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HPV testing as a screen for cervical cancer

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The lifetime cumulative risk of HPV infection is greater than 80%

Introduction

Cervical cancer is a leading cause of death in women worldwide, with 530 000 new cases and 275 000 deaths worldwide each year.[1] Cervical cancer is preventable because it has a long pre-invasive phase and is easily identified by clinical and histopathological examination.

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.[2]

SOURCES AND SELECTION CRITERIA

Medline, CINAHL, Embase, the Cochrane library, and PubMed were searched between 1 January 1990 and 30 June 2014 with no language restrictions. Search terms included "HPV", "human papillomavirus", "HPV test", "HPV screening", "HPV vaccination", "cervical neoplasia", "cervical carcinogenesis", "cervical cancer screening", "cervical cancer prevention", "cervical cytology", "Papanicolaou smear", and "Pap smear". References identified from relevant articles were also searched. Articles were selected if they contained level I or level II-1-3 evidence. Publications from low and middle income countries that reported case series were included.

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.[1] 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%.[3] 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.[4] 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.[3]

HPV subtypes

More than 130 HPV genotypes have been identified and can be subdivided into mucosal and cutaneous. HPV infections of the genital tract have been subclassified into those associated with benign (low risk) and malignant (high risk) genital tract disease.

Almost all types of cervical cancer—squamous cancer, adenosquamous cancer, and adenocarcinoma—are now thought to be associated with HPV infections. HPV-16 and HPV-18 are associated with 70% of all invasive cervical cancers.[7]

Acquisition and natural course

HPV infection is sexually transmitted. 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] Persistent infection is defined as the presence of high risk HPV for longer than two years.

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.

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.

Immunosuppression promotes persistent HPV infection. HIV coinfection may also promote HPV related malignant transformation at a molecular level.

Genetic predisposition may play a role in the development of persistent HPV infection.[16 17]

Oral contraceptives and hormone replacement therapy may upregulate HPV viral expression.[7] Active smoking and passive smoking are significant risk factors, (odds ratio 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. 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. An abnormal cytologic report requires biopsy and confirmation by histology. Cytologic and histologic diagnoses agree in about 50% of cases. The sensitivity of

It cannot be assumed that all HPV screening tests are comparable

cytology has been reported as 78% (range 30-87%), with a specificity of 62% (61-94%).[20]

HPV testing was originally used as reflex testing (after the cytologic diagnosis of atypical squamous cells) to help triage atypical Pap smears to colposcopy or to close follow-up. HPV testing was not recommended by the American Congress of Obstetricians and Gynecologists (ACOG) when a gross cervical lesion was present or when a cytologic report was severely abnormal.[24] HPV testing followed cytology as the next generation of testing in asymptomatic women with visually normal cervices to predict future risk of cervical cancer.

Rationale for HPV testing in cervical cancer screening

The sensitivity of cytology varies between countries and cytology laboratories and depends on the medical infrastructure of a particular region.

The false negative rate of Pap smears is hard to adequately measure but has been cited as 50%.[20] 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.[20]

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%.[25]

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.[26]

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%.[27] 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.[27] 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.[28]

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 30 years. 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).[22]

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.[29]

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.[32 33] 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. 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.

Most assays target the L1 or the E6/E7 region of the HPV genome.[34] 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.

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.[34]

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. 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.

Accuracy of HPV tests

Several trials have sequentially evaluated different HPV tests. Five HPV tests were evaluated in post-treatment follow-up in the Scottish Test of Cure Study (STOCS-H).[35] 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. Sensitivities of HC2, Cobas, BD HPV test, Aptima, and RealTime ranged from 93.5% to 96.3% compared with 88.9% for cytology.[36]

In the US, the 2014 FDA advisory panel recommended the Cobas 4800 System as the only HPV test for primary screening.[2] 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.[37] 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%.[38]

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

Study types

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 (see thebmj.com) summarizes the studies that have shown that HPV testing has a higher sensitivity than cytology for the detection of high grade pre-invasive disease.[26-50]

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.[54] One caveat is that 30% of cervical cancers are associated with HPV subtypes other than HPV-16 and HPV-18.[55] 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.[55]

Summary of HPV testing strategies

Testing for high risk HPV is more sensitive than cytology at identifying pre-invasive but not invasive cervical cancer in cross sectional studies. High risk HPV testing gives superior predictive information about future risk of cancer.

However, two cohort studies identified concerns that 21-64% of women who were positive for high risk HPV subtypes were lost to follow-up.[42 43]

Use of HPV testing for follow-up after treatment for cervical disease

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]

30% of cervical cancers are associated with HPV subtypes other than HPV-16 and HPV-18

HPV testing can be used as a test of cure because it has a sensitivity of 85-97%.[35] Knowledge about the subtype of high risk HPV helps predict the subsequent risk of CIN 3. 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]

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]

The observation that HPV 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]

HPV testing in resource poor environments

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]

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

Table 2 | Primary screening guidelines

Age range (years)	Cervical cancer/ 100 000 US women	Guidelines			
		IARC[74]	ASCCP 2012[29]	WHO[75]	FDA advisory panel 2014[2]
0-20	0.1	No screening	No screening	No screening	No screening
21-25	4.5	No screening	Cytology every 3 years	No screening	Follow ASCCP guidelines
25-30	4.5	Cytology every 5 years, HPV preferred	Cytology every 3 years	No screening	Primary HPV test (Cobas) or as co-test with cytology
31-39	13.9	Cytology every 5 years, HPV preferred	Cytology every 3 years cytology or cytology with HR-HPV every 5 years	Cytology, HR-HPV, or VIA (primary HPV best) every 3-5 years	Follow ASCCP guidelines
40-49	16.5	Cytology every 5 years, HPV preferred	Cytology every 3 years cytology or cytology with HR-HPV every 5 years	Cytology, HR-HPV, or VIA (primary HPV best) every 3-5 years	Follow ASCCP guidelines
50-64	15.4	Cytology every 5 years, HPV preferred	Cytology every 3 years cytology or cytology with HR-HPV every 5 years	Cytology, HR-HPV, or VIA (primary HPV best) every 3-5 years	Follow ASCCP guidelines
>65	14.6	Stop screening at 60-64 years	Stop screening	Not covered	Follow ASCCP guidelines
65-69	27.4				
70-79	23.7				
80-84	22.9				
>85	18.9				

ASCCP= American Society for Colposcopy and Cervical Pathology; FDA=Food and Drug Administration; HR-HPV=high risk human papillomavirus; IARC=International Agency for Research on Cancer; WHO=World Health Organization.

of infrastructure and the need for skilled cytotechnologists. Programs have been developed where slides can be processed and screened on site during a clinic session.[69]

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 41.4% 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=0.0001 and P=0.0031, for cervical and vaginal specimen comparisons for the careHPV test, respectively).

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).[72 73] HPV-16 and HPV-18 vaccination has reduced CIN 3 by 17-33%, and colposcopy and treatment by 10% and 25%, respectively.[72] 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 74] 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.[29] 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.[12] 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 modeling 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.[76]

The newest FDA advisory approves the use of the Cobas 4800 System for primary HPV screening after age 25 years.[2] 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.

HPV-16 and HPV-18 vaccination has reduced CIN 3 by 17-33%, and colposcopy and treatment by 10% and 25%, respectively