

## MINI REVIEW

# CERVICAL CANCER CONTROL, PRIORITIES AND NEW DIRECTIONS

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**Cervical cancer is caused by infection with a range of high risk "oncogenic" human papillomavirus (HPV) types, and it is now accepted that >99% of cervical cancer is initiated by HPV infection. The estimated lifetime risk of cervical cancer is nevertheless relatively low (less than 1 in 20 for most community based studies). Although sensitivity and specificity of the available diagnostic techniques are suboptimal, screening for persistent HPV infection is effective in reducing the incidence of cervical cancer. Infection can be detected by molecular techniques or by cytological examination of exfoliated cervical cells. Persistent infection is the single best predictor of risk of cervical cancer.<sup>1</sup>**

**The latest findings of HPV and cervical cancer research need to be widely disseminated to the scientific and medical societies that are updating screening and management protocols, public health professionals, and to women and clinicians. This report reviews current evidence, clinical implications and directions for further research in the prevention, control and management of cervical cancer. We report the conclusions of the Experts' Meeting at the EUROGIN 2003 conference.**

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**Key words:** cervical neoplasia; human papillomavirus; screening; colposcopy; HPV vaccine

### EPIDEMIOLOGY

Human papillomavirus (HPV) infection is a common, sexually transmitted infection and both women and men are rapidly exposed to the virus after the onset of sexual intercourse. The risk of infection is increased by the number and risk behavior of sexual partners, and the duration of the infection is contingent on the HPV type. HPV 16 stands out with a median duration around 16 months and other high-risk viral types show a mean duration of around 8 months, whereas low risk types consistently display a duration of around 4–5 months.<sup>2,3</sup> Both women and clinicians need to be aware that most infections have a benign clinical course and resolve spontaneously. The estimated 3–10% of women in different populations who cannot clear the viral infection and become persistent HPV carriers constitute the high-risk group for progression to cervical cancer. The pooling of International Agency for Research on Cancer case control studies has provided generic and type specific risk estimates for 18 HPV types. The adjusted odds ratios (OR) for HPV DNA detection (factor by which the reference risk of cervical cancer is multiplied if HPV DNA is detected) for any single type was odds ratio (OR) = 172.6 (95% confidence interval [CI] = 122.2–243.7). Type specific risk estimates were as follows: HPV 16: OR = 435; HPV 18: OR = 248; HPV 45: OR = 198; HPV 31: OR = 124; HPV 33: OR = 374; HPV 35: OR = 74;

HPV51: OR = 67; HPV 52: OR = 200; HPV 58: OR = 115; HPV 59: OR = 419. The risk for any given high-risk type was not statistically different from the risk reported for HPV 16, and the risk related to the presence of multiple HPV types in the specimen was no different from the risk linked to a single HPV type. The standard estimates of the attributable fraction (AF) %, (the proportion of disease that is related to HPV DNA) derived from these and most other studies range from 90–98% compared to a prevalence of 5–20% from cervical specimens of women identified as epidemiological controls.<sup>4</sup> The association is equally strong for squamous cell cancer and cervical adenocarcinomas. Detailed investigation of the few cervical cancer specimens that appear as HPV DNA negative strongly suggest that these are largely false negatives. Consequently, the claim has been made that HPV infection is the first necessary cause of a human cancer ever identified, and provides a strong rationale for the use of HPV tests in screening programs and for the development of HPV vaccines.<sup>5</sup>

The IARC studies also reported the contribution of additional factors to the risk of cervical cancer in HPV carriers. Among HPV-positive women, any use of oral contraceptives was associated with a significant increase in risk (OR = 1.47 [1.02–2.12]), use for <5 years was not related to cervical cancer (OR = 0.77 [0.46–1.29]) but the risk increased significantly for 5–9 years of use (OR = 2.72 [1.36–5.46]) and for 10 or more years (OR = 4.48 [2.24–9.36]).<sup>6</sup> If the interaction between HPV and hormonal contraceptives is confirmed, this would support the introduction of HPV tests in the screening protocols of long-term users of oral contraceptives. HPV-positive women who reported 7 or more full term pregnancies had a 4-fold increased risk of cervical cancer as compared to similar HPV-positive women that were nulliparous (OR = 3.8, [2.7–5.5]). Smoking was associated with a 2-fold statistically significant increased risk of cervical cancer with a significant dose response, and an independent risk factor for cervical cancer.<sup>7</sup> HPV-DNA positive women who were seropositive for type-2 herpes simplex virus (HSV-2) or Chlamydia trachomatis antibodies were also at a moderately increased risk. The literature,

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Received 28 July 2003; Accepted 1 August 2003

DOI 10.1002/ijc.11530

however, is not fully consistent on these additional factors. Host factors that determine the course of HPV infection are largely unknown. Although natural history studies in males are rarer, there is consistent evidence that HPV is transmitted sexually, and that circumcision protects against persistent infection. Circumcision also reduces the risk of cervical cancer among spouses.<sup>8</sup>

#### SCREENING AND DIAGNOSIS

The objective of cervical cancer screening is to prevent the occurrence of and death from cervical cancer by detecting and treating high-grade squamous intraepithelial lesions (HSIL), which are precursor lesions for invasive cancer. The risk of cervical cancer in screened populations is determined by the sensitivity of the method utilized and the interval at which that method is applied. The most widely used screening approach to detect HSIL is conventional cervical cytology, followed by investigation of positive women with colposcopy and directed biopsy. Although cytology based screening programs in Europe, North America and Australia have been followed by a substantial reduction in mortality from cervical cancer, a wide range of values has been reported for the sensitivity and specificity of conventional cytology tests in different settings. In a meta-analysis of 62 studies of cytology conducted between 1984–92, the mean sensitivity was 58% (range = 11–99%) and mean specificity 68% (range = 14–97%).<sup>9</sup> Thus, cervical cancer screening protocols that utilize cervical cytology as the sole primary screen will continue to have Pap diagnoses that have an unclear meaning. The majority of these women will be normal, whereas 6–11% will have CIN 2/3 and approximately 1/1,000 will have cervical cancer. The relative insensitivity of conventional cytology means that frequent testing is required for optimal cancer protection, compromising cost efficiency and prompting the investigation of approaches that may be applied at greater intervals with similar safety. Discussions of the relative effectiveness of different methods must take into account both test sensitivity and intervals.<sup>10</sup> In general, the most cost effective regimen is to use the most sensitive possible test at the longest possible interval. This approach relieves the system of the costs of evaluation and treatment of large numbers of abnormal screening tests that in most cases represent low grade transient abnormalities, whose recognition adds greatly to expense without increasing cancer protection. Although the Pap test is significant from its historical perspective, its impact on the incidence of cervical cancer, and its position as the most widely used cancer screening test in the world, recent publications suggest that the sensitivity of the Pap smear is 50–60%, with the relative proportion of sampling to screening errors being about 2:1. Smears may be further complicated by high unsatisfactory rates, preparation artefacts that hinder computer analysis, and the inability to pursue additional testing. There is a growing body of evidence that liquid-based cytology addresses the limitations of the smear method, improves detection of cervical lesions, and facilitates technologies such as imaging and ancillary testing.<sup>11–13</sup> Recent government sponsored reviews by the National Institute for Clinical Excellence in the United Kingdom<sup>14</sup> and the Agency for Health Care Policy Research<sup>15</sup> in the United States concluded that liquid-based cytology is a cost-effective alternative to conventional smear-based cytology, and should be adopted.

The knowledge gained during the last 20 years on the necessity for persistent high-risk HPV to establish and maintain CIN3 and eventual progression to cervical cancer has provided the basis for evaluation of the clinical utility of testing for cancer-associated HPV types.<sup>16</sup> Primary screening studies involving more than 40,000 women worldwide have also demonstrated HPV testing to be more sensitive than cytology alone, and that the combination provides a negative predictive value of >99%.<sup>17–20</sup> The result has been recent approval in the US of combined testing for women over the age of 30. As HPV testing provides assessment of the current risk as well as the risk of subsequent development of high grade CIN and given the known natural history of HPV-induced cervical precancers, a minimum screening age of 25, or 8 years from first intercourse is indicated. In

women with a negative HPV test and a normal cytologic screen, the screening interval could be safely extended to 8–10 years without compromise of cancer prevention, but in countries where annual or biennial screening is the norm, the widening of screening intervals to 3–5 years may be more acceptable.

#### LOW RESOURCE SETTINGS

Cervical cancer screening programs are rare in developing countries, and, in those few countries where a program has been introduced, a substantial reduction in the incidence and mortality from cervical cancer has yet to be observed. The constraints of cytology based screening in low-resource settings have prompted the evaluation of alternative methods including visual inspection of the cervix after application of 3–5% acetic acid (VIA) and Lugol's iodine (VILI), and HPV testing to detect cervical neoplasia. VIA and VILI are simple, inexpensive, low-technology methods that require minimal infrastructure, no laboratories for reporting, and a short training period (1–2 weeks) for health professionals. As the results are available immediately, further diagnostic investigations (colposcopy and biopsy for test positive women), and planning and treatment are possible during the same visit. This avoids recall of women for procedures, resulting in logistic advantages, better compliance and cost savings. The sensitivity of VIA for high-grade lesions and invasive cancer ranged from 70.9–82.6% and the specificity ranged from 64.1–86.5% in cross-sectional studies in Zimbabwe,<sup>21</sup> China<sup>22</sup> and India.<sup>23</sup> In the Indian study, the sensitivity and specificity of VILI to detect high-grade lesions and invasive cancer were 87.2% and 84.7% respectively. The major disadvantage of VIA and VILI is the low specificity of these tests, and it remains to be seen whether specificity can be improved by further developments in test definitions and training strategies. To date, there is no universally accepted uniform definition of test results for VIA and VILI, and given the considerable variation in the way these tests are applied and interpreted in different settings, standard definitions and approaches are urgently needed. Nevertheless, a VIA/VILI-based screening programme may be more readily integrated into primary care health services in less developed regions. Model-based simulations of cost effectiveness indicate that cervical cancer screening strategies that incorporate VIA or HPV DNA testing and eliminate colposcopy may be attractive alternatives to cytology-based screening programs in low-resource settings.<sup>24,25</sup>

#### MANAGEMENT

Colposcopy is an integral part of the management of women presenting with abnormal cervical cytology and those with lesions in the lower genital tract indicative of intraepithelial neoplastic disease. Various protocols exist for cervical abnormalities and these are detailed in the flow chart in the ASCCP guidelines.<sup>26</sup>

Colposcopy as a subjective modality has a sensitivity for the detection of intraepithelial disease in the range of 60–75%.<sup>27</sup> When employed with exfoliative cytology or HPV DNA testing or both, this sensitivity can be increased into the range of >90%. The sensitivity of a combined Pap and HPV testing strategy is further enhanced by referring immediately to colposcopy all women with LSIL or HSIL cytology regardless of HPV result. Women who are ASC-US HPV negative should have repeat cytology in 1 year, whereas those who are ASC-US HPV-positive are best managed by colposcopic evaluation.

HPV testing lacks the interlaboratory/interobserver variability of cervical cytology and has been demonstrated in the ALTS trial and other studies to be an efficient and sensitive management option for women with equivocal cytology.<sup>28,29</sup> It is the preferred management option for women with an equivocal Pap derived from a liquid-based Pap sample as the HPV test can be obtained directly from the remaining liquid media without having the patient return to obtain the specimen. A single HPV test at 12 months is more sensitive than 2 repeat Paps for the detection of CIN3 in the post-colposcopy management of women in a number of clin-

ical settings.<sup>30</sup> Women with equivocal cytology who are HPV-positive should be referred for colposcopic evaluation, whereas HPV negative women with equivocal cytology can be reassured.

#### PROPHYLACTIC AND THERAPEUTIC VACCINES

Development of prophylactic vaccines for HPV is warranted because HPV infection precedes and plays a central role in the development of cervical cancer.<sup>31</sup> Although around 15 HPV types are thought to cause cervical cancer, a vaccine containing HPV 16 would prevent around half of the cases of cervical cancer, a vaccine containing types 16 and 18 would prevent two-thirds, and a vaccine against the 6 most prevalent types would prevent four-fifths of cases. In animal models, virus-like particle (VLP) prophylactic vaccines protect against virus challenge. The efficacy of several papillomavirus vaccines in animal models has stimulated the development of human papillomavirus vaccines and testing in clinical trials.<sup>32</sup> The vaccines currently in phase II/III trials are sub-unit vaccines composed of VLPs obtained by self-assembly of the major capsid protein L1. These VLPs do not contain the viral genome and therefore cannot spread infection. Nevertheless, they morphologically and antigenically resemble natural virions and consequently induce a potent neutralizing immune response in recipients. It has been shown that neutralizing antibodies can block HPV infection *in vivo* and *in vitro*.<sup>33</sup> Conformational epitopes present in VLPs appear critical for the induction of neutralizing because in animal models vaccination with denatured L1 protein failed to protect against virus challenge.

Clinical trials show encouraging results with no severe side effects.<sup>34</sup> HPV L1 VLP vaccines are highly immunogenic stimulating B and T cell responses and generating high titers of neutralizing antibody.<sup>35</sup> Data from all clinical trials indicate that antibody levels fall after the booster injection but persist for at least 18 months post vaccination.<sup>36</sup> Preliminary results suggest that protection is type specific. HPV vaccine prevents the establishment of HPV infection and thereby has the potential to prevent cervical cancer.

Development of therapeutic vaccination for HPV is also warranted because there are an estimated 5 million women worldwide already infected with HPV, and whose infection will lead to invasive cervical cancer. Tumors and their precursor epithelial lesions including persistent HPV infection express papillomavirus non-structural proteins. In animal models, therapeutic vaccination controls growth of tumors expressing papillomavirus non-structural proteins. Animal studies have shown, using transplantable tumor models whose relevance to human cervical cancer is probably minimal that: papillomavirus non-structural proteins are immunogenic and safe as vaccine components; almost any viral protein together with almost any adjuvant system will protect against transplantable tumor challenge; almost any viral protein, together with some adjuvant systems not yet licensed for use in man, will cure mice of early stage established tumors,<sup>37</sup> and a range of immunological mechanisms (antigen-specific and innate) are effective at producing cure. Better animal models involving animal papillomavirus infection have shown that a combination of viral non-structural proteins (E2, E6, E7) can control development of lesions if given early after viral challenge because papillomavirus infections resolve without intervention in the 2 commonly used models (cow and dog) but these models are of limited predictive value for persistent cervical infection. Similar results in the cottontail rabbit are seen only if immunization is achieved before disease development.<sup>38</sup> Early phase human trials have shown that E6 and E7 proteins, and some peptides derived from these proteins, are immunogenic in man. These proteins can be safely delivered to patients with cervical cancer and cervical precancer, using a range of adjuvants and delivery systems including viral vectors, peptides and proteins.<sup>39</sup> Evidence for therapeutic efficacy is limited to studies on virus load or viral persistence. No Level 1 evidence of efficacy in randomized placebo controlled trials is yet available

#### FURTHER RESEARCH

Additional research is currently being conducted on the mechanisms by which an extremely common and largely trivial infection may occasionally induce, in the absence of early detection and treatment, a fatal invasive cancer. The following areas of research were highlighted at the EUROGIN conference. Evaluation of biomarkers that will further stratify HPV-positive women for risk for progression to CIN3 and cervical cancer may provide useful insights. Other issues include the role of known and suspected environmental co-factors, the determinants of immune response to the viral infection, the interaction between the host and the virus, and the relevance of the different strains and variants of the HPV viral types. Also of great interest are investigations that attempt to elucidate the risk factors leading to persistent HPV infection with highly oncogenic HPV types. The HPV DNA male carrier remains an orphan condition of potential importance in the epidemiological chain of HPV and cervical cancer, and the efficacy of prophylactic vaccines in males should be evaluated. The role of HPV in non-genital cancer sites is also a promising field, with skin cancer and cancers of the upper aro-digestive tract as some of the major candidates. Developments in the technology to measure HPV antibody response in serum specimens would facilitate the evaluation of vaccination studies, and might foster additional epidemiological research on HPV infection and human cancer.

Appropriate consideration is required on how new technologies evaluated for adoption will improve cervical screening programs in terms of traditional measures (sensitivity, specificity, positive and negative predictive values) and cost effectiveness, including analysis of quality of life issues. Evidence is not yet available about the efficacy of alternative screening methods in reducing cervical cancer incidence and mortality. The time course of cervical carcinogenesis and the legal milieu in developed countries, combined with the relative rarity of cervical cancer means that RCTs with cervical cancer as an endpoint will never be done. Recognition of the value of surrogate endpoints is therefore essential to research. Results from randomized intervention trials may be conducive for introducing these interventions in public health programs, provided that the mandates of evident benefit to patients, providers and payers are also demonstrated. Because good quality cytology screening is associated with a significant reduction in disease burden, it is likely that screening tests with a similar accuracy profile to that of conventional cytology are likely to be associated with a similar reduction in disease burden in screening programme settings, provided that appropriate service delivery conditions are met.

Although most of the current research has focused on assessing new approaches to determine if the benefits of these technologies justify added costs, the outcomes of the analysis will be affected by the thresholds of evidence that are deemed acceptable, the cost assumptions, testing intervals, compliance with testing, the potential of combining multiple technologies and the benefits assigned to improved quality of life. The complexity of this analysis has led to divergent results, and evaluation of the cost-effectiveness and clinical utility of liquid-based cytology and HPV testing in primary screening needs to be completed in multiple international settings. Computer assisted imaging of the Pap sample may significantly improve laboratory workflow and the ability of abnormalities to be detected by screening. Further research is also needed in understanding the interrelationship between morphologic examination and the presence of HPV within cells, or the molecular alterations in the cell that arise from HPV infection.

In low-resource settings, continued evaluation of how to best utilize the information available about HPV to reduce the risk of cervical cancer should include identification of low-cost and safe treatment options for HPV-positive women, and assessment of vaccine acceptance, distribution, funding and administration for each country in preparation for vaccine approval.

More extensive trials of prophylactic vaccines are necessary to confirm efficacy, the existence or not of cross-protection, and the duration of protection. For therapeutic vaccines, better animal

models of persistent viral infection are required. Phase I human trials are expensive and no validated surrogate markers of vaccine efficacy have been defined as yet. Ethically acceptable trial designs, that would be feasible and affordable in the real world, for demonstrating efficacy of an HPV therapeutic vaccine are also needed. Lastly, more extensive trials of non-specific immunotherapy for AIN and VIN seem justified as these diseases are recurrent and have a poor response to existing therapies.

#### CONCLUSIONS

The practical conclusion from prevalence and natural history studies is that both HPV based preventive strategies, whether for screening or vaccination, should effectively target all cases of cervical cancer. The practical conclusions from the equivalence of risk estimates for each of the 15 high risk types indicate that group

testing of clinical specimens for a cocktail of high risk types should be sufficient for screening and patient management. Individual typing remains necessary in research settings and for studies evaluating therapeutic or preventive type-specific HPV vaccines.

The goal of cervical cancer control is attainable, but requires coordination of resources and sharing of information. Patients, providers and payers must all perceive the benefit of the new system *to them* or change will not take place. Schemes that cannot be widely implemented are of very modest utility. Education of each group in both high and low resource settings is therefore essential if improvements in practice are to move from speculation and research into widespread clinical practice. The most important point remains that women and clinicians need to be informed of the usually benign nature of an HPV infection to minimize the anxiety that may accompany a positive HPV test result, and this should be at the forefront of all communication messages.

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