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Review

Cervical screening in the 21st century: the case for human papillomavirus testing of self-collected specimens

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Abstract

Cervical screening by Pap smear involves a high rate of false negatives, necessitating frequent testing. Because women do not like the sampling procedure, many avoid being screened. Testing for the causative high-risk human papillomavirus (HPV) types, by PCR or other technologies, on self-collected (tampon) samples permits women to be monitored non-invasively. The high negative predictive value of HPV testing means a greater interval between tests, and thus reduces costs. HPV testing lends itself to primary screening. A kit for self-collection and return to a testing laboratory, followed by practitioner notification and follow-up if required, should result in wider participation. The higher accuracy of HPV testing should lead to improved cervical cancer prevention.
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Keywords: cervical cancer; cervical screening; human papillomavirus; hybrid capture; polymerase chain reaction; specimen self-collection; tampon.

Introduction

Half a million new cases of cervical cancer are diagnosed world-wide each year, resulting in a quarter of a million deaths (1). Incidence overall is second only to breast cancer. In the USA cervical cancer is the 14th most common cancer, with 12,000 new cases (10 per 100,000 women) and 4000 deaths in 2003, with 5-year survival of 66% (1). In Europe it is the 10th most common cancer. It accounts for 8% of all cancers in the female population of developed countries, and 25% in developing countries (2). Unlike many other cancers, moreover, cervical cancer and pre-cancer primarily affect women in their most productive years (ages 30–50 years) (3). The lifetime risk of cervical cancer is 1 in 20, and was predicted as 2.9% by modelling of data in The Netherlands, with a peak at age 48 years (4). Notably, it is the only cancer that is almost completely preventable by regular screening, which can reduce the risk to 0.4% (4).

Here we argue the case for human papillomavirus (HPV) detection as a primary cervical screening test.

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Pap smear for cervical screening

Background

Cytological screening by the Papanicolou (Pap) smear has been in common use for over 50 years (5). In any cytological screening programme, most women test normal. The 6%–11% who test positive for abnormal cells are referred for colposcopy to obtain a biopsy to assist in diagnosis prior to removal of cells. Many smears show minor cytological abnormalities, which are much more common than either low-grade squamous intraepithelial lesions (LSIL) or high-grade squamous intraepithelial lesions (HSIL). Most positive Pap smear results do not identify women who will develop HSIL. In particular, atypical squamous cells (ASC) of undetermined significance (ASCUS) according to the Bethesda System (6) have an especially low positive predictive value (PPV) for >HSIL [where PPV is the probability that the patient has the condition when restricted to those patients who test positive; thus PPV=TP/(TP+FP), where TP is the number of true positive results and FP the number of false positive results]. The reproducibility, and thus reliability, of ASCUS is low and not all women with minor abnormalities are referred for colposcopy. Unless a high-grade lesion is found, excision is generally avoided. Nevertheless, unnecessary colposcopies, with associated costs, are unavoidable in the absence of more informative triage information. Alarmingly, however, a California study found 28% of women with cervical cancer had had only ''normal'' results from regular screening (7).

Sensitivity of Pap test

Certain criteria have been invoked (6) to help improve the specificity of referral, while attempting to maintain a high sensitivity level (where sensitivity is the probability of a positive test result given that the target is present, and specificity is the probability of a negative result given that the target is absent (8)). In a population screened, the risk of cervical cancer is

dependent on the sensitivity of the test used and how often it is applied. A meta-analysis of 62 studies found sensitivity of cytology to be 58% (range 11%–99%) and specificity to be 68% (range 14%–97%) (9). It concluded that not only is the Pap smear only moderately accurate, but it also does not achieve concurrently high sensitivity and specificity (10). For example, specificity in the 90%–95% range corresponds to sensitivity in the 20%–35% range. A more recent meta-analysis that was confined to 12 studies that met rigorous inclusion criteria has shown that the sensitivity of the Pap smear, using LSIL as the threshold, was 77% (10). Clearly, cervical screening programmes that rely on cytology alone lead to diagnoses that are equivocal.

Low sensitivity increases frequency of testing Because the Pap smear is so insensitive, a woman must be tested frequently in order to provide optimum protection. This compromises cost-efficiency and encourages the search for approaches that can be used less often, but that result in at least similar safety. Both of these factors have to be taken into account when assessing the relative effectiveness of different methods (11). For maximum cost-effectiveness, the most sensitive test must be utilised over the longest possible interval. This averts the cost impact of evaluation and treatment of large numbers of abnormal screening results that mostly represent low-grade abnormalities of a transient nature, which adds greatly to expense without increasing protection from cervical cancer. Despite the Pap smear having historical significance, being credited with reducing cervical cancer incidence in the population over previous decades, and being the most widely used test, not only is its sensitivity low, but the relative proportion of sampling to screening errors is 2:1. Pap results also suffer from high ''unsatisfactory'' rates and preparation artefacts.

Liquid-based methods

Liquid-based cytological methods have been introduced over the past decade to overcome some of the problems (12–14), and in the USA and UK these have been recommended for adoption as a cost-effective alternative to smear-based cytology. Although the most widespread, the ThinPrep™ Pap Test (Cytyc Corporation, Boston, MA, USA), has the potential to reduce the rate of unsatisfactory smears, there is no evidence that it improves screening results (15, 16).

TruScreen device

A device, known in its current version as TruScreen (Polartechnics, Sydney, Australia), has been in use for over a decade, with recent entry into China and other large second-world markets. The device is placed on the cervix and sends electrical and optical signals to a computer to ascertain abnormalities (17). While this can give a result in real time and has enormous potential in field testing in remote regions and developing countries, it is no more accurate than a Pap smear, with sensitivity for histologically confirmed HSIL lesions of 70% and 69% by TruScreen and Pap, respectively (18). Used as an adjunct to the Pap smear, sensitivity was increased to 93%. For LSIL, sensitivities of TruScreen, Pap and combined testing were 67%, 45% and 87%, respectively.

Screening based on HPV testing

Background

Over the past two decades, considerable evidence has emerged in support of testing for the causative agent in cervical cancer, namely high-risk types of HPV (1, 19, 20). HPV detection has been proposed for both primary screening (21), triage of equivocal Pap smears (22, 23), and follow-up of patients after treatment (24–26). High HPV prevalence and sexual transmission mean a high rate of HPV infection in young people soon after commencement of sexual activity. There are over 40 anogenital HPV types. These may confer negligible through to substantial risk of cervical cancer. Of the over 15 high-risk types, the most common is HPV16, which has a median duration of infection of 16 months, whereas for other high-risk types (HPV18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, 82) the average period of infection is 8 months, as observed in sexually active college women in the Bronx, New York (27), while for low risk types, 4–5 months is the norm (28, 29). Although most HPV infections resolve spontaneously, 3%–10% of women do not clear the infection (30). In a Swedish study the 5-year HPV clearance rate was 92%, with HPV16 the only type that persisted, and HPV16 persistence was associated with HSIL (31). Persistence of HPV infection is, moreover, the single best predictor of risk of cervical cancer (30). Thus, HPV infection by itself is a poor predictor of a future intraepithelial lesion.

A study of 8656 Danish women found that 17.7% of younger and 24.5% of older cytologically normal women who were HPV-positive had an abnormal Pap smear within 5 years, and risk of HSIL or cancer within 10 years was 13.6% and 21.2%, respectively (32). Thus a positive HPV test in a woman with a negative Pap smear is a valuable predictor of a future high-grade lesion (32).

Pooling of data from the International Agency for Research on Cancer from 11 case-control studies in nine countries gave an adjusted odds ratio (OR) for risk of cervical cancer if HPV DNA is detected of 173 [95% confidence interval (CI) 122–244] (33). Individual risk estimates for 10 relevant HPV types are shown in Table 1. The risk for any given type did not differ from the risk conferred by HPV16. Moreover, infection with multiple HPV types did not further increase the risk. Using a detection method involving polymerase chain reaction (PCR; details of which are discussed in a later section), HPV DNA was found in 91% and 98% of cases by two different PCR-based tests (viz. MY09/11

Table 1 Risk of cervical cancer according to HPV type.

Type	16	18	45	31	33	35	51	52	58	59
Odds ratio	435	248	198	124	374	74	67	200	115	419

and GP5+/6+, respectively) (33). In contrast, HPV prevalence in control women was only 13% (5%–20%) (33). Association with HPV is, moreover, similarly strong for squamous cell carcinoma and cervical adenocarcinoma (34, 35). The most common HPV types in patients, in descending order of frequency, were 16, 18, 45, 31, 33, 52, 58 and 35 (33).

Does the causative role of high-risk HPVs in cervical cancer (36) mean that HPV detection should be used as the basis of screening programmes, especially given the low reliability of Pap smears? In this regard, data obtained to date are primarily from cross-sectional studies. This does not, however, allow adequate evaluation of the effectiveness of different screening strategies or of the preinvasive stages of cervical cancer development. The European Commission (EC) stated in Council Recommendation of 2 Dec 2003 (2003/878/EC) item 25 ''No screening test other than those listed in the Annex [Pap smear] is scientifically justified to be offered to people with no symptoms in an organised population-based programme before it has been shown in randomised controlled trials to decrease disease-specific mortality in particular''.

Randomised controlled trials of screening for HPV

Six large-scale randomised controlled trials (RCTs) are currently under way in the UK, Canada, The Netherlands, Italy, Finland and Sweden to establish the performance of HPV testing and decide whether it should be added to screening programmes or whether it should replace cytology as a primary screening test for cervical cancer (21). Results are starting to emerge from some of these RCTs and final results are expected to be published in 2007.

In the Italian RCT, involving 5808 women, HPV testing alone with cytology triage was found to be 1.6-fold more sensitive than conventional cytology, and PPV was only slightly lower than conventional cytology (37). In women aged <35 years, relative PPV was better than in older women. The HPV approach thus avoided an increase in false positives, even in the age group of women with high infection rates. Primary HPV testing was thus advocated as a feasible alternative to conventional cytology for screening of women under 35 years of age (37). The study found that only women with persistent infection need be referred for colposcopy (37). High-risk HPV clearance precedes cytological regression by 3 months (38). One question that remains, however, is to what extent so-called persistence of HPV reflects a lack of clearance as opposed to clearance and reinfection.

In the Swedish study, 28% of cytologically negative women who went on to exhibit persistent high-risk HPV had confirmed ≥HSIL by colposcopy at follow-up (19 months), with PPV of 29% (39). Interim data from the Finnish study of 200,000 women showed more positive women in the HPV arm (40). The study in Amsterdam, involving 5-year follow-up of 44,102 women after initial testing by PCR for 14 high-risk HPV types, revealed that one or more types were present in 2154 subjects (41). In the UK study, based in Manchester, 24,510 women had liquid-based cytology and HPV testing at entry, revealing infection rates of 40% for those aged 20–24 years, 28% for ages 25–30 years, 18% for 30–34 years, down to 6% after age 60 (42). The authors questioned the recommendation of primary screening with HPV testing in combination with cytology triage, since high-grade dysplasia was observed as commonly in women under 30 years of age as in 30–49-year-olds (42). Results of follow-up screening of these women at 3 years are due soon. The Canadian Cervical Cancer Screening Trial (CCCaST) involves 9667 women aged 30–69 years recruited in Montreal and St. John's from 2002 to the end of 2004 (43). At entry, an abnormal Pap smear was noted in 2.8%, 6.1% had a positive HPV test, and 1.1% had both. Results were to be reported after 12–18-month follow-up.

A decision on implementation of HPV testing awaits publication of these RCT results. The EC requirement that HPV testing show a decrease in mortality before being sanctioned as a screening test in clinical practice is perhaps excessive, given the established role of HPV and the reliability of HPV testing. Other considerations such as the advantages, clinical effectiveness, cost benefit, shortcomings and other consequences are discussed in the present review. Although somewhat limited at present, once completed, the results of the RCTs will greatly add to current predictions.

What if HPV testing is adopted?

If HPV testing is shown to reduce prevalence of >HSIL in subsequent screening, this should lead to implementation of more effective screening policy, thus reducing mortality and morbidity (21).

HPV tests have high sensitivity and predictive value for HSIL, with two large studies in the USA showing, moreover, that detection of HPV DNA should improve the management of patients with minor abnormalities (44, 45). Furthermore, American Society of Colposcopy and Cervical Pathology Consensus Guidelines strongly endorse the use of HPV testing for women with ASCUS as an essential triage tool in the work-up prior to assessment by colposcopy (46).

Triaging with HPV has shown that 98% of women with HSIL are positive for HPV (47). In contrast, only 74% of those with LSIL were HPV-positive (47). The rate of HPV in women with a negative colposcopy result was only 34%. It was found that triage by HPV of women with an ASCUS-AGUS (atypical glandular cells of undetermined significance) cytology report would have spared 59% from having a colposcopy

and 47% from having a biopsy. These are major reductions. It would, moreover, have missed only 5% of HSIL. In ASCUS-AGUS patients the PPV for HSIL would have increased from 14% to 34%. In the case of women with LSIL, 17% would have been spared colposcopy, and in those with HSIL this would be 4%. Importantly, no HSIL would have been missed (47).

The poor specificity of the Pap test for colposcopy means an excessive number of referrals and biopsies (47). Whereas the annual rate of cervical cancer in the USA and UK is approximately 1 in 10,000, and of pre-cancer is 1 in 1000, approximately 1 in 10 women will test positive for HPV (48).

A large proportion of HSIL thus does not progress to carcinoma. However, there is no way of knowing, based on the Pap smear result, which will and which will not, so that all are nevertheless treated. Much of the treatment is therefore unnecessary. Moreover, HPV testing may be a useful alternative to 6-month repeat cytology for women with ASCUS. Failure to clear a HPV infection would merit cytological examination or even colposcopy.

Role of HPV in cancer at other sites

It is worth noting that high-risk mucosal HPVs, most notably HPV16, also have a causal role in 20%–30% of head and neck cancers (49, 50), the sixth most common cancers in the USA. These have a mortality of $>50\%$, which has not improved over the years (51). Interestingly, HPV DNA can be found in plasma from cervical cancer patients (52). In addition, HPV has been located, and may contribute to cancer, in breast tissue, suggesting acquisition from sexual activity (53, 54) or carriage in the bloodstream from an infected cervix (55, 56). A role for HPV in breast cancer remains controversial, however.

Meaning of a HPV result and preferred screening strategy

The number of women with normal cytology despite being positive for HPV (so-called false-positives) is of concern. In 2293 Belgian women aged 20–50 years with normal cytology, the prevalence of high-risk HPV did not decline until after age 35 (57). It was suggested that delaying primary screening for HPV from 30 to 35 years of age would decrease by 50% the number of normal women having a transient HPV infection (57).

The combination of HPV testing and cytology has a negative predictive value [ratio of negative results to the test(s) used to all-screen negative results] of $>99\%$ (58–61). Such a high value is to be expected for any test for a low-prevalence disease such as SIL, since most of the screen negatives will be true negatives in a low-prevalence environment. The extremely high negative predictive value of the combination of a negative HPV result and a normal Pap smear should allow considerable widening of the screening interval (62, 63). Under such circumstances the screening interval can be safely extended to 8–10 years with little compromise in prevention of cancer (64). However, in countries where screening is annual or once every 2 years, increasing this to every 3–5 years might be more acceptable (64). Because a HPV result provides information about the current risk as well as the risk of developing HSIL (i.e., the natural history of time to pre-cancer), it has been suggested that age 25 or 8 years from first intercourse be the minimum for screening (65). For women aged ≥ 30 years, primary screening every 2 or 3 years by HPV test plus cytology, or cytology with reflex HPV DNA testing for equivocal results, has been regarded as providing greater cancer reduction at lower cost than an annual Pap smear (66). For post-colposcopy management a HPV test at 12 months is more sensitive than two repeat Pap smears for HSIL detection. In low-resource countries, moreover, HPV testing is attractive (67, 68).

Of course, once a positive HPV test result is obtained, a decision must be made about whether to refer the woman for treatment or continue monitoring the infection by repeated tests at more frequent intervals.

It has also been argued that high prevalence of HPV infection, the presence of transient infections and implementation of a new test without sufficient data to change the screening interval will mean that large numbers of women may end up with positive test results, leading to an increase in the number of referrals.

Thus, to summarise: (i) 20 years of research has shown that persistent high-risk HPV is involved in HSIL and progression to cervical cancer; (ii) testing for cancer-associated HPV types has clinical utility in screening (69); and (iii) HPV testing is more sensitive than cytology alone, as shown in primary screening studies involving $>40{,}000$ women world-wide (58–61), and HPV testing improves diagnostic accuracy (35). Not surprisingly, combined testing (i.e., HPV testing and a Pap smear) has been approved by the FDA in the USA for women aged >30 years (70). A quality assurance programme has, moreover, confirmed HPV testing to be robust, with high reproducibility in different laboratory settings (71). Clinician guidelines on HPV testing have been released recently by the US Centers for Disease Control and Prevention (72). Results from RCTs are likely to lead to endorsement of this test. The RCT findings may assist in deciding which women who test positive should be followed up.

Cost-effectiveness

Another issue is improving the cost-effectiveness of screening programmes. In this regard, the relatively low cost that is possible with HPV testing by PCR makes detection of HPV desirable on economic grounds. The estimated reduction in lifetime risk of cervical cancer varies from 81% to 93%, depending on the screening frequency, type of cytology, and test strategy. In one study (66) it was found that screening women of all ages every 3 years with liquid-based cytology, and 3-year screening by a HPV DNA test plus cytology of women aged >30 years provide

equivalent or greater benefits than an annual conventional cytology test. The incremental cost-effectiveness was calculated as US$95,300 and US$228,700 per year of life gained for each of these, respectively. If annual screening was by HPV testing plus cytology instead, this would add only a few hours to life expectancy with a cost-effectiveness ratio of >US$2,000,000 per year of life gained.

Costs of different tests and procedures vary from country to country, and are particularly high in the USA. Moreover, the relative costs between different tests can also vary. The costs of long-standing procedures such as colposcopy and biopsy are unlikely to decrease, whereas the cost of PCR-based testing should decline continually over time, thus making this test increasingly attractive. Importantly, the sensitivity of HPV for HSIL is very high (44, 45, 47).

Other risk factors

Less than 1% of women who are infected with high-risk HPV will go on to develop cervical cancer. However, there is no way at present of distinguishing women who will develop the disease from women who will not. Nevertheless, there are factors associated with elevated risk. Risk is increased when oral contraceptives have been used for more than 5 years: for 5–9 years, OR=2.7, and for >10 years, OR=4.5. For a woman who smokes, the risk is increased 2–3-fold and is dose-dependent (73, 74). The risk is 1.8-fold higher for ≥7 compared to 1–2 full-term pregnancies (75). Impairment of host immunological responses is associated with persistence of HPV infection (76). High viral load is associated with a considerable increase in risk of HSIL (30, 74). In one study, the risk increased 43-fold for high viral load compared with 3-fold for low viral load (77). Moreover, 25% of women with high HPV16 load prior to age 25 went on to develop cervical carcinoma in situ within 15 years (77). Women with HSIL all had higher viral load (by type-specific quantitative real-time PCR) than 33% of women with normal cytology (78). Risk is also slightly higher for seropositivity for herpes simplex type 2 (HSV2) or *Chlamydia trachomatis* antibodies. History of chlamydia infection doubles the risk of persistent HPV infection, possibly via induction of a chronic inflammatory state (79). A similar increase in risk applies to HSV2 infection (80). Impairment of the host immunological response also appears to be associated with persistence of HPV infection (76).

HPV is transmitted sexually and if the male is uncircumcised the occurrence of persistent HPV infection and cervical cancer in the spouse is elevated by 5.6-fold, with the rate increasing with the number of sexual partners the man has had (81). There is, moreover, no consistent evidence that condom use is associated with lower HPV positivity (81, 82). Skin-skin contact, including foreplay, is capable of passing on HPV. Risk of LSIL, HSIL and cervical cancer were, however, somewhat reduced by condom use (82).

Testing for HPV mRNA

Expression of the HPV oncogenes E6 and E7 is an indicator of active infection. A kit for the detection of E6/E7 mRNA for types 16, 18, 31, 33 and 45 is now available commercially (Pre-Tect HPV-proofer®, Norchip, Klokkarstua, Norway). In a recent survey of women under 30 years of age, 14.5% were positive by Pre-Tect HPV-proofer® compared with a 20.8% positivity rate by type-specific PCR and 2.8% by cytology (83). These results suggest that RNA testing might be useful for triaging of HPV DNA-positive women. In another small study, RNA testing was found to be less sensitive but more specific than HPV DNA for the presence of disease in HPV-positive cytologically normal women and was also associated with persistent infection (84). RNA testing may also be useful for triaging of women with equivocal cytology. The ability to differentiate active from latent infections through RNA technology is an important advance. At this stage the ability to screen for only five HPV mRNAs is a limiting factor, although a broader spectrum test covering 15 HPV types is under development by GenProbe (San Diego, CA, USA).

Adjunct biomarker: $p16^{INK4a}$

High-grade cervical dysplasia results from deregulated expression of the HPV oncogenes (E6 and E7). Interaction between E6 and E7 and tumour suppressors p53 and retinoblastoma (pRb), respectively, promotes the chromosomal instability that sets the cell on a course towards cancer (85). The disruption of pRb by E7 leads to overexpression of the cyclin-dependent kinase inhibitor $p16^{INK4a}$ (CDNK2A) through a feedback mechanism (86). High and stable expression of E6/E7 oncoproteins correlates with high expression of $p16^{INK4a}$, independent of viral load (87). Immunohistochemistry for $p16^{INK4a}$ is a sensitive means of identifying dysplastic cells in histological slides, as well as in conventional smears or liquid-based cytology preparations (88, 89). This suggests that $p16^{INK4a}$ immunostaining may help to avoid ambiguities in the interpretation of cervical cytology samples, thus leading to more rapid diagnosis. The availability of commercial $p16^{INK4a}$ cytology and histology kits may facilitate automated screening of cytological slides. Although this could improve cytological screening, it adds to the workload and cost.

Management

Management of moderate to severe dysplasia typically involves colposcopy and diagnostic biopsy (46). Colposcopy is, however, subjective and its sensitivity for detection of intraepithelial disease is 60%–75% (90). Sensitivity can be increased to >90% when colposcopy is used with cytology or HPV testing or both. Whereas it is recommended that women who have ASCUS but are HPV-negative have repeat cytology

within 1 year, those with ASCUS who are HPV-positive may need to be managed with colposcopy.

One advantage of HPV testing is that it lacks the variability observed for cytology between different laboratories and observers (44, 45). It is, moreover, the preferred option for a woman with an equivocal cytology result. The absence of high-risk HPV in a cervical sample with equivocal cytology means the woman is highly unlikely to go on to develop cervical cancer, thus dampening any enthusiasm for aggressive management (91, 92). In one study, the 33% of cytologically normal women with HPV load less than that observed in all HSIL women could be managed less aggressively (78). HPV can, moreover, be tested for in the sample left over from a liquid-based specimen. After colposcopy, subsequent management by a single HPV test at 12 months is more sensitive than two repeat Pap smears (93).

HPV detection techniques

Hybrid Capture II

At present the only widely available commercial HPV test approved by the FDA for clinical use is the Hybrid Capture II assay (HCII; Digene Corporation, Gaithersburg, MD, USA) (94). It is based on hybridisation of DNA probes to 13 HPV types. Hybridisation probing is an old technology, requires relevant laboratory expertise and is fairly expensive (95). The sensitivity of HCII depends on signal amplification (95). Crosswell contamination may, however, influence marginally positive test values (96). Results that are borderline require retesting by an alternative technology such as PCR (97).

Polymerase chain reaction

In 1988 PCR was first reported for HPV detection (98–101). PCR is a Nobel Prize-winning technique that amplifies nucleic acid sequences in the HPV genome and this approach has become widespread. A WHO study across 29 laboratories in 12 countries of 24 coded samples containing HPV16 and 18 alone or with five other HPV types found general consistency of detection and typing (102). Virtually every biomedical or biotechnology laboratory in the world uses PCR for one application or another, and its commercial use is set to rise markedly after the core technology of PCR, owned by Hoffmann-La Roche, came off patent in the USA on March 28, 2005.

In 2003 a commercial PCR kit – the Amplicor HPV test – was launched by Roche (Alameda, CA, USA). This involves a pool of primers that give a ~165-bp amplicon from the L1 region of the same 13 high-risk HPV types as HCII. β-Globin DNA is included as a positive control. The test shows whether one of these 13 HPV types is present, but not which one. It shows promise as a screening tool, being sensitive, specific, feasible, and easy to handle (103). Its specificity (95%) was similar to colposcopy (96%) in one study, which found that it is comparable to other HPV tests that involve PCR, and to HCII, in detecting SIL in management of women with an abnormal Pap smear (103). Both Amplicor and HCII appear suited to routine screening (104). Just as an abnormal colposcopy and HSIL result, this HPV PCR test was regarded as a powerful independent predictor of HSIL, making it suitable as a replacement for cervical cytology in the management of women with an abnormal Pap smear (103).

Roche have also produced a linear array kit (LA-HPV) that permits HPV typing for 37 genotypes. This compares favourably with Roche's PGMY primer-based line blot assay, but with a higher detection rate (105). It is, however, expensive.

An earlier PCR test, the E6 E7 HPV Geno-Kit High Risk kit (Amplimedical SpA, Bioline Division, Turin, Italy) (106) has also been developed. Other commercial methods – the INNO-LiPA and Amplisense HPV typing – have been compared with GP5+/6+ PCR enzyme immunoassay-reverse line blotting (PCR-EIA-RLB), showing high agreement for HPV typing for single infections, but much less when multiple types were present (107).

There are numerous variations on the theme of HPV PCR. Which of these is preferable remains unresolved. A recent review suggested that PCR directed at the oncogenic E6/E7 region of the viral genome offers a number of advantages (108). Nevertheless, a PCR assay that amplifies the whole of the E6 region and the N-terminal part of E7 has been found to give results equivalent to the MY09/11 PCR test, which is directed at the L1 (''common'') region (109). Thus, the various approaches may be comparable overall. A testing algorithm that combines broad-spectrum and type-specific PCR can, moreover, be used to increase accuracy, especially for low-concentration HPV types in mixed infections (110).

The detection of HPV-related product after PCR can involve various techniques. Merely a difference in size of PCR products can be used, with one assay able to discriminate 15 high-risk HPV types after early region amplification (111). A recent approach is MassArray technology in which, after real-time competitive PCR and primer extension, matrix-assisted laser desorption/ionisation-time of flight (MALDI-TOF) mass spectrometry separation of products on a matrix-loaded silicon chip array is used to detect as few as several initial molecules (112). The Luminex xMAP system-based suspension array is another high-throughput method that was found to simultaneously discriminate 18 high-risk from 8 low-risk HPV types in L1 consensus PCR products (113). Another approach to typing of multiplexed HPV PCR products involves type-specific oligonucleotides coupled to fluorescently labelled polystyrene beads. This can rapidly and sensitively detect up to 100 HPV types simultaneously (114). There is also a recent one-tube multiplex PCR assay, directed at the E6 region, for detection of 25 HPV types (115). This Templex assay is a multiplex procedure in which the target is enriched initially, then SuperPrimers produce an excess of single-stranded reverse product, which is detected on the Luminex platform.

There have also been advances in DNA extraction procedures. An example is the automated Roche MagNA Pure LC method, which is superior to manual methods in HPV testing protocols (116).

In view of the important clinical information conveyed by HPV viral load (77), determination of the latter is desirable in any diagnostic procedure (117).

Incidence of HPV

Pooling of data from 11 studies in nine countries found HPV rates of 13.4% and 15.6% by the MY09/11 and GP5+/6+ methods of PCR, respectively (33). Values were 90.7% and 96.6% in those with cervical cancer. Incidence varies greatly between populations, depending on the extent of sexual exposure, number of partners, age of coitarche and the test used. In both Boston (118) and Portland (119), the HPV rate in cytologically normal women was 13% by MY09/11, but in HSIL was 81% and 72%, respectively. In relation to the sexual exposure issue, HPV incidence by PCR in female students at the University of California (Berkeley) was 46% (120), and, similarly, was 43% over 3 years in cervicovaginal lavage samples from college women in New York (27). In Antwerp, Belgium, 14% of women who attended their general practitioner for a Pap smear had HPV, compared with 34% in prostitutes who attended a sexually transmitted infection (STI) clinic (121). High rates are also seen in HIV-positive women (122).

HPV incidence varies with age. In Amsterdam, women aged 25–29 years had the highest rate (19.6%), dropping to 4.3% after age 30, with the reduction in high-risk HPV types, since low-risk types persisted (123). In Germany, the rate after age 30 was 6.4% (124). In France, rates varying from 7.3% (125) to 20% and 25% (by HCII and PCR, respectively) have been reported (126). Marital status affects the rate, HPV being twice as common in single than in married women in a Turin study, with a rate of 8.8% overall (127). Rates in Brescia by MY09/MY11 and hybrid capture were 6.6% (128). In Greece, 2.9% had HPV, compared with 1.7% who were Pap-positive; a normal smear and positive HPV test result were observed in 2.3% (129).

HPV incidence in South American countries is approximately 14% (130). In Argentina, the rate was 3% in women claiming no previous sexual activity and 18% in the sexually active (131). In Bogata, the rate was 14.8% (9% high-risk and 3% low-risk), being 26% for age <20, 2.3% in 45–54-year-olds, and 13% after age 55, with incidence peaking before 25 years and then declining to a minimum after age 65 (132).

The fact that HPV does not disappear completely in old age was indicated by autopsies of elderly women in Japan, which showed 5.4% had LSIL and 0.6% HSIL (133). In Hong Kong, HPV prevalence was 4.2% (134) and in central China was 5.9% in normal subjects, increasing through the various grades of SIL to over 90% in cervical cancer, with 79.6% having HPV16 (135). Interestingly, an Asian profile of HPV types was observed in Western Australia, where the rate of infection was 27% (136).

HPV is absent in virginal women, including those who use tampons or have had digital penetration (137).

Self-sampling

Although women are encouraged by governments and health authorities to undertake regular Pap smears, the procedure involved in collecting a sample requires the intervention of a medical practitioner. It causes discomfort, is embarrassing and inconvenient, and conflicts with the personal beliefs of some women. Even attending a doctor to discuss sexual matters can be confrontational. Non-participation in screening programmes is consequently higher among certain ethnic and religious subsections of the community (138, 139), as well as in women of poorer socioeconomic status and other women deemed at risk. Apart from this, the Pap smear result can be a false negative if the affected region of the cervix is missed (i.e., inadequate sampling) or the cytological interpretation is incorrect.

If a woman is infected with HPV, the viral DNA will be present in cells that are shed from the surface epithelia of the cervix and vagina. Thus, testing for HPV in such exfoliated cells is an alternative way of screening, as shown in a number of studies, with good results. In early work, collection of exfoliated cells involved cervicovaginal lavage (140–145). A lavage can be self-administered, but its value is limited due to patient compliance (142). Subsequent studies found that tampon samples also contained an adequate amount of exfoliated cells for HPV detection (146, 147). Such sampling can be carried out by the woman herself, since the skill required is how to insert a tampon, something that most women are experienced in. This can facilitate expanded screening of women and identify infected women without gynaecological intervention. It would enable participation by women from geographic localities lacking gynaecological services, as well as those who would otherwise be unable or unwilling to attend for a Pap smear. Even women who do attend for their regular Pap smear would likely prefer to take their own specimen, rather than visit their doctor. Self-collection increases uptake (148, 149) and could make up for loss of diagnostic accuracy, if any.

Results from tampons correlate well with results from cervical scrapes (147). A comparison of cervicovaginal lavage and vaginal tampons found a correlation of 88% (146). For vulvar or self-administered vaginal swabs and cervical scrapes, the correlation was 90% (120). In one study, HPV was detected significantly more often in tampons than in lavages (146). Sensitivity for tampon testing was 94% and specificity was 81% (146). Both cervicovaginal lavage and tampon specimens lend themselves well to PCR as the HPV detection method (145). Only 1% of samples were unsuitable for PCR, and only 12% required

DNA extraction for efficient amplification, as shown using the β-globin gene as a control (146). Tampons (147) and lavages (140) give a higher yield of cells and DNA than scrapes. Tampons and lavages do not sample the same site as a cervical scrape; rather, they collect mainly squamous epithelial cells from the walls of the vagina, together with shed cervical cells. This matters little, as genital HPV infection, if present, is likely to be widespread in the anogenital epithelium (120, 147) and the sample would, of course, include cervical cells. It should also avoid missing a restricted site of infection. The medical attendant's charge is also avoided if the woman carries out the collection herself.

A study in Melbourne found positivity for HPV by PCR was 73% for conventional cervical scrapes and 69% for specimens from tampons, which the women were asked to insert and immediately withdraw, then place in a sterile specimen jar to bring to the doctor (147). In a Canadian study, HPV was detected more in self-collected vaginal swab specimens (65%) than in swabs collected by physicians (53%), suggesting that the former are of greater value (150). In women from the Eastern USA, agreement between clinician- and self-collected Dacron swabs was 88%, making the latter a technically feasible alternative to collection of cervical cells by a clinician (151). Agreement was also observed in a Brazilian study that used a collection brush (152). A study in Australia of women living in remote areas found tampon samples tested by PCR were an acceptable and sensitive sampling modem for detection of various STIs (*Neisseria gonorrhoeae*, *Chlamydia trachomatis* and *Trichomonas vaginalis*), being superior to conventional methods (153). This should also apply to HPV.

In a meta-analysis of 12 studies involving self-collected samples (150), pooled data from six studies in which the subjects used Dacron or cotton swabs or cytobrushes for collection showed a sensitivity of 0.74 (95% CI 0.61–0.84) and a specificity of 0.88 (95% CI 0.83–0.92) for HPV testing compared to the reference standard of clinician-collected specimens for HPV testing. The diagnostic OR was 22 and area under the receiver operating characteristic curve was 0.91. The receiver operating characteristic curve is the most comprehensive method of assessing screening test performance. This is because it demonstrates the dynamic trade-off between sensitivity and specificity, since the threshold for designating a result as being ''positive'' is moved. With adjustment in trade-off between sensitivity and specificity, the operating point on the receiver-operating characteristic moves along the curve. For tampons, sensitivity ranged from 0.67 to 0.94 and specificity from 0.80 to 0.85. Not included in these figures was a very large study involving 1194 participants that had a specificity of 1.0 (no false positives) (154). In that study, sensitivity of cervical pre-cancer or cancer detection using self-obtained samples for HPV testing was 96%, compared to 79% by Pap smear. Including this study increased the diagnostic OR of the meta-analysis to 36. Seven of the studies that used PCR for HPV detection (142, 146, 147, 155–158) had sensitivity ranging from 0.63 to 1.00 and specificity spanning 0.80–1.00. Five tested for HPV by hybrid capture (154, 159–162), with sensitivity of 0.56–0.93 and specificity of 0.79–1.00. The authors of the meta-analysis concluded that self-collection may be an appropriate alternative for low-resource settings or patients reluctant to undergo pelvic examination (150). In our view, self-collection could become the main collection method.

Another potential sampling approach is urine self-collected as a dry paper smear for ease of transport, storage and direct HPV PCR testing. The results were not quite as good, only matching those from a cervical scrape (163).

As an extension of the concept of self-collection, we present in Figure 1 a protocol whereby a woman can purchase a tampon-based kit from a pharmacy or other suitable retail outlet, collect a specimen at her own convenience in private, then either mail this to the clinical pathology laboratory or place it into a suitable receptacle, such as a collection box at the pharmacy or supermarket, with samples collected by a courier and transported to the laboratory for HPV testing. The result would then be sent to her and/or a doctor she nominates on a form that accompanies the sampling kit.

Implication of vaccines for HPV testing

In the future it is hoped that HPV vaccination will eliminate cervical cancer. Since HPV is the most common STI not just in women, but also in adolescent girls [16% in one US study (164)] a mass prophylactic HPV vaccine should target those younger than 14–17 years, since some girls in this age range already have HSIL (165). Thus, vaccination should preferably take place prior to earliest sexual debut (166, 167) (i.e., be administered to children). This may meet parental

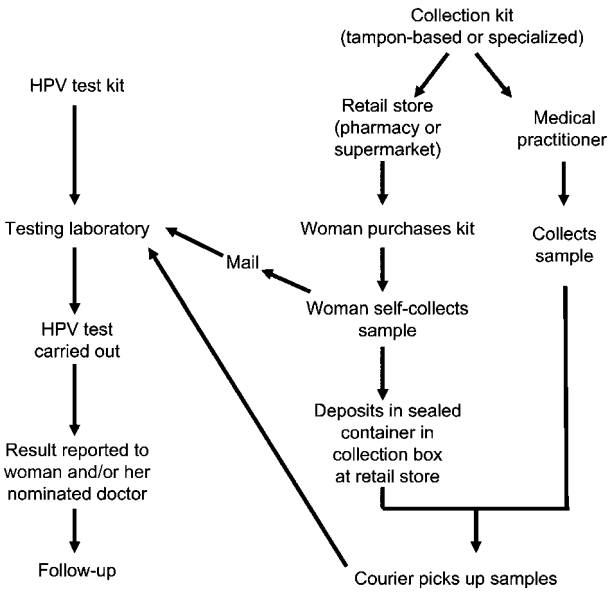

Figure 1 Flow diagram showing a possible cervical screening strategy that could be used to improve participation by women, enhance accuracy and reduce unnecessary clinical intervention.

resistance, however (168). It will, moreover, require, at least in the UK, education about the role of HPV in cervical cancer and the safety of vaccines (169). Vaccination will also need to be directed against every high-risk HPV type.

Although, theoretically, a vaccine to type 16 should prevent approximately 50% of infections, to types 16 and 18 combined, 71%, and to the seven most prevalent HPV types, 87% world-wide (170), one issue is the possibility of replacement, whereby the rarer HPV types not vaccinated against gradually replace the currently more common HPV types in the population. Moreover, concurrent infection by multiple HPV types occurs more often than by chance (171). There are nonetheless some data to indicate that prevention of HPV16 is not likely to promote the risk of infection with other types and that HPV types act as independent STIs (172). Different HPV types might also convey different levels of risk.

Vaccination of males may be crucial for inducing ''herd immunity'' (48, 167), especially as uptake by females will fall short of 100% (48). There are no clinical trial data, however, on the effectiveness of such male vaccination.

In mid-2006, Merck (Whitehouse Station, NJ, USA) released its Gardisal subunit-quadrivalent vaccine comprising virus-like DNA-free particles of self-assembled L1 protein of the high-risk HPV16 and 18 and the common low-risk types 6 and 11 that usually cause noticeable warts. Not far behind, Glaxo-SmithKline (Middlesex, UK) has developed a bivalent vaccine to types 16 and 18. Thus, there is still some way to go to achieve vaccination against all HPV types (166, 173). Even if this were to be effectively implemented, it will take many years for vaccination to clear high-risk genital HPV from the population (48). Thus, vaccines will have no impact on the need to screen for many years. The wide publicity given to a HPV vaccine could in fact increase cervical cancer rates because women may erroneously believe the vaccine to be therapeutic and thus forgo screening (174). Although HPV16 has traditionally been the most common HPV type in the general population (33, 170), new data from the US Centers for Disease Control, in which overall population prevalence of 23 high-risk HPV types in females aged 14 to 59 years was 15.2%, has shown that HPV16 (1.5%) was below the frequency of types 53, 52, 59, 66 and 61 (frequencies: 2.8%–1.6%); HPV18 was 0.8% (175). HPV16 is nevertheless more likely to persist to cancer, so the long-term implications of this new data for the current vaccination programme remain to be seen. Identification of a therapeutic vaccine to HPV is an important research area at present (48). This is, however, quite a challenging endeavour for many biological reasons (48) and early results have been disappointing (176).

Expert opinion

We have discussed the distinct advantages of HPV screening over conventional methods such as cytology. These include high test sensitivity, reliability, molecular testing based on detection of viral DNA, and the fact that HPV tests are direct, in that they determine the presence of the causative virus, rather than relying on an indirect and potentially subjective assessment of cytological appearance. HPV could be adopted as a stand-alone test, and, if positive, other tests such as $p16^{INK4a}$ or cytology could then be employed to increase specificity (48). HPV testing lends itself to protocols whereby women collect their own specimen. Such an approach should be welcomed by most women and therefore greatly improve participation in screening. As a result, we foresee the HPV route as a means of further reducing cervical cancer incidence, with little or no increase, and most likely a decrease, in overall costs of detection and treatment of women who test positive. The lower cost will be because (i) testing needs to be done less often, and (ii) of the ever-diminishing expense of reagents and equipment for PCR, which we believe will supersede older technologies based on DNA hybridisation. Moreover, with advances in automation of liquid handling, DNA extraction, real-time PCR and its miniaturisation, computer technology, and overall speed and complete enclosure of the process as a ''sample in one end, result out the other end'' set-up, we are moving towards a ''one-hour photo'' model of viral diagnostics.

Outlook

The well-recognised reliability of HPV testing contrasts with the notorious unreliability of the Pap smear. Now that core PCR technology is off patent, as will be the original application patent for use of PCR in HPV testing in 2008, we envisage an enormous expansion in technology for better HPV tests in the lucrative arena of cervical screening. Speed, accuracy, reliability, familiarity, acceptability, suitability for self-collection of samples, and an expected continual decline in costs in this highly competitive market for clinical diagnostic testing point to HPV becoming the primary means of screening within a few years. Already HPV testing is approved and reimbursed by medical insurance and/or governments for women over 35 years of age and for follow-up of those who have been treated for cervical abnormalities. This is the thin edge of the wedge that is now set to expand to direct primary testing of all women.

Highlights

HPV testing of self-collected specimens is the way of the future. This is because of:

- Accuracy and reliability of DNA testing by methods such as PCR;
- The longer interval between tests that this implies;
- Applicability of HPV testing to DNA in cells from samples collected for liquid-based cytology or dislodged from a tampon used by the woman herself and mailed to the testing laboratory;

- Convenience and ease of sample collection by the woman herself or her medical practitioner;
- Scope for automation, speed, lower cost, and communication of results electronically; and
- Suitability to the lifestyle and busy schedule of the modern woman.

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