

## Recent advances in the molecular epidemiology of cervical cancer

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Cervical cancer is one of the most common neoplastic diseases affecting women, with a combined worldwide incidence that is second only to cancer of the breast. In developing countries, however, cervical cancer ranks as the most important of all female neoplasms, whereas in western developed nations it ranks as the tenth most common malignant disease [Parkin et al, 1993]

In North America, the overall 5-year survival rate for women with cervical cancer is 65%, lower than that for breast cancer [Holleb et al, 1991]. Taken together, the proper diagnosis, treatment, and follow-up of the patients with cervical cancer or its precursor lesions place an important burden on the public health system, particularly in developing countries.

Epidemiologic studies conducted during the past three decades have consistently indicated that cervical cancer risk is strongly influenced by measures of sexual activity, both of the woman and her partner [Franco, 1991]. Other risk factors include tobacco smoking, parity, use of oral contraceptives, conditions leading to immunodepression, diet low in beta carotene and vitamins C and A, and infrequent pap smear testing [Brinton, 1992; Munoz and Bosch, 1992]. While there is still some debate in the literature as to the actual importance of the latter variables, no one questions the validity of the findings for sexual activity. As a result. In the past 20 years laboratory scientists and epidemiologists have tried to identify the sexually-transmitted agent or agents that act as the intermediate cause of cervical cancer.

Most of the research conducted during the 60s and 70s attempted to search for an etiologic role for the herpes simplex viruses (HSV). Although HSV has proven to be carcinogenic in vitro and in vivo clinical studies eventually demonstrated that only a fraction of cervical carcinomas contained traces (viral DNA) of HSV infection and epidemiologic studies failed to demonstrate an association between HSV and cervical cancer [reviewed in Franco, 1991]. However, the most

important reason for the attention to be diverted from the HSV was the emergence in the 1980s of papillomaviruses (HPV) as the most likely cause of cervical cancer [Zurhausen, 1991].

Clinical and sub-clinical HPV infections are the most common sexually-transmitted diseases today. There are over 70 HPV types defined on the basis of DNA homology [Vonkrogh, 1991]. Two major groups are defined according to their epithelial affinity: the ones infecting the dry skin and those infecting the moist mucosal areas of the body. Genital types are typically divided into three groups based on the frequency of association with malignant tumors, and thus, the presumed oncogenic potential. The low risk group includes types 6, 11, and 42-44, which are common low-grade SIL (LG-SIL), less so in high-grade SIL (HG-SIL), and practically nonexistent in cancer specimens. The intermediate risk group is comprised of types 31, 33, 35, 51 and 52, whose combined frequencies of association increase within the SIL spectrum, but decrease in carcinomas. The high risk group includes HPV types 16, 18, 45 and 56, which are strongly associated with carcinomas and exhibit diverse behavior with respect to HG-SIL. The latter group seems, in fact, to be composed of two different subgroups: high-risk/HPV 16, which seems to be equally predictive of HG-SIL and cancer, and high risk/HPV 18/45/56, which are strongly associated with cancers but somewhat uncommon in HG-SIL [Lorincz et al, 1992].

Interestingly, some inconsistencies emerged in early molecular epidemiology studies of HPV and cervical cancer that used first generation DNA hybridization methods to detect the virus. Contrary to expectations, these studies found that cervical HPV infection was not associated with sexual activity variables [Villa and Franco, 1989; Reeves et al, 1989; Kjaer et al, 1990], a paradoxical finding considering that cervical cancer risk is strongly associated with sexual behavior. As shown subsequently by us and others [Franco, 1991, 1992;

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Schiffman and Schatzkin, 1994], by measurements errors of HPV infection status were the cause for these incoherent findings. Techniques to detect the presence of HPV in cervical cells have involved considerably, from simple scoring of cytologic signs of HPV to immunocytochemical staining, nucleic acid hybridization methods, and more recently, the polymerase chain reaction (PCR). Modern PCR protocols based on the so-called consensus primers (Because they amplify defined regions in genes that are highly conserved across HPV types) have sensitivity and specificity for epidemiologic studies [Gregoire et al, 1989; Manos et al, 1990; Vanderbrule et al, 1990]. Use of PCR has minimized the problem of misclassification of viral status in epidemiologic studies.

A consensus panel convened by the World Health Organization's International Agency for Research on Cancer (IARC) has recently concluded that there is now compelling evidence, both from the biologic and from the epidemiologic standpoints, to consider HPV infection as cause of cervical cancer [Franco, 1992]. A variety of case-control and cohort studies conducted in the last five years have consistently shown that HPV infection is the strongest risk factor for cervical cancer, with relative risks (RR) in the 20-70 range. Such a magnitude is higher than that for the association between smoking and lung cancer and is second only to that for the association between the chronic carrier state of hepatitis B infection and liver cancer, causal relations in cancer that are no longer challenged [Franco et al, 1994].

Cervical HPV infection detected by DNA hybridization techniques is found in 15%-40% of asymptomatic women of reproductive age [Franco, 1991]. Interestingly, however, when additional cervical specimens are taken from these women in follow-up surveys the majority of the infections are harbored to the same HPV type in subsequent specimens [Moscicki et al, 1992,1993; Hildesheim et al, 1994; Franco

et al, 1994]. In addition, prospective epidemiologic studies have indicated that the risk of subsequent cervical neoplasia seems to be proportional to the number of specimens testing positive for HPV [Koutski et al, 1992]. These findings suggest that only persistent infections of the cervical epithelium may be the ones eventually triggering carcinogenic development. Considering that there is now an ongoing debate concerning whether HPV testing should be added to existing cervical cancer screening programs [Nuovo and Nuovo, 1991; Reid and Lorencz, 1991; Johnson, 1995], it is imperative that issues related to viral persistence be addressed by epidemiologic studies.

The difficulty however, resides in defining persistence. Viral typing plays a useful role in this regard, by providing a first screen of multiple specimens from the same woman, thus indicating which cases can be safely ruled out as being transient infections because different HPV types were found in consecutive specimens. But, how would we interpret cases with concordance of types during follow-up? For instance, would two HPV-16 (a common genital type) positive specimens taken a few months apart from the same woman indicate persistent infection, or could they represent two consecutive transient infections with HPV-16? We have recently proposed a solution to this problem [Franco, et al, 1994] by demonstrating the applicability of DNA sequencing techniques to detect molecular variants of the HPV in the epidemiologic studies of the natural history of cervical neoplasia. These variants are identified on the basis of mutational patterns in the DNA, thus allowing interpretation of persistence on firmer grounds. Ongoing cohort studies incorporating detection of molecular variants of HPV should eventually answer questions related to the applicability of HPV testing as a screening tool for cervical cancer.