Published in final edited form as:

Best Pract Res Clin Obstet Gynaecol. 2012 April ; 26(2): 233–242. doi:10.1016/j.bpobgyn.2011.11.001.

New Technologies for Cervical Cancer Screening

Alaina J. Brown, M.D.¹ and Cornelia L. Trimble, M.D.^{1,2,3}

¹Department of Gynecology and Obstetrics, The Johns Hopkins Medical Institutions, Baltimore, MD, USA

²Department of Oncology, The Johns Hopkins Medical Institutions, Baltimore, MD, USA

³Department of Pathology, The Johns Hopkins Medical Institutions, Baltimore, MD, USA

Abstract

New technologies for cervical cancer screening seek to provide an accurate, efficient, and cost-effective way of identifying women at risk for cervical cancer. Current screening uses HPV DNA testing combined with cytology and requires multiple visits at a great cost to the patient and the society. New methods for screening include HPV diagnostics (detection of either the presence of HPV or integration of the virus into the host cell), proliferation, and detection of epigenetic changes, either in the host or virus. These methods show promise in changing the way that current cervical cancer screening is undertaken in both low and high-resource settings.

Epidemiology of Cervical Cancer

Although we have known how to screen for squamous cervical carcinoma (SCCx) since the 1940s, it is still the second most common cancer diagnosed among women worldwide [1]. Virtually all SCCx are caused by persistent infection with Human Papillomavirus (HPV), most commonly HPV types 16 and 18 [6]. In high-resource settings, such as North America, in the last half-century, screening strategies that identify cervical high-grade squamous intraepithelial lesions (HSIL) have reduced the incidence and mortality of SCCx by greater than fifty percent. However, current technologies are relatively inefficient at identifying individuals at risk for disease and require longitudinal testing over a woman's lifetime. This type of screening is not feasible in low-resource settings. Accordingly, on a global scale, SCCx is the third most common cause of cancer-related death in women, resulting in 309,800 deaths worldwide in the year 2007 [1].

This cancer is preventable because effective screening strategies that identify the precursor lesion may allow the disease to be cured. The two major histologic types of cervical cancer include SCCx and adenocarcinoma. SCCx is the most common type, representing 70% of cases [2]. Adenocarcinoma, which is more commonly associated with HPV type 18, comprises approximately 25% of cases. In North America, the incidence of adenocarcinoma appears to be rising [39]. Adenosquamous carcinoma is the least common and comprises approximately 3-5% of cases [2].

In high-resource settings, cervical cancer is the seventh most common female cancer [1]. In the United States, the annual incidence of SCCx is 12,200 women and the annual mortality is 4,210 women [3]. Because of differences in access to medical care, cervical cancer is disproportionately diagnosed in minorities and amongst women of low socioeconomic

Corresponding author: CL Trimble Phipps 255 600 North Wolfe St. Baltimore, MD 21287 phone: (410) 502-0512 fax: (410) 502-0621 ctrimbl@jhmi.edu.

Conflict of interests: Neither CLT nor AJB have any financial conflicts of interests to declare.

status. According to the American Cancer Society, in the United States, the incidence of disease in African American females is 10.8 cases of cervical cancer per 100,000 women [4]. The incidence of disease in Hispanic females is 12.7 cases of cervical cancer per 100,000 women [5]. In contrast, the incidence of disease in white females is 8.2 cases per 100,000 women [4]. Globally, cervical cancer is much more common in low-resource settings when compared to high-resource settings. Eighty percent of the 555,100 new cases worldwide per year are diagnosed in low-resource settings[1]. Because disease is not diagnosed until it is late-stage, and because treatment also requires infrastructure and resources, more than 85% of the 309,800 SCCx deaths in the year 2007 occurred in low-resource settings [1].

Etiology of Cervical Cancer

Persistent mucosal infection with an oncogenic (high-risk) HPV genotype, including types 16, 18, 33, 45, 31, 58, 52 and 35, is the most significant cause of cervical cancer. HPV types 16 and 18 are the genotypes most commonly associated with disease, and are identified in 70% of SCCx cases [6]. HPV infection is transmitted by direct contact, and is common among sexually active men and women. The estimated prevalence of infection ranges from 50-80% [7]. Risk factors for developing cervical disease include age of sexual debut, number of sexual partners, prolonged use of oral contraceptive pills, high parity, cigarette smoking, co-infection with HIV or other sexually transmitted infections, and chronic immunosuppression [8].

While HPV infection causes cervical cancer, most HPV infections do not lead to cervical cancer. HPV infection is easily and silently transmitted, as it does not cause symptoms. Approximately 90% of HPV infections resolve within several months of initial infection [7]. Persistent viral infection is the single biggest risk factor for the development of high-grade dysplasia and progression to cervical cancer.

Transient HPV infections correlate with low-grade squamous intraepithelial lesion (LSIL) cytology or cervical intraepithelial neoplasia 1(CIN1) histology. Persistent oncogenic HPV infections correlate with high-grade intraepithelial lesions (HSIL) cytology or CIN2/3 histology. Persistent infections are associated with integration of the viral genome into the host genome and subsequent transformation. After viral integration, two viral gene products, E6 and E7, are expressed, both of which are necessary but not sufficient for disease initiation and persistence. These oncoproteins bind to and disrupt the function of tumor suppressor genes p53 and the retinoblastoma protein (pRB), respectively. Disruption of these genes causes blocked apoptosis and cell cycle arrest, leading to dysplasia [9, 10]. The expression of viral oncoproteins in dysplastic epithelial cells, and the indolent biology of intraepithelial HPV lesions together present many opportunities to prevent the development of SCCx by performing routine screening.

Current Cervical Cancer Screening Methods

The goal of cervical cancer screening is to identify patients at risk for the development of disease – that is, those with the immediate precursor lesion, high grade squamous intraepithelial lesions.. Current screening for cervical cancer is highly dependent upon the type of resources available in the population being screened. In high-resource settings, routine screening includes pap smears over the course of a lifetime to evaluate for cervical dysplasia. Depending on the age of the patient, evaluation may or may not include screening for high-risk HPV. If abnormal cytology is detected, then the patient may either have more frequent pap smears, or may be referred to colposcopy for further evaluation. This type of screening allows for close evaluation of the cervix and early excision of high-grade

dysplasia in appropriate cases. The American Congress of Obstetricians and Gynecologists (ACOG) currently recommends that cervical cytology screening begin at age 21 and thereafter be repeated every two years for women ages 21-29, and every 3 years for women 30 and older who have had three prior normal pap smears. More frequent screening is recommended for immunosuppressed patients, women infected with human immunodeficiency virus (HIV), women exposed to diethylstilbestrol in utero, and women previously treated for CIN 2, CIN 3 or cancer. Screening may be discontinued in women age 65-70 with three prior consecutively normal pap smears, and no abnormal pap smears over a period of ten years [11].

In addition to repetitive cytology screening, many providers in high-resource settings implement concurrent testing for oncogenic HPV DNA in women with either an atypical squamous cells of undetermined significance (ASCUS) pap smear or among women who are over age 30. Three types of tests to detect oncogenic HPV DNA are FDA-approved. The Hybrid Capture 2 test, approved by the FDA in 2003, detects 13 oncogenic HPV types (16/18/31/33/35/39/45/51/52/56/58/59/68) using full genome probes complementary to HPV DNA, specific antibodies, signal amplification, and chemiluminescent detection. The Cervista HPV HR test, approved by the FDA in 2009, detects 14 high-risk HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) using a signal amplification method for detection of specific nucleic acid sequences. This method uses a primary reaction that occurs on the targeted DNA sequence and a secondary reaction that produces a fluorescent signal. These two tests have two limitations. First, neither test differentiates between single HPV genotype infections and multiple concurrent HPV genotype infections. Second, neither test quantitates viral load. The third, and newest HPV DNA test, Cervista HPV 16/18, was approved by the FDA in 2009, and detects only HPV 16 and 18, the genotypes most commonly associated with cancer, using a similar method to the Cervista HPV HR assay [6]. Among women with HSIL cytology, HPV 16 is detected in 45.4% , and HPV18 in 6.9% [6].

Detection of oncogenic HPV with HPV DNA screening tests is an effective strategy in the triage of cytology interpreted as atypical squamous cells of undetermined significance (ASCUS). A substantial literature suggests that in women over age 30, HPV testing may be a cost-effective and accurate means of primary screening. Cuzick et. al [12] retrospectively examined HPV testing and cytology samples in 60,000 European and North American women between the ages of 30-60 years. HPV DNA testing was more sensitive in detecting cervical intraepithelial neoplasia grade 2 or 3 (CIN2+) than cytology (96.1% vs. 53.0%), but less specific (90.7% vs. 96.3%). The sensitivity of HPV testing was similar among different areas of Europe and North America, whereas the sensitivity of cytology in these areas varied [12]. Another study evaluating HPV testing and pap smear cytology in 10,154 Canadian women ages 30-69 identified sensitivities and specificities similar to those demonstrated in the Cuzick study [12, 13]. In the Canadian cohort, the sensitivity of HPV DNA testing for identifying CIN2+ was 94.6% (95% CI (84.2-100)) and the specificity was 94.1% (95% CI (93.4 to 94.8)). In contrast, the sensitivity of Pap smear was significantly lower (55.4% (95% CI, (33.6 -77.2) $p = 0.01$). However, the specificity of Pap smears was similar to HPV testing (96.8% (95% CI (96.3-97.3) $p < 0.001$). The sensitivity of both tests used concurrently was 100% with a specificity of 92.5% (Table 1). Because these screening methods are complementary, many high-resource settings have implemented algorithms that incorporate both. The use of cytology and HPV detection has reduced the incidence of cervical cancer in the United States from 14.8 per 100,000 in 1975 to 6.8 per 100,000 in 2008 [14].

Despite the effectiveness of using cytology and HPV DNA testing to detect disease, it is expensive and cumbersome. Many women undergo repetitive pap smears and colposcopy

for evaluation of low-grade dysplastic lesions that are likely to resolve over time. Repetitive clinic visits and testing not only places a psychological burden on the patient, but also places economic strain upon the society providing the screening. In the United States alone, it has been estimated that six billion dollars per year are spent on evaluation of low grade lesions [15].

Screening for cervical cancer is restricted by the financial resources and the social infrastructure of the society being screened, relying on methods that are low-cost and require few visits to the clinic. Accordingly, alternative methods of screening that may be implemented quickly and cheaply, such as visual inspection alone or visual inspection with a magnifying device, are currently used in low-resource settings.

Visual inspection involves evaluation of the cervix with the naked eye, using either dilute acetic acid solution (VIA) or Lugol's solution/iodine (VILI) to identify cervical lesions. VIA has a sensitivity of 79% (95% CI 73-85%) and a specificity of 85% (95% CI 81-89%) for the detection of CIN2+ lesions [16]. The use of VILI can increase sensitivity marginally, by 10%, and does not change the specificity [16]. The use of a magnifying device to aid in evaluation of the cervix has similar sensitivity and specificity to VIA alone [16]. The sensitivity and specificity of visual detection are dependent on the skill of the provider and vary widely.

Although these methods are imperfect, they may decrease rates of cervical cancer in low-resource settings. Using computer models, Goldie et. al [17] analyzed screening strategies among women ages 35-39 in India, Kenya, Peru, South Africa, and Thailand. They estimated that one-time screening of women at age 35, using either visual inspection of the cervix or high-risk HPV testing, could reduce the lifetime risk of cancer by 25-36%, at a cost of less than \$500 per year of life saved. Using this model, two screenings at ages 35 and 40 resulted in a relative reduction in lifetime risk by approximately 40 percent. Visual inspection, in combination with testing for oncogenic HPV may be used in screen-and-treat programs, which incorporate immediate excision of cervical lesions.

In a large prospective study in rural India, Sankaranarayanan et al [18] evaluated the effectiveness of three different screening tools: one time high-risk HPV screening, visual inspection, and cytologic testing in 131,746 women between the ages of 30 and 59 years old. In this cohort, a single round of HPV testing led to significant reduction in the incidence of stage II or higher cervical cancer (1 per 1000 in the HPV testing group versus 2.5 per 1000 in the control group). A reduction in cervical cancer mortality was also seen in the HPV testing group. In contrast, neither cytology nor VIA resulted in a significant reduction in either the incidence of advanced cancer or mortality when compared to controls. This study illustrates the potential effectiveness of one-time screening in unscreened populations with a high incidence of disease, but also emphasize the importance of using a reproducible, objective test, such as detection of oncogenic HPV genotypes, as opposed to subjective exams that are critically dependent on the skill of the provider.

New Cervical Cancer Screening Methods

An ideal screening method would allow for the efficient and inexpensive screening of all women regardless of their social situation. Methods meeting these criteria would not only allow for effective screening to take place in low-resource settings but could also decrease the overall fiscal burden that current cervical cancer screening methods place on high-resource health care systems. Several new approaches are currently being developed. These screening methods may be considered in three broad areas: HPV diagnostics (detection of either the presence of HPV or of viral integration into the host genome), biomarkers of

cellular proliferation, and detection of epigenetic changes, either in the host or virus. Several of these methods show promise to improve cervical cancer screening in both low and high-resource settings.

Screening Methods Utilizing HPV Diagnostics

Current recommendations of the American Society for Colposcopy and Cervical Pathology (ASCCP) state that women ages 30 years and older who have normal cytology but are high-risk HPV DNA positive may benefit from genotyping assays for the presence of HPV 16 and 18. Women in whom HPV 16 and 18 is detected should be referred for colposcopy. If other high-risk types are demonstrated, but no HPV 16 and 18 is detected, the patient should be followed with repeat cytology and testing for high-risk HPV DNA in 12 months [24]. The ASCCP guidelines state that it is also acceptable to observe women with negative cytology who are high-risk HPV DNA positive with repeat cytology and high-risk HPV DNA screening in 1 year. In general, testing for HPV DNA is not a useful screening strategy in either women younger than 30 years of age or those with abnormal cytology. HPV infections in women less than 30 years of age are transient and likely to regress over time. HPV testing in women with abnormal cytology is redundant because it will demonstrate the presence of oncogenic HPV [24].

In women 30 years or older, identification of oncogenic HPV DNA is currently being implemented in high-resource settings to function as a primary screening test, simultaneously with a pap smear [19, 20]. The presence of HPV DNA in cervical samples of women 30 years of age and older is likely to reflect persistent infection, in contrast to cytology that may reflect transient abnormalities. HPV DNA testing provides a quantitative means of HPV detection, compared to the evaluation of cellular changes in cervical cytology, which is more subjective. HPV DNA testing is also performed as a reflex test on any ASCUS pap smear. By directing the management of ASCUS cytology and triage of women 30 years of age and older, HPV testing has saved both patients and the health care system a significant amount of time and resources. Despite the overall success of this strategy in identifying CIN2+, the system remains cumbersome, requiring multiple visits. Cost-benefit analyses in high-resource setting suggest that high-risk HPV DNA testing alone may replace cytology as the primary means of cervical cancer screening in women 30 years of age and older [19].

While screening for oncogenic HPV DNA is useful in a high-resource setting, the costs and time involved in running the currently available tests restrict their use in low-resource settings. A rapid, low-cost oncogenic HPV DNA screening test that could be used in low-resource settings has the potential to greatly decrease the worldwide incidence of cervical cancer. One assay currently under development is the careHPV assay (QIAGEN, Gaithersburg, MD, USA) which uses a signal-amplification assay that detects 14 different high risk HPV DNA types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68), requires only 25×50 cm of work space, does not require electricity or running water, and takes approximately 2.5 hours to perform [21]. This assay time of 2.5 hours, as compared to the approximately 6 hours required for HC2 high-risk HPV testing, allows for evaluation and treatment the same day if needed.

The careHPV assay has been evaluated by Qiao et. al [21] in China in a prospective cohort of 2,388 women ages 30-54 who had not previously been screened for cervical cancer. In this study, women self-collected a careHPV vaginal sample and then underwent provider directed careHPV testing, HC2 testing, visual inspection by a midwife, and digital colposcopy by a physician with guided cervical biopsies as indicated. Using CIN2+ as the reference standard, the sensitivities and specificities of the careHPV test were 90.0% (95%

CI 83.0–97.0) and 84.2% (95% CI 82.7–85.7), respectively, on provider-collected cervical specimens, and 81.4% (95% CI 72.3–90.5) and 82.4% (95% CI 80.8–83.9), respectively, on patient-collected vaginal specimens [21]. These methods were both superior to visual inspection, which had a sensitivity of 41.4% (95% CI 29.9–53.0) and a specificity of 94.5% (95% CI 93.6–95.4). There was no significant difference in the incidence of CIN2+ between provider and patient collected samples [21]. While this approach provides logistical and economic advantages, currently, there are no plans to make it available in high-resource settings.

Another strategy using HPV diagnostics for screening involves identification of specific oncogenic HPV genotypes. Currently available assays detect a pool of 13 to 14 oncogenic HPV DNA types, but do not specify how many HPV genotypes or which genotypes are present. Given the transient nature of many HPV infections, many women may have detectable HPV DNA, but may be at low-risk for disease [22]. Currently, Cervista is the only FDA-approved HPV genotyping test that identifies only HPV 16 and 18. Many additional HPV genotyping assays are not currently FDA-approved, but are available for use outside of the US [23]. (Table 2)

Quantitating HPV viral load appears, on the surface, to be a rational strategy to identify women at risk for persistent HPV infection and progression to high-grade dysplasia. A correlation between HPV 16 viral load and high-grade dysplasia is supported by the literature, but the association between viral load and dysplasia is not as apparent in the other oncogenic HPV types. Moberg et. al [25] examined 2,747 archived pap smear specimens and found that the risk of CIN3 correlated with HPV16 viral load. They did not observe a strong relationship with increasing viral load for other HPV types such as 18, 31, and 45. Similar results were found by Gravitt et. al in a cross-sectional and prospective study of 2,000 HPV-infected women [26]. Given the differences in the type of assays used to quantify the presence of the HPV virus, these viral load studies are currently of limited clinical application. Some assays are unable to normalize against the number of cells in the sample. Accordingly, a high viral load could represent many cells with few virions each or a few cells containing many virions. An inaccurate description of the viral biology and the possible implications for the host could result from this discrepancy. Additionally, some HPV viral load assays such as HC2 report a threshold that does not make a distinction between different HPV types. Overestimation of the presence of oncogenic HPV may result. Despite these caveats, the development of HPV viral load assays that may reliably be used as an adjunct screening tool to identify women at increased risk of progression to CIN 2+ and cervical cancer remains a promising tool in cervical cancer screening.

Screening for HPV integration into the host genome is a subcategory of HPV diagnostics. HPV integration is a key molecular event in the transition from an innocuous HPV infection to one that has oncogenic potential. HPV integration results in increased expression of the viral E6 and E7 proteins. Increased expression of these proteins ultimately results in the disruption of host cell proteins, p53 and pRb [27]. Tests that detect the integration of HPV into the host cell and corresponding risk of CIN 2+ or cancer are in development, and may provide a useful way of screening women at risk for cervical cancer. Studies have demonstrated that viral integrants are detected in 100% of HPV 18 positive and 70-80% of HPV 16 positive cases of cervical carcinoma [28, 29]. A smaller subset of HSILs (15%) and 0% of LSILs contain transcriptionally active viral integrants [27].

Detection of p16(INK4a) correlates tightly with viral integration. In a normal cell, p16 blocks cyclin-dependent kinases (CDK) 4/6. Increased expression of the E6 and E7 oncogenes disrupt cell cycle regulation, resulting in cell cycle progression. In the normal cell, cell cycle progression is activated by CDK 4/6 and in part regulated by p16. However,

because in HPV-transformed cells, cell cycle activation is caused by E7 and not by CDK 4/6, p16 has no effect on the cell cycle activation. Increased expression of p16 in cells driven by viral oncogene-mediated cell-cycle dysregulation can be detected through cellular immunostaining [32].

A review by Tsoumpou et. al [32] examined 61 studies which evaluated the presence of p16 in different cytologic and histologic specimens. In their study, detectable p16 expression was associated with increasing severity of dysplasia. Among normal cytologic samples 12% (95% CI: 7–17%) had detectable p16. Forty-five percent of ASCUS (95% CI: 35–54%), 45% of LSIL (95% CI: 37–57%) and 89% of HSIL samples (95% CI: 84–95%) had detectable p16 expression. A similar trend was identified in histological samples. Two percent of normal biopsies (95% CI: 0.4–30%), 38% of CIN1 (95% CI: 23–53%), 68% of CIN2 (95% CI: 44–92%) and 82% of CIN3 (95% CI: 72–92%) had detectable p16 staining [32]. While these data are promising, current usage of the p16 biomarker is limited due to variability depending on the stains used. This is particularly true for low-grade lesions, where the percentage of cytological samples with detectable p16 ranges from 10% to 100% for ASCUS and from 10% to 86% for LSIL. Similarly, p16 staining in histological samples of CIN1 biopsies range from 0–100% [32]. Future research must determine methods to standardize p16 immunostaining.

Researchers are currently evaluating other biomarkers to help identify HPV integration into the host genome. One such approach is quantification of high-risk HPV messenger RNA (mRNA). Currently available high-risk HPV tests detect the presence of potentially carcinogenic HPV DNA, but do not evaluate the transcriptional activity of the viral DNA. High-risk HPV mRNA assays provide indirect functional information about the transcriptional activity of the virus by evaluating the activity of E6 and E7. Detectable transcripts of HPV correlate with the oncogenic potential of the particular virus [20]. Castle et. al [30] identified a correlation between the detection of HPV E6 and E7 mRNA and the severity of cervical dysplasia. They evaluated 531 liquid cytology samples using a prototype assay that collectively detected E6 and E7 mRNA for 14 oncogenic HPV genotypes. Ninety-four percent of women (46 of 49 patients) with CIN3 and all the women in their group with cancer (5 of 5 patients) tested positive for high-risk HPV E6 and E7 mRNA activity.

Molden et. al [31] evaluated the effectiveness of HPV DNA detection to mRNA detection in predicting risk of CIN2+ in a prospective study of 77 Norwegian women older than 30 years of age with ASCUS or LSIL cytology. They performed subsequent cytology and biopsies on these women two years after initial HPV DNA and mRNA screening. Women with an ASCUS/LSIL pap and a positive high-risk mRNA test were 69.8 times (95% CI = 4.3, 1137.3) more likely to be diagnosed with CIN2+ within two years as women with the same cytology and a negative high-risk mRNA test. When compared to mRNA testing, detectable HPV DNA in the same group of women had a 10-fold lower predictive value for CIN2+ within two years of initial evaluation [31].

Because the correlation between HPV mRNA and high-grade dysplasia is a biologically plausible biomarker of risk, HPV mRNA detection may improve the specificity in the evaluation of women with ASCUS and LSIL paps smears [31]. Because many have lesions that will not progress to CIN3 or invasive cancer, currently, these women present a treatment dilemma. No reliable methods exist to identify those lesions that are likely to regress. As a result, these women are monitored with serial colposcopic exams at a great expense to patients and the healthcare community. Detection and quantification of mRNA transcripts in these women may further refine current broad spectrum high-risk HPV DNA typing by allowing physicians to know whether or not the virus is actively replicating E6

and E7 oncogenes. mRNA transcript assays show great promise for being able to stratify the risk of progression to high-grade dysplasia in women with abnormal cytology.

The E6 strip test is also a biomarker that indicates viral integration. Schweizer et al [33] evaluated the correlation of the HPV E6 test (Arbor Vita Corporation, Fremont, CA) which takes an hour to perform, and detects the HPV-E6 oncoprotein of HPV types 16,18 and 45, with detection of oncogenic HPV DNA in cytologic samples. They also evaluated the correlation between the HPV E6 strip test and the histologic detection of low-grade and high-grade CIN. Their study demonstrated that 51 of 75 (68%) women with CIN3+ had a positive HPV E6 strip test. None of the 16 samples with normal or CIN1 histology tested positive.

Screening Strategies Identifying Epigenetic Changes

Aberrant methylation of tumor suppressor genes is a known cause of cell cycle dysregulation. Many genes are currently being evaluated as potential methylation biomarkers for cervical cancer, but assay reliability for these methylation markers is highly variable. Some promising candidate genes include DAPK1, CADM1, and RARB [34]. One study by Feng et. al [35] examined the usage of three methylation biomarkers (DAPK1, RARB, CDH13, and TWIST1) in Senegal, a low-resource setting. These researchers examined the feasibility of using these markers for a urine based cervical cancer screening method. They analyzed the urine samples of 129 Sengalese women age 35 or older. One hundred and ten of these women had biopsy proven cervical dysplasia or cervical cancer (CIN1, n=9, CIN2/3 n=29, invasive cervical cancer n =72). Nineteen had no evidence of dysplasia or cancer. They noted hypermethylation of at least one of the four genes in the urine samples of 62% of women with invasive cervical cancer, 29% of CIN2/3 and 4% of patients with CIN1 or normal histology. These results were lower than the sensitivity obtained by testing urine for the presence of high-risk HPV DNA (70% of invasive cervical cancer, 59% of CIN 2/3, 44% of CIN-1, and 11% of women negative for cervical neoplasia on biopsy), but suggest that methylation biomarkers may have future clinical utility in low-resource settings.

Another area of biomarker research is in the use of telomerase RNA component (TERC) identification by fluorescence *in situ* hybridization (FISH). Most cervical cancers have an extra copy of the long arm of chromosome 3 and as a result demonstrate amplification of TERC (present on chromosome band 3q26) which appears to play a key role in progression from low-grade dysplasia to cancer [36]. Many studies indicate that TERC identification may become a useful screening tool for cervical cancer. A prospective study by Andersonn et. al [36] found a correlation between increasing TERC detection in cytology specimens and higher grade of dysplasia. In this study, 78 liquid based cytology samples were evaluated for TERC amplification. These initial samples were followed by repeat pap smears and histological evaluation. TERC amplification was positive in 7% of normal histological samples, 24% of CIN1, 64% of CIN2, 91% of CIN3 and 100% of invasive cancer samples. Heselmeyer-Haddad et. al [37] performed a retrospective analysis of 59 pap smears with known histological correlations to evaluate the correlation between TERC amplification and cervical dysplasia. They demonstrated that progression to cervical cancer is never seen without TERC amplification and that conversely, specimens without extra copies of TERC were likely to undergo spontaneous regression of HPV infection. In their study, detection of TERC predicted progression of CIN1/2 to CIN3 after a follow up of two months to three years with 100% sensitivity and 70% specificity. Obvious limitations of this screening method include the costs and technical skill required for FISH testing.

Screening Methods Utilizing Proliferation Markers

Other biomarkers under early evaluation for cervical cancer screening include CDC6 and MCM5. These proteins are present in normal cells only during the activation of the cell cycle and help form pre-replicative DNA complexes during the G1 phase. They are absent from the cell during quiescence and differentiation. Dysplastic cells have unregulated cell cycles and as a result, CDC6 and MCM5 reflect cell proliferation [38]. Studies indicate that CDC6 may be a biomarker of high-grade and invasive lesions of the cervix, with limited use in low-grade dysplasia. MCM5 appears to be a biomarker that is expressed independent of high-risk HPV infection and may in the future serve as a useful marker for both HPV-dependent and HPV-independent cervical dysplasia [38].

The Future of Cervical Cancer Screening

New screening methods for cervical cancer are greatly needed, as all current screening methods require an infrastructure for testing and for management of abnormal results. Because of the costs and manpower required for the implementation of an infrastructure, few women in low-resource settings have access to screening for cervical cancer. Future screening methods must address the need for an efficient, cost-effective screening tool that quickly, accurately, and cheaply identify women at risk for HPV-associated malignancies.

References

1. Global Cancer Facts and Figures 207. Available from: <http://www.cancer.org/acs/groups/content/@nho/documents/document/globalfactsandfigures2007rev2p.pdf>
2. SEER data for 2000-2004. Available from: <http://seer.cancer.gov/>
3. Cancer Facts and Figures 2010. Available from: <http://www.cancer.org/acs/groups/content/@nho/documents/document/acspc-024113.pdf>
4. Cancer Facts and Figures for African Americans 2009-2010. Available from: <http://www.cancer.org/acs/groups/content/@nho/documents/document/cffaa20092010.pdf>
5. Cancer Facts and Figures for Hispanic/Latinos 2009-2011. Available from: <http://www.cancer.org/acs/groups/content/@nho/documents/document/ffhispanicslatinos20092011.pdf>
6. Bosch FX, et al. Epidemiology and natural history of human papillomavirus infections and type-specific implications in cervical neoplasia. *Vaccine*. 2008; 26(Suppl 10):K1–16. [PubMed: 18847553]
7. Stanley M. HPV - immune response to infection and vaccination. *Infect Agent Cancer*. 5:19. [PubMed: 20961432]
8. Bosch FX, de Sanjose S. The epidemiology of human papillomavirus infection and cervical cancer. *Dis Markers*. 2007; 23(4):213–27. [PubMed: 17627057]
9. Schiffman M, et al. Human papillomavirus and cervical cancer. *Lancet*. 2007; 370(9590):890–907. [PubMed: 17826171]
10. Narisawa-Saito M, Kiyono T. Basic mechanisms of high-risk human papillomavirus-induced carcinogenesis: roles of E6 and E7 proteins. *Cancer Sci*. 2007; 98(10):1505–11. [PubMed: 17645777]
11. *Cervical Cytology Screening*, in *Practice Bulletin # 109*. American Congress of Obstetricians and Gynecologists. 2009
12. Cuzick J, et al. Overview of the European and North American studies on HPV testing in primary cervical cancer screening. *Int J Cancer*. 2006; 119(5):1095–101. [PubMed: 16586444]
13. Mayrand MH, et al. Human papillomavirus DNA versus Papanicolaou screening tests for cervical cancer. *N Engl J Med*. 2007; 357(16):1579–88. [PubMed: 17942871]
14. Howlader, N., et al., editors. SEER Cancer Statistics Review 1975-2008. National Cancer Institute; Bethesda, MD: 2011.

15. Follen M, Richards-Kortum R. Emerging technologies and cervical cancer. *J Natl Cancer Inst.* 2000; 92(5):363–5. [PubMed: 10699061]
16. Arbyn M, et al. Pooled analysis of the accuracy of five cervical cancer screening tests assessed in eleven studies in Africa and India. *Int J Cancer.* 2008; 123(1):153–60. [PubMed: 18404671]
17. Goldie SJ, et al. Cost-effectiveness of HPV 16, 18 vaccination in Brazil. *Vaccine.* 2007; 25(33): 6257–70. [PubMed: 17606315]
18. Sankaranarayanan R, et al. HPV screening for cervical cancer in rural India. *N Engl J Med.* 2009; 360(14):1385–94. [PubMed: 19339719]
19. Cuzick J, et al. Overview of human papillomavirus-based and other novel options for cervical cancer screening in developed and developing countries. *Vaccine.* 2008; 26(Suppl 10):K29–41. [PubMed: 18847555]
20. Gravitt PE, et al. New technologies in cervical cancer screening. *Vaccine.* 2008; 26(Suppl 10):K42–52. [PubMed: 18847556]
21. Qiao YL, et al. A new HPV-DNA test for cervical-cancer screening in developing regions: a cross-sectional study of clinical accuracy in rural China. *Lancet Oncol.* 2008; 9(10):929–36. [PubMed: 18805733]
22. Munoz N, et al. Epidemiologic classification of human papillomavirus types associated with cervical cancer. *N Engl J Med.* 2003; 348(6):518–27. [PubMed: 12571259]
23. Schutzbank TE, Ginocchio CC. Assessment of clinical and analytical performance characteristics of an HPV genotyping test. *Diagn Cytopathol.*
24. ASCCP. HPV Genotyping Clinical Update. 2009. Available from: <http://www.asccp.org/ConsensusGuidelines/HPVGenotypingClinicalUpdate/tabid/5963/Default.aspx>
25. Moberg M, Gustavsson I, Gyllensten U. Type-specific associations of human papillomavirus load with risk of developing cervical carcinoma in situ. *Int J Cancer.* 2004; 112(5):854–9. [PubMed: 15386375]
26. Gravitt PE, et al. High load for most high risk human papillomavirus genotypes is associated with prevalent cervical cancer precursors but only HPV16 load predicts the development of incident disease. *Int J Cancer.* 2007; 121(12):2787–93. [PubMed: 17722112]
27. Pett M, Coleman N. Integration of high-risk human papillomavirus: a key event in cervical carcinogenesis? *J Pathol.* 2007; 212(4):356–67. [PubMed: 17573670]
28. Cullen AP, et al. Analysis of the physical state of different human papillomavirus DNAs in intraepithelial and invasive cervical neoplasm. *J Virol.* 1991; 65(2):606–12. [PubMed: 1846186]
29. Pirami L, Giache V, Becciolini A. Analysis of HPV16, 18, 31, and 35 DNA in pre-invasive and invasive lesions of the uterine cervix. *J Clin Pathol.* 1997; 50(7):600–4. [PubMed: 9306943]
30. Castle PE, et al. A cross-sectional study of a prototype carcinogenic human papillomavirus E6/E7 messenger RNA assay for detection of cervical precancer and cancer. *Clin Cancer Res.* 2007; 13(9):2599–605. [PubMed: 17473189]
31. Molden T, et al. Predicting CIN2+ when detecting HPV mRNA and DNA by PreTect HPV-proofer and consensus PCR: A 2-year follow-up of women with ASCUS or LSIL Pap smear. *Int J Cancer.* 2005; 114(6):973–6. [PubMed: 15645423]
32. Tsoumpou I, et al. p16(INK4a) immunostaining in cytological and histological specimens from the uterine cervix: a systematic review and meta-analysis. *Cancer Treat Rev.* 2009; 35(3):210–20. [PubMed: 19261387]
33. Schweizer J, et al. Feasibility study of a human papillomavirus E6 oncoprotein test for diagnosis of cervical precancer and cancer. *J Clin Microbiol.* 48(12):4646–8. [PubMed: 20926711]
34. Wentzensen N, et al. Utility of methylation markers in cervical cancer early detection: appraisal of the state-of-the-science. *Gynecol Oncol.* 2009; 112(2):293–9. [PubMed: 19054549]
35. Feng Q, et al. Promoter hypermethylation of tumor suppressor genes in urine from patients with cervical neoplasia. *Cancer Epidemiol Biomarkers Prev.* 2007; 16(6):1178–84. [PubMed: 17548682]
36. Andersson S, et al. Detection of genomic amplification of the human telomerase gene TERC, a potential marker for triage of women with HPV-positive, abnormal Pap smears. *Am J Pathol.* 2009; 175(5):1831–47. [PubMed: 19880826]

37. Heselmeyer-Haddad K, et al. Genomic amplification of the human telomerase gene (TERC) in pap smears predicts the development of cervical cancer. *Am J Pathol.* 2005; 166(4):1229–38. [PubMed: 15793301]
38. Murphy N, et al. p16INK4A, CDC6, and MCM5: predictive biomarkers in cervical preinvasive neoplasia and cervical cancer. *J Clin Pathol.* 2005; 58(5):525–34. [PubMed: 15858126]
39. Wang SS, Sherman ME, Hildesheim A, Lacey JV Jr, Devesa S. Cervical adenocarcinoma and squamous cell carcinoma incidence trends among white women and black women in the United States for 1976-2000. *Cancer.* 2004; 100(5):1035. [PubMed: 14983500]

Practice Points

- Current cervical cancer screening methods are restricted by the region in which they are implemented
- New methods attempt to effectively, efficiently, and cheaply screen populations regardless of their resources
- New screening methods are broken into three broad areas: HPV diagnostics (either detection of the presence of HPV or integration of the virus into the host cell), proliferation, and detection of epigenetic changes (either in the host or virus).

Research Agenda

- Development of effective screening tests that may be used in low-resource settings
- Improvement of assays for detection of HPV viral load
- Improvement of strategies to detect viral integration

Summary

New methods of cervical cancer screening show great promise in allowing all women, regardless of socioeconomic status, to undergo evaluation for cervical cancer. These screening strategies focus on identification of oncogenic HPV infection and viral activity. They are broken into three broad areas: HPV diagnostics (either detection of the presence of HPV or integration of the virus into the host cell), proliferation, and detection of epigenetic changes (either in the host or virus). Many of these methods are in the early stages of development, but p16 evaluation and E6 testing strips show great promise. Through the implementation of new screening methods, practitioners hope to further refine and streamline the evaluation of women at risk of developing cervical cancer.

Table 1

Sensitivity and testing for Pap smear and HPV DNA testing in the detection of CIN2+

Test	Sensitivity	Specificity
Pap smear	53-55.4%	96.3-96.8%
High-risk HPV DNA testing	94.6-96.1%	90.7-94.1%
Pap smear + high-risk HPV testing	100%	92.5%

Cuzick J, Clavel C, Petry KU, Meijer CJ, Hoyer H, Ratnam S, Szarewski A, Birembaut P, Kulasingam S, Sasieni P, Iftner T. Overview of the European and North American studies on HPV testing in primary cervical cancer screening. *Int J Cancer*. 2006;119(5):1095.

Mayrand MH, Duarte-Franco E, Rodrigues I, Walter SD, Hanley J, Ferenczy A, Ratnam S, Coutlée F, Franco EL, Canadian Cervical Cancer Screening Trial Study Group.

Human papillomavirus DNA versus Papanicolaou screening tests for cervical cancer *N Engl J Med*. 2007;357(16):1579.

Table 2HPV Genotyping tests¹

HPV genotyping test	HPV types detected
Cervista® HPV 16/18 (Hologic, Inc; Marlborough, MA) ²	HR HPV types 16 and 18
Digene HPV Genotyping PS Test (Qiagen; Hilden, Germany)	HR HPV types 16, 18, and 45
Roche LINEAR ARRAY HPV Genotyping Test (Roche; Basel, Switzerland)	37 LR and HR HPV types
Innogenetics INNO-LiPA HPV Genotyping Extra (Innogenetics; Gent, Belgium)	28 LR and HR HPV types
SPF10 Line Probe Assay HPV-typing System (Roche; Basel, Switzerland)	Recognizes most genital tract HPV types
Papillocheck1 (Greiner Bio-One; Frickenhausen Germany)	18 HR and 6 LR HPV types
RealTime High Risk HPV Assay (Abbott Laboratories; Abbott Park, IL)	HPV types 16 and 18
HPV Genotyping LQ Test (Qiagen Inc; Valencia, CA)	18 HR HPV types
Seeplex HPV4A ACE (Seegene; Rockville, MD)	HPV types 16 and 18
CLART HPV 2 (Genomica; Madrid, Spain)	35 LR and HR HPV types
GenoFlow HPV Array (DiagCor; North Point, Hong Kong)	33 LR and HR HPV types
HPV Typing (molGENTIX; Barcelona, Spain)	15 LR and HR HPV types

HPV, human papillomavirus; HR, high-risk; LR, low-risk.

²FDA-approved test.

¹Schutzbank TE, Ginocchio CC. Assessment of clinical and analytical performance characteristics of an HPV genotyping test. *Diagn Cytopathol*. 2011 Apr 6. doi:10.1002/dc.21661. [Epub ahead of print]