

High-risk human papillomavirus screening and testing with immunohistochemical surrogate biomarkers: an alternative to polymerase chain reaction

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The vacuolisation and ballooning of cervical squamous cells, presently known as koilocytosis, was first described in 1957 by FW Stewart, who defined it as “wartlike atypia” (a hint towards a possible viral link). The presence of viral particles in the nuclei of epithelial cells of condylomata was first documented electron microscopically by AEG Dunn and NM Ogilvie in 1968. Similarities between condylomata and cervical precancerous lesions led to the hypothesis that the papovavirus causing condylomata may be a contributory factor in cervical carcinogenesis. This hypothesis was confirmed in 1978 by Laverty et al, with the electron microscopic identification of viral particles consistent with a papillomavirus, in a preinvasive lesion in an immunosuppressed woman.¹

Koilocytosis is a response of squamous cells to human papillomavirus (HPV) infection, but is not HPV type-dependent.¹ In addition, HPV infection occurs in the absence of koilocytosis. Sometimes, the only abnormality is nuclear enlargement. There may be no detectable changes at all.² In other words, morphology (histopathologic or cytopathological) may be inconclusive at times, while inter-observer variability has an influence too.³

From a diagnostic and prognostic point of view, it is optimal to know the types of HPV implicated in a specific lesion. Over the last two decades, ample evidence has accumulated on the role of high-risk HPVs (mainly 16 and 18, as well as 12 other potential ones), in preinvasive and invasive cervical lesions.⁴ Since conventional morphological criteria are unable to identify the presence or absence of HPV and their specific type, with certainty, it is important to use ancillary methods. HPV-deoxyribonucleic acid (DNA) types can be identified by Southern blot, polymerase chain reaction (PCR), in situ hybridisation (ISH) with

DNA probes, hybrid capture, and enzyme-linked immunosorbent assay.⁵⁻¹³ The oncoproteins produced by HR-HPV can be identified by immunohistochemistry (IHC), either on paraffin-embedded tissue sections, or on liquid cytology preparations.¹⁴ However, liquid-based cytology is not used extensively in the South African public health sector because it bears a higher cost than conventional cytology. Furthermore, conventional cytology may be problematic with regard to antibody staining, and would not achieve the same technical robustness of other approaches.

High-risk-HPV oncoproteins and their action on the cell cycle

The HR-HPV 16 and 18 oncoproteins, E6 and E7, are produced respectively by the corresponding E6 and E7 genes. The E6 protein binds to, and degrades, the host cell protein, p53 (a homolog of its precursors, p63 and p73), produced by the p53 gene. The p53 gene is a major role player in cell cycle control and genomic stability. The p53 protein is a transcription factor and apoptosis (programmed cell death) regulator. The E6 protein is capable of selectively degrading the p53 gene, leading to the inactivation of the p53 through gene mutation, and neutralization of the wild-type p53 protein. This leads to the inactivation of cellular negative regulatory proteins, and to uncontrolled cell replication and genomic deregulation. The presence of p53 gene mutation correlates inversely with the presence of HR-HPV. Some functional p53 protein may remain in HPV-infected cells, but at low levels.^{9,15-16}

The retinoblastoma protein (pRb) pathway is another cell regulatory pathway of utmost importance. By binding to the transcription factor E2F, the pRb arrests the transcription of cell proliferation genes, and blocks the gene encoding for the cyclin-dependent

kinase inhibitor p16^{INK4A} (in short, p16). The HR-HPV produced oncogenic E7 protein selectively degrades the pRb, leading to the inactivation of cellular negative regulatory proteins in synergism with E6. When E2F is released from pRb, the blocking effect on the transcription of the p16 gene is lost, and results in accumulation of p16 protein in the cells.¹⁶

The surrogate markers for HR-HPV highlight the effects of the oncogenic proteins, E6 and E7, namely the loss of control of cell replication, apoptosis, and genomic stability. As opposed to the identification of HPV DNA/mRNA, IHC identifies the presence of HR-HPV by surrogate markers. A large number of publications have validated HPV-HR surrogate markers against HPV-DNA detection.^{9-10,12-13,15,17-20}

Immunohistochemical surrogate markers of high-risk HPV

A panel of monoclonal antibodies, used as surrogate markers, is now available, mainly cluster designation (CD)40, p16, p53, and Ki-67.

Cluster designation 40

CD 40, alternatively called p50, a member of the tumour necrosis factor family, is crucial in regulating cellular and humoral responses of apoptosis, inflammation, immune regulation, and oncogenesis. Although less researched than the other Mabs, it is overexpressed in HPV-infected lesions, cancer cells, and invasive squamous cell carcinoma.²¹⁻²⁴

P16

P16 has been extensively investigated. It labels cervical dysplastic squamous and glandular cells with a sensitivity of 99.9%, and a specificity of 100%, both immunohistochemically and immunocytochemically. It has also been shown to be able to separate low- and high-grade lesions, and identify histologically or cytologically classified low-grade lesions that are susceptible to cancer progression. The method has been validated against ISH, and has been shown to be superior to ISH. Increasingly, reports show that with p16, IHC HR-HPVs are also identified in vulvar preinvasive and invasive lesions, penile cancer, and head and neck cancers.^{14,18,25-34}

P53

So far, P53 has received less attention than p16 in cervical pathology.^{12,16,35-38} P53 mutation, resulting in the inactivation of the wild-type p53, is the most frequent genetic event in cancer. Wild-type p53 gene is an important tumour suppressor, apoptosis inducer, and cell-cycle controller. P53 is inactivated through

binding to, and sequestration by, the E6 oncoprotein. Its mutation may occur during cervical carcinogenesis, through downstream mutagenesis. The pathogenesis of dysplasia occurs through the inactivation of the protein produced by the gene, rather than at the level of the genomic mutation. The mutated p53 leads to a malignant phenotype. The prevalence of p53 mutation in cervical cancer is less than 10%. This makes it the less suitable surrogate marker. The commercially available p53 Mabs are directed against the mutant and wild-type. Hence, choice of the right Mab is crucial.

Ki-67

Ki-67 is an antigen that corresponds to a nuclear non-histone protein, expressed by cells in the proliferative phases G1, G2, M and S. On its own, it reflects the cell turnover rate only. It is usually associated with other markers.^{27,36-40} This makes its use less attractive outside of a research context. If used, it should be used in conjunction with other markers, such as p16.

It should be emphasised that IHC should always, as far as possible, correlate with morphology, especially in equivocal cases. On the other hand, when morphology and IHC are discordant, the interpretation should be cautious, repeated, and reviewed by a second pathologist.

Cost and benefits of surrogate markers

Recently, the South African HPV advisory board published guidelines for screening and testing.⁴¹ The emphasis on the need for screening and testing is indisputable. However, the place of hybrid capture PCR in the algorithm is disputable. First, it would be unpractical in terms of equipment, trained personnel, and cost. A recent South African study on the feasibility and financial implications of pap smear screening (National Health Laboratory Service current cost of R65, or US\$9) combined with HPV-DNA PCR, estimated the cost per PCR test at US\$300 (\pm R100).⁴² However, the current cost in the South African public health sector is around R900. A new test (careHPV[®]) has been developed to detect 14 high-risk types of HPC. It was shown to have a sensitivity of 90%, and a specificity of 84.2%.⁴³⁻⁴⁵ The current cost is reported to vary, in the range of US\$20-30 (\pm R140-200).⁴⁴

In the public sector, the cost of IHC is R335 (\pm US\$48). In our laboratory, an average of 10 preinvasive cervical lesions are diagnosed weekly. P16 immunophenotyping could be added to the existing IHC work load easily, at a much lower cost than PCR, and at least pending the availability of careHPV[®].

Currently, IHC is routinely used for hormone receptor immunophenotyping of breast cancer,

typing of lymphomas, and identification of the Kaposi's sarcoma virus. It is estimated that, in South Africa, HPV 16 and 18 are present in 26.6% of LSIL/CIN1 (low-grade squamous intraepithelial lesion/cervical intraepithelial neoplasia 1), and 58.4% of HSIL/CIN2-3.⁴⁶ The high burden of disease that relates to cervical diseases warrants the implementation of HR-HPV surrogate marking. However, the practicality of this approach in the South African public health sector has not yet been determined. For instance, the limited availability of liquid-base cytology would restrict the implementation of p16 IHC to biopsy specimens. The interpretation of p16 IHC lends itself to the same interobserver variability potential as cytology. None the less, IHC has been shown to be a highly valuable asset in diagnostic histopathology. P16 IHC is unlikely to become the preferred screening method for HR-HPV. It is a surrogate marker of HR-HPV, indicating that a specific woman is at risk of preinvasive and invasive cervical disease.

It is well established that only 10% of HR-HPV carriers will develop invasive cancer, regardless of the method used to diagnose the presence of HR-HPV. Whatever the diagnostic tool, the importance of the diagnosis lies in its ability to detect a risk factor, and the ensuing management and follow-up. P16 IHC could be one of these tools, for instance, in cases of low-grade intraepithelial lesions infected by HR-HPV,⁴⁷ and pending new and more affordable, as well as generalisable, methods. Flow diagrams for the management of preinvasive lesions have their merits, but should be implementable. In settings where opportunistic screening remains the primary method of cervical pathology detection, several years are needed before flow diagrams will be able to replace the current methods. Meanwhile, p16 immunophenotyping, at the time of preinvasive lesion diagnosis, would have the potential merit to screen women at risk, and possibly to avoid hysterectomies based on the histopathologic report of the initial punch or cone biopsy.

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