

Beyond the Pap Test: New Techniques for Cervical Cancer Screening

Minimizing false-negative results

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Cervical cancer continues to be a major cause of death among women worldwide.¹ It is as deadly as it is avoidable. Each year in the United States, about 13,000 new cases of cervical cancer are diagnosed, and more than 4,000 women die of the disease.²

The primary screening test for cervical cancer is the Papanicolaou (Pap) test, which was introduced in the 1940s.³ Its use resulted in a 70% decline in invasive cervical cancer incidence during the next several decades. The decreases in the incidence as well as the mortality from invasive cervical cancer have been so dramatic that the Pap test is one of the few interventions to receive an "A" recommendation rating from the US Preventive Services Task Force. This rating (which indicates that good evidence exists to support the recommendation that the intervention be specifically considered in a periodic health examination) was given to the Pap test even though its effectiveness has not been demonstrated by randomized trials.

Yet, disappointingly, the number of cervical cancer

ABSTRACT: Although most cases of invasive cervical cancer occur in the unscreened population, nearly one third of cases can be attributed to screening failure. New screening technologies are aimed at decreasing the 30% false-negative rate in screened women. Although computerized screening techniques have not been widely implemented, the use of liquid-based cytology is increasing. Direct visual inspection with acetic acid wash, speculscopy, or cervicography may be used to augment conventional cervical cytology; however, more studies are needed to fully evaluate their role in cervical cancer screening. Testing for HPV infection, which has been implicated as a cause of cervical cancer, may become useful in triaging certain patients with abnormal cervical cytology. (*Women Health Primary Care* 2001;4(12):753-758)

cases that are diagnosed in the United States has remained constant in the past 10 years, and recent evidence suggests that the incidence in white women younger than 50 years might be increasing.⁴ The estimated lifetime risk of cervical cancer is about 3.7% in unscreened women, compared with 0.3% in women who have annual

Pap tests. Even though most cases of cervical cancer occur in patients who have not undergone screening, an estimated 6,000 cases occur annually in patients who have had infrequent screening examinations.⁴ A recent report from the Agency for Healthcare Research and Quality (AHRQ) noted that the accuracy of the Pap smear is overestimated. Based on a review of unbiased studies and published meta-analyses, the sensitivity of conventional Pap smear screening is close to 50%.⁵

Is it time to consider new technologies for cervical cancer screening? Approximately 30% of invasive cervical cancer cases result from screening failure: either unsatisfactory collection of the sample or misinterpretation of cervical cytology.⁶ About two thirds of false-negative Pap tests result from sampling error and one third, from detection error. New technologies are directed at reducing the false-negative rate.

In this article, we will review these new approaches to cervical cancer screening, including liquid-based cytology (which aims primarily to reduce sampling

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error) and computerized screenings (which target detection error). We will also discuss adjunctive measures that can be used with cytology, such as human papillomavirus (HPV) testing.

COLLECTION TECHNIQUES

ThinPrep®: The ThinPrep system is a liquid-based technique for evaluating cervical cytology (Table 1). A specimen is collected from the cervix using a broom-like device or using an endocervical brush and plastic spatula (which is used to remove the cells from the cytobrush after collection). The specimen is then placed into a fixative solution, which suspends the cells. After transfer to the laboratory, the solu-

tion is agitated to break up cellular clumps; disperse blood, inflammatory cells, and debris; and mix the cellular sample. A predetermined number of cells are drawn onto a filter membrane, which is then applied to a glass slide in a monolayer and evaluated by the cytopathologist.

The major advantage of this collection technique is a slide with less mucus, less debris, and a decreased number of cellular clumps. Compared with conventional cytology collection techniques, ThinPrep results in a lower rate of slides that are unsatisfactory or that are adequate but limited by artifact.

ThinPrep also improves the de-

tection rate of low-grade squamous intraepithelial lesions (LSIL) and high-grade squamous intraepithelial lesions (HSIL).⁷ The improvement in sensitivity initially appeared to be greater in populations with a low incidence of cytologic abnormalities⁵; however, more recent data suggest an increased detection rate of HSIL and cancer in high-risk populations as well.⁸

Following a cytologic diagnosis of atypical squamous cells of undetermined significance (ASCUS) using conventional collection methods, the incidence of biopsy-confirmed cervical intraepithelial neoplasia (CIN) is 30% when a reactive process (ie, benign cellular changes) is favored and 44% when a neoplastic process is likely. The corresponding rates are 16% and 56%, respectively, after an ASCUS diagnosis with ThinPrep.⁹ The sensitivity and specificity for detecting glandular abnormalities may be greater with ThinPrep than with conventional cytology, as documented by recent reports.¹⁰

Based on a review of the available studies, the AHRQ concluded that the ThinPrep technique improves the sensitivity of the conventional Pap test by at least 60%.⁵ This report also noted that too few studies exist to determine the impact of ThinPrep on specificity and cost-effectiveness in routine cervical cancer screening.

CytoRich®: Another liquid-based technology, CytoRich, uses a collection technique similar to that employed by ThinPrep.¹¹ Unlike ThinPrep, however, CytoRich is approved for use only with a broom-like device. Mixing the solution separates clumps of cells. Centrifuging against a density gradient separates debris and inflammatory cells. Cells are suspended and allowed to settle onto a slide by gravity. The slide is then stained and evaluated by a cytopathologist. This process reduces the number of contaminating cells on the

Table 1. New techniques for cervical cancer screening

Technique	Comment
ThinPrep® (liquid-based technique for specimen collection)	Produces a slide with less mucus, less debris, and a decreased number of cellular clumps Lowers the rate of slides that are unsatisfactory or that are adequate but limited by artifact
CytoRich® (liquid-based technique for specimen collection)	Reduces the number of contaminating cells on the slide and the number of unsatisfactory slides
Computer-assisted automated Pap test rescreening	Targets abnormal slides to be manually reviewed by the cytopathologist
AutoPap® (computer-assisted screening)	May be used for primary screening as well as rescreening May identify 70% to 80% of slides with abnormalities that were missed by manual screening
Acetic acid wash	Used with unaided visual inspection or low-magnification examination of the cervix to augment conventional cervical cytological screening
Speculoscopy	Uses a chemoluminescent light to illuminate the cervix after the cytologic specimen has been obtained
Cervicography	Provides a photographic image to be analyzed by an expert (the low specificity results in referral of a high proportion of women for colposcopy)
Hybrid Capture® II	Screens for the presence of human papillomavirus Can be used with liquid-based cytology (reflex testing), or specimen can be obtained with a separate swab

slide and the number of unsatisfactory slides and results in increased sensitivity.^{12,13}

DETECTION TECHNIQUES

Quality control regulations mandated by the Clinical Laboratory Improvement Amendment of 1988 require that 10% of cytology slides classified as normal be manually rescreened by a cytopathologist. Various selection processes may be used. Computer-assisted automated Pap test rescreening targets abnormal slides to be manually reviewed by the cytopathologist.

AutoPap[®], an algorithm-based decision-making technology, identifies for rescreening slides exceeding a certain threshold for the likelihood of abnormal cells. Thus, in contrast to a random rescreening process, which arbitrarily selects 10% of slides for review, the population of slides selected by AutoPap has a greater proportion of abnormal specimens. At a sort rate of 10% to 15%, this slide population should contain 70% to 80% of the slides containing abnormalities missed by manual screening.⁵

AutoPap is a sensitive rescreening modality and has been approved by the Food and Drug Administration (FDA) for primary screening. It has been shown to be superior to conventional Pap test screening in identifying ASCUS, LSIL, and HSIL.^{14,15} In one study, 97.8% of the specimen slides that had been designated by AutoPap for no further review during primary screening were found to contain normal cytology during a manual rescreening; ASCUS was detected on 0.8% of the slides during rescreening and benign cellular changes on 1.4%. These results indicate that the false-negative rate with AutoPap is low.¹⁶ However, larger studies from several centers are needed to confirm these results.

Screening with the new collection and detection devices may reduce death rates from cervical can-

cer to a greater degree than does screening with the conventional method, regardless of screening interval; however, this is unproven. When costs for year-of-life saved were evaluated, costs were lower for new technologies when screening was performed every three years, compared with every two years by conventional cytology. When added to an annual cancer screening protocol, new technologies do not appear to be cost-effective

for liquid-based cytology are higher than the charges for conventional testing.

OTHER TECHNIQUES

Various combinations of monolayer and computer-assisted techniques are being developed to reduce the false-negative rates that result from collection and detection errors, but none have yet been approved by the FDA for primary screening.

Table 2. How effective is HPV testing for detecting CIN3+?*

Triage test threshold	Sensitivity (%)	Referral rate (%) [†]	Positive predictive value (%)	Negative predictive value (%)
HPV testing with HC II	96.3	56.1	10.0	99.5
HSIL+ cytology	44.1	6.9	37.5	96.5
LSIL+ cytology	64.0	26.2	14.3	97.1
ASCUS+ cytology	85.3	58.6	8.5	97.9

HPV, human papillomavirus; CIN3+, cervical intraepithelial neoplasia grade 3 or worse disease; HC II, Hybrid Capture[®] II; HSIL+, high-grade squamous intraepithelial lesions or worse disease; LSIL+, low-grade squamous intraepithelial lesions or worse disease; ASCUS+, atypical squamous cells of undetermined significance or worse disease.

* Based on the combined HPV triage and immediate colposcopy arms of the study.

[†] Percentage of the study population that would have been referred for colposcopy based on the use of a particular triage test threshold.

Adapted from Solomon et al. *J Natl Cancer Inst.* 2001.³²

cer. However, more studies with longer follow-up are needed to fully analyze the cost-effectiveness of liquid-based technology. Furthermore, these technologies do not address the serious problem of the unscreened patient population.

Because of the increased sensitivity of liquid-based cytology and the ability to do reflex HPV testing (see discussion on page 756), liquid-based cytology is gaining widespread use. For populations without trained cytopathologists and cytologists, however, liquid-based cytology may not be available. Furthermore, in some communities, its availability may be limited by cost considerations, since the charges

Cytec CDS-1000[™]: Still under development, Cytec CDS-1000 is an automated liquid-based cytology system that uses the ThinPrep process. It is designed to screen and print 48 potentially abnormal fields, 24 fields of cell clusters, and eight normal fields on a monitor. The fields are then reviewed manually by trained technicians.

AutoCyte[®] system: This screening technique consists of an automated microscope and a computer-controlled slide handler. It uses slides that are prepared by the thin-layer cytology systems (CytoRich method). The AutoCyte system can screen up to 300 slides within 24 hours. For each abnormal slide, the most significant abnormal cellular

features and the interpretation of each are captured, stored, and processed by a series of algorithms. The images are presented for review by a cytopathologist. A recent study of the AutoCyte system correlated cytologic findings with biopsy results and revealed a 31% improvement in the detection of all squamous intraepithelial lesions and invasive cancer, compared with detection by conventional techniques.¹⁸

AcCell™: A prescreening system scheduled to enter clinical trials soon, AcCell is designed to be unattended. It is capable of pro-

However, most patients with HPV infection or even CIN grade 1 do not develop cancer or high-grade lesions. It may be chronicity of infection with high-risk HPV types that defines patients at highest risk for CIN grade 3 and cervical cancer.

cessing 1,000 slides per 24 hours, at a rate of 30 to 40 slides each hour.

AcCell includes knowledge-based pattern-recognition algorithms, a special purpose algorithmic computer, and a multi-spectral microscope. A series of narrowly defined optical wavelengths are used to optimize the visual contrast of cellular components. AcCell uses a two-stage procedure (an interactive image review preceded by a threshold protocol) to increase the accuracy and speed of image processing. The threshold protocol removes 50% of the normal slides. The remaining slides are then reviewed by a cytopathologist as a ranked series.

Optical and photographic screening: Several other technologies may be employed to augment conventional cervical cytological screening. Optical and photographic screening tests include acetic acid wash, speculscopy, and cervicography.

◆ Unaided visual inspection of the cervix or low-magnification examination of the cervix after an acetic acid application has improved sensitivity but has low specificity for detecting cervical abnormalities. In one study of 2,827 patients, the rate of detecting cervical disease was im-

proved by 30% over Pap tests alone.¹⁹

These findings have been confirmed by other investigators.²⁰ However, the possibility of false-positive results may limit the usefulness of this procedure.

◆ Speculscopy uses a chemoluminescent light to illuminate the cervix after the cytologic specimen has been obtained. Speculscopy is designed as a screening tool and not a diagnostic test. The results of speculscopy-aided screening appear to correlate well with colposcopic findings in patients with ASCUS.²¹ In combination with the Pap test, speculscopy improves the yield of biopsy-proven cervical pathology.^{22,23}

Fluorescence spectroscopy has the potential to improve the detection of preinvasive cervical neoplasia.²⁴ Trimodal spectroscopy combines intrinsic fluorescence, reflectance, and light scattering to provide complementary information about the biochemical and morphologic changes that occur during the development of cervical neoplasia. In a preliminary study, differences in normal cytology, precancerous lesions, and cervical cancer were noted by investigators using trimodal spectroscopy.²⁵

◆ Cervicography utilizes a photographic image that may be sent to an expert for review, diagnostic confirmation, or consultation. To date, the usefulness of this method as a screening or triage tool has not been studied in a large enough series.²⁶

ADJUNCTIVE MEASURES

HPV testing: The association between certain HPV types (16, 18, 31, 33, 35, 45, 51, 52, and 56) and the development of CIN and invasive cervical cancer is well established.²⁷ However, most patients with HPV infection or even CIN grade 1 do not develop cancer or high-grade lesions. It may be chronicity of infection with high-risk HPV types that defines patients at highest risk for CIN grade 3 and cervical cancer.^{28,29}

The Hybrid Capture® system (approved by the FDA in 1995) can identify high-risk HPV types. A specimen from liquid-based cytology is denatured to free DNA strands. The HPV DNA probe for both high-risk and low-risk HPV is then mixed. The hybrids that form are collected in a capture tube, rinsed, and bound with a chemoluminescent agent, which allows the type and amount of HPV to be determined. The recently developed Hybrid Capture II system is 10 times more sensitive than the first-generation system for detect-

ing high-risk HPV.³⁰

The ASCUS/LSIL Triage Study (ALTS) Group³¹ is prospectively addressing the role of HPV DNA testing as a triage strategy in women with ASCUS/LSIL. The initial data from this trial suggest that the detection of HPV in patients with LSIL on Pap smears adds little to management because the tests of most of these patients are HPV-positive.

In a separate report, HPV typing (as compared with a repeated single cytology) was helpful in triaging patients who have ASCUS (Table 2).³² Thus, patients with a negative HPV screening test result can be followed with routine cytology, whereas patients with test results that are positive for HPV require further evaluation.

HPV vaccine: Of the women who develop cervical cancer in the United States, 50% have never been screened, and an additional 10% have not had a Pap test within five years of diagnosis.³³ Epidemiologic data from many countries and population groups have identified HPV in up to 95% of women with cervical cancer. High-risk HPV DNA has been detected in up to 93% of patients with high-grade dysplasia.^{34,35} The virus is implicated in the deregulation of cell division, which results in malignant transformation.

Given the inability to cytologically screen all women for cervical cancer, eradicating the virus may be a more effective worldwide strategy for eliminating the disease. Animal studies using viral-like particles (VLPs) have generated high levels of neutralizing antibody.³⁶ HPV 16 VLPs and VLPs that incorporate the E7 oncoprotein, along with L1 and L2 capsid proteins, are now undergoing clinical trials as a preventive vaccine.²⁹ A 40-fold increase in serum antibody titer (compared with titers observed in natural infection) has been demonstrated in adults who were

vaccinated with HPV 16 L1 VLP.³⁷ The vaccines were well tolerated. Most cervical cancer deaths occur in women from developing countries that may lack the resources for widespread screening. Therefore, an effective HPV vaccine to prevent cervical cancer may have a major public health impact. 🌐

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