Recent Advances in Cervical Cancer Screening

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Centers for Disease Control and Prevention (CDC) estimates that about 11,967 new cases of HPV-associated cervical cancer are diagnosed in the United States each year. More black and Hispanic women get cervical cancer than women of other races or ethnicities, possibly because of decreased access to Pap testing or follow-up treatment.

Based on solid evidence, cervical cancer screening and protection against HPV infection by vaccination against HPV types 16/18, use of barrier contraceptives, and sexual abstinence decreases cervical cancer incidence. Also avoidance of active and passive cigarette smoking, high parity, and long term use of contraceptives decreases the risk of cervical cancer.

In this article, we aim to review the preventative and screening methods for cervical cancer. Discussion of the latest Cervical Cancer Screening Guidelines chart given by CDC that compares recommendations from the American Cancer Society, Preventive Services Task Force, and the American College of Obstetricians and Gynecologists is done.

With the FDA approval of the first HPV test for primary cervical cancer screening on April 25th, 2014, clinicians now have 3 different first line screening options, the Pap test, co-testing with Pap and HPV tests, and HPV testing as a stand-alone test. Specifically, the Roche Cobas® HPV Test was approved for primary screening for cervical cancer as a stand-alone test.

Clinical trials that evaluate cancer-screening methods are taking place in many parts of the country. Ongoing trials on the development of Human Papillomavirus Type 16 E7- specific Human Immunologic Assays in Non-HLA2 Type Human Being, Molecular markers in Cervical Cancer Screening, and multispectral digital colposcopy are going on. These improvements in screening strategies along with therapeutic and preventive methods contribute significantly to the control and prevention of cervical cancer.

Key Words: HPV, cancer screening guidelines, HPV test, cervical cancer

INTRODUCTION

Cervical cancer incidence and mortality have markedly declined in the United States since the introduction of cervical cancer screening in the 1950s and 1960s. However, cervical cancer still remains an important public health issue.

The incidence of cervical cancer has declined since 1957 when cervical pap smears were introduced but this decline tapered off during 2006 to 2010, mainly among younger women. The rates were stable in women younger than 50 years of age and declined by 3.1% per year in women over 50 years. From 2006 to 2010, death rates had plateaued despite aggressive screening protocols among women younger than 50. About 12,360 cases of invasive cervical cancer were diagnosed and 4020 deaths reported in 2014 alone.

While primary prevention would be ideal in the control of cervical cancer, it has practical challenges. Although human papilloma virus (HPV) vaccination is safe and effective, there are challenges in its implementation. According to teen vaccination coverage data from CDC, there is a persistent coverage gap between HPV vaccination and other vaccinations recommended for adolescents to protect adolescents from HPV-related cancers.

Screening techniques include cytology, visual inspection with 3%-5% acetic acid and magnification, Lugol’s iodine, colposcopy, HPV testing, and a combination of these methods. Since the implementation of Pap test, various modifications have been made to improve the sensitivity and specificity. The advent of HPV DNA testing is having a tremendous impact on the way that screening for cervical cancer is conducted. Screening strategies are constantly being revised. Recently, E6/E7 based mRNA studies and p16 immuno tests have been introduced that target the molecular alterations associated with transformation rather than simply detecting high risk HPV (hr-HPV) infections. mRNA, and p16 transformation studies are more specific than HPV DNA test.

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CERVICAL CANCER SCREENING - EVOLUTION

The initial and most important step in cervical cancer screening was the introduction of Papanicolaou (Pap) testing. Implementation of Pap testing resulted in declined incidence of cervical cancer between 1955 and the mid-1980s. With the improvements in diagnostic testing and treatment protocols, the incidence of cervical cancer has further decreased. The Bethesda System for reporting cervical cytology was developed at an NCI workshop in 1988, and was first used in 1991. In 2001, it was further updated to improve the utility and understandability of results.

The clinical performance of the cytology-based screening technology has limitations. The sensitivity of the conventional Pap test for the detection of high-grade lesions has a wide range from 30% to 87%. To overcome this limitation, liquid based cytology (LBC) was developed. In LBC, the sampling technique involves use of a cytobrush which is rotated by 360 degrees five times around the cervix and the exfoliated cells in the cytobrush are stirred in a proprietary solution. This sampling method reduces specimen inadequacy by 80% and the specimen can be used for HPV, chlamydia and gonorrhea testing in addition to cytological examination. The ThinPrep Pap test was approved by FDA in 1996 and three years later, SurePath Pap test was also approved. Women now benefit from a lower inadequacy rate of cervical samples since the switch from conventional methods to LBC method. According to some studies, the clinical sensitivity of LBC in the detection of high-grade lesions has increased from 88% to 93% compared to conventional tests. Other advantages of LBC include improvement in sample adequacy, reproducibility, and ability to support HPV co-testing.

Automation of manual screening of cytology slides is another advancement that occurred over the past 20 years. This method screens the slide automatically and presents a number of fields that are reviewed by the cytotechnologist, thereby reducing screening errors and increase productivity. Two automated screening systems have been developed, which are the BD FocalPoint GS Imaging System and the ThinPrep Imaging System. The BD FocalPoint system uses SurePath liquid-based cytology and the ThinPrep imager uses ThinPrep liquid-based cytology. FDA approval of these two imaging systems for primary cervical screening is based on evidence that they are capable of detecting an equivalent or higher percentage of high-grade dysplasia than manual screening.

Molecular and epidemiologic studies have demonstrated strong association between high-risk strains of HPV and cervical carcinoma. Tests for high-risk HPV DNA have been developed for use on cervical samples. Studies show that the high-risk HPV tests can improve the sensitivity of the Pap test to greater than 95%. These improvements in cytologic screening techniques as well as the introduction of HPV DNA testing has greatly facilitated the identification of women at high risk for cervical cancer and the revision of screening strategies.

Of recent, several biomarkers that play an important role in the cervical cancer development are being studied. There markers include p16INK4a (CDKN2A), survivin (BIRC5), metalloproteinase 9 (MMP9), topoisomerase 2 alpha (TOP2A), minichromosome maintenance 5 (MCM5), and MKi67 proteins (MKi67).

HPV TESTS

Large randomized trials conducted in many parts of the world suggest the use of HPV tests as a primary screening tool. There are validation guidelines and laboratory guidelines suggested for HPV testing. The sensitivity of the candidate test for ≥ CIN2 should be at least 90% of the sensitivity of the reference assay which is Hybrid Capture 2 HPV DNA test. The specificity of the candidate test for ≥ CIN 2 should be at least 98% of the specificity of the reference assay.

Evidence shows that HPV testing generally has a higher sensitivity but lower specificity than does cytology in the detection of CIN2 and CIN3. Among women older than 30 years, cytology had a specificity of 97% compared with 94% for HPV testing. The specificity of HPV DNA testing would be even lower among women younger than 30 years, who have more transient HPV infections. To improve specificity and minimize over-treatment with HPV DNA testing, the suggested approaches are (1) triage HPV-positive results with cytology or another more specific molecular assay and (2) further workup pursued only after two sequential positive HPV tests. The reason for the lesser sensitivity of HPV testing for invasive cervical cancers is explained. The invasive cells have major molecular rearrangements and the viral DNA load is lower. Many countries are considering HPV testing as a primary screening tool followed by triage with Pap test. FDA approved HPV tests are compared below in Table 1.

CLINICAL UTILITY AND SCOPE OF HPV DNA TESTING

There is variation in interpretation of ASCUS Pap smears among cytopathologists. 2.5 million ASCUS Pap results are reported every year in the United States. HPV DNA testing is utilized in this scenario to avoid unnecessary colposcopy procedures. Patients with ASCUS who turn out to be positive for high-risk HPV DNA go for colposcopy and those who are negative have repeat Pap tests at 6 months and 12 months. If the repeat Pap tests are also negative, patients revert back to the routine screening guidelines.

The combination of the high sensitivity of HPV DNA testing and the high specificity of cytology can increase the screening interval in women tested negative with cytology and HPV DNA tests. Such a combined test was approved by the FDA in 2003 for primary screening of low risk women aged ≥ 30 years every 3 years. Large-scale studies have provided solid evidence for the existence of HPV-negative cervical cancers. Co-testing with cervical Pap cytology and FDA-approved hrHPV tests will maximize the detection of cervical cancers.
<table>
<thead>
<tr>
<th>FDA approved HPV assays</th>
<th>Clinical Indications</th>
<th>Detection method</th>
<th>HPV typed detected</th>
<th>Collection and processing of specimens</th>
<th>Limitations</th>
<th>Advantages</th>
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<tbody>
<tr>
<td>Hybrid Capture 2 HPV DNA test</td>
<td>1. Reflex testing of patients with ASC-US, to determine the need for referral to colposcopy. 2. In conjunction with routine Pap testing of women over age 30 to adjunctively screen for the presence or absence of high-risk HPV types</td>
<td>Qualitative detection using In vitro nucleic acid hybridization assay with signal amplification using microplate chemiluminescence</td>
<td>13 high-risk HPV types 6, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68</td>
<td>The HC2 DNA Collection Device or HC Cervical Sampler (cervical broom) with samples deposited in either Digene Transport Medium or a Cytyc PreservCyt vial</td>
<td>Cross-reactivity of its probe cocktail with untargeted HPV types resulting in inaccurate results. Lack of an internal control to evaluate specimen adequacy or the presence of potentially interfering substances.</td>
<td>Most frequently used diagnostic HPV test worldwide. The recommended reference assay.</td>
</tr>
<tr>
<td>Cervista HPV HR Test</td>
<td>Same as HC2</td>
<td>Qualitative detection using invader chemistry. It is a signal amplification method for detecting specific nucleic acid sequences utilizing isothermal reactions.</td>
<td>14 high-risk HPV types -16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68</td>
<td>Collected in PreservCyt solution of the ThinPrep Pap Test preservation system, using a broom-type device or endocervical brush/spatula.</td>
<td>Potential cross-reactivity with HPV types 67 and 70, which give positive results</td>
<td>Can be used with cervical specimens collected in ThinPrep PreservCyt solution.</td>
</tr>
<tr>
<td>Cervista HPV 16/18 Test</td>
<td>Same as HC2</td>
<td>Same as HPV HR Test- Invader chemistry</td>
<td>Qualitative detection of DNA from HPV types 16 and 18</td>
<td>Same as those for HPV HR.</td>
<td>Cross-reactivity to high levels of HPV high-risk type 31. Very low levels of infection or sampling error may cause a false-negative result.</td>
<td>Same as HPV HR test</td>
</tr>
<tr>
<td>Cobas 4800 HPV test</td>
<td>1. To triage ASC-US positive women who are ≥ 21 years, to screen women ≥ 30 years for high-risk HPV genotypes 16 and 18 along with cytology, 2. Primary HPV screening for cervical cancer in women 25 and older in the United States</td>
<td>Polymerase Chain Reaction (PCR) and nucleic acid hybridization methods for the detection of 14 high-risk (HR) HPV types in a single analysis.</td>
<td>The test specifically identifies HPV types 16 and 18 while concurrently detecting the 12 remaining high-risk types (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68) at clinically relevant infection levels</td>
<td>Cervical specimens collected in Cobas Pcr Cell Collection Media (Roche) or ThinPrep PreservCyt solution.</td>
<td>Limited literature on the analytical and clinical validation of the Cobas 4800 HPV test.</td>
<td>The Cobas HPV test has high quality, is automated. Approved for primary screening for cervical cancer as a stand-alone test.</td>
</tr>
<tr>
<td>Aptima HPV assay</td>
<td>Same as HC2</td>
<td>Transcription mediated amplification-based assay.</td>
<td>Detection of E6/E7 mRNA transcripts of 14 high-risk HPV types -16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68</td>
<td>Collected in ThinPrep Pap test vials containing PreservCyt solution or the Aptima Cervical Specimen Collection and Transport Kit.</td>
<td>No discrimination between the 14 high risk HPV types.</td>
<td>No cross-reactivity with any tested high-risk HPV types or with normal flora and opportunistic organisms that may be found in cervical samples. At the CIN3+ end point, the assay is equally sensitive (95 percent) as HC2 but more specific than HC2</td>
</tr>
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</table>

The first, and currently only FDA approved test for primary HPV screening for cervical cancer in women 25 and older in the United States is the Cobas® HPV Test, which is manufactured by Roche Molecular Systems, Incorporated, Pleasanton, California. As with any laboratory test, the sensitivity of HPV testing is not 100%. A subset of squamous and glandular carcinomas such as gastric type adenocarcinoma may not be detected by HPV testing. A recent United States cancer registry study found that 9.4% of cervical cancers were HPV negative and an additional 3.2%...
contained rare HPV subtypes. Quality assurance of HPV testing and evidence based algorithms for the follow up of HPV testing should be developed more thoroughly.\textsuperscript{45}

**CURRENT GUIDELINES FOR SCREENING**

Table 2 is a comparative table with cervical cancer screening guidelines for average-risk women by American Cancer Society (ACS), American Society, for Colposcopy and Cervical Pathology (ASCCP), and American Society for Clinical Pathology (ASCP), American College of Obstetricians and Gynecologists (ACOG) and US Preventive Services Task Force (USPSTF) released in 2012.

<table>
<thead>
<tr>
<th>Cervical Cancer Guidelines</th>
<th>American Cancer Society (ACS), American Society for Colposcopy and Cervical Pathology (ASCCP), and American Society for Clinical Pathology (ASCP)\textsuperscript{40} 2012</th>
<th>U.S. Preventive Services Task Force (USPSTF) 2012</th>
<th>American College of Obstetricians and Gynecologists (ACOG)\textsuperscript{47} 2012</th>
</tr>
</thead>
<tbody>
<tr>
<td>When to start screening\textsuperscript{48}</td>
<td>Age 21.</td>
<td>Age 21.</td>
<td>Age 21 regardless of the age of onset of sexual activity.</td>
</tr>
<tr>
<td>Cytology (conventional or liquid based)</td>
<td>21-29 yrs.</td>
<td>Every 3 years.</td>
<td>Every 3 years.</td>
</tr>
<tr>
<td></td>
<td>30-65 yrs.</td>
<td>Every 3 years.</td>
<td>Every 3 years.</td>
</tr>
<tr>
<td>HPV co-test (cytology + HPV test administered together)</td>
<td>21-29 yrs.</td>
<td>Not recommended.</td>
<td>Not recommended.</td>
</tr>
<tr>
<td></td>
<td>30-65 yrs.</td>
<td>Every 5 years.</td>
<td>Every 5 years.</td>
</tr>
<tr>
<td>Primary HPV testing</td>
<td>For women aged 30-65 years, screening by HPV testing alone is not recommended in most clinical settings.</td>
<td>Recommend against screening for cervical cancer with HPV testing (alone or in combination with cytology) in women aged &lt;30 years</td>
<td>Not addressed.</td>
</tr>
<tr>
<td>When to stop screening</td>
<td>Aged &gt;65 years with adequate screening history\textsuperscript{*}</td>
<td>Aged &gt;65 years with adequate screening history.</td>
<td>Aged &gt;65 years with adequate screening history.</td>
</tr>
<tr>
<td>Screening post-hysterectomy</td>
<td>Total hysterectomy - stop screening.</td>
<td>Stop screening.</td>
<td>Stop screening.</td>
</tr>
<tr>
<td></td>
<td>Supra-cervical hysterectomy - continue screening according to guidelines.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Need for a bimanual pelvic exam</td>
<td>Not addressed in 2012 guidelines but was addressed in 2002 ACS guidelines.\textsuperscript{49}</td>
<td>Addressed in USPSTF ovarian cancer screening recommendations.</td>
<td>Addressed in 2012 well-woman visit recommendations.\textsuperscript{47} Aged &lt;21 years- &quot;external-only&quot; genital examination is acceptable. Aged ≥21 years- complete pelvic examination is a shared decision between the patient and her health care provider. Annual examination of the external genitalia should be continued</td>
</tr>
<tr>
<td>Screening among those immunized against HPV 16/18</td>
<td>Routine Screening according to the age.</td>
<td>Routine Screening.</td>
<td>Routine Screening.</td>
</tr>
</tbody>
</table>

\textsuperscript{*}Adequate screening is defined as three consecutive negative cytology results or two consecutive negative co-tests within 10 years before stopping the screening, with the most recent test/co-test performed within 5 years. Women aged >65 years who have a history of CIN2, CIN3, or AIS should continue screening for at least 20 years after regression or adequate management.

These guidelines do not apply to women who have had high-grade precancerous cervical lesion (CIN 2 or 3) or cervical cancer, women with in utero exposure to diethylstilbestrol, or women who are immunocompromised, or are HIV positive. These recommendations apply to women who have a cervix, regardless of sexual history. No distinction should be made in screening guidelines or management whether conventional cytology or liquid-based cytology is used.

**CURRENT GUIDELINES FOR SCREENING**

With better molecular insights into alterations induced by HPV E6 and E7 oncogenes, biomarkers can be used as effective triage tools. Since the altered expression of these biomarkers result in cellular neoplastic transformation, they have improved specificity over high-risk HPV testing. Studies show that p16/Ki-67 dual staining on suspicious cervical cells has improved validity in the identification of
high-grade cervical cancer precursors compared to hr-HPV tests. It results in 50% reduction in colposcopy referral for ASCUS and LSIL patients compared with hr-HPV triage.53

A recent study by Bierkens et al54 shows that methylation levels of two genes CADM1 and MAL increases with the grade of dysplasia and are highest in carcinomas. Also, the methylation levels increased with the duration of hr-HPV infections.

CONCLUSION
The Cytotechnology Education and Technology Consortium in 2014 stated that cervical cancer screening in the United States remains opportunistic, but lacks uniform test accessibility, patient compliance and an organized national program.55 Non-attendance is an important problem concerning the effectiveness of cervical screening programs.56-58 Innovative programs to increase screening rates are carried out by the National Breast and Cervical Cancer Early Detection Program. Women who have no regular source of health care, women without health insurance, and women who immigrated to the United States within the past 10 years are at high risk because of the underutilization of screening methods.59 The cervical screening methods should remain readily accessible and affordable for all women.

HPV DNA testing has a tremendous impact in both developed and developing countries considering the reproducibility and sensitivity of HPV tests. Research is going on to study if hr-HPV DNA testing can be done as primary testing method, because a negative HPV test result offers extended period of safety over negative cytology results.60 Incorporation of hr-HPV tests in cervical screening programs can increase public awareness of the association between hr-HPV and cervical cancer, which may lead to higher utilization of prophylactic HPV vaccine.

In summary, recent advances in cervical cancer screening is one of the important multi-dimensional approach to the prevention of cervical cancer. In addition to implementation of new screening methods, evaluation of their adherence and success over time will help maximize the benefits of cervical cancer prevention strategies.

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CONFLICT OF INTEREST
The authors have no conflict of interest to disclose.

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25. Bigras G, de Marval F. The probability for a Pap test to be abnormal is directly proportional to HPV viral load: results from a Swiss study.


