Human papillomaviruses and cervical screening

Cervical screening aims to reduce deaths from cancer of the cervix by detecting and treating pre-malignant disease, and our national screening programme is a success story. Our experience mirrors that of Finland and Iceland where organised screening programmes were introduced much earlier and where dramatic falls in mortality from cervical cancer have already been achieved. Screening currently relies on cervical cytology, and the Papanicolaou (Pap) smear, which have been used since the latter's introduction in the 1940s. Why then is there a need to consider alternative screening strategies? The answer to this can be found by considering the large group of women who are found to have low-grade smear abnormalities. These women, who are mainly young, constitute the majority of those found to have an abnormal smear and yet most of them will have either no underlying disease or low-grade changes which have the potential to regress. This is the rationale behind the current recommendation that such smears be repeated after six months and referral for colposcopy only then be made if the abnormality persists. This policy would not be contentious were it not for the fact that studies have consistently shown that up to one third of this group will have high-grade intraepithelial neoplasia (CIN 2/3). Furthermore two longitudinal randomised studies on the management of these women have recently concluded that a policy of early referral for colposcopy is the preferred option. If we adopted this policy change, not only would colposcopy clinics be stretched to the limit, but a significant number of women with no disease or trivial changes would also be subject to a psychologically stressful procedure and possible overtreatment. We need therefore to be more selective and identify better those women likely to have underlying high-grade CIN. Hence the interest in secondary screening techniques.

Although evidence of a direct causation is incomplete, we do know that human papillomaviruses (HPV) play a central role in the genesis of CIN and cervical cancer. Since 1983 molecular probes have been available for the detection of HPV and it has been shown that high-risk HPV types (particularly HPV 16, 18, 31, 33) are found in the overwhelming majority of cervical cancers and CIN 3 lesions. If these agents are suspected so strongly as being the major cause of CIN and cervical cancer, then surely screening for them offers an alternative to the Pap smear for detecting women at risk. However, despite the availability of rapid accurate tests for the virus, interest in utilising them for mass screening has been hampered until recently by the experience of studies in the mid to late 1980s. In terms of testing for HPV, Southern blot hybridisation remains the gold standard with sensitivity of 95% and specificity of 98%. It is, however, complicated and laborious and has no use in a clinical situation. Filter in-situ hybridisation (FISH) was developed as a simple rapid test for HPV in the mid 1980s. Studies which utilised FISH can be largely discounted because of the poor specificity (less than 50%) of the test and that no true distinction could be made between the main low-risk types (HPV 6/11) and the high-risk types (HPV 16, 18). The polymerase chain reaction (PCR) is in theory a very simple technique which can generate large amounts of target DNA by using known oligonucleotide primers and heat stable DNA polymerase. It is an exquisitely sensitive technique, yet it is this very sensitivity that was also its drawback in the early days of PCR. Contamination of the specimens in the laboratory is a major problem which can lead to nonsensical results such as finding HPV DNA in distilled water! Some early PCR papers seemed to indicate that HPV infection (even with high risk types) was almost ubiquitous and have since been retracted. Strict laboratory technique with the use of both positive and negative controls has overcome these early problems, but scepticism based on the early work remains in the minds of many clinicians when considering the value of HPV testing in a clinical situation. The sensitivity of PCR throws up another problem: as minute quantities of viral DNA can be detected, is a positive result for high-risk HPV based on detection of a tiny amount of DNA as clinically significant as a positive result from a larger amount of DNA? A consideration of some of the more recent papers may provide an answer to that question and also as to whether HPV detection is likely to be of use as a mass screening tool.

There is now increasing evidence from a number of small studies that quantitative or semi-quantitative HPV DNA detection may be a useful triage in deciding which women with low-grade cytological abnormalities need colposcopy. Two studies from London have looked for the presence of high-risk HPV types in cervical smears by PCR from 133 and 200 women respectively. Both studies used a very simple method of semi-quantitation which relied on a visual comparison of the amplified DNA product. As the cellular content of a smear can vary greatly, some estimate of the amount of human DNA in each sample for amplification must be made to standardise the results. This was clear from only one of those studies. Furthermore the diagnostic endpoint used in the studies was not consistent. Both used a combination of excision of the transformation zone, directed punch biopsy and colposcopic opinion alone. However, those reservations aside, both studies concluded that intermediate or high amounts of HPV DNA were significantly associated with high-grade CIN and that HPV testing could be a useful addition to, but not a replacement for, cervical cytology.
A different test known as hybrid capture has been similarly evaluated. This technique is a simple non-radioactive solution hybridisation test. The target molecule is a single stranded DNA which hybridises to RNA probes. These dimers are captured on a plastic tube by immobilised antibodies. The bound hybrids are reacted with an alkaline phosphatase RNA-DNA antibody and chemoluminescent substrate is cleaved emitting light proportional to the amount of hybrid captured. This is therefore a quantitative test for HPV DNA. Two studies from the USA of 217 and 311 women respectively concluded that hybrid capture was a useful triage in women with abnormal squamous cells of undetermined significance (ASCUS) and low-grade cytological abnormalities.9,10

The studies mentioned above have utilised HPV detection as a secondary screening technique. In such a situation the women tested have already been identified as being of higher risk by the primary test. What about using HPV detection as a primary screening tool? The problem here is that because of the much lower disease prevalence in the general population the predictive value of a positive test is greatly diminished. However, a recent study by Cuzick et al of over 2000 women using HPV detection in primary screening found that 44% of high-grade lesions were detected by HPV testing alone (that is, negative cytology) but that 25% of high-grade lesions were negative for the types of HPV being tested.11 Again the take-home message was that HPV testing could augment but not replace conventional cytology. The problem of the poor predictive value of a positive test is much less in older women where the proportion of positive tests representing invasive disease increases sharply. Studies have shown that the prevalence of HPV declines with age and maturing host immunity of the infection, and that persistence of infection is the key to an increased risk of developing CIN and cervical cancer.12 It may be therefore that primary screening for HPV in this group may prove cost effective.

The question of cost should not be overlooked and, as there is an immense market to be tapped, inevitably there will be pressure from companies producing HPV detection kits to introduce widespread testing. It may be that HPV testing will enable us to rationalise the screening programme and increase the screening interval for those women deemed to be at low risk. This would to some extent offset the extra costs but it still remains to be proven. Aside from the cost implications for health care services, we should not underestimate the psychological impact on women on being told that they have acquired a sexually transmitted infection which renders them at increase risk of cancer, even though this infection will be a transient phenomenon in a large proportion of cases.

In the immediate future we will be evaluating not only HPV DNA detection, but also serological testing for capsid antigen.13 This should add further pieces to the jigsaw in our understanding of cervical cancer by enabling large longitudinal cohort studies to be carried out. If such studies confirm that high-risk HPV types are the cause of cervical cancer then the long term goal of using vaccination as primary prevention can then be addressed. So to conclude, it seems from a number of small studies that HPV detection may be useful in the cervical screening programme to triage women with low-grade smear abnormalities, although there is, as yet, no evidence that it can replace conventional cytology. However, before we embrace wholesale HPV screening in the UK we need confirmation of its value from large prospective randomised trials on the management of women with low-grade cytological abnormalities.

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