HPV testing as a screen for cervical cancer

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ABSTRACT

Human papillomavirus (HPV) has been identified as a necessary factor in the development of pre-invasive and invasive cancers of the lower genital tract, of which cervical cancer is the most prevalent. A molecular understanding of malignant transformation and epidemiologic information has led to the development of many strategies for detection and early intervention. Newer tests for oncogenic subtypes of HPV have made it possible to predict the risk of future development of cervical cancer. This review summarizes the current understanding of HPV related disease and examines the role of HPV testing as a screening tool for cervical cancer. It summarizes the data from prospective and randomized controlled trials on HPV screening from Europe and North America and includes smaller studies from low and middle income countries where cervical cancer is the most common cancer in women.

Introduction

Cervical cancer is a leading cause of death in women worldwide, with 530,000 new cases and 275,000 deaths worldwide each year. Cervical cancer is the third most common cancer worldwide; it is the sixth most common cancer in women in developed countries and the second most common cancer in resource limited countries. Cervical cancer is preventable because it has a long pre-invasive phase and is easily identified by clinical and histopathological examination. The incidence of this disease is therefore directly related to a nation’s medical infrastructure and the resources available for population-wide screening and the treatment of identified cancers. The goals of screening programs for cervical cancer include the identification and treatment of true precursors of cervical cancer.

This review summarizes the current knowledge of human papillomavirus (HPV) related premalignant and malignant disease and the studies that have led to the recommendation of HPV testing as a primary screening test.

Incidence and prevalence

In the United States the overall incidence of cervical cancer is 7.5 cases per 100,000 women. In countries with the highest incidence, rates range from 37.8 per 100,000 in Fiji to 75.9 per 100,000 in Malawi. HPV infection is one of the most common and contagious infections in the world. The lifetime cumulative risk of HPV infection is greater than 80%. Most genital HPV infections are transient, with a persistence rate (presence of HPV for more than two years) of less than 10% for infections with a high risk subtype, which are associated with pre-invasive lower genital tract disease and invasive cancer. The proportion of HPV infections that are high risk subtype versus low risk subtype varies by age and geography; for example, adolescents may be at equivalent risk for low and high risk infections but high risk HPV infections constitute 50-80% of infections in women over age 30 years.
Acquisition and natural course
HPV infection is sexually transmitted. The virus is spread by skin to skin contact. Trauma may play a role in the differential location of HPV related disease. Most infections are transient and will clear within about eight months, especially in women under 30 years. Viral load is usually reduced to undetectable levels by two years.8

In a cohort of 1052 women aged 21-55 years who were followed and tested for HPV at least three times over two years, the rate of regression or persistence of HPV was related to the concomitant cytologic finding.9 The rates of persistence of HPV by cytology were 6.5%, 1.5%, 11.4%, and 0.8%, respectively, for the following cytologic findings: normal, low grade squamous intraepithelial lesion (LSIL), high grade squamous intraepithelial lesion (HSIL), and invasive squamous cell cancer. These persistence rates reflect the fact that women with preinvasive and invasive lesions were treated, whereas women with normal cytology were just monitored. Conversely spontaneous regression was 56.8%, 14.8%, and 0.2% for lesions that were found to be normal, LSIL, and HSIL, respectively. No invasive cancers spontaneously regressed. The time of transformation to invasive cancer ranges from 12 months to 10 years.10

Persistent infection is defined as the presence of high risk HPV for longer than two years. In a cohort of 354 women with high risk HPV infections, 9.3% with persistent infections developed cervical intraepithelial neoplasia 3 (CIN 3; high grade squamous intraepithelial lesion, severe dysplasia) over a median follow-up of 33 months.11

The prevalence of HPV in postmenopausal women ranges from 14% to 38%. HPV infection is more likely to be persistent in women over the age of 65 years, so a positive HPV test is more likely to be clinically significant. In a study of 260 menopausal women, 14% tested positive for HPV. Half of them had oncogenic subtypes and most of these women had persistent infections.7 Because persistent HPV infections are directly linked to cervical cancer, the age specific incidence rates of cervical cancer guide decisions about screening.

A study published in 2014 recalculated the incidence of cervical cancer in older women after correcting for hysterectomy rates in the United States.12 The age specific prevalence of hysterectomy increased with age, with the highest rates being in women aged 75-84 years. The uncorrected cervical cancer rates plateaued at ages 40-44 years, at 15.6 cervical cancer cases per 100 000 women. However, after correcting for hysterectomies the incidence of cervical cancer steadily increased until 65-69 years, at which point it was 27.4 cases per 100 000 women. The corrected incidence rates for older women aged 70-79 years, 80-84 years, and over 85 years are 23.2-24.2 per 100 000, 22.9 per 100 000, and 18.9 per 100 000, respectively.

Role of HPV in malignant transformation
HPV infects the basal cells of the squamous epithelium. The viral E6 and E7 gene products interact with and inhibit host tumor suppressor gene products, p53 and retinoblastoma protein, respectively. These proteins are important for cell cycle control and apoptosis, and inactivation of these genes, which occurs with high risk HPV infections, can induce malignant transformation.13

Although high risk HPV infection is a prerequisite for cervical cancer, several other cofactors are needed for malignant transformation to occur. Persistence of high risk HPV infection at one and two years after initial infection is highly predictive of a lifetime risk of pre-invasive and invasive cervical neoplasia. The HPV genotype seems to be the most important factor in persistence, with HPV-16 and HPV-18 being the most likely to persist.14

Immunosuppression promotes persistent HPV infection. HIV coinfection may also promote HPV related malignant transformation at a molecular level. Women with HIV have a twofold to fivefold increased risk of HPV infection compared with HIV uninfected women, and they have a threefold to fivefold increased likelihood of developing CIN, recurrent lesions, and cervical cancer.15

Genetic predisposition may play a role in the development of persistent HPV infection. The role of the HLA genes in protecting against or increasing the risk of persistence has been studied intensively.16 17 The results have been variable and differentially associated with ethnic group. In the analysis of the entire HLA region, DQ/DR genes were found to be associated with the development of cervical cancer, whereas the HLA-DRB*1*13 group was linked to protection against cervical cancer.

Oral contraceptives and hormone replacement therapy may upregulate HPV viral expression.7 Active smoking and passive smoking are significant risk factors, with an odds ratio of 3.7 and 2.1, respectively, for the development of squamous cell cancer of the cervix.18

Other cofactors that may play a role in persistent infection include host factors, such as age and genetics, and external cofactors, such as nutrition and environment.

Screening tests for cervical cancer
Screening tests have traditionally identified an existing pre-invasive or invasive lesion.

The simplest and cheapest method—visual inspection with acetic acid (VIA)—is used in many mass screening programs in low income countries. When a 3-5% solution of acetic acid is applied to the cervix, normal dense lesions can be seen as acetowhite. The test has a specificity of 82% (range 64-98%) and sensitivity of 84% (66-96%) owing to a high false positive rate.19

The Papanicolaou (Pap) smear or cervical cytology has been the mainstay of cervical cancer screening for 60 years. The advantages of cytology are its simplicity and low cost. An abnormal cytologic report requires biopsy and confirmation by histology. Cytologic and histologic diagnoses agree in about 50% of cases. The sensitivity of cytology has been reported as 78% (range 30-87%), with a specificity of 62% (61-94%).20 However, these results were published more than two decades ago so they should be interpreted with caution. No recent studies have assessed the accuracy of cytologic testing and directly compared its sensitivity and specificity with current tests.

A case-control study comprising 1062 women who died from cervical cancer and 10 494 women without cervical cancer evaluated the risk of death based on
The false negative rate of Pap smears is hard to adequately measure but has been cited as 50%. Few large studies have been performed because most women who are screened do not undergo confirmatory colposcopy after a normal Pap smear. The sensitivity of cytology to detect high grade lesions ranges from 55% to 94%, depending on the laboratory, the experience of the cytologist, the adequacy of the sample, and the fixation technique.

One meta-analysis of 94 studies of conventional Pap smears and three studies of liquid based cytology gives a range of sensitivities from 30% to 87%.

A population based study found that 30-60% of women who presented with an invasive cervical cancer had normal Pap smears three to five years before diagnosis. This suggests that the negative predictive value of cytology is of short duration and that the test should be repeated at least every three years.

By contrast, the negative predictive value of HPV testing is high but it lacks specificity. The false negative rate for HPV polymerase chain reaction (PCR) analysis for detection of the presence of a cervical HPV related lesion is low at 0% (95% confidence interval 0% to 0.047%), and the specificity is 60.7%. The sensitivity of HPV testing is about 90%. Polymerase chain reaction (PCR) testing for HPV DNA had a very low false negative rate for predicting HPV related lesions of the cervix in a community based population. One study showed that women who tested negative for high risk HPV subtypes remained at low risk for the development of pre-invasive cervical lesions over a study period of five to 18 years.

There are three main strategies for HPV testing. These strategies have been examined in North America and Europe. The first—primary cytology with HPV triage—is the current approach for women under 36 years of age. This strategy is good in a population with a high prevalence of HPV. Current recommendations are to use the HPV test as reflex testing for atypical squamous cells of undetermined significance (ASCUS).

The second is primary HPV testing with cytology or colposcopy triage. This approach has low specificity unless used in a population with a low prevalence of HPV. The third strategy, which is currently used for women over 30 years, is to test with cytology also to test for HPV.

Despite the differences in sensitivity and specificity between screening tests, most cervical cancers occur because screening was not performed, rather than a failure of screening to detect the cancer. Sixty per cent of women with cervical cancer in the US have never had a Pap smear or have not had one in more than five years.

In low resource environments almost all women with cervical cancer have never been screened. Cytologic screening is significantly more effective at preventing cervical cancers and increasing survival rates than no screening at all—where intervention occurs only when a woman has abnormal symptoms. Several cohort studies have shown that the incidence of cervical cancer falls after the institution of screening. For instance, Japan instituted cytologic screening for women over 30 years in 1982. Since then mortality from cervical cancer has fallen by 50%—the incidence rate of invasive cervical cancer decreased from 13.4 to 7.2 per 100,000 women.
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from 1975 to 1998. The Japan Collaborative Cohort Study analyzed 63,541 women, aged 30-79 years, who were free from any cancer history at enrollment. An age-adjusted Cox model indicated significantly lower cervical cancer mortality rates in women who had Pap smear screening compared with those who did not (hazard ratio 0.30, 0.12 to 0.74). For women who were screened versus those who received treatment for cervical cancer only when they had symptoms, the proportion of women with screen detected invasive cancer who were cured was 92% (75% to 98%) versus 66% (62% to 70%) for unscreened women, a significant difference in cure rate of 26% (16% to 36%).

In summary, cytologic screening (Pap smear) was the first cervical cancer screening test to reduce the incidence and mortality of cervical cancer. Recent prospective studies from Japan and Sweden have confirmed that its use significantly reduces the incidence of cervical cancer. However, the sensitivity of this test to detect high grade lesions ranges from 55% to 94%, depending of the laboratory and the expertise of the technologists. HPV testing was first used as a triage test of mildly abnormal cytologic findings and is now used concurrently with Pap smear testing or as a primary screening test. HPV testing has increased the sensitivity of detecting abnormal lesions but has a lower overall specificity.

Types of HPV tests

Two general types of HPV tests are available: those that report the detection of high risk subtypes, without specifying which ones, and those that report the presence of HPV-16 and HPV-18. HPV can be detected through HPV DNA testing, RNA testing, and the detection of cellular markers of HPV associated malignant transformation.

A control for specimen adequacy is necessary for a negative HPV test to be meaningful. β globin is commonly measured by gel electrophoresis, enzyme linked immunosorbent assay (ELISA), or PCR to check for adequate epithelial cellularity. Specimens for HPV tests are obtained using a swab, cervical brush, or tampon, which is then placed in HPV transport test medium. Before HPV testing, the material from the swab has to be reconstituted in a liquid medium.

In the US, no Food and Drug Administration approved tests are available for head and neck, anal, or penile specimens, and there are no FDA approved tests that use serum or blood.

One hundred and forty eight different HPV tests are commercially available worldwide, and another 44 tests are variants of these. Most assays target the L1 or the E6/E7 region of the HPV genome. It cannot be assumed that all HPV screening tests are comparable. Some HPV tests that are currently available do not have a control for epithelial cellularity, and a test can be falsely negative if the sample has sparse or insufficient squamous cells.

In 2005, the World Health Organization established a worldwide network to standardize the quality of HPV laboratory testing, and it periodically reports on the proficiency of various HPV tests.

It is important to measure viral load so that the clinical significance of a positive HPV DNA test can be determined. A viral load of less than 10 HPV genomic equivalents is not clinically significant and probably reflects a transient infection. A viral load of greater than 10 genomic equivalents reflects the existence of dysplastic changes or a high risk of developing dysplasia.

There are two main methods of testing for HPV DNA, and all commercial DNA HPV tests use one of these two techniques. Signal amplification uses hybridization in the liquid phase. The second technique, target amplification, uses gene amplification with PCR. Detection of particular genotypes requires amplification followed by hybridization with specific probe types. In addition, quantitative detection of viral HPV DNA can be used to assess viral load. A third approach is the detection of mRNA encoding proteins E6 and E7 of high risk HPV subtypes.

Specific HPV tests

In the US and Europe four types of HPV tests are approved for primary screening: the hybrid capture 2 (HC2), Cervista HPV HR, Cobas 4800 System, and Aptima mRNA. These tests are approved for primary screening (as a co-test with a Pap smear) in women over 30 years and for reflex testing after a positive ASC-US result in women 21 years or more.

DNA tests

The HC2 assay (Qiagen, Gaithersberg, MD, USA; previously Digene Corp), which was approved by the FDA in 2003, is a nucleic acid hybridization assay that quantitatively detects HPV subtypes. It identifies the presence of 13 high risk HPV types.

The Cervista HPV HR test (Hologic), which was approved in 2009, detects HPV-66 in addition to the 13 subtypes detected by HC2. Another Cervista test (Cervista 16/18) that detects the HPV-16 and HPV-18 high risk subtypes is also available. It is approved for use only in women 30 years or more as a follow-up test after a positive HPV screen for the 14 high risk HPV types. The HPV 16/18 test is intended to be used adjunctively with cytology as follow-up to a less specific HPV test.

The Cobas 4800 System (Roche Molecular Systems, Alameda, CA USA) detects HPV-16 and HPV-18 and also provides a pooled result for another 12 high risk subtypes. The test simultaneously detects HPV-16 and HPV-18, along with HPV-31, HPV-33, HPV-35, HPV-39, HPV-45, HPV-51, HPV-52, HPV-56, HPV-58, HPV-59, HPV-66, and HPV-68. It is based on PCR, which is a sensitive technique, and can detect fewer than 10 copies of HPV DNA in a background of 1000 human cell DNA equivalents.

RNA tests

The Aptima mRNA test (Gen-Probe, San Diego, CA, USA) identifies E6 and E7 RNA, and it detects 14 high risk subtypes of HPV. This test has recently received FDA approval for cervical HPV testing. It has a similar sensitivity to HC2 of 100% but is more specific 84%.

Accuracy of HPV tests

Several trials have sequentially evaluated different HPV tests. Five HPV tests (HC2, RealTime HR-HPV, Aptima, Cervista HPV HR, and Cervista 16/18) were evaluated
in post-treatment follow-up in the Scottish Test of Cure Study (STOCS-H). Sensitivity was 100%, with all assays able to detect CIN 2 at six months after treatment. Specificity ranged from 75% to 84%. Another comparison study evaluated seven tests in 1099 women with abnormal smears: HC2, Cobas, RealTime, BD HPV test, PreTect Hvp-Proofer assay (NorChip), Apta, and p16 cytology. Sensitivities of HC2, Cobas, BD HPV test, Apta, and RealTime ranged from 93.5% to 96.3%, respectively, compared with 88.9% for cytology.16

While these studies found similar performance among the five tests, in the US, the 2014 FDA advisory panel recommended the Cobas 4800 System as the only HPV test for primary screening.7 This was based on the results of the ATHENA (Addressing THE Need for Advanced HPV diagnostics) trial, which showed that the Cobas 4800 System was more accurate than HC2.17 For CIN 3 the sensitivity and specificity of the Cobas 4800 System were 93.5% and 69.3%, respectively, compared with 91.3% and 70% for HC2.2

Overall, HPV screening tests that detect L1 DNA have a high negative predictive value but less than a 50% positive predictive value for the determination of CIN 2 and greater. The addition of tests for E6 and E7 mRNA improves the positive predictive value to 78%.18

In the setting of an HPV prevalence of 20.6%, PCR for HPV DNA had a false negative rate of zero and a 61.8% specificity for identifying HPV related lesions. That is 61.8% of women whose biopsies were negative were HPV DNA negative.2

**HPV as a primary screening test**

Although the prevention of cervical cancer should be used to evaluate the efficacy of a screening test, most studies use CIN 2 and CIN 3 to assess either equivalency of HPV with cytology or the predictive value of HPV testing. The important question is for how long does a negative HPV test predict the absence of cervical cancer? Three main types of study have been used to evaluate HPV testing: randomized studies, population based cohort studies, and cross sectional studies. It is difficult to summarize these studies because of the different study designs, time frames over which the populations were evaluated, and comparison groups.

**Study types**

Population based cohort studies and prospective randomized testing with long term follow-up give predictive information about future risk of cervical cancer and provide the highest level of evidence for the value of HPV testing compared with cytology.

Observational studies either directly compare HPV testing with cytology at one point in time to provide information on sensitivity and specificity of testing, or they follow a cohort over time.

Many studies have shown that HPV testing, alone or combined with cervical cytology, is more sensitive than cervical cytology alone at detecting high or low grade cervical histopathology. Table 1 summarizes the studies that have shown that HPV testing has a higher sensitivity than cytology for the detection of high grade pre-invasive disease.20,33

**Randomized studies**

Many studies have shown that HPV testing, alone or combined with cervical cytology, is more sensitive than cervical cytology alone at detecting high or low grade cervical histopathology. A randomized trial of HPV testing as the initial screen and cytology as triage versus cytology alone enrolled more than 100,000 women. Detection of CIN 2 was significantly higher for HPV DNA screening with cytology triage compared with conventional screening (relative rate 1.39, 1.03 to 1.88). Relative rates of detection of CIN 1 and CIN 3+ were not significantly different. The specificity of HPV testing with cytology triage was similar to conventional screening in all age groups, although in women aged 35 years or more specificity was higher for HPV testing with cytology triage than for conventional screening. The positive predictive values for HPV DNA screening with cytology triage were consistently higher than those for conventional screening. Interestingly, the detection of invasive cancer was the same in both arms. A trial of 60,901 women with both liquid based cytology and Cobas HPV testing showed that the Cobas HPV test was more sensitive than cytology for the detection of CIN 3 and cancer.51 In another randomized study of more than 18,000 women, HPV testing increased the detection of CIN 2+ compared with cytology alone.52

A summary of four European randomized trials (Swedescreeen, POBASCAM, ARTISTIC, NTCC) of HPV testing versus cytology examined the overall outcomes.39 HPV testing provided 60-70% greater protection against invasive cervical cancer over 6.5 years of follow-up. Each study compared cytology plus HPV testing with cytology alone.45 46 49 52

In all studies, women with HPV positive, cytology negative results underwent repeat HPV and cytology six to 18 months later before referral to colposcopy. There was an increased cumulative detection of CIN 2 and CIN 3 compared with cytology alone. In this population, however, the incidence of invasive cervical cancer was low—only 107 cervical cancers were identified in 176,464 women screened and followed over 6.5 years.

By contrast, the ARTISTIC study, which compared cytology with or without HPV testing, found similar overall rates of CIN 3 or invasive cancer in both groups over two rounds of screening.77 Cytology picked up equivalent rates of high grade lesions to HC2 testing in screened populations. This suggests that in well screened low prevalence populations, HPV and cytology are equally effective screening tools but in unscreened populations, HPV testing gives a higher yield for abnormal cervical findings.

Unlike the ARTISTIC study, a randomized trial of HPV versus cytology as a primary screening tool found that initial screening for HPV with and without cytology reduced the occurrence of invasive cervical cancer at the second round of screening.46

The ATHENA study examined the predictive value of Cobas to identify the risks of the development of CIN 2 and CIN 3.13 Absolute risks of CIN 2 by Cobas were 14% for all high risk HPV subtypes, 24% for HPV-16, and 4.3% for HPV-18 versus 0.75% for HPV negativity. The relative risk for CIN 2 and CIN 3 in women who were positive for high risk HPV was 18.6 and 29.7, respectively. A follow-up report of the ATHENA study evaluated 10 screening
strategies and determined that HPV testing with immediate referral to colposcopy gave the highest sensitivity of 89% but also the highest false positive rate of 38%.\(^{41}\)

**Cohort studies**
The Canadian Cervical Cancer Screening Trial (CCaST) followed 10,154 women aged 30-69 years with both HPV testing and conventional cytology.\(^{42}\) Sensitivity for CIN 2 and higher was 94.6% for HPV testing versus 55.4% for cytology. Specificity was 94.1% for HPV testing versus 96.8% for cytology.

The feasibility and efficacy of HPV testing as a primary screen in women aged 30-65 years over a three year time interval was evaluated.\(^{42}\) Women who tested positive (1646/23,739 women; 6.9%) were referred for a Pap smear. Women with Pap smears showing ASCUS or worse were referred to colposcopy. Women who tested HPV negative were rescreened every three years. The colposcopy rate per 1000 women with an HPV positive test was 19.36 compared with 14.54 for older Pap smear data. The detection rate of high grade cervical cancer was 6.58 compared with 2.37 for these historic controls (rate ratio 2.78). The investigators identified a serious problem with follow-up, however. Only 46% of HPV positive women received a Pap smear and only 24% underwent colposcopy. In a community setting, there is a risk of loss to follow-up with HPV testing.

A 13 year follow-up of a nested case-control study in Sweden (12,527 women) compared HPV testing with cytology every three years. It found a similar detection rate for new CIN 2 (3.2%) and CIN 3 (1.9%) in both groups,\(^{43}\) although HPV testing allowed earlier detection. Thus, the sensitivity of HPV based screening after five years was the same as for cytology based screening after three years. Women with a negative HPV test had a low risk of developing CIN, and the authors concluded that with HPV testing screening could be reduced from every three years to every five years. In a population based screening study, 40,000 women aged 30-59 years were randomized to cytology alone or

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**Table 1 | Summary of trials evaluating cytology versus HPV testing for cervical cancer screening**

<table>
<thead>
<tr>
<th>Study</th>
<th>Country</th>
<th>No of women</th>
<th>Age (years)</th>
<th>HPV test</th>
<th>Strategy</th>
<th>Follow-up</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pan 2014 (^{36})</td>
<td>China</td>
<td>25,404</td>
<td>15-59</td>
<td>HC2</td>
<td>Cytology, cytology+HPV (GP/5+/GP/6+) imaging co-testing; HPV alone; pooled analysis of 3 cross sectional population based studies</td>
<td>None beyond colposcopy and biopsy</td>
<td>Cytology+HPV co-testing had highest sensitivity and NPV does not allow assessment of lead time detection of CIN by cytology v HPV</td>
</tr>
<tr>
<td>Elfstrom 2009 (^{36})</td>
<td>Sweden</td>
<td>12,527</td>
<td>23-60</td>
<td>GP 5+/6+ PCR HPV</td>
<td>Cytology v cytology/HPV every 3 years; ages 23-50; every 5 years; ages 51-60</td>
<td>13 years cumulative incidence of CIN 2/3 same for HPV+cytology and cytology, HPV testing allows earlier detection</td>
<td></td>
</tr>
<tr>
<td>Mayrand 2007 (^{36})</td>
<td>Canada</td>
<td>10,154</td>
<td>30-69</td>
<td>HC2</td>
<td>Cytology v HPV test</td>
<td>3 years</td>
<td>HPV more sensitive and less specific than cytology for CIN 2/3</td>
</tr>
<tr>
<td>Cox 2013 (^{36})</td>
<td>USA</td>
<td>47,000</td>
<td>&gt;21</td>
<td>Cobas</td>
<td>10 screening strategies</td>
<td>1 year</td>
<td>HPV alone with colposcopy for HR-HPV most sensitive with highest false positive rate</td>
</tr>
<tr>
<td>Louanto 2014 (^{27})</td>
<td>Canada</td>
<td>23,739</td>
<td>30-65</td>
<td>HC2</td>
<td>HPV screen; if positive then Pap smear; if abnormal then colposcopy</td>
<td>3 years</td>
<td>Detection of HSIL increased 3-fold. Time to colposcopy with HPV decreased from 11 months to 3 months; follow-up poor, only 46% of HR-HPV women had cytology; only 24% of HR-HPV women had colposcopy</td>
</tr>
<tr>
<td>Castle 2012 (^{28})</td>
<td>UK</td>
<td>19,512</td>
<td>16-94</td>
<td>HC2 and prototype Cervista 16/18</td>
<td>Cervical Lavage tested retrospectively for HR-HPV women followed prospectively with routine cytologic screening</td>
<td>18 years</td>
<td>HPV testing predicted who developed CIN 3 10-18 years later</td>
</tr>
<tr>
<td>Kati 2011 (^{36})</td>
<td>USA</td>
<td>315,061</td>
<td>&gt;30</td>
<td>HC2</td>
<td>Co-testing and assessment of risk of CIN 3</td>
<td>6 years</td>
<td>Prevent CIN 3 similar for HPV and cytology; HPV predicts future risk</td>
</tr>
<tr>
<td>Zarri 2013 (^{37})</td>
<td>Italy</td>
<td>11,895</td>
<td>25-64</td>
<td>HC2</td>
<td>HPV screen; if positive then Pap smear; if abnormal then colposcopy</td>
<td>2 years</td>
<td>7% HR-HPV; compliance 78.6%; referral to colposcopy 4.6%; CIN 2 detection 4.5%</td>
</tr>
</tbody>
</table>

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*ASCSUS= atypical squamous cells of undetermined significance; CIN=cervical intraepithelial neoplasia; HC2=hybrid capture 2; HPV=negative predictive value; Pap=Papanicolaou; HPV=human papillomavirus; HR=high risk; PCR=polymerase chain reaction.\(^{44}\)*

Note: PCR based assays that use the general primer mediated 5+/6+ (GP/5+/GP/6+) systems to amplify sequences from the L1 region of the HPV genome.

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**Randomized studies**

<table>
<thead>
<tr>
<th>Study</th>
<th>Country</th>
<th>No of women</th>
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<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stoler 2013 (^{36})</td>
<td>USA</td>
<td>47,008</td>
<td>&gt;21</td>
<td>Cobas v HC2</td>
<td>ASCUS Pap smears, colposcopes, biopsies, HPV test</td>
<td>None</td>
<td>Cobas test comparable to HC-2</td>
</tr>
<tr>
<td>Kitchener 2009 (^{36})</td>
<td>UK</td>
<td>24,510</td>
<td>20-64</td>
<td>HC2</td>
<td>Cytology v cytology/HPV</td>
<td>2 years</td>
<td>Co-testing did not detect higher rate of CIN 2/3</td>
</tr>
<tr>
<td>Rijkstra 2012 (^{24})</td>
<td>Netherlands</td>
<td>44,938</td>
<td>29-56</td>
<td>GP 5+/6+ PCR HPV</td>
<td>Cytology v cytology/HPV</td>
<td>5 years</td>
<td>Earlier detection with HPV but no difference in incidence of CIN 3</td>
</tr>
<tr>
<td>Leinonen 2009 (^{36})</td>
<td>Finland</td>
<td>108,425</td>
<td>25-65</td>
<td>GP 5+/6+ PCR HPV</td>
<td>Cytology v cytology/HPV</td>
<td>HPV detected more CIN 2; no difference in cervical cancer rate</td>
<td></td>
</tr>
<tr>
<td>Oglvie 2012 (^{36})</td>
<td>Canada</td>
<td>18,648</td>
<td>25-65</td>
<td>HC2</td>
<td>Cytology v different referrals to colposcopy</td>
<td>HPV arm had increased CIN 2 detection v cytology alone</td>
<td></td>
</tr>
<tr>
<td>Ronco 2010 (^{36})</td>
<td>Italy</td>
<td>94,370</td>
<td>25-69</td>
<td>HC2</td>
<td>Round 1: cytology v cytology/HPV test; round 2: HPV test alone</td>
<td>3 years</td>
<td>Detection ratio of CIN 2 in women aged 25-34 in round 1 and 0.64 in round 2</td>
</tr>
<tr>
<td>Ronco 2014 (^{36})</td>
<td>Sweden, Netherlands, UK, Italy</td>
<td>176,464</td>
<td>20-64</td>
<td>HC2 and GP 5+/6+ PCR HPV</td>
<td>HPV v cytology; pooled analysis of 4 randomized trials</td>
<td>6.5 years</td>
<td>HPV screening gives 60-70% increased protection</td>
</tr>
</tbody>
</table>

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*HPV+cytology and cytology; HPV testing HPV+cytology and cytology; HPV testing

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However, two cohort studies identified concerns that 21-64% of women who were positive for high risk HPV subtypes were lost to follow-up. 62 63

Use of HPV testing for follow-up after treatment for cervical disease
Disease recurs in 5-20% of women treated for high grade cervical dysplasia. 56 Observational studies show that HPV testing is as specific after the treatment of CIN as it is when used as a triage tool, identifying residual and recurrent pre-invasive disease more efficiently than cytology. 57 On the basis of case series, the ideal time to repeat HPV testing after a cone biopsy is 18-24 months. 58

HPV testing can be used as a test of cure because it has a sensitivity of 85-97%. 59 Knowledge about the subtype of high risk HPV helps predict the subsequent risk of CIN. In a 14 year follow-up of a 12,527 women in a Swedish cohort, women who were positive for HPV-16, HPV-18, HPV-31, or HPV-33 had a 14 year cumulative incidence for developing CIN 3 of greater than 28%. This figure was 14-18% for women who were positive for HPV-35, HPV-45, HPV-52, or HPV-58, and less than 10% for those who were positive for HPV-39, HPV-51, HPV-56, HPV-59, HPV-66, or HPV-68. 59

HPV testing after treatment for high grade cervical lesions is predictive of risk of recurrence, persistent infections, and time to next recurrence. In a study of 58 paired cervical cone biopsies, 95.9% of women had persistent high risk HPV infection. Of these, 74.5% were HPV-16 and HPV-18 positive. The time between the first and second cone biopsy was shorter for women over 40 years (median time 2.6 years) than for those under 40 years (6 years) and for women with HPV-16 and HPV-18 infections (1.8 years) than for those with other high risk subtypes (3.8-8.2 years). 60 These data reinforce the importance of age and HPV subtype in persistent HPV infections.

Accuracy of HPV testing
Poor specificity and correspondingly poor positive predictive value limits the use of HPV testing alone as a primary screening test, particularly in younger women. HPV testing has better specificity in women over 30 years than in younger women. In addition, HPV infection in older women is more likely to be persistent and is therefore more likely to be clinically significant. 61

False negative HPV testing has been evaluated in retrospective studies. 62 63 64 Women with HPV negative ASC-H (atypical squamous cells rule out high grade dysplasia) have a 2% risk of developing invasive cancer in the next five years. Women who have HSIL on cytology but a negative HPV test still have a 29% risk of developing CIN 3 and a 7% risk of developing invasive cancer in the next five years. 65 Women who have HSIL on cytology who also have a falsely negative HPV test or whose cancer is not related to HPV will be missed by an HPV only screening protocol.

The HC2 test has a false negative rate of 1-5% in CIN 2/3. False negative rates are higher for small lesions and in women over 40 years. The observation that HPV testing plus HPV testing using PCR. 66 At five years, all women had a repeat Pap smear and HPV test. HPV testing led to earlier detection but not a reduced incidence of high grade cervical lesions.

A population study based in clinical practice of women over age 30 years used HPV and cytology in various combinations. It found a five year incidence of cervical cancer of 3.2 per 100,000 women in those who were both HPV and cytology negative, 3.8 per 100,000 in women who were HPV negative and 7.5 per 100,000 in women who were negative for cytology. 67 Of note, 17 of the 27 women with adenocarcinoma were positive on HPV testing but negative on cytology, which confirms the difficulty in detecting adenocarcinoma with cytology.

The most compelling predictive data on HPV testing come from a study that looked at 19,512 women who were followed with Pap smear for up to 18 years. All women had undergone cervical lavage, which was retrospectively tested for high risk HPV. HPV testing for HPV-16 and HPV-18 predicted who would develop CIN 3 10-18 years later. 68

Cross sectional studies
A meta-analysis of 25 studies, 24 of which were cross sectional, showed a sensitivity of HC2 HPV testing of 90% for detecting CIN 2 compared with cytology, but specificity was lower than for cytology (86.5% vs 91.9%). 69

A pooled analysis of 13 cross sectional studies from China directly compared four screening strategies: cytology alone, cytology with HPV triage for ASCUS results, HPV testing with cytology triage for positive HPV results, and cytology with HPV co-testing. 70 Cytology with HPV co-testing had the highest sensitivity (99.3% for CIN 3) and negative predictive value but the lowest specificity (76%) and positive predictive value (71%). Referral to colposcopy ranged from 9.6% for HPV testing alone with cytology triage to 25% for co-testing. The standard protocol of cytology alone with HPV triage for ASCUS led to a sensitivity of 95%, specificity of 91.3%, positive predictive value of 16.2, negative predictive value of 99.9, and a 10.5% referral rate to colposcopy.

Other strategies for HPV screening
Another triage strategy to reduce colposcopy referrals is the genotyping of high risk HPV to identify HPV-16 or HPV-18 when high risk HPV testing is positive but cytology results are negative. Women with HPV-16 and normal cytology have a 10% risk of developing CIN 3. 71 One caveat is that 30% of cervical cancers are associated with HPV subtypes other than HPV-16 and HPV-18. 72 Therefore, clinical judgment (cytology, clinical symptoms, and examination) should be used when deciding which HPV-16, HPV-18 negative women to refer to colposcopy. Conversely for women with ASCUS cytology who are positive for any HPV subtype, the five year cumulative risk of CIN 3 and cancer is 6.8%. This group should be referred for colposcopy without the need for genotyping. 73

Summary of HPV testing strategies
Testing for high risk HPV is more sensitive than cytology at identifying pre-invasive but not invasive cervical lesions.
prevalence has two peaks—women under 30 years and those in their mid-50s—has led to the concept of HPV latency with later reactivation of infection. According to this hypothesis, HPV infections can be dormant in patients with normal immunity but be reactivated at a later age. Women with latent infections will have a negative HPV test.62

The detection of high risk HPV can vary with the menstrual cycle,63 so there is a risk of missing an HPV infection when using a single DNA test. In one study, 33 women aged 22-53 years took vaginal swabs twice weekly for 16 weeks. A significant short term variation in positivity was noted, with an estimated risk of missing 24% of HPV positive smears.64 Neither vaginal sex nor condom use during follow-up was associated with recurrent viral detection or loss of detection.

**HPV testing in resource poor environments**

Cervical cancer is the second most common cancer in women in developing countries and in low and middle income countries, where 85% of deaths from cervical cancer occur.1

The prevalence and distribution of HPV subtypes varies geographically and ethnically.65 The International Agency for Research on Cancer (IARC) HPV prevalence survey, which tested women from 26 regions for HPV subtype infections, reported a high prevalence of HPV in sub-Saharan Africa, Latin America, and India. These regions have the highest rates of cervical cancer worldwide.66

In a retrospective evaluation of 8977 paraffin specimens of invasive cervical cancer from six continents, high risk HPV subtypes were identified using PCR analysis.67 The prevalence of HPV-16 associated cancer was 66-72% in Europe and North America, 59% in South America and Oceania, 60% in Asia, and 48% in Africa. The greatest proportion of HPV-18 associated cervical cancers (23%) occurred in Africa. Ten per cent of cervical cancers in Africa and 4-7% of cancers on other continents were associated with high risk HPV-45.

An international cost effectiveness analysis evaluated several strategies for cervical cancer screening in Thailand, India, Peru, Kenya, and South Africa.68 It found that the lifetime risk of cervical cancer was reduced by 25-35% in women who are screened once in their lifetime, at around age 35 years, with VIA or HPV testing. The risk is reduced by 40% if women are screened twice.

More than 90% of women in low and middle income countries have never had a Pap smear because of a lack of infrastructure and the need for skilled cyto technologists. Programs have been developed where slides can be processed and screened on site during a clinic session.69

In a meta-analysis of 11 African and Indian cohort and cluster randomized trials of VIA followed by colposcopy and biopsy, VIA had a sensitivity of 79% and a specificity of 85% for the detection of CIN 2 or greater.70 In a seven year follow-up in which more than 80 000 women were randomized to VIA versus health education, the incidence of cervical cancer decreased significantly by 25% in the VIA group compared with education alone (incidence hazard ratio 0.75, 0.55 to 0.95) and mortality hazard ratio 0.65 (0.47 to 0.89).71 Women in rural India were randomized to HPV testing with HC2 or no screening, cytological analysis, or VIA. In eight years of follow-up, there were 34 deaths from cervical cancer in the HPV screened group versus 54 deaths in the cytology group, 56 deaths in the VIA group, and 64 in the no screen group (hazard ratio 0.52, 0.33 to 0.83).72

In general, HPV tests are too expensive for low resource settings. However, the careHPV (Qiagen, Gaithersberg, MD, USA) kit costs just $5.00 (£3.3; €4.4). In a randomized study of 2388 women aged 30-54 years, VIA, cytology, HPV testing with HC2 and careHPV was performed. Colposcopy with biopsy was done as needed. The sensitivities and specificities, respectively, of detecting CIN 2/3 were 61.4% and 94.5% for VIA, 85.3% and 87.5% for cytology, 97.1% and 85.6% for HC2, and 84.3% and 87.7% for careHPV (areas under the curve significantly different, $P/=0.001$ and $P/=0.003$, for cervical and vaginal specimen comparisons for the careHPV test, respectively).

The IARC is creating an electronic directory of global screening programs through its screening group (http://screening.iarc.fr/cervicalindex.php). As more complete data on HPV prevalence by region and screening programs emerge, it will be possible to design low cost screening and vaccination programs that are tailored to different regions and ethnic groups.

**HPV vaccination and the future of HPV testing**

The current duration of protection is 8.4 years for the bivalent vaccine (HPV-16 and HPV-18) and five years for the quadrivalent vaccine (HPV-6, HPV-11, HPV-16, and HPV-18).73 HPV-16 and HPV-18 vaccination has reduced CIN 3 by 17-33%, and colposcopy and treatment by 10% and 25%, respectively.74 A nine valent prophylactic vaccine (HPV-6, HPV-11, HPV-16, HPV-18, HPV-31, HPV-33, HPV-45, HPV-52, and HPV-58) is currently being developed and tested. This new vaccine will extend protection against oncogenic HPV subtypes.

**Guidelines**

Table 2 summarizes guidelines and current data on rates of cervical cancer by age group.

WHO has published guidelines for cervical cancer screening and a guide to care.75 These guidelines are mindful of resource poor regions and are pragmatically focused on women aged 30-50 years. WHO recommends primary screening with HPV testing, if possible, over VIA or cytology. Several algorithms are given for the management of positive high risk HPV results but immediate treatment with cryotherapy of loop electrosurgical excision is encouraged.

The 2012 guidelines from the American Cancer Society, US Preventive Services Task Force, and the American College of Obstetricians and Gynecologists and the European guidelines from the IARC continue to be the standard and accepted guidelines for cervical cancer screening in North America and Europe, respectively.2 76 Screening should start at age 21 years. Cytologic screening alone should be performed every three years. For women aged 30-65 years, either cytologic screening every three years or cytology and HPV co-testing every five years if the results
are negative is recommended. Women should discontinue screening at age 65 years if they have had three negative Pap smears or two negative Pap and HPV tests in the preceding 10 years. Women who have been treated for pre-invasive disease should continue to be screened annually for at least 20 years after treatment. These screening guidelines do not apply to high-risk women with a history of lower genital tract neoplasia or other risk factors for malignant transformation, such as immunosuppression (for example, women with HIV infection) or a history of diethylstilbestrol exposure.

Given that the new data on the prevalence of cervical cancer (adjusted for women who have had a hysterectomy) show increased rates of cervical cancer in women over 65 years, the age to stop cervical cancer screening must be reconsidered. Careful prospective documentation of the incidence of cervical cancer after age 65 years will guide future recommendations.

Concern has been raised that reducing the frequency of co-testing with a Pap smear and HPV testing will increase the incidence of cervical cancer. A modelling study showed that the recent US Preventive Task Force guideline revision (co-testing every five years instead of every three years) would lead to an extra one in 369 women who follow screening guidelines developing cervical cancer.

The newest FDA advisory approval uses the Cobas 4800 System for primary HPV screening after age 25 years. The FDA panel suggests that women who test positive for HPV-16 or HPV-18 should have an immediate colposcopy. It recommends cytology triage first for the other 12 high risk HPV types. The panel stresses that its recommendation does not change current medical practice guidelines for cervical cancer screening.

**RESEARCH QUESTIONS**

What is the incidence of cervical cancer in women over 65 years who have not undergone hysterectomy?

What factors lead to persistent high risk human papillomavirus (HPV) infections and how can this be measured?

What is the ideal follow-up for a high risk HPV positive test?

How should guidelines change for women who have received HPV vaccination?
Follow-up.


