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THE HEALTH PROFESSIONAL'S HPV HANDBOOK 2

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HPV HANDBOOK

2: CURRENT EVIDENCE-BASED APPLICATIONS

Editors-in-Chief
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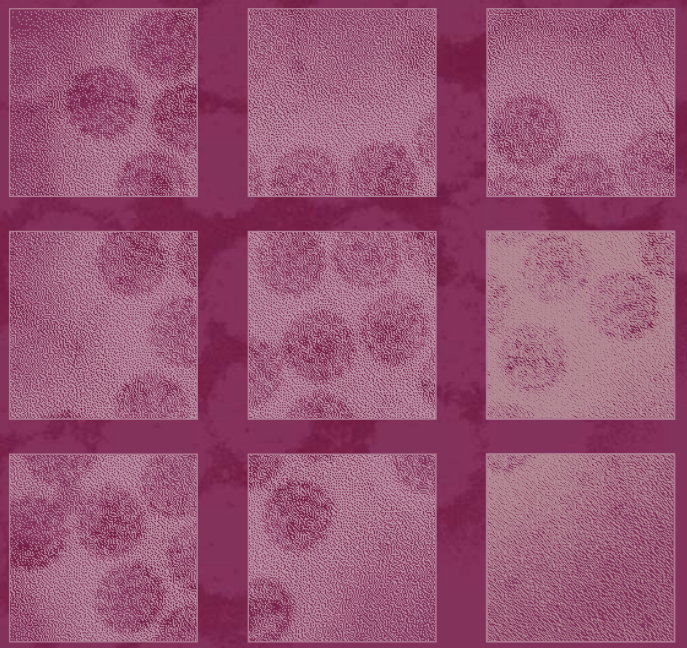

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Contents

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Introduction to the HPV Handbook series

These compact, illustrated handbooks are concise but comprehensive resources that introduce medical students, general medical practitioners and gynaecologists to the significance of the human papillomaviruses in the etiology of cervical cancer. All chapters are fully referenced and written by experts in the field.

Handbook 1: *Human Papillomavirus and Cervical Cancer*, introduces the human papillomaviruses that are responsible for genital warts or cervical cancer. The chapters review virus structure, the epidemiology of HPV, the latest advances in HPV vaccination and new markers for cervical disease.

Handbook 2: *Current Evidence-based Applications*, describes the implications of implementing HPV testing for the management of women with various degrees of dysplasia, and discusses HPV testing for post-treatment follow-up. It also provides an overview of the current status of HPV testing as a tool for cervical cancer screening.

Handbook 3: *HPV and Cervical Cancer*: *Public Health Perspectives*, examines the benefits and drawbacks of cervical cytology and HPV testing as part of an organized screening programme to prevent cervical cancer.

1. Human papillomavirus testing for the management of equivocal cytology (ASCUS)

K Ulrich Petry

- Pap smear screening to detect all true precursor lesions has limited sensitivity
- The management of mild to moderate lesions is particularly challenging

The equivocal Pap smear – a major clinical and economical challenge for cervical cancer screening programmes

The incidence of cervical cancer in most industrialized countries has declined since the initiation of screening programmes based on the Pap smear. Although anticipated as a successful strategy, in general, the concept of preventing cancer by detection and treatment of preinvasive lesions in the general population has been proven to be sufficient and cost-effective only in the case of prevention of invasive carcinoma of the uterine cervix. Optimal screening programmes for other diseases (e.g. HIV screening of blood donors) detect or exclude disease with specificity and sensitivity rates close to 100%. Furthermore, this success is achieved with almost all test results being either clearly positive or negative; e.g. for HIV screening, the frequency of equivocal test results is 0.1–0.01%. To be an efficient method of cancer prevention, cytology should ideally detect all true precursor lesions of cervical cancer with an accuracy that is as high as that for screening blood donors for HIV antibodies. However, this expectation has not been fulfilled for at least three reasons (see box).

Limitations of cytology in screening and cancer prevention

- The accuracy of cytology is far from perfect
- A lack of consensus on what constitutes a true precursor of cancer
- The number of equivocal Pap smear results by far exceeds the number of women with clinically relevant disease

The imperfect accuracy of cytology

The definitive diagnosis of cervical neoplasia depends on the histological identification of neoplastic cells in tissue sections, whereas cytology uses the degree of dyskaryosis in single cells or clumps of cells recovered from the cervix to interpret the grade of the underlying neoplastic lesion. Even under the best conditions, this methodological difference must result in disagreement between the cytological diagnoses of cervical neoplasia and the histological confirmation of disease. Furthermore, in routine practice the performance of the Pap smear test is flawed by the inherent sampling and interpretation errors that are associated with cytology as a diagnostic method. Menstruation, ovulation, inflammation and pregnancy are merely a few of many more frequent conditions that regularly influence Pap smear screening results, even in the absence of technical sampling or interpretation errors. All these factors contribute to a reduction in the accuracy of Pap smear screening, by increasing the likelihood of false-positive as well as false-negative smears, and by increasing the number of equivocal results.

No consensus on what constitutes a true precursor of cancer

The role of cervical intraepithelial neoplasia grade III (CIN 3) as a true cancer precursor with a confirmed high risk of progression to invasive disease is undisputed.[1,2] Surgical treatment of CIN 3 is an accepted standard of care, and conization and loop electric excisional procedure (LEEP) are the most common procedures. In contrast, there is significant controversy concerning the management of borderline atypia, CIN 1 and even CIN 2 lesions. It is well known that the majority of CIN 1/2 cases will regress spontaneously, with only a minority progressing to CIN 3 or invasive cancer.[3] Borderline atypia, mild and moderate dysplasia are common in young women of reproductive age and, as incidence rates are rising in many countries, conservative management of such lesions could help to reduce the significant costs of surgery and avoid the complications associated with invasive treatment of the uterine cervix.[4] While this would appear to be an

attractive option, it has been established that a significant proportion of women with a cytological diagnosis of mild or moderate dysplasia have underlying disease of a more severe nature. Such women, should they be followed with cytology alone, would have a 16- to 47-fold increased risk of developing invasive cervical cancer.[5] To conclude, there is little agreement on how severe a cellular atypia has to be in order to justify a definition as precursor, and even less consensus on the appropriate management of borderline atypia, as well as mild and even moderate dysplasia.

Equivocal Pap smears greatly exceed the number of women with clinically relevant disease

Between 6% and 7% of the estimated 50 million Pap smears taken each year in the US are reported as abnormal. However, before the ALTS-trial results were published in 2000 and 2001, there was no agreement on the appropriate management of the approximately 3 million women with equivocal cytological abnormalities (ASCUS – atypical squamous cells of undetermined significance; and LSIL – low-grade squamous intraepithelial lesions). In the US, $2.5 billion per year is spent on the management of ASCUS, which exceeds the cost of treating all women with high-grade neoplasia. Although there are no equivalent data from some countries, it is reasonable to assume that the situation in most member states of the European Community is similar.

Importantly, participants in screening programmes expect results either to exclude disease by normal findings or to prove the presence of disease by atypical findings. Equivocal results are not readily understood and are often perceived as cancer until proven otherwise. Equivocal Pap smears can therefore cause severe psychological stress to the women affected, in the absence of appropriate counselling and management strategies, and incur significant costs.

Management of equivocal Pap smears

Any effective management strategy of equivocal cytology needs to address the heterogeneity of epithelial changes that may result in borderline atypia; the high rate of spontaneous remission of these minor cellular changes; and, finally, the increased risk of underlying high-grade neoplasia that accompanies this result. Kinney *et al*., 1998, demonstrated that the majority of histologically confirmed high-grade neoplasias were not diagnosed as high-grade lesions on cytology.[6] Instead, approximately two-thirds of high-grade neoplasia cases were classified either as ASCUS or as LSIL on cytology. Other investigators confirmed that an equivocal result corresponds to a greater-than-background risk for incident or prevalent invasive cervical carcinoma.

For the attending clinician, the problem associated with equivocal cytology is to efficiently identify the minority of women with either prevalent CIN 3 or cancer, or a high risk of developing them during follow-up. Therefore, to be clinically useful, any management strategy for equivocal Pap smears must be able to assist with the identification of women with underlying disease, whilst not unduly increasing either patient stress or patient management costs. The currently available options are repeat Pap smear, immediate colposcopy or HPV testing. Other options are under investigation and these include microsatellite instability, $p16^{INK4A}$ and telomerase immunostaining.

Repeat Pap smear

Theoretically, equivocal Pap smears without neoplasia should return to normal cytology on follow-up, while those with underlying high-grade neoplasia should result either in persisting, equivocal or obviously atypical smears, when controlled by repeat cytology 3–6 months after the initial Pap smear. If this were true in practice, repeat cytology would be an inexpensive and attractive option for secondary screening, but most studies have reported high rates of false-positive and false-negative results, together with persisting equivocal results for women without colposcopically identifiable neoplasia.[7] Several

studies have examined the performance of repeat cytology for the follow-up of women with equivocal Pap smears, and the reported sensitivity rates for the detection of underlying clinically relevant disease were in the range 60–85%, with specificities ranging from 77% to 96%[8–10] (Table 1).

Table 1 Performance of repeat Pap smear testing in triaging equivocal smears

Trial	Sensitivity (%)	Specificity (%)	HSIL (CIN 3) prevalence[a] (%)	Referral to colposcopy (%)
Cox 1995[8]	60 (38)[b]	77 (96)[b]	6.7	31 (12)
Manos 1999[9]	76.2	–	6.7	38.9
Solomon 2001[10]	85 (59.2)	–	11.4 (5.1)	58.6

[a]Calculations based on equivocal (low-grade) smears as positive
[b]Disease defined as any grade of intraepithelial neoplasia

To achieve a reduction of equivocal cytology findings, one option is the implementation of stricter definitions of borderline atypia that reclassify very mild cellular changes in the 'normal' category. However, as high-grade disease may underlie even minimal cellular changes, such an approach would increase the overall false-negative rate of cytology within the mass screening programme. The magnitude of the reduction of equivocal cytology results, as a consequence of stricter definitions, would correlate with the increase of false-negative smears.[11]

Colposcopy

Colposcopy with histological assessment by directed punch biopsies is the gold standard procedure for the evaluation of cytological diagnoses of high-grade neoplasia, and for the further investigation of persistent LSIL in North America and some, but not all member states of the European Community.[12] Although colposcopy was developed in Germany by Hans Hinselmann, in 1925, *diagnostic* cold-knife conization, rather than colposcopy with guided biopsies, is the standard for histological assessment of atypical Pap smears in

Germany and some of its neighbouring German-speaking countries. However, this is an inpatient procedure with significant morbidity that cannot be justified for the follow-up of equivocal cytology. Given the high number of equivocal Pap smears, combined with the small number of women who will have underlying clinically relevant lesions, the direct and indirect costs of immediate colposcopy with histological evaluation are too high to recommend it as the best diagnostic option for equivocal Pap smear results.

To reduce the number of referrals to colposcopy among women with equivocal smears, a second test (or possible further tests) should be used to identify women with underlying high-grade neoplasia. The process of repeat screening, within an already screened group of individuals, is defined either as secondary screening or triaging.

Methods that have been used and evaluated in the triage of equivocal cytology include cervicography, repeat Pap smear test, human papillomavirus (HPV) testing, HPV DNA integration, aneuploidy measurement, polar probe, immunohistochemistry to detect p16 or other potential markers of cervical neoplasia.

Cervicography, visual inspection aided by acetic acid (VIA)

Cervicography apparatus consists of a 35mm camera body, with a 50mm extension ring and a 100mm macro lens, together with a ring strobe light. After visualization of the cervix with a self-retaining speculum, the cervix is cleaned and moistened with acetic acid. Photographs are taken before and after application of acetic acid, and the cervicograms sent to an expert for evaluation.

Several studies have investigated cervicography as a method of primary or secondary screening. Its sensitivity for high-grade cervical neoplasia has been found to be generally high, but its specificity too low to recommend its use in already established primary screening programmes. However, studies evaluating naked-eye examinations of the cervix after acetic acid application, in previously unscreened populations in Africa and Asia, concluded that this method was a

feasible and adequate means of preventing cervical cancer in Third World countries with a high prevalence of this malignancy.

In countries within the European Community, cervicography has a very limited role, although it may have potential application in triaging young women (20–30 years of age) with equivocal smears.

HPV DNA testing

HPVs are the causative agent of virtually all cervical cancers. Genital HPV types are very common, with prevalence rates of up to 30% among 20–25-year-old women in some populations, although the majority of these infections are transient and resolve spontaneously within 2 years. Multiparity, long-term hormonal contraception, malfunction of the cellular immune response, and other co-factors that have yet to be identified may result in the persistence of HPV. Only persistent HPV infections are capable of inducing the transforming steps within the host cell that may finally lead to carcinogenesis (Figure 1).

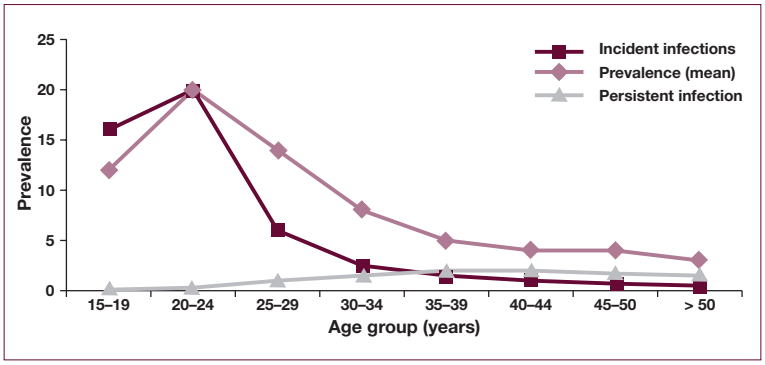

Figure 1 Age distribution of HPV infection

HPV infection is a unique risk marker; its absence virtually excludes any risk of developing high-grade pre-invasive or invasive cervical neoplasia because high-risk (HR) HPV-negative individuals lack the causative disease agent. As such, HR HPV testing has been proposed

for the triage of women with equivocal cytology, on the basis that those who test negative are at virtually no risk of having underlying clinically relevant lesions (and could therefore be returned to the routine screening pool), whereas those testing positive could be referred to colposcopy for further follow-up. However, a single HPV test cannot distinguish between transient and persistent infection, and even the persistence of HR HPV infection is not proof of underlying high-grade neoplasia. Therefore, HPV testing is a promising risk indicator within primary screening programmes, especially of low-risk populations, while its role in the triage of LSIL and equivocal Pap smears is limited, although its usefulnesss in some indications has been confirmed in several trials.

A number of trials have compared the efficacy of HPV testing and repeat Pap smears for the triage of women with equivocal smears (Table 2).

Table 2 Performance of HPV testing in triaging equivocal smears

Trial	Sensitivity (CIN 3) (%)	Specificity (%)	HSIL (CIN 3) prevalence (%)	Referral to colposcopy (%)
Cox 1995[8]	93.3	71.3	6.7	37.4
Manos 1999[9]	89.2	–	6.7	39.5
Solomon 2001[10]	95.9 (96.3)	–	11.4 (5.1)	56.1
Petry 2003[11]	100	90.9	1.8	10.8
Lin 2003[13]	100	74.4	36.5	52.7

The ASCUS/LSIL Triage Study (ALTS) was a multicentre, randomized trial that compared three different management strategies for the detection of underlying CIN 3 among 3488 women with a diagnosis of ASCUS:

(1) Immediate colposcopy

(2) Triaging for colposcopy based on HPV testing (hc2) and thin layer cytology

(3) Triaging for colposcopy based on repeat cytology alone

The highest number of cases of underlying CIN 3 ($n = 77$) was detected in the HR HPV testing arm, followed by 59 cases in the immediate colposcopy arm and 44 cases among women with conservative (repeat Pap smear) management. Compared with immediate colposcopy, triaging based on HPV testing reduced the number of referrals to colposcopy by 44%. Overall, the sensitivity of HR HPV testing for CIN 3 was 96.3%, while repeat Pap smears showed a sensitivity of 44.1% for HSIL+; 64.0% for LSIL; and 85.3% for ASCUS+ cytology.[10]

On the basis of the ALTS-trial findings, and of the meta-analysis of a further five published trials on the management of women with ASCUS, the ASCCP Consensus Conference 2001 in Bethesda, USA, recommended that 'A program of repeat cervical cytological testing, colposcopy or DNA testing for HR HPV types are all acceptable methods for managing women with ASC-US (rating A1). When liquid-based cytology is used or when co-collection for HPV DNA testing can be done, reflex HPV DNA testing is the preferred approach'.[12]

Another important recommendation of the 2001 Bethesda meeting was the revision of the ASCUS classification that was divided in ASC-US and ASC-H = atypical squamous cells, high-grade lesion cannot be ruled out. The consensus recommendation for ASC-H was immediate colposcopy.

It is important to note that the conclusions of the ALTS trial and the recommendations of the ASCCP were based on a US interpretation of

ASCUS and a US cost structure. While ALTS may demonstrate what can be achieved, the results should be interpreted in the context of the cytological practice and cost structures pertaining to each individual European state, as these are likely to differ from the US. The efficacy of HPV testing depends on several factors that will vary between different health systems. The 44% reduction in the number of referrals to colposcopy reported in the ALTS-trial represents a cost saving in the US health system, with its high cost of colposcopy, but a larger reduction may be needed in most European countries, to compensate for the cost of HPV testing.

However, in a primary screening trial in Hannover and Tübingen, in Germany (the HAT-trial), 167 of 8101 (2.1%) participants had equivocal (Pap IIw) smears. This figure is far lower than that reported in the ALTS trial, and it is likely that the Pap IIw category is not a direct correlate of ASCUS. This view is supported by the low prevalence of HR HPV in the Pap IIw category – only 10.8%, compared with 56% in the ASCUS category in the ALTS trial. In this study, no cases were observed among 149 (89.2%) women with equivocal smears and a negative HPV test result, and all women with underlying CIN 2/3 were HR HPV-positive.[11] Importantly, these findings indicate that over 75% of the women in Germany with a Pap IIw result would be HR HPV-negative, and could therefore be returned to routine screening without further follow-up. While the economic calculations have yet to be completed, it is likely that HPV testing would be able to provide a positive cost benefit within the German healthcare system, whilst providing a substantial degree of reassurance for the large majority of women with an equivocal Pap smear.

It is important to note that the HAT trial was restricted to women over the age of 30 years, which is one probable reason for the lower prevalence of HR HPV, in comparison with the ALTS trial, another reason, among others, being the differences in the classification of Pap IIw versus ASCUS smears. However, regardless of the prevalence of HPV in the respective populations, the negative predictive value of HPV testing in both studies was virtually identical, and this characteristic of HPV testing appears to be independent of subject age.

Therefore, while the cost benefits achieved by the healthcare system might vary by age group, the protective effect and the clinical interpretation of a negative HR HPV test will remain the same for all age groups.

Immunohistochemistry and other molecular diagnostic methods

Several other techniques that may provide more accurate cellular indicators of high-grade neoplasia than HPV DNA testing are currently under evaluation. The ability of HR HPV types to cause neoplasia is mainly determined by the activity of their transforming E6 and E7 proteins. Expression of E6/E7 mRNA varies with the severity of the lesion and is higher in high-grade lesions than low-grade lesions.[14]

The process of HPV-induced cell transformation results in overexpression of the p16^{INK4A} protein. Immunohistochemistry staining for p16^{INK4A} in cervical histology slides stained neoplastic cells reliably, allowed a more accurate grading of intraepithelial neoplasia than the conventional hematoxylin-eosin staining, and reduced inter- and intra-observer variability among pathologists. To date, only a few investigations have investigated the use of p16^{INK4A} to identify individual dyskaryotic cells in conventional Pap smears or liquid cytology, but all high-grade lesions were detected with this method.[15,16]

Mini-chromosome maintenance proteins (MCMs) are required to initiate cell division. As high-grade CIN cells never leave the mitotic cycle, MCMs are abundant and found in all cellular compartments in neoplastic epithelium. Antibodies to MCMs showed a high degree of sensitivity and specificity for the detection of CIN in Pap smears.[17,18] Although promising, these preliminary results need to be confirmed by larger studies.

Conclusions

At present, there are three evidence-based options for the management of equivocal Pap smears: conservative follow-up by repeat Pap smears, immediate colposcopy or DNA testing for HR HPV. Management by repeat cytology, although an accepted standard in most European countries, has been demonstrated to have suboptimal sensitivity for the detection of underlying clinically relevant disease. As such, cytology for the follow-up of equivocal Pap smears must be repeated at least every 6 months, and the patient referred to colposcopy for further investigation if the equivocal cytology persists for more than 12 months after the first Pap smear, or otherwise immediately if the cytological diagnosis worsens. If the repeat cytology option is selected, the clinician needs to consider three factors:

(1) The level of anxiety experienced by the patient. For a particularly anxious patient, the prolonged uncertainty is likely to prove unnecessarily stressful, and either HPV testing or immediate colposcopy may be a more attractive option for her, even if she is required to pay for these procedures herself.

(2) The likelihood of loss to follow-up. If the clinician judges that the patient is unlikely to return for follow-up cytology, he or she is ethically obliged to recommend a more definitive diagnostic procedure, such as colposcopy, with the option of immediate treatment ('see-and-treat') for any lesions judged to be clinically important.

(3) The cost and inconvenience to the patient, including both the time required to attend the clinic and the indirect costs, such as loss of salary for absences from work, the costs of child care, etc.

Immediate colposcopy with directed biopsy of any clinically suspicious lesions is a safe option, with respect to the detection of any underlying clinically relevant disease. However, it is likely to be the most expensive option for the healthcare system. It is also associated with an increased possibility of overtreatment, and the accompanying

likelihood of increased morbidity for the patient. Following the 2001 Consensus Guidelines, immediate colposcopy is required for all equivocal smears that are classified as ASC-H, or where other risk factors are present (Figure 2).

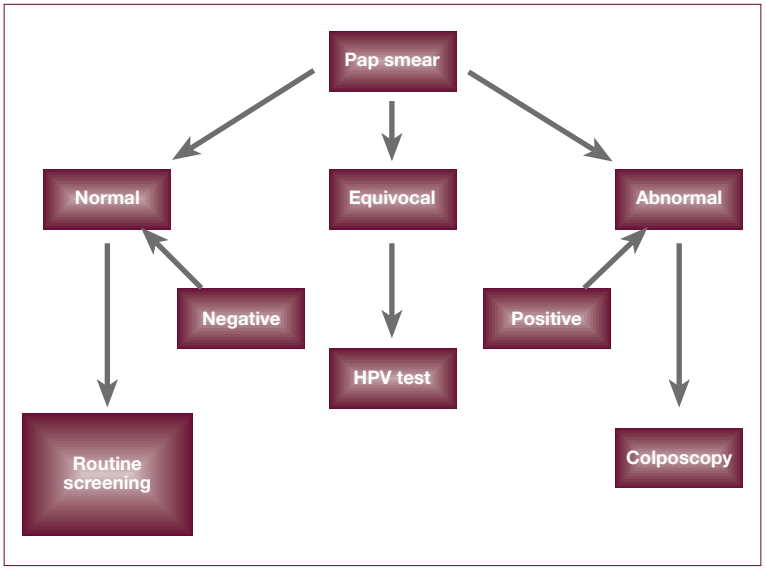

Figure 2 Concept of triaging equivocal cytology results based on HPV testing; equivocal smears that are HPV-positive are treated as abnormal

HPV testing, as currently practiced, is most useful for distinguishing which women with equivocal cytology are at virtually no risk of having underlying disease. In the ALTS trial, approximately half of the women with ASCUS were HR HPV-negative, and could have been safely returned to the routine screening pool with no further follow-up, and with the reassurance that they had no clinically relevant cervical dysplasia. In the US, this would have provided a cost saving for the healthcare system, together with a substantial degree of reassurance, as well as a cost saving for the women involved. While the cost-benefit analyses should be repeated for European healthcare systems, the

reassurance that accompanies a negative HR HPV test will remain valid, and apply to women of any age group. Likewise, the clinician needs to consider three factors:

(1) Although a negative HPV test excludes almost any risk of high-grade cervical neoplasia in all age groups, the performance of HPV DNA testing is flawed by the high rate of transient HPV infections among young women (18–29 years). Repeat cytology might be the better choice in order to follow up borderline cytology in the youngest age groups (<25 years). If HPV testing is used to triage newly diagnosed equivocal smears before the age of 30 years, a repeat HPV test, 6–12 months after the initial test, is recommended. Only where there is persisting detection of DNA of HR HPV types should women be transferred for colposcopy (Figure 3).

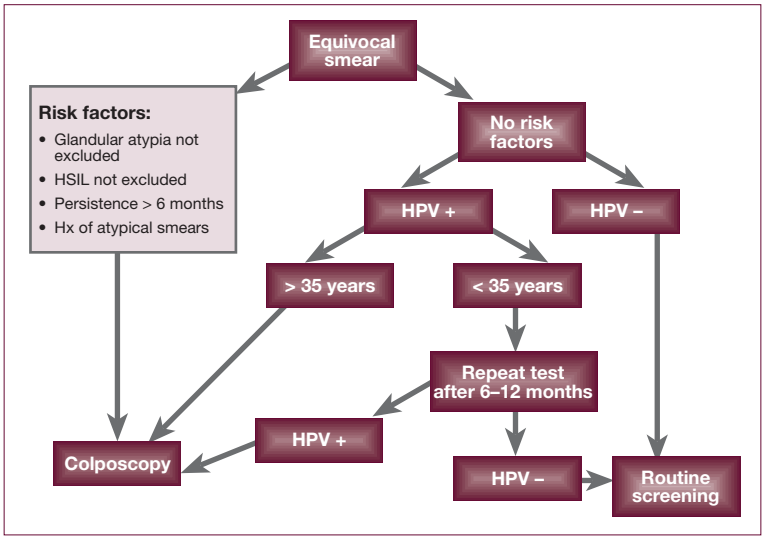

Figure 3 Recommended management of equivocal smears

(2) Following the flow chart in Figure 3, the level of anxiety and the risk of loss to follow-up will be reduced among HR HPV-negative women of all age groups, and among HR HPV-positive patients who are 29 years of age and above. However, anxious HPV-positive women in the 18–29 years age-group may suffer from the prolonged observation period. Immediate colposcopy will be preferable for some of these women.

(3) Testing positive for HR HPV may alarm women, even if the colposcopical evaluation shows no evidence of disease. Among such women, the risk of overlooked disease and incident high-grade disease is low (approximately 5% within 3 years). A follow-up based on cytology and HPV testing should eliminate almost any risk of developing invasive disease, and detailed counselling should transform unjustified anxiety into compliance.

Summary

- HPV testing can determine which women with equivocal cytology have virtually no risk of underlying disease
- In the absence of risk factors, referral of all women with equivocal Pap smears for colposcopy with histological assessment appears to be neither cost-effective nor necessary
- When liquid-based cytology or co-collection for HPV DNA testing is used, reflex HPV testing is preferred over triage of women with equivocal smears for colposcopy
  - Among women ≥30 years of age, HPV DNA testing is superior to repeat cytology in identifying the minority of high-grade cervical neoplasia cases among women with ASCUS, borderline atypia or related categories of equivocal Pap smears
- Although a negative HPV test excludes almost any risk of high-grade cervical neoplasia in all age groups, the utility of HPV DNA testing is limited in young women (18–30 years of age)
  - Repeat cytology may be the better choice of test, in order to follow up borderline cytology results in the youngest age groups
  - If HPV testing is used to triage newly diagnosed equivocal smears in women <30 years of age, we recommend a repeat HPV test, 6–12 months after the initial test
  - Only where there is persisting detection of DNA of HR HPV types should women be transferred for colposcopy

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2. HPV testing for the management of women with atypical glandular cells (AGC)

Walter Prendiville

- Atypical glandular cells (AGC) hidden in the endocervical canal may be precursors for adenocarcinoma *in situ* and invasive adenocarcinoma
- An AGC diagnosis is less common than that of atypical squamous cells of undetermined significance but carries a higher risk of carcinoma
- Detection of AGC during cervical screening is problematic
  - The clinical significance of AGC remains poorly understood and patient management has not been standardized
  - The accurate recognition and classification of AGC by cytology remains difficult
- The role of human papillomavirus DNA testing in the management of women with AGC is under active investigation

Introduction

While the cytological criteria for adenocarcinoma *in situ* (ACIS) are relatively well described, the broad spectrum of other benign and dysplastic changes remains ill-defined. To address this issue, the term 'atypical glandular cells of undetermined significance' (AGUS) was introduced at the 1988 Bethesda Conference.[1] In 2001, this term was revised to 'atypical glandular cells' (AGC), in order to clearly distinguish this condition from ASCUS (atypical squamous cells of undetermined significance). The Bethesda terminology for subclassification is shown in the box below.

Bethesda terminology

- **1988: AGUS** – 'atypical glandular cells of undetermined significance'. Morphological changes that are suggestive of the benign reactive process but insufficient for the diagnosis of ACIS
- Endocervical
- Endometrial or extrauterine adenocarcinoma
- Adenocarcinoma, not otherwise specified (NOS)

2001: AGC – 'atypical glandular cells'

- NOS
  - Endocervical
  - Endometrial
  - Glandular
- Favor neoplasia
  - Endocervical
  - Endometrial
- Endocervical ACIS
- Adenocarcinoma

The reported frequency of AGC varies from 0.11 to 2.5%.[2–7] Many countries are experiencing a trend of increased incidence, which may be a consequence of an increase in the prevalence of human papillomavirus (HPV) infection, or of improvements in screening, or of the growing awareness of AGC as a possible diagnosis.[8] In addition, the introduction of new sampling devices has significantly improved the detection of AGC lesions.

The incidence of an associated premalignant or malignant condition in women with a diagnosis of AGC NOS is 9–41%, and 96% in women with AGC favor neoplasia.[1,9] Although screening based on cytology improves the likelihood of the disease being detected at an early stage,[10,11] diagnosis remains problematic. Difficulties in diagnosis are mainly due to factors that affect cell morphology (see box).

Issues in diagnosis

- Sampling of normal cells high in the endocervical canal could result in cells showing morphology that could be confused with an AGC diagnosis
  - Nuclear pleomorphism, nuclear crowding
  - Architectural effects; palisading, feathering and rosette formation
- Intrauterine devices may affect cell appearance
- The clean 'punched out' appearance of large vacuolated cells helps discriminate these from malignant vacuolated cells

Retrospective studies of slides obtained from women who developed ACIS or adenocarcinoma have demonstrated that the sensitivity of cervical smears for AGC remains relatively low – between 45% and 72%.[12–14] This was demonstrated by the finding that the organized screening programme in The Netherlands has been less successful in reducing mortality for AGC than for squamous lesions; after adjustment for age, stage and lymph node involvement, the relative

risk of death was 1.6 times higher (95% CI: 1.2–2.1) for patients with adenocarcinomas than for patients with squamous cell carcinoma.

HPV testing for AGC

The development of highly sensitive molecular methods for the detection of high-risk (HR) HPV represents a considerable advance in the evaluation and treatment of patients with ASCUS, and has greatly aided the identification of squamous intraepithelial lesions. The majority of lesions resulting in an AGC diagnosis are of squamous origin (with or without glandular involvement), and HR HPV has been associated with the development of adenosquamous tumours and with mucinous adenocarcinomas.[15] Only rare histological variants of cervical adenocarcinoma appear to be unrelated to HPV infection.[15] HR HPV testing may therefore prove useful in the management of women with AGC.

There is supporting evidence for this view from a population-based, case–control study in 150 women with ACIS and 650 controls, in which the presence of HPV DNA type 16 and 18 was demonstrated in 87% of ACIS archival samples, using an assay for virus-like particles.[16] It was reported that 46.5% of samples were positive for type 18 alone, and 15.5% for type 18 in combination with types 16 or 72. The age-adjusted relative risk of ACIS associated with HPV 18-positivity was 3.3 (95% CI: 2.2–4.9); no increased risk was associated with HPV 16-positivity (the type most commonly associated with squamous lesions), perhaps indicating possible differences in etiology between squamous lesions and adenocarcinomas.[16] The figures for HPV prevalence in this study may be an underestimate, due to the limitations of the capture enzyme-linked immunosorbant assay (ELISA) for testing potentially degraded archival material. Long-term oral contraceptive use was also implicated as a risk factor, in addition to HPV infection, in this study.[16]

The sensitivity of HPV testing to identify adenocarcinoma has been confirmed in a review of 137 Pap smears, in which HPV testing successfully identified all the five women known to have developed ACIS.[17]

Since HPV is more commonly found in association with endocervical rather than endometrial lesions, HPV testing can also be used to confirm the site of origin. For example, when the hybrid capture DNA test (designed to detect the 13 most common HR types) was used in a study, 39 (78%) endocervical adenocarcinomas contained HR HPV DNA, compared with only one (2.0%) endometrial adenocarcinoma.[18] This ability to distinguish HR HPV types may prove useful in women already identified as having AGC favor neoplasia, and who therefore have a high risk of malignancy.

In order to determine what proportion of adenocarcinomas are caused by infection with HR HPV types, a systematic analysis was performed on specimens derived from cases of ACIS and adenocarcinoma.[19] In this comprehensive study, archival formalin-fixed specimens of indisputable ACIS ($n=65$) and adenocarcinoma ($n=77$) of the cervix were tested for HR HPV DNA by GP5+/6+ PCR enzyme immunoassay (EIA) and type-specific E7 PCR for 14 HR HPV types. All of the ACIS samples and 94% of the adenocarcinoma samples were positive, supporting the rationale for HPV testing in these disorders.

Further studies have demonstrated that the most prevalent HR HPV genotype in adenocarcinoma is type 18, followed by type 16 and 45.[19,20–23] This finding contrasts with the pattern observed in squamous lesions, in which type 16 is the most prevalent HPV genotype, and perhaps indicates that differing mechanisms of carcinogenesis occur in glandular versus squamous cells. The prevalence of HR HPV combined with specific $p16^{INK4A}$ (a potential marker of HR HPV pathogenic activity) expression points to HR HPV as the main causative agent for ACIS and adenocarcinoma of the cervix.[19,24]

Conclusions

- Use of improved cytological criteria and HPV DNA testing may permit improved management of women with AGC
- HPV testing may prove beneficial in women identified as having AGC favor neoplasia (and who therefore have a high risk of malignancy), in order to ascertain whether abnormal cells are of endocervical or endometrial origin
- Since ACIS and nearly all cervical adenocarcinomas are HR HPV-positive, the incorporation of HR HPV testing in cervical cancer screening programmes is likely to decrease the incidence of cervical adenocarcinoma markedly

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3. HPV testing for the management of low-grade squamous intraepithelial lesion

Heather Evans and Patrick G Walker

- Management of women with a diagnosis of low-grade squamous intraepithelial lesion (LSIL) is problematic because of the difficulty in distinguishing between lesions that will progress and those that will spontaneously regress
- The English cervical screening programme revised guidelines suggest that, ideally, women should be referred for colposcopy after the first positive test for mild dyskaryosis, but it remains acceptable to recommend a repeat test. Women must be referred after two positive tests for mild dyskaryosis
- Alternative, less expensive but equally effective management strategies continue to be evaluated
- HPV testing for triage of women with LSIL is not considered to be a viable strategy

Introduction

In the UK, 4.5 million Pap smears are taken each year, of which 2.4% (108,000 per year) are diagnosed as mild dyskaryosis.[1] The relationship between cytology and histology is shown in Table 1. A cytological diagnosis of low-grade squamous intraepithelial lesion (LSIL) may be accompanied by a wide range of disease states, including: no abnormality; koilocytosis (benign cellular changes after human papillomavirus [HPV] infection); underlying LSIL; and HSIL (high-grade squamous intraepithelial lesion).

Table 1 Relationship between cytology and histology nomenclature

Old terminology[2]	Histology	Histological grade[3]	Cytology[4]
Mild dysplasia	CIN 1	L-CIN	LSIL
Moderate or severe dysplasia	CIN 2 / CIN 3	H-CIN	HSIL

CIN: Cervical intraepithelial neoplasia; L: Low-grade; H: High-grade; SIL: Squamous intra-epithelial lesion

The importance of follow-up is emphasized by a study that showed that 33% of women with mild dyskaryosis will subsequently show cervical intraepithelial neoplasia (CIN) 2/3 histology after a single, careful repeat smear.[5] A repeat smear protocol correctly identified 82% of all CIN lesions and 93% of CIN 2 and 3 lesions, and the overall false-negative rate was 24%, although most missed lesions were low grade. To ensure safe practice for women who have a mildly dyskaryotic smear followed by a negative smear, the authors of this study recommended further repeat cytology.

Current recommendations for the use of HPV testing

Until recently, the UK National Health Service Cervical Screening Programme (NHSCSP) recommendations specified that two mildly

dyskaryotic smears were required before referral to colposcopy. This was subsequently amended (2003) to suggest that best practice, where resources permit, should be referral after a single mild abnormality. For women with borderline nuclear change, analogous to atypical squamous cells of undetermined significance (ASCUS), the potential of HPV testing is being evaluated.

Potential benefits of HPV testing

- Less invasive diagnostic testing
- Avoidance of unnecessary treatment
- Fewer medical complications
- Reduced patient anxiety and inconvenience if referral is recommended

The causative role of HPV in cervical carcinogenesis supports the use of HPV testing for triage of women with smears suggestive of mild dyskaryosis, on the basis that those who are high-risk (HR) HPV-positive are more likely to have underlying HSIL, whilst those who are HPV-negative are at low risk of having clinically significant disease.

Table 2 Prevalence and genotype distribution according to grade of lesion

	Low-risk HPV: Types 6, 11 (%)	High-risk HPV: Types 16, 18 +/– 33 (%)	'Novel' types (%)	Multiple types (%)	Reference
Low-grade CIN	19	29	19	22	6
High-grade CIN	–	88	–	7	6

The NHSCSP has recently carried out a pilot study of the introduction of liquid-based cytology in the national programme. This study also examined the possibility of reflex HPV testing on samples from women with borderline nuclear changes (i.e. ASCUS) or mild dyskaryosis (LSIL) as a form of triage.

In the NHS HPV triage pilot study (see box), HPV testing is replacing the assessment of low-grade abnormalities by colposcopy. Women whose index smear shows borderline nuclear changes or mild dyskaryosis will be tested subsequently for HR HPV types. If tested positive, each case will be referred immediately to colposcopy. If tested HPV-negative, each will have a second smear after 6 months, as is the current recommended practice, but a second HPV test will also be performed at this timepoint. If the cytological abnormality has either persisted or progressed to a higher-grade lesion, or if the woman is found to be HPV-positive, she will be referred for colposcopy. If the abnormality has regressed and the woman is again HPV-negative, she will be returned to routine screening.

HPV triage pilot study, UK

(Norfolk and Norwich Hospital; Southmead Hospital, Bristol; Royal Victoria Infirmary, Newcastle)

The study is designed to evaluate:

- The extent to which HPV testing in women with low-grade cytological changes reduces the need for colposcopy
- The positive predictive value of the HPV test in women with low-grade smear abnormalities, and the negative predictive value for women with persistent mild dyskaryosis
- The public acceptability of HPV testing as part of the screening programme, including an assessment of the anxiety experienced by patients returned to normal recall after a negative HPV test (despite an earlier abnormal smear), as well as the anxiety caused to patients whose HPV test is positive
- The prevalence of HPV infection in the UK population with low-grade abnormalities
- The impact of the introduction of HPV testing on a laboratory

During the course of the pilot study, the Bristol and Norwich hospitals slightly revised their HPV triage. For women who tested positive for

HPV following a low-grade smear, only those aged 35 years and over were referred to colposcopy immediately. Younger women were asked to return for a repeat smear and HPV test 6 months later, and the referral was made on the basis of this second test. This modification was introduced because of an excessive number of colposcopy referrals, and is based on the finding that older women with HPV are more likely to need treatment than younger women with HPV. Women who tested HPV-negative were managed in the same way as the original pilot protocol.

The UK National Institute of Clinical Excellence (NICE) has recommended the implementation of liquid-based cytology (LBC) in England and Wales, a policy that has been endorsed by the Department of Health. After evaluating the results from the pilot sites, NICE has concluded that LBC is more cost-effective than conventional screening. LBC reduces the rate of smears that are unsatisfactory or inadequate for reporting to 1.6% (from 9.0%, as occurs with conventional screening). This improvement benefits women, in turn, by reducing anxiety, uncertainty and the need for repeat smears. LBC may also reduce specimen interpretation time. The final evaluation report on the cost and effect on screening programmes of HPV triage is still awaited at the time of writing.

Assessment of HPV testing

Two major studies have used the FDA-approved Hybrid Capture 2 (hc2) HPV test to assess the role of HPV testing for triage of women with LSIL on cervical cytology.[7,8]

The first of these was a multicentre study conducted by the US National Cancer Institute (The Atypical Squamous Cells of Undetermined Significance/Low-grade Squamous Intraepithelial Lesions Triage Study [ALTS] Group).[7] Four clinical centres, in different areas of the US, participated in a randomized clinical trial of the use of HPV DNA testing in women with cytological evidence of LSIL.

A total of 642 women referred with LSIL had analysable HPV test results. The mean chronological age (24.9 years) and age at first coitus (16 years) were similar among the four clinical centres. HPV DNA was detected in cervical samples from 532 (82.9%) of the 642 women. This high frequency of HPV-positivity was confirmed by PCR assay. Of a subset of 210 paired specimens tested by hc2 and PCR, 81.4% were positive by both methods.

The very high percentage of women with LSIL (diagnosed from Pap smears) testing positive for HPV DNA indicates that there is a limited potential for HPV testing to direct decisions about the clinical management of women with LSIL. The estimated cost of HPV testing of all women with a cytological diagnosis of LSIL would outweigh the savings gained from avoiding colposcopy for only 20–27% of women.

The second of these studies, by Lin *et al*., 2000, showed that HPV testing may be of most use in screening older women with LSIL.[8] In this investigation, 119 women over 50 years of age (median age 62 years) were referred for colposcopy (with Pap smears reported as ASCUS or LSIL) to two hospitals in Taiwan. The results of cervical histology according to cytology and HR HPV testing are shown in Table 3.

Table 3 Cervical histology results in a study of older women in relation to cytology and HR HPV profiles

	Histology					
HPV status/ cytology	**Normal**	**CIN 1**	**CIN 2**	**CIN 3**	**Cancer**	**Totals**
HPV+/ASCUS	6	6	13	11	3	39
HPV–/ASCUS	30	5	0	0	0	35
HPV+/LSIL	2	11	9	10	2	34
HPV–/LSIL	7	4	0	0	0	11
Totals	45	26	22	21	5	119

For those with ASCUS or LSIL, the rates of all grades of CIN were significantly higher among HPV-positive women than HPV-negative

women where no high-grade disease was found. Therefore, the addition of an HR HPV DNA assay to the cytological examination would appear to provide excellent sensitivity and negative predictive value for the detection of high-grade CIN or cancer in older women with minimally abnormal Pap smears (ASCUS or LSIL), when considered either separately or as a group. In this population of women over the age of 50 years, approximately a third were HPV-negative and could have safely avoided colposcopy.

The results of this study show the greater precision of HPV testing in distinguishing women likely to develop high-grade CIN, compared with other investigations in which HPV testing was used as an adjunctive test in cases of triage for mildly abnormal cytology. This difference is largely due to the selection of study subjects (referral age) and the use of an overall diagnosis, rather than the initial colposcopic biopsy for histological results.

Management of LSIL confirmed on biopsy

There is no clear consensus on what action should be taken when LSIL is confirmed on histology as low-grade CIN, and whether one should treat the abnormality or manage it conservatively by observation only. An audit by the British Society of Colposcopy and Cytopathology (BSCCP) in 1996 showed that 53% of units would treat CIN 1 and 47% would observe.

Shafi *et al*., 1995, discussed the management of low-grade lesions, and the question of whether to treat or to follow up,[9] suggesting that if surveillance is impossible, all cases of CIN should be treated; however, if women were prepared to undergo surveillance, then this could be offered. Treatment for women under observation would be suggested if they continued to have an abnormality persisting for 2 years, or if the lesion worsened in grade or size.

The dilemma of deciding how to treat low-grade lesions arises from the uncertainty of whether the lesion will progress, regress to normal or persist as a low-grade lesion. Conflicting results, in terms of

patterns of progression, regression and persistence, have been reported (Table 4).[2,10,11]

Table 4 Patterns of HPV persistence in mild dysplasia/CIN 1

	Regressed (%)	Persisted (%)	Progressed (%)	CIS/invasive (%)	Reference
Very mild dysplasia[a] (4 months)	1	78	21	1	2
Mild dysplasia[b] (4 months)	0	69	31	0	2
Mild dysplasia[b] (36 months)	0	4	34	62	2
Mild dysplasia[b]	62	22	–	–	10
CIN 1	11	63	26	–	12
CIN 1	60	30	3	1	12

[a]Very mild dysplasia: defined as atypical cells of dysplastic type confined to the superficial and intermediate layers

[b]Mild dysplasia: where less than 10% of the abnormal cells are of the basal type

Knowledge of HR HPV status could alter how women with confirmed LSIL are managed, if it enabled the prediction of patterns of progression and regression. In a study by Campion *et al*., 1986, 100 women with cytological and colposcopic evidence of mild cervical atypia consistent with CIN 1 were followed up.[12] HPV type 16 was reported in 85% of the cases with progressive disease, indicating that the detection of HPV 16 may be a non-invasive way of identifying women at high risk of rapid progression of mild atypia to CIN 3.

However, these results were not supported by Downey *et al*., 1994, who examined the relationship between HPV type 16 and the potential for progression of minor-grade cervical disease.[13] Of 95 women who had been referred to colposcopy with smears suggestive of mild dyskaryosis and HPV type 16 DNA-positivity, 37 had histologically proven CIN 1; 12 had koilocytosis only; and 43 had no abnormality.

Follow-up was for 70 months. Among the total group, the probability of remaining free of high-grade cervical disease was 0.71. There was no difference in the probability of remaining disease-free between HPV 16-positive and HPV 16-negative women, nor was there a significant difference in disease-free probability when the group results were stratified by HPV 16 viral load. These data suggest that a histological diagnosis of minor-grade cervical disease is a better long-term predictor of disease progression than HPV 16-positivity.

In a literature review on the spontaneous evolution of intraepithelial lesions according to lesion grade and HPV genotype, Syrjanen, 1996, concluded that the risk for progression was particularly high in HPV 16-induced lesions.[14] However, this observation was applicable only to a large series of women, and was of little or no help in predicting disease outcome in individual women.

More data are therefore needed in order to define the precise role of HPV testing in the management of women with histologically confirmed LSIL, and large trials are required. Such women should be tested for HR HPV at entry to the study and be followed up

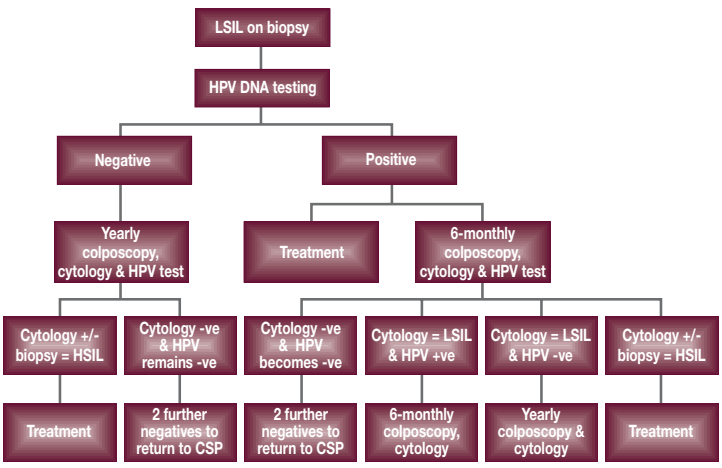

Figure 1 Management of women with biopsy-confirmed LSIL

conservatively with colposcopy, cytology and HPV testing at 6-monthly intervals, over a period of 36 (and possibly up to 60) months. A suggested management algorithm is shown in Figure 1.

While HPV testing may not be appropriate for all women with LSIL, the US ALTS trial results have enabled the American Society for Colposcopy and Cervical Pathology (ASCCP) to make recommendations for HPV testing for the management of specific subsets of women.

American Society for Colposcopy and Cervical Pathology recommendations on the use of HPV testing:

- Selected postmenopausal women with LSIL, selected adolescents with LSIL and women referred to colposcopy for LSIL and having A CIN 1:
  - May be followed up without initial colposcopy using a protocol of
    - (a) repeat cytology at 6 and 12 months or
    - (b) HPV testing at 12 months and referral to colposcopy if positive, and return to routine screening (12 months) if negative

Post-treatment LSIL

Treatment of HSIL and some cases of LSIL is justified in order to pre-empt cancer. However, while a single loop excision of the transformation zone (LLETZ) appears to be a safe procedure (with no effect on menstruation or fertility, compared with controls[15]), it is important to avoid unnecessary repeat treatment, particularly of low-grade lesions in young women. Possible risks associated with repeat excision include cervical stenosis which, if complete, may prevent sperm from entering the endometrial cavity; a potential risk of secondary infection leading to ascending infection of the tubes; and a change in the physical characteristics of cervical mucus, which could lead to fertility problems. Shortening of the cervix increases the

likelihood of it becoming incompetent during pregnancy, with a potential risk of late miscarriages. Therefore, repeat treatment should be avoided, through the use of better post-treatment screening methods.

Data are emerging to support the inclusion of HR HPV testing for monitoring women initially treated for CIN 2/3,[16] as described more fully in Chapter 4. The role of HPV testing could easily be expanded to include those treated for CIN 1 (LSIL), because it would be important, in any group, to avoid repeat treatment. For those with LSIL confirmed by cytology or histology, knowledge of their HPV DNA-negativity would allow follow-up with yearly cytology, colposcopy and HPV testing. If women were HPV DNA-positive, close follow-up with cytology, colposcopy and HPV testing might be offered. A suggested post-treatment algorithm is shown in Figure 2.

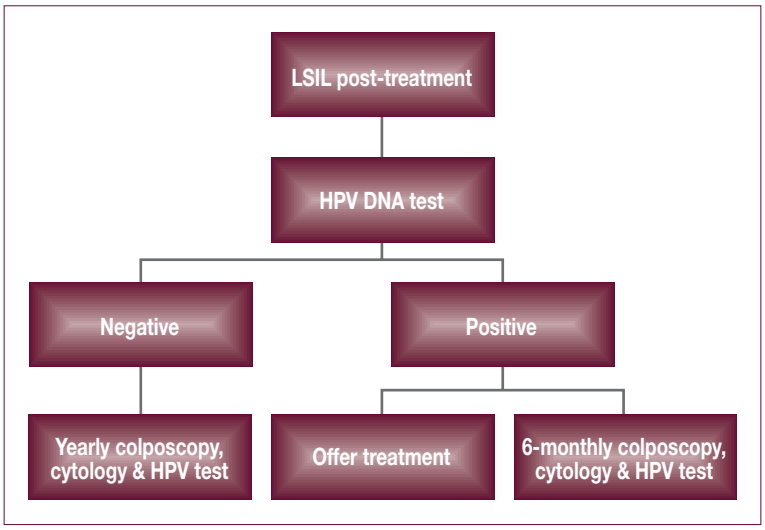

Figure 2 Post-treatment management of women with LSIL

Conclusions

- HPV DNA testing has limited potential for women with LSIL because of the very high incidence of HPV-positivity
- In an older age group (>50 years of age), where the percentage of HPV-positivity is likely to be much lower, there may be a role for HPV testing as recommended by the ASCCP
- The addition of a HR HPV DNA assay to cytological examination appears to provide excellent sensitivity and negative predictive value for the detection of high-grade CIN or cancer in older women with minimally abnormal Pap smears (ASCUS or LSIL)
- Further studies are needed to define the precise role of HPV testing in the management of women with histologically confirmed LSIL
  - Those who are not HR HPV-positive could be managed more conservatively with yearly colposcopy and cytology visits
  - Those who are HR HPV-positive could be offered either early treatment with earlier return to the cervical screening programme or 6-monthly colposcopy and cytology follow-up

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4. The role of viral HPV testing in post-operative follow-up

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- Women who have been treated for cervical intraepithelial neoplasia (CIN) have a fivefold increased risk of developing invasive cervical cancer compared with the general population
- The major factors that influence recurrent/residual disease are positive endocervical margins and age > 50 years
- The follow-up of women treated for CIN is fraught with difficulty, since cytology and colposcopy are relatively insensitive techniques
- Because of these limitations, human papillomavirus (HPV) testing has been proposed for use during post-treatment follow-up
- Preliminary evidence consistently supports the use of high-risk HPV testing as post-operative follow-up

Introduction

The viral origin of cervical cancer is now well established, and high-risk (HR) human papillomavirus (HPV) DNA has been found in virtually all cervical cancers,[1,2–4] and high-grade pre-cancerous lesions. Cervical cancer screening in most Western nations is performed using the Pap smear, although research over the last 10 years has revealed that the Pap smear has a number of limitations: lack of sensitivity, poor quality control (across much of Europe) and a high rate of equivocal/atypical squamous cells of undetermined significance (ASCUS). HPV testing has been proposed for both primary screening and for the follow-up of equivocal Pap smears. More recently, HPV testing has also been proposed for use in the post-treatment follow-up of patients with cervical intraepithelial neoplasia (CIN).

Treatment of CIN

In Europe, high-grade pre-cancerous lesions (CIN 2/3) are usually treated by LLETZ (large loop excision of the transformation zone), laser ablation or cold-knife conization. Having undergone treatment, patients must then be carefully followed up. For women who have been treated for CIN, the risk of developing invasive cervical cancer is five times that of the general population. Cervical cancer after treatment for CIN can arise from two sources: residual disease or recurrent disease. These two terms are often confused in the medical literature (see box)

For the purposes of this review, the terms ‘residual disease’ and ‘recurrent disease’ are used synonymously. Residual disease is used here to mean the presence of CIN at any time after treatment.

Hysterectomy can confidently quantify immediate residual CIN, and has revealed an unexpectedly high incidence of residual disease (23–34%).[5] This contrasts with the relatively low incidence of CIN found in larger, longer-term follow-up studies of treated patients whose uteri were left intact,[6–8] and may indicate that hysterectomy is more useful if delayed (Table 1).

Terminology

- Residual disease refers to CIN that has not been eradicated at treatment and which will usually be revealed by cytology or colposcopy during the first year of follow-up
- Recurrent disease occurs after eradication of CIN; it is reasonable to assume that disease is recurrent if it occurs ≥ 3 years after treatment and was not previously seen during careful follow-up, but it is never certain that recurrent disease is not 'residual'

Table 1 Post-treatment occurrence of CIN in women with intact uteri

Subjects (*n*)	Follow-up (years)	Abnormality rate (%)	Invasive lesions (*n*)	Reference
394	10	4.4[a]	2	9
3560	12	NA	6	6
NA	2	2.1[b] 16.5[c]	NA	7

[a]Annual abnormality; [b]after complete excision; [c]after incomplete excision; NA: not available

Factors influencing the residual disease rate

The probability of having residual disease after treatment depends on several factors. If the endocervical margin is involved, the risk is much greater, and ranges from 16% to 50%.[8,10] In contrast, the residual disease rate when the excision appears complete with free margins is only 2–3%.[8,11,12] Clearly, having positive margins does not mean that a woman should be re-treated, as the majority of women do not harbour residual disease and would, therefore, be subjected to unnecessary intervention and possible cervical functional morbidity. However, these women may warrant particular attention with a more rigorous follow-up protocol. Furthermore, women with negative margins also have a risk of residual disease, and should not be dismissed as free from the risk of subsequent disease reappearance.

Key risk factors for residual disease

- Age > 50 years
- Positive endocervical margins

In a study by Flannelly *et al*., 2001, age was shown to be a particularly important post-treatment risk factor; of a total of six cases of invasion after LLETZ, five occurred in women over 60 years of age.[6] This group, therefore, advocated routine re-treatment, rather than surveillance, in women over 50 years of age with CIN at the original margins of excision. This study had the following limitations: disease was histologically apparent in only half of the specimens, and in only 755 of 3560 patients was it clear to the pathologist which margin was involved. In the follow-up protocol, patients were offered a cytological examination at 3 months and, if the results were normal, they were discharged from the clinic and advised to have annual cytological examinations as community screening over the next 5 years. Ultimately, only 60% of the patients had three or more cervical smear samples taken. Despite these criticisms, the discovery that age > 50 years is an independent risk variable was an important finding. There is less supportive evidence concerning other risk factors that have been found to be associated with residual disease following treatment. The clinical utility of these additional risk factors is relatively small, in that they do not clearly identify the woman who actually has disease, nor do they confidently rule it out for any individual. Rather, they modify the risk of finding residual CIN. Consequently, most colposcopists implement strict follow-up cytology and colposcopy regimens – a course of action that is time-consuming for the clinician, and worrying for the patient who perceives a genuine, continuing risk. Clearly, a definite marker of residual disease would be of immense clinical value.

Follow-up after treatment

Current follow-up protocols are based on the Pap smear and colposcopy, and results may be influenced by morphological changes associated with the healing process. For example, in the study by Gardeil *et al*., 1997, colposcopy did not detect any additional residual disease that had not already been discovered by cytology.[8] Indeed, the British Society for Colposcopy and Cervical Pathology (BSCCP) recommends that routine post-treatment follow-up should be cytological, and has stated that there is no evidence to recommend mandatory colposcopic follow-up. Similarly, there is good evidence that cytology after treatment is less sensitive and less specific in the detection of CIN than when used prior to treatment[13–16] (see box).

Post-treatment colposcopy may not detect residual malignant cells, due to:

- Morphological changes:
  - Inflammatory
  - Fibrotic
  - A shift in the squamocolumnar junction
- The small size of residual lesions

In recognition of these limitations, follow-up protocols recommended by national societies tend to be exhaustive and demanding of patients' and colposcopists' time. Their demands produce a heavy clinical load, often in services that are already overburdened by the needs of 'new' referrals. For example, in France the first follow-up assessment is recommended between 3 and 6 months after treatment, and includes a smear and colposcopy with directed biopsies and/or an endocervical curettage. The French Colposcopy Society recommends that this assessment is repeated after 6–12 months, and the patient returned to annual cytological follow-up only after all these tests prove negative.[17] However, even frequent repetition cannot overcome the inherent limitations of these tests, and lengthy protocols may lead to a proportion of women being lost to follow-up.

Role of viral testing for post-treatment follow-up

In untreated women who spontaneously clear their HPV and cytological abnormality (which the majority do), Nobbenhuis *et al*., 2001, have shown that viral clearance precedes the return to cytological normality by 3 months.[18] Several authors have now shown that a distinct decrease in viral infection occurs after treatment of cervical lesions. Kucera *et al*., 2001, studied the evolution of viral infection after excision of the transformation zone in 119 patients, all of whom had CIN and were HPV-positive. After this treatment, only 6% of patients had persisting HPV infection 12 months later.[19]

How valuable is HR HPV-positivity, after treatment for CIN, as an index of its clearance? The available evidence is encouraging, and is discussed below.

HPV status versus margin status

Preliminary evidence indicates that HR HPV status is useful as an index of disease clearance. In a study by Lin *et al*., 2001, half of the women whose hysterectomy specimens were HPV-positive had residual CIN after initial excision, whereas none of those with HPV-negative hysterectomy specimens had CIN.[20] Jain *et al*., 2001, reported the rates of occurrence of residual lesions observed in post-conization hysterectomy specimens, in relation to the histologic status of the resection margins and the presence of post-operative viral DNA (Table 2).[21] In this prospective follow-up study, 79 HR HPV-positive patients all underwent conization for CIN 3, followed by an HPV test and finally, after 6–8 weeks after conization, a hysterectomy. These authors reported that 59% (47/79) of the patients had involved margins and 40% (32/79) had residual lesions.

In this study, the use of HR HPV testing was significantly more effective than either resection margin status or Pap smear results for the detection of residual lesions; the sensitivity of the post-treatment HR HPV test and the negative predictive value were both 100%.[21] Notably, the high rate of post-operative viral infection (53%) is

probably associated with the short time-interval (6–8 weeks) between conization and viral sampling.

Table 2 Residual disease according to resection margins, HPV and Pap smear results after conization

	Residual lesions absent		Residual lesions present		Total
	n	%	*n*	%	*n*
Free margins	31	97	1	3	32
HPV–	25	100	0	0	25
HPV+	6	86	1	14	7
Involved margins	16	34	31	66	47
HPV–	10	100	0	0	10
HPV+	6	16	31	84	37
Normal Pap smear	10	67	5	33	15
Abnormal Pap smear	6	19	26	81	32
Total	47		32		79

Nagai *et al*., 2000, also found that HR HPV testing could reveal all cases of post-treatment residual disease, although the test had a relatively poor specificity at 8 weeks.[22] In this small ($n=58$) study, these authors also documented a 100% negative predictive value and a decline in HPV-positivity from 97% to 20%, 8 weeks after treatment. Bollen *et al*., 1999, reported very similar results in their even smaller study ($n=43$).[16] Two additional retrospective case–control studies demonstrated that HR HPV testing recognizes all residual disease.[14,23] The addition of HPV testing to cytology increased the sensitivity without reducing specificity.

Finally, Nobbenhuis *et al*., 2001, compared the efficacy of HPV testing to that of the Pap smear for the detection of recurrent disease.[24] In this prospective study, 184 patients treated for high-grade lesions were followed up with Pap smears and HPV testing at 3, 6, 9, 12 and 24 months. The viral infection rate declined from 98.4% pretreatment to 26.1% after 3 months of treatment. The recognition rate for severe CIN was 15.8%, with a median time to diagnosis of 6 months. The authors reported that HR HPV testing had a significantly higher

sensitivity, compared with Pap smears, at 3 and 6 months after treatment (90% vs 62%, respectively, at 6 months), although the difference in sensitivity tended to disappear over time. The specificities of the two techniques were similar (92% and 91%, respectively, at 6 months). All patients who were HPV-positive and without disease recurrence at 3 months, became HPV-negative, after a median of 8 months. The negative predictive value of the two tests used in combination was very high (99%). However, other studies have indicated that the negative predictive value of HPV testing, while still higher than that of cytology, was not 100%. The currently available data indicate that a negative predictive value approaching 100% can be achieved using a combination of HPV testing and a Pap smear.

Conclusions

- Preliminary studies indicate that HR HPV status is a significantly more effective marker for the detection of residual lesions than either resection margin status or Pap smear result
- The best time to perform HPV testing is 6 months or more after treatment
- As with other applications of HPV testing, the unprecedented high negative predictive value is its strongest asset
- A negative predictive value of almost 100% can be achieved by combining HPV testing with a Pap smear
- A negative smear and a negative HPV status would confer a high degree of confidence to both patient and clinician, enabling the patient's discharge from colposcopic surveillance and an earlier reversion to routine cytology-screening intervals

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5. HPV for cervical cancer screening

Joakim Dillner

- Incorporation of human papillomavirus (HPV) testing into cervical cancer screening programmes requires a full assessment of the following factors:
  - Quality-control criteria used in tests
  - Age of target population
  - Follow-up and longitudinal performance
  - Results of cost–efficacy modelling for HPV screening programme design
- The potentially important roles of HPV testing as triage for specimens with borderline cytology, as primary screening, and to manage women after treatment, are discussed

Introduction

The causative relationship between human papillomavirus (HPV) and cervical carcinoma has provided the incentive to target HPV in the prevention of cervical cancer. This chapter considers the use of HPV testing in cervical cancer screening, and defines the studies needed to assess whether its increased sensitivity will result in an overall improvement of the screening programme, bearing in mind its increased costs and typically lower specificity.

HPV tests: Principles and laboratory practice

Quality-control criteria

Reproducibility

HPV DNA tests have been improved greatly in recent years, since early assays were shown, by careful validation, to have commonly produced misclassified results.[1] Misclassification can lead to serious underestimation of relative risks, as shown by a comparison of two similar studies performed in the same laboratory (one using moderately reproducible technology, the other carefully validated PCR technology), each of which resulted in a completely different conclusion: their reported estimates of the relative risk for cervical intraepithelial neoplasia (CIN) in cases of HPV-positivity were 2.3 and >10, respectively.[2]

Despite improved test reproducibility, clinicians and investigators should remember that positivity may not always reflect a true infection. A blinded reanalysis of the same set of samples, on two different occasions, should be used to assess current standards of testing.

Sensitivity and specificity

Assessment of sensitivity and specificity is not straightforward, as clinical measures are dependent on a known 'gold standard' that should be applicable to (and accordingly, be a true reflection of) the real-life clinical situation. For the purpose of evaluating clinical practice, it is more appropriate to consider test performance in relation

to the desired properties of the test, rather than to some form of laboratory standard. In this context, the desired property is that testing should reduce the risk of cervical cancer. HPV-negative women should have a low risk of developing cervical cancer, the duration of which will determine the testing frequency and general cost–efficacy of a screening programme. HPV-negative women have a very low risk of having high-grade CIN or cancer at the time they are tested, and HPV testing has now also been shown to have long-term predictive value for the future occurrence of high-grade CIN or cervical cancer,[3,4] further supporting its cost-effectiveness.[5] HPV-positive women have a high risk of developing cervical cancer. Ideally, a treatment/surveillance option that reduces the risk for cervical cancer should be offered to women who test positive. Currently, treatment is offered to women with cytological abnormalities, a strategy with a proven effect on prevention of cancer. Women testing HPV-negative after treatment can expect to be protected against recurrence of cervical neoplasia. So far, follow-up studies reported in the literature have mostly used general primer PCR.[6]

From these considerations, there are several major HPV DNA tests that may be considered appropriate for use in screening (Table 1).

In summary, although HPV testing in general has improved greatly in performance, the principal candidate tests for potential use in HPV screening have not been evaluated for this purpose to a similar extent. For both research studies and clinical use, continuous quality-control monitoring is essential, and further improvements to reduce inter-test discrepancies are desirable.

Table 1 Characteristics of HPV DNA testing methods

Test	Current availability of test kit[a]	Methodology	Advantages	Disadvantages
hc2	Available	RNA–DNA hybridization and sensitive detection of the formed DNA/RNA hybrids	Suitable sensitivity for detection of high-grade CIN and CxCa[7,8]	Cross-reactivity with additional HPV types[9]
GP5+/GP6+ EIA	Unavailable	PCR (140 bp amplicon of L1 gene) then enzyme immunoassay	High specificity and sensitivity; rapid; little interlaboratory variation; high reproducibility[10–12]	Concordance with other PCR methods (e.g. MY09/11); requires standardized protocols and validated reagents;[10] less sensitive for detecting HPV types 53 and 61[13]
PGMY09/11	Unvailable	PCR (450 bp amplicon of L1 gene) then reverse probe hybridization	Equal sensitivity for detection of high-grade CIN and CxCa as hc2	Less sensitive for detecting HPV types 31 and 52
SPF10	Available	PCR (65 bp amplicon of L1 gene) then reverse probe hybridization	Highly sensitive	High analytical sensitivity does not necessarily translate into increased clinical sensitivity
PreTect HPV Proofer	Available	NASBA detection of oncogene E6 and E7 RNA	–[a]	–[b]
Roche AMPLICOR HPV Test	Available	PCR (165 bp amplicon of L1 gene) then MWP format detection by cocktail probe hybridization	Preliminary results indicate very high sensitivity for detection of high-grade CIN	A high proportion of cytologically normal women who have not developed CIN on follow-up test weakly positive

[a]at time of writing (2004); [b]clinical studies still ongoing at time of writing; NASBA: Nucleic acid sequence-based amplification

Follow-up and longitudinal performance

For screening programmes in general, the longitudinal performance indicators are the most relevant ones to consider, as they determine the duration of the protective effect – and therefore the length of the screening interval and cost–efficacy of the entire programme.

In studies that have followed HPV DNA-positive women for up to 10 years, results have consistently shown that the positive predictive value of HPV testing is much increased when the risk of developing a high-grade CIN during the subsequent 10 years – not merely at the time of diagnosis – is considered.[14] These observations support the rationale for proposing HPV testing in screening programmes with a longer screening interval, compared with screening programmes based only on Pap smear testing. However, the HPV test has a substantial longitudinal predictive value for the future development of high-grade CIN, which can also give rise to management dilemmas. Categorizing a previously healthy woman as at high-risk for developing cancer (and therefore requiring repeated tests at frequent intervals) causes considerable psychological stress. For primary HPV screening to be a realistic option, the group of women requiring follow-up should be small, and the duration of intensive follow-up, after confirmation of HPV-positivity, should not be unreasonably long. The longer a healthy HPV-positive woman is classified as a high-risk patient for cancer, the greater the negative effect of primary HPV screening and the associated costs. Clearance of the infection is highly desirable.

Several studies have now shown conclusively that conventional treatment (conization) of HPV-positive CIN results in a clearance of the infection.[15–21] Whether the infection is still present after treatment enables prediction of the risk of recurrence of their CIN lesions.[6,18] Follow-up will therefore not be necessary for life, but only until HPV-positive women become HPV-negative. It may be possible to augment clearance of HPV infection (in the absence of cytological abnormalities) by some form of treatment. With the current status of our knowledge, the clinician would need to monitor HPV-positive patients either until cervical lesions develop (then treat by currently established protocols), or until the women become HPV-negative.

In summary, further research is necessary to better define the longitudinal performance indicators (sensitivity, specificity, positive and negative predictive values) of HPV DNA testing, as well as of combined HPV DNA testing and cytology. New methods for the specific treatment of HPV infection are required, with the aim of minimizing the time needed for follow-up of HPV-positive women with intensified screening. Research to determine algorithms for the optimal follow-up of HPV-positive women is also desirable.

Using cost–efficacy modelling to design HPV screening programmes

In a pioneering series of studies by van Ballegooijen *et al*., mathematical modelling was used to address two questions: which programme designs are likely to be the most cost-efficient and in what critical areas of uncertainty is research most needed.[5,22,23] The longitudinal performance of the HPV test was shown to be critical for achieving cost-efficiency, because the cost and lower specificity of HPV screening has to be compensated for by a longer screening interval, in order to be cost-effective.

The 'Europe Against Cancer' sponsored trial, initiated in Sweden, has included a component for the mathematical modelling of cost–efficacy.[24] Swedish population-based registry data, accumulated over 30 years of organized screening, has provided reliable input data to the model. As emphasized above, critical input data may differ between populations, and it is uncertain whether the data are applicable outside Sweden. The addition of HPV testing aims to increase the sensitivity of screening for CIN 2/3 and cancer *in situ*, and to reduce costs by increasing the screening interval for women with negative combined test results. Details of the study are as yet unpublished, at the time of writing, but its principal features and findings are summarized here (see box).

The results are in line with those from other modelling studies performed in the Netherlands and in the UK. Similarly, the estimates of

the cost savings achieved by the screening programme concord with data from another Nordic country (Finland) that also has an organized screening programme and comparable levels of medical costs.[25]

Cost–efficacy modelling (Sweden)

- Routine cytological screening was compared with 1) no screening and 2) HPV DNA testing as an adjunct to cytology, in women over 32 years of age
- Input data of the longitudinal performance of HPV DNA testing was derived from screening for type-specific HPV persistence using PCR
- Analysis used a Markov model of a hypothetical population of 100,000 women
- Routine cytology screening resulted in considerable lifespan extension and resource savings, compared with no screening
- Add-on of HPV DNA testing without changing cytology screening increased costs and had insignificant effects on life-expectancy
- Combined cytology and HPV DNA testing every 9 years had measurable gains in life-expectancy and resource savings, compared with routine cytological screening

In summary, design of HPV screening trials, screening policies and/or policy evaluation studies should be based on cost–efficacy modelling studies, specific to each population to be targeted for screening.

Use of HPV testing in HPV triaging of equivocal smears

At the time of writing, the meta-analysis by Arbyn *et al*., 2004,[26] is the most recent systematic comparative review of HPV testing and repeat Pap testing for triage. This survey demonstrated the improved accuracy of HPV testing (using the Hybrid Capture 2 [hc2] assay), in comparison with the repeat Pap smear, for the detection of high-grade intraepithelial neoplasia of the uterine cervix among women with equivocal cytological results.

In summary, substantial evidence supports the use of well-validated HPV testing for detection of high-grade intraepithelial neoplasia of the uterine cervix among women with equivocal cytological results in organized screening as an evidence-based policy.

Use of HPV testing in primary screening

The most effective action for improving cervical cancer prevention is to ensure a high attendance rate of the population in screening programmes.[27,28] The next most effective action is to ensure that women with cancer or precursor lesions who do attend will not receive a false-negative diagnosis.[27,28]

Over 95% of cervical cancers and high-grade CIN lesions are HPV-positive, and HPV DNA testing has a superior sensitivity, compared with the Pap smear, for the detection of prevalent high-grade CIN.[4] Similarly, since most adenocarcinomas are HPV-positive, HPV testing should improve detection rates, as cytological screening is of limited use in detecting cervical adenocarcinoma.

Trials of HPV DNA testing were compared in the systematic review by Lörincz and Richart, 2003.[4] These authors concluded that HPV DNA testing was a more sensitive indicator for prevalent high-grade CIN than either conventional or liquid cytology. A combination of HPV DNA and Pap smear testing resulted in an almost 100% sensitivity and negative predictive value. The specificity of the combined tests was

slightly lower than the specificity of the Pap test alone, but this shortcoming could potentially be offset by the resultant greater protection from neoplastic progression and cost savings that would be afforded by the extended screening intervals. One 'double-negative' HPV DNA and Pap test provides better prognostic assurance against the risk of future CIN 3 than three subsequent negative conventional Pap tests.

Target age groups for HPV screening programmes

The greatest cost benefit from introducing a standard Pap smear screening programme occurs when a country changes from a no-screening policy to one of a lifetime cytological test. This single test, at the optimal age of about 35 years, is estimated to contribute 25% of the protective effect of an entire screening programme (with smears every third year).[27] By analogy, the greatest relative benefit of HPV screening is expected when a country changes its programme from one of no HPV testing to one of a lifetime HPV test. The optimum timepoint of this lifetime test should be sufficiently late in life, to ensure that most HPV exposures have already occurred, but sufficiently early, such that the risk of invasive cervical cancer having already developed is low.

The HPV infection rate is high in younger age groups, and decreases with age.[29,30] By 35 years of age, the prevalence of infection with oncogenic HPV types is 1–8%.[12,18,31–34] For example, the Europe Against Cancer sponsored trial in Sweden of nationwide, population-based HPV screening (of 12,527 B35-year-old Swedish women) reported a joint prevalence of 7.3% for the 14 most common oncogenic HPV types (6, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68).[34] This finding indicates that the optimal time to begin HPV screening should be between 30 and 40 years of age. The cost-effectiveness of HPV testing would be poor in women aged < 30 years, because HPV is more common and cervical cancer is rarer than in older age groups. In addition, variations in HPV prevalence between

different countries, or between distinct regions within countries (e.g. between urban and rural regions), could result in a variation in cost-effectiveness between populations. HPV epidemiological studies should be population-based, large-scale studies; use established reference methods for HPV testing; and target age groups in which HPV screening has highest specificity (i.e. 30–60 years of age).

In summary, research is needed to determine the age-specific epidemiology of HPV infections in different populations. Cost–efficacy modelling studies should be repeated for different populations, accounting for differences in associated costs, HPV infection rates of different HPV types, and background rates of other risk factors for cervical cancer.

Possible strategies for improving HPV test specificity

Table 2 summarizes strategies that could improve the specificity of HPV testing for primary screening programmes.

It has been established that HPV persistence is a necessary risk factor for HPV-associated cancers, and that repeated HPV testing improves specificity without significant loss of sensitivity. Performing two HPV tests separated by a 1-year interval will, among 35-year-old women, identify about half of those initially HPV-positive women as being persistently positive.[34] However, repeat testing has not yet been widely evaluated in practice, or in cost–efficacy models.

Several methods for triaging in the event of an HPV-positive test have been proposed, and further assessment of their usefulness is warranted. Such research should be guided by cost–efficacy modelling studies, which are better able to assess which performance indicators should be scrutinized, in order for a triaging test to be a practical proposition.

Table 2 Strategies for improving HPV test specificity

Strategy	Comments	References
Repeat HPV testing	Infections normally clear within 1–2 years; women who develop CIN or CxCa are persistently positive for HPV DNA in repeated tests	3,12,18, 35–39
Testing for high viral load	Restricting analyses to samples with high viral load may increase the predictive value of the test	40, 41
HPV serology	Able to predict the risk of invasive cervical cancer up to 15 years before diagnosis; positive serology is associated with progression to CIN 3 among HPV DNA-positive women	42–44
Testing for HPV integration	May increase the predictive value that the HPV-positive sample is derived from a sample that contains CIN or cervical cancer	45
Inclusion of other risk factors	Meta-analysis indicates that the presence of *HLA haplotypes* DQw3 and DR15/DQ6 increase the risk of both CIN and cervical cancer *Smoking* is a risk factor for invasive cervical cancer, but not HPV persistence, and may be inversely correlated with persistence A history of infection with *Chlamydia trachomatis* may predict an increased risk of cervical cancer among HPV DNA-positive women	46 38 47

Primary HPV screening in general

Epidemiological studies show that the cervical cancer protective effect of cervical screening is very strong,[28] but cervical screening had not been evaluated by randomized trials before its implementation. Consequently, this has delayed the rational implementation of cervical screening programmes, and has engendered a long-standing debate on the true efficacy of screening.[48] New primary screening methods should be fully assessed, using randomized trials to determine effects at the population level. However, randomized studies are very costly, and their results slow to emerge, and possibly outdated when finally

obtained. In addition, their conclusions may not be fully applicable to the public healthcare setting.[49]

Primary HPV screening

- Appropriate settings/systems
  - Organized screening systems are clearly superior to opportunistic screening for reduction of cervical cancer incidence
  - New technologies should be evaluated for their suitability within organized, population-based call and recall screening systems
- Appropriate study design
  - Randomized trials
  - Mathematical modelling
  - Randomized healthcare policy

Mathematical modelling is an alternative that can provide more rapid answers.[50] Whilst modelling studies have provided valuable information on the potential benefits of HPV screening, it is disconcerting that different modelling studies produce substantially different results.[51–53] Part of the reason for these discrepancies, though only part, is the diverse use of different values for the input variables, most notably for the estimates of cost, progression/regression rates and test sensitivity/specificity. These values are usually obtained from the scientific literature, and published studies vary greatly in quality and healthcare setting.

An alternative type of evaluation is the randomized healthcare policy, in which the new policy is introduced only partially – e.g. in some geographical regions or certain birth cohorts only, rather than in the general population. In this strategy, research funds for randomized trials are not required, and the results are closely applicable to the real-life healthcare policy, and not merely to the research setting. This approach has been successfully applied in the Finnish mammography programme.[49]

In summary, a judicious combination of randomized trials, modelling studies and randomized healthcare policies is recommended. Modelling should be used to investigate optimal settings and study designs that can then be incorporated in randomized trials with intermediate endpoints (such as protection against high-grade CIN). Assessment of factors that influence intermediate endpoints can then be used in further modelling studies, in order to estimate their effects on late endpoints (such as mortality) and/or to design randomized healthcare policies.

HPV testing in post-treatment follow-up of CIN

The standard treatments for CIN are various kinds of cervical surgical procedures.

Several studies have found that HPV DNA is cleared after effective treatment for CIN, and that persistence of HPV DNA predicts recurrence.[17,18,54,55] These findings suggest that HPV DNA testing could provide a useful means of evaluating different treatment modalities.

The meta-analysis of Paraskevaidis *et al*., 2004,[6] identified 11 studies (eight retrospective; three prospective) that evaluated the use of HPV testing after conservative treatment for CIN.[15,17,18,20,56–62] The total number of women included in these studies was 900, of whom 678 (75.3%) were considered as having a successful treatment, compared with 222 (24.7%) who were considered treatment failures.

Heterogeneity across these studies was marked (Table 3). In addition, duration of follow-up varied from 3–6 weeks[59] to 206 months in some individuals,[15] and a wide variety of methods was used to confirm or exclude recurrent disease. The specificity of HPV testing ranged from 44%[15] to 95%.[17] HPV DNA was detected pre-operatively in 718 of the 873 women (82.2%). The sensitivity of the pre-operative HPV test was found to be particularly high in studies that included only high-grade lesions, where HPV DNA was detected in 176 out of 181 cases (97.2%).

Table 3 Principal features of studies evaluated in the meta-analysis of Paraskevaidis *et al*., 2004[6]

Grade of initial lesion	Treatment	Test schedule	Test method	Reference
Variable	Excision only	Pre- and post-operative	PCR	17
Variable	Excision only	Pre- and post-operative	PCR	60
Variable	NA	Post-operative	PCR	15
Variable	Excision and destruction	Pre- and post-operative	PCR	61
Variable	Excision and destruction	Pre- and post-operative	PCR	56
Variable	Excision and destruction	Pre- and post-operative	PCR	20
Variable	Excision only	Pre- and post-operative	hc2	58
High-grade only	Excision only	Post-operative	PCR	62
High-grade only	Excision only	Pre- and post-operative	PCR	18
High-grade only	Excision only	Pre- and post-operative	hc2	59
High-grade only	Excision only	Pre- and post-operative	PCR	57

hc2: Hybrid Capture 2 assay; PCR: Polymerase chain reaction; NA: Not available

Among the 672 women in whom the treatment was considered successful, the post-operative HPV DNA test was reported as negative in 566 (84.2%), and positive in 106 (15.8%). In contrast, among the 204 cases that were considered as treatment failures, only 35 cases (17.1%) had a negative post-operative HPV DNA test, whereas 169 cases (82.8%) tested positive.

In summary, the meta-analysis showed that post-treatment HPV testing can quickly and efficiently detect treatment failure.

Timing of post-treatment HPV testing

The optimal timing of post-treatment HPV testing has been explored in several studies, notably Elfgren *et al*., 2002.[63] A substantial proportion of women showed clearance as early as 3 months, and significant clearance was also evident between 3 and 6 months. After 6 months, the clearance rate slowed. An extensive evaluation of possible post-treatment testing options in the Dutch screening programme (that previously used post-treatment cytology at 6, 12 and 24 months) found evidence to suggest that double testing with cytology and HPV at 6 months and 24 months would be the best option.

In summary, improved follow-up regimens are needed, and the use of post-treatment HPV testing should be explored when designing new follow-up regimens after CIN treatment. There is evidence to support the use of a double cytology and HPV test, 6 months after treatment, to improve safety of post-treatment follow-up. While there is evidence to suggest that the subsequent follow-up of women who test doubly negative (for both HPV and cytology) should be less intense, there is insufficient information to indicate which specific follow-up regimen should be used. Implementation with careful monitoring and/or randomization is recommended. Further research on the long-term protection of HPV-negativity, as well as of joint cytology and HPV-negativity, is warranted.

Conclusions

- HPV testing has advanced enormously in performance
- A combination of HPV DNA and Pap testing has almost 100% sensitivity and negative predictive value when used as primary screening
- To minimize the duration of follow-up in HPV-positive women, new methods of treating HPV infection are needed
- A double cytology and HPV test, 6 months after treatment, should improve the safety of post-treatment follow-up

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