options as well. The detection of HPV in trophoblasts has resulted in the speculation that earliest HPV infection might occur prior to implantation, possibly by fertilization of the oocyte by an HPV-carrying spermatozoon. In various series, HPV DNA has been detected in 8–64% of the semen samples of asymptomatic males. Also, seminal plasma and spermatozoa have been shown to contain HPV DNA, and transcription of HPV 16 E6 and E7 has been demonstrated in spermatozoa. Thus, theoretically, the subsequent virus spread might originate from embryos after fertilization.[1]

An accurate elaboration of the predominant modes of HPV acquisition in children is of particular importance because of the implications that the diagnosis of genital wart might have for the need for child-protection measures to be undertaken.[16,17]

Questions for the future

The evidence implicating that infection with high-risk genital HPVs may occur early in life and remain persistent for a considerable period of time is of considerable importance for HPV vaccination strategies.[1,2] Although sexual transmission occurs in both children and adolescents, neonatal infections seem to

be predominantly of types 16 and 18 and persist for short periods in the genital area. The reported discordances in HPV types between neonates and older children have yet to be explained. The majority of genital warts in children are related to HPV 6 and 11. Acquisition of HPV during childhood and adolescence is not an immediate cause of severe morbidity. Future studies should include large-scale longitudinal designs to test and follow-up neonates with careful attention to the issues of sexual abuse in order to confidently diagnose and appropriately treat HPV infections in a child. The role of age and immunity remains confusing, which supports the need for studies to examine cervical immunity more closely. Perinatal acquisition of HPV also has implications for future prophylactic HPV vaccination trials, especially when 12–34% of the pregnant women in some populations may harbor genital HPV infection.[3,13]

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EFFICACY OF A BIVALENT L1 VIRUS-LIKE PARTICLE VACCINE IN PREVENTION OF INFECTION WITH HUMAN PAPILLOMAVIRUS TYPES 16 AND 18 IN YOUNG WOMEN: A RANDOMISED CONTROLLED TRIAL.

Harper DM, Franco EL, Wheeler C, Ferris DG, Jenkins D, Schuind A, Zahaf T, Innis B, Naud P, De Carvalho NS, Roteli-Martins CM, Teixeira J, Blatter MM, Korn AP, Quint W, Dubin G. Lancet 2004;364(9447):1757–1765.

This report presents data from a randomised, double-blind controlled trial designed to assess the efficacy, safety, and immunogenicity of a bivalent HPV-16/18 L1 virus-like particle vaccine for the prevention of incident and persistent infection with these two HPV types and their associated cervical lesions. A total of 1113 women between 15 and 25 years of age were randomised to receive three doses of either the vaccine or the placebo on a 0-, 1-, and 6-month schedule in North America and Brazil. Women were regularly assessed for HPV infection for up to 27 months. The bivalent HPV vaccine was efficacious in preventing incident (92% efficacy) and persistent (100% efficacy) cervical infections with HPV-16 and HPV-18, as well as their associated cytological abnormalities and precancerous lesions (93% efficacy). The vaccine was generally safe, well tolerated, and highly immunogenic. Vaccination against such infections could prevent development of up to 70% of cervical cancers worldwide.

AGAINST WHICH HUMAN PAPILLOMAVIRUS TYPES SHALL WE VACCINATE AND SCREEN? THE INTERNATIONAL PERSPECTIVE

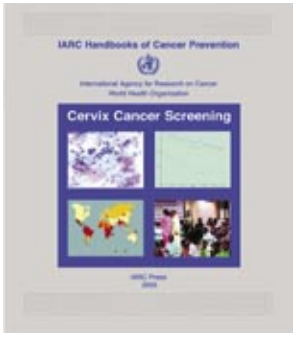
Muñoz N, Bosch FX, Castellsague X, Díaz M, de Sanjosé S, Hammouda D, Shah KV, Meijer CJ. Int J Cancer 2004;111(2):278–285.

This is a report of a pooled analysis of data from a number of studies coordinated by the International Agency for Research on Cancer (IARC) that included 3607 women with cervical cancer recruited in 25 countries. HPV DNA was detected in 96% of specimens. The 15 most common HPV genotypes were, in descending order of frequency, 16, 18, 45, 31, 33, 52, 58, 35, 59, 56, 39, 51, 73, 68, and 66. Higher than average proportions of type 16 were found in northern Africa, of type 18 in south Asia, of type 45 in sub-Saharan Africa, and of type 31 in Central/South America. A vaccine including types 16 and 18 could potentially prevent 71% of cervical cancers worldwide. A vaccine containing the seven most common HPV types would prevent about 87% of cervical cancers worldwide, with little regional variation. The impact of modifying the number of types in the screening cocktail tests would be small and probably irrelevant for screening programs.

KEY PUBLICATIONS

IARC HANDBOOKS OF CANCER PREVENTION VOL. 10: CERVIX CANCER SCREENING

February 2005. 302 pages, numerous b/w and color charts, figures, graphs and tables. ISBN 92 832 3010 2



Cervical cancer is still one of the most common forms of cancer among women, despite the success of screening for precursors of the disease using the widespread cytological procedure, the Papanicolaou (or Pap) test. This volume reviews what is known about the occurrence, natural history and causes of cervical cancer, before describing the established methods and newer variants and approaches for screening that are now being introduced, tested or investigated. Based on an international meeting of experts, the volume concludes with their evaluation of the evidence on the efficacy of screening for cervical cancer by the various techniques as well as their relative appropriateness depending on the resources available and competing priorities. It also provides recommendations for the public health implementation of screening, including the frequency of screening and the age groups that should constitute the target population, and the identification of areas for further research. Readership: Public health planners and managers, gynaecologists, cancer screening personnel.

LIQUID-BASED CYTOLOGY IN CERVICAL SCREENING: AN UPDATED, RAPID, AND SYSTEMATIC REVIEW AND ECONOMIC ANALYSIS.

Karnon J, Peters J, Platt J, Chilcott J, McGoogan E, and Brewer N. Health Technol Assess 2004;8(20):iii,1-iii,78.

This report reviews the cost-effectiveness of using liquid-based cytology (LBC) in cervical screening. From the evidence available, it is likely that the LBC technique will reduce the number of false-negative test results as well as the incidence of invasive cervical cancer. There is now more evidence showing that the use of LBC screening will also reduce the number of unsatisfactory specimens and the time needed to obtain the smear samples. Analyses based on natural history models of disease, conducted in this study, show that LBC is consistently more cost-effective than conventional Pap smear testing over the same screening interval.

EUROGIN 2004

INTERNATIONAL EXPERT MEETING WAS HELD IN NICE

OCTOBER 21-23, 2004 **WWW.EUROGIN.COM**

The meeting was attended by over 350 participants and had an eminent international faculty.

During this meeting, a wide range of topics were covered, each session being carefully designed and with specific objectives, reflecting the multi-disciplinary nature of this event. Apart from the substantial amount of new original research data presented as free communications and in Highlights of Research sessions, several important messages concerning the current state and future directions in cervical cancer prevention were conveyed by the invited keynote speakers. Importantly, if nothing further is done to prevent cervical cancer, it has been estimated that there will be one million women developing the disease annually by 2050, a figure that is double current estimates. As is happening today, the poorest parts of the world will be the worst affected. Thus, the translation of scientific knowledge into effective control measures is an absolute imperative, as concluded by Dr. P. Boyle (International Agency for Research on Cancer, IARC). These same issues were also addressed by the Congress President, Dr. Monsonego (EUROGIN), in his welcome address.

It is also clear that the highly effective organized screening programs of the developed European countries are not feasible in developing countries, where the prospects for effective cervical cancer control seem gloomy even in the foreseeable future. As emphasized by several speakers (Dr. Sankaranarayanan, Dr. Shastri, and many others), there is an urgent need for alternative diagnostic tests suitable as screening tools. Such optional measures include screening with acetic Acid (VIA), visual screening with Lugol's iocline (VILI) and HPV testing. We heard from Dr. Lörincz that Digene (US) is currently designing a simple, relatively rapid, and affordable batch-based diagnostic HPV DNA test (the dcHPV test) for use in low-resource settings. When available, wide dissemination of this new test should allow millions of women to benefit by a reduction in their risk of cervical cancer and disease mortality. Improved diagnosis based on liquid-based cytology (LBC) or molecular biomarkers of Cervical Intraepithelial Neoplasia (CIN) (as tested in several reported studies), might contribute to the same direction in countries with resources for implementation of these technologies.

The possibility to prevent cervical cancer in the future by prophylactic HPV vaccines was extensively discussed in several sessions. Dr. J. Schiller gave an update of the ongoing phase III efficacy trials of HPV L1 Virus Like Particles (VLP)-based vaccines (Merck, GlaxoSmithKline and the National Cancer Institute), for which data on long-term type-specific HPV protection and related CIN lesions will be available within the next two years. One of the companies (Merck) expects to launch commercial sales of their tetravalent vaccine (HPV 6,11,16 and 18) already in 2006. However, several important issues need to be seriously discussed, not least, how to solve the question of access to the vaccine in the developing world.

The main goal of the EUROGIN 2004 meeting was to present comprehensive approaches to cervical cancer control, to pinpoint the recent advances made, and to exchange information at a specialist level with regard to early detection, new diagnostic and therapeutic procedures, including HPV vaccination, recommendations for clinical practice, new directions for research, and enlarging the discussion beyond the medical and scientific aspects. Several sessions discussed the social and psychological impact of HPV test results in the context of screening programs as well as the prospects of HPV vaccine acceptability.

The Congress Summary Reports is now published (Monsonego J. Gynecologic Oncology 2005;96:830-839). The next opportunity will be EUROGIN International Congress in Paris, 23-26 April 2006.

J. Monsonego, MD
Chairman of the Scientific Programme.

POSITIONS AND OPPORTUNITIES

TWO TENURE-TRACK FACULTY POSITIONS IN DERMATOLOGY

Applications are invited for a Molecular Epidemiologist (A) and an Immunologist (B). Candidates (Ph.D., M.D.) should have postdoctoral research experience with publication history, current grant support, and expertise in analyzing susceptibility to viral diseases (A) / multichannel flow-cytometry (B). Qualified investigators are expected to develop an extramurally funded research program and contribute to a group effort focused on all aspects of human papillomavirus infections. Applications: Curriculum vitae, research interests, and three references. Contact: Walter G. Hubert, Ph.D., Department of Dermatology, University of Arkansas for Medical Sciences, Mail Slot 576, 4301 W. Markham St., Little Rock, AR 72205.

ENGENDERHEALTH

Amy E. Pollack, MD, MPH
President, EngenderHealth.
New York, USA.

What does EngenderHealth do?

EngenderHealth has been working since 1943 to make reproductive health services safe, available, and sustainable in the world's poorest countries. We support family planning, maternal health care, and HIV/AIDS prevention and treatment where basic health care needs are most urgent. We work in over 40 countries in Africa, Asia, and Latin America.

What is EngenderHealth's work in cervical cancer?

Even though the Pap test has successfully reduced cervical cancer morbidity and mortality in developed countries, this approach has not led to similar reductions in low-resource settings because it is inaccessible, unaffordable, and impractical. Since 1995, EngenderHealth has collaborated with Columbia University and the University of Cape Town, South Africa, to investigate alternative screening methods that might be suitable for low-resource settings. In 1999, EngenderHealth and four other organizations launched the Alliance for Cervical Cancer Prevention (www.alliance-cxca.org), a major initiative to reduce cervical cancer worldwide, funded by the Bill & Melinda Gates Foundation. We work to overcome barriers to cervical cancer prevention in developing countries, where women are most affected.

Currently, EngenderHealth is analyzing data from our most recent clinical trial in South Africa, assessing the safety and effectiveness of screening by visual inspection with acetic acid (VIA) or HPV DNA testing followed by cryotherapy treatment as indicated, done by mid level clinicians. Our work is helping to identify approaches to make screening more accessible and effective in regions where the infrastructure associated with Pap screening is not viable.

Additionally, we're working with health authorities in Bolivia, Bulgaria, India, and Romania to assess the cervical cancer prevention services in place, and provide technical assistance, ensuring that client and provider perspectives and needs are incorporated into the design of programs.

Since men's attitudes and behaviors are important factors that can inhibit or encourage women's screening, EngenderHealth works to involve men as supportive figures in the process.

What are your key findings?

We have demonstrated that HPV DNA testing and VIA have test performance characteristics as good as, or better than, Pap smears, and that these alternative screening methods are more cost effective than Paps in a low-resource setting like South Africa. The preliminary data from our current study indicate that a two-step "Screen and Treat" approach is safe and effective, reducing the number of clinic visits required to detect and treat precancer, and minimizing attrition at follow-up, with the potential to decrease cervical cancer morbidity and mortality.

How will the development of an effective HPV vaccine affect your work?

We are very excited about a vaccine to prevent HPV. However, there are some caveats. Most importantly, it will not be available for quite some time. Also, the vaccine will be given to young people before they become sexually active, so it will not affect the millions of women who are or will become infected with HPV before it is available. They will need screening for many years to come. And, while the vaccine will protect against some strains of HPV that cause most cervical cancers, there are others that will not be prevented. Our work in screening and pre-cancer treatment will continue to be essential for many years to come.

HPV DETECTION IN BONE METASTASIS OF SQUAMOUS CELL CARCINOMA 11 YEARS AFTER A DIAGNOSIS OF CERVICAL CARCINOMA

Background

Human Papilloma Virus (HPV) has been detected in virtually all cervical carcinomas.[1] HPV detection by molecular techniques has been applied mainly in screening settings and in the triage of borderline cytological abnormalities. Its usefulness in the diagnosis of the origin of distant metastases has not yet been fully assessed.[2–5]

Material and Methods

A 53-year-old patient, with a previous history of invasive squamous cell cervical cancer (stage 1b) that was diagnosed and surgically treated with a total hysterectomy 11 years ago, underwent computerized tomography (CT) exploration due to pain in the pelvic-bone area. Routine follow-ups had not shown any indication of recurrence of disease. The CT scan revealed a 6-cm lesion in the iliac bone, clearly identifying bone destruction, as well as adjacent soft tissue extension (Figure 1). A fine needle aspiration (FNA) was performed on the lesion and a cytology sample was collected. A cellular smear and a paraffin cell block were prepared with the cell aspirate. Squamous cell carcinoma was diagnosed after microscopic examination of the aspirated sample (Figure 2).

Due to the possible cervical origin of the bone metastases, HPV-detection and -genotyping were performed on the DNA extracted from sections cut from the paraffin blocks of both the cervical tumor and the iliac bone metastases FNA sample. The primary cervical tumor had been resected in another hospital and the archived paraffin block was requested from their Pathology department (Figure 3).

Nucleic acid extraction: DNA was extracted from 10-μm sections of paraffin-embedded material of both samples using a commercial kit (HPPPT, Roche).

Polymerase Chain Reaction (PCR) was applied with two different sets of consensus primers for HPV detection; the PGMY 09/11 and the GP5+/6+. Genotyping of the HPV was performed by reverse hybridization with type-specific probes.[6,7]

1. The PGMY 09/11 primers were used to amplify a 450 base-pair (bp) fragment of HPV L1 gene. Reverse hybridization was subsequently carried out for genotyping.[6] Biotinylated PCR products were hybridized to 27 immobilized probes (9 low-risk HPV types and 18 high-risk HPV types) and detected using streptavidin-horseradish peroxidase conjugate. Subsequently, a chromogenic solution was used to produce a signal that can be visually interpreted on the membrane that is displayed in a strip format.

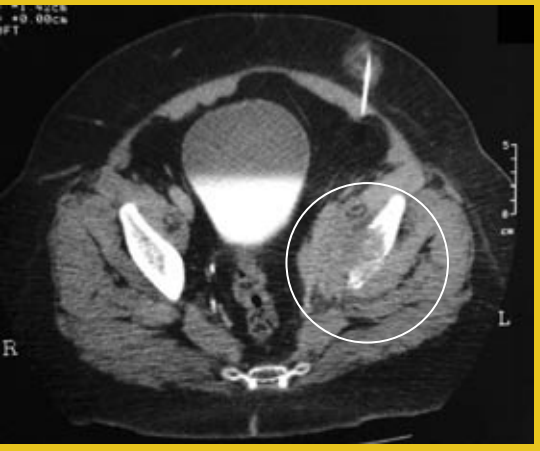
2. The General Primer GP5+/6+ PCR, amplifying a 150-bp fragment of the HPV L1 gene, was used, and reverse hybridization was subsequently applied for genotyping.[7] 37 Different HPV oligonucleotide probes (23 low-risk types and 14 high-risk types) were covalently bound to a negatively charged nylon membrane. The heat-denatured biotinylated PCR product was pipetted perpendicularly to the already immobilized probes into the parallel slots of a miniblotter. Subsequently, antibiotin conjugate followed by ECL solution were applied.

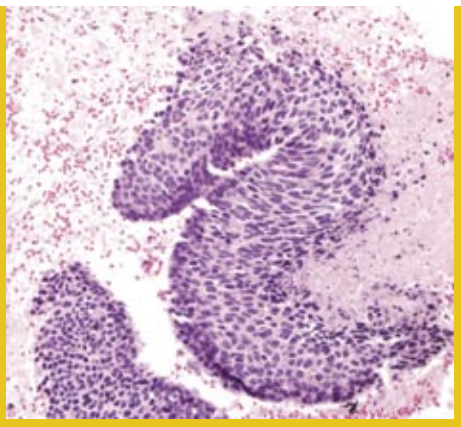
3. Both techniques use β-globin PCO3/PCO5 primers for amplification as an internal control for checking DNA quality. The resulting 209-bp fragment was visualized in a 1.5% agarose gel electrophoresis.

Results

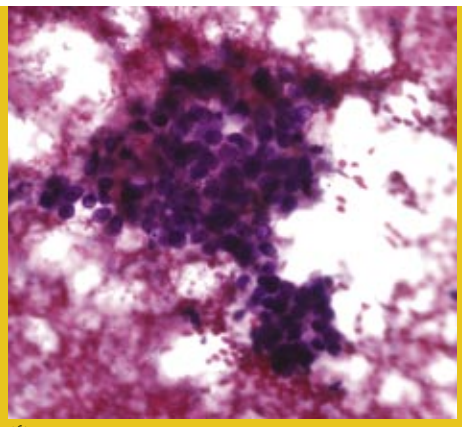
β-Globin amplification was detected in both the original cervical cancer sample and in the FNA from the metastatic lesion. The paraffin-extracted DNA samples from both cervical carcinoma and pelvic

Figure 1
CT *scan showing the destructive iliac bone lesion and soft tissue mass.*





(*a*)



(*b*)

Figure 2
Cell block (*a*) *and smear* (*b*) *corresponding to the bone fine needle aspiration* (FNA), *both showing squamous cell carcinoma with necrosis.*

J. Klaustermeier BS,[1] M. Hurtado MD,[1] M.Olivera,[1] E. Andia MD,[1] S. Marín MD,[1] M.J. Panades MD,[2] B. Lloveras MD, PhD[1]

[1] Institut Català d'Oncologia/Hospital Universitari de Bellvitge, Barcelona, Spain.
[2] Hospital Arnau de Vilanova, Lleida, Spain.

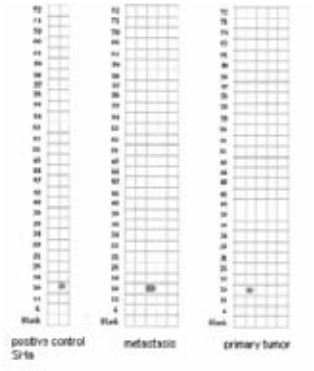


Figure 4
Reverse line-blot hybridization of GP5+/6+ PCR *products from primary tumor and metastic tumor as well as positive control, all showing* HPV 16 *signals.*

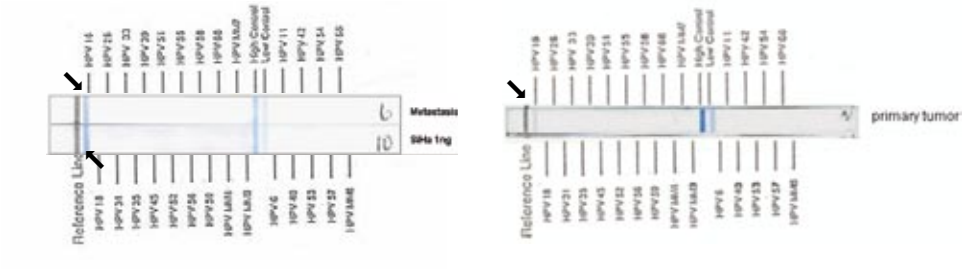


Figure 5
Linear array depicting an HPV-16*-positive signal* (*arrows*).

bone metastases contained HPV 16 DNA. Concordant results were obtained from both the HPV-PCR techniques (Figures 4 and 5). These results provided strong evidence as to the cervical origin of the bone metastases.

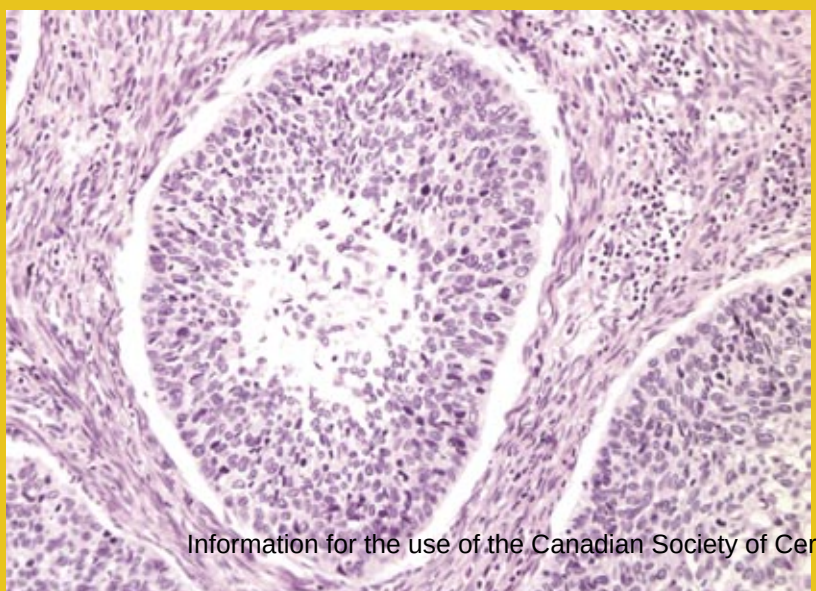
Discussion

HPV-detection and -typing technologies have developed greatly since the early epidemiological studies showing the role of HPV in cervical cancer etiology. At present, these technologies are being used in cervical cancer screening programs, in the triage of women with undetermined cytologic atypia (ASC-US), and in the follow-up of patients after treatment for cervical lesions. However, situations in which HPV testing could contribute diagnostic information are infrequent and poorly defined.

A clinical dilemma concerning the origin of the tumor developed when metastatic squamous cell carcinoma was diagnosed 11 years after the initial diagnosis of cervical cancer as, during this period, there had been no apparent indication of relapse. The appearance of iliac pain was the first sign that led to the identification of a destructive bone lesion which was then visualized on the CT scan. Squamous cell carcinoma with necrosis was diagnosed from the FNA sample of the cellular smear as well as from the cell block. The material from paraffin blocks was used for DNA extraction, and HPV-detection and -typing were performed with the objective of confirming metastasis from the primary cervical cancer. The detection of HPV 16 DNA in both samples using two different PCR techniques yielded valuable information for clinical evaluation.

This is just one example of the diagnostic application of HPV-detection and -typing in metastatic cancers of unknown origin.

Figure 3
Tissue section from the primary cervical tumor and an HE *stained slide showing invasive squamous cell carcinoma.*



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INTERNATIONAL AGENDA

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The 8th International Gcc Dermatology & Venerology Confrence

Contact: Ayman H. Hassanein, MD
Tel: +96 59 718 019
E-mail: q8dvc@yahoo.com

Las Vegas, USA

13th - 17th March 2006

American Society of Colposcopy and Cervical Pathology's 2006 Biennial Meeting

Venue: JW Marriott Las Vegas Resort, Spa and Golf
Web: www.asccp.org/biennial.shtml

Torino, Italy

5th - 8th April 2006

19th European Congress of Obstetrics and Gynaecology

President: A. Van Assche, C. Benedetto
Venue: Lingotto Conference Centre
Tel: + 39 011505900
E-mail: info@mafservizi.it
Web: www.ebcog2006.it

Vienna, Austria

6th - 8th April 2006

Clinical Dermatology 2006

Contact: Organising Secretariat, CCT Postgraduate Education Limited, 50-52 Union Street, London SE1 1TD United Kingdom
Tel: +44 171 407 9731
E-mail: d2000@cctltd.u-unet.com

Paris, France

23rd - 26th April 2006

Eurogin 2006

Venue: Palais des Congrès
Contact: Peter Mattonet
Tel: + 33 1 44 40 01 20
E-mail: admin@eurogin.com
Website: www.eurogin.com

Maratea, Italy

24th - 27th May 2006

VII European Course of Cervical Pathology and Colposcopy

Contact: Roberto Piccoli
Tel: 00 390 815 585 090
E-mail: rpiccoli@unina.it

Prague, Czech Republic

1st - 7th September 2006

23rd International Papillomavirus Conference and Clinical Workshop

Venue: Hilton Hotel Prague
Organizers: Hamsikova E. Smith E. Turek L. Vonka V.
Tel: +1 319 339 7177 / +420 221 977 273
E-mail: tomas.maxa@czech-in.cz
Web: www.ipvconference2006.org