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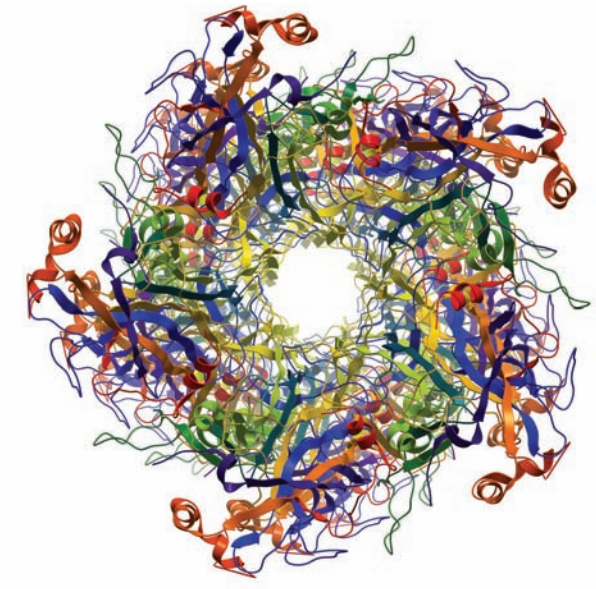

Cover: Major Capsid Protein of Human Papilloma Virus

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FEATURES

CONTINUING EDUCATION

DEPARTMENTS

PRODUCT FOCUS

MARKETPLACE

EXECUTIVE SNAPSHOT

Unmet clinical needs in cervical cancer screening

By Jianyu Rao, MD, Luisa Escobar-Hoyos, MSc, and Kenneth R. Shroyer, MD, PhD

The incidence of cervical cancer has dropped dramatically due to the success of the Papanicolaou (Pap) test, (the cytologic examination of cervical cells) in the detection of high-grade premalignant lesions that can be treated before they progress to invasive cervical carcinoma. Although the Pap test is the most effective tool ever deployed for cancer screening, it has been limited by problems of low sensitivity for high-grade premalignant lesions of the cervical mucosa.

Epidemiologic and molecular studies over the past three decades have firmly established that human papillomavirus (HPV) infection is the etiologic agent for virtually all cases of cervical squamous cell carcinoma (SCC) and also for the vast majority of cases of endocervical adenocarcinoma. Although the development of HPV-based test strategies can be used to enhance sensitivity for the detection of clinically significant lesions, HPV testing has been less effective as a primary screening assay in patient populations that have a high prevalence of HPV infection, including adult women under age 30 in both industrialized nations and many third-world countries. In addition, the incidence of death due to cervical cancer has not changed in many developing countries due to difficulties in introducing cost-effective and highly sensitive and specific cervical cancer screening programs that do not require a large clinical laboratory infrastructure and trained cytotechnologists to analyze results. Hence, there is a significant need for a new generation of molecular tests that can augment the existing Pap test, and potentially replace the HPV or Pap tests as the frontline screen in developing countries.

The biology of cervical cancer

Cervical cancer is highly curable when found early because it is usually a very slow-growing cancer, generally taking two years or more to progress and begin to spread to surrounding tissue. Invasive SCC comprises 75 percent to 77 percent of cases of malignant epithelial neoplasms of the cervix and is the most thoroughly investigated of cervical cancers.[1] In addition to SCC, invasive adenocarcinoma is also caused by HPV-mediated transformation and accounts for nearly 15 percent of cases of cervical carcinoma.

With exceedingly rare exceptions, virtually all cervical SCCs and the great majority of endocervical adenocarcinomas result from persistent HPV infection that has not been cleared by the patient's immune system. Cervical SCC does not arise *de novo* but is preceded by premalignant high grade squamous intraepithelial lesions (HSILs) that are composed of immortalized, clonal cell population that have not yet gained the potential to invade the underlying cervical stroma.[2-4]

Although cervical cancers originate from cells with pre-cancerous changes, epidemiologic studies suggest that only a minority of women with pre-malignant lesions of the cervix will develop cancer (10 percent to 30 percent, depending on regional influences). The prevalence of infection is age-related, with approximately 25 percent of women in their 20s in the United States harboring asymptomatic infections at any one time, but decreasing in prevalence as a woman ages. While most cases of HPV infection spontaneously regress within one to two years, in some cases, a transforming event occurs, frequently as a result of integration of the high risk HPV viral genome into the genome of cervical basal epithelial cells, leading to the loss of normal cell cycle regulatory mechanisms and the development of premalignant lesions of the squamous and/or glandular mucosa.[5,6]

Based to a large extent on the recognition that most cases of HPV spontaneously clear without treatment, the American College of Obstetrics and Gynecology, the American Cancer Society, and a number of other organizations recently recommended a reduction in the number of screening tests in young women. Recognition that most cases of HPV infection are transient has also highlighted the limited specificity of HPV testing for clinically significant lesions that require therapeutic intervention, reflecting clinically false-positive test results.[1,7] Although the recent introduction of the HPV vaccine will ultimately reduce the mortality associated with cervical cancer in vaccinated patient populations, early detection of cervical cancer by routine testing is likely to continue to be the most important aspect of cervical cancer management over the next several decades.[8]

Current cervical cancer screening methods and the need for improvements

The Pap test, first developed in the 1940s, has remained the gold-standard screening test for cervical cancer. When it is detected early, cervical cancer is among the most treatable of all cancers. In the U.S., deployment of the Pap test to detect premalignant lesions has reduced the incidence of cervical cancer by more than 75 percent over the past 30 years. The American Cancer Society estimates that in 2015 approximately 12,900 new cases of invasive cervical cancer will be diagnosed, and about 4,100 women will die from it. Notably, according to the World Health Organization (WHO), in 2008, there were more than 530,000 new cases of cervical cancer worldwide and 275,000 deaths from cervical cancers. More than 90 percent of them were recorded in developing countries. In Africa, 75,000 new cases were recorded in 2008 and 50,000 women died.[9] Women are dying in the developing world from a disease that has been largely eliminated in women in the U.S.

In recent years, incremental improvements in cervical cancer testing have been made to offset the limitations of the Pap test: the low sensitivity for detecting significant high-grade and above lesions[10]; the need for in-laboratory analysis with trained cytotechnologists; and test inaccessibility in the developing world. To date, more than 100 strains of the HPV virus have been de-

continued on page 10

Continuing Education

LEARNING OBJECTIVES

Upon completion of these articles, the reader will be able to:

1. Discuss reasons which describe the current and unmet clinical needs in cervical cancer screening.

2. Describe the current screening methods for cervical cancer and why there is a need for improvement on these techniques.

3. Identify the ways in which cervical cancer screening lacks in developing counties and describe what is needed for a clear and concise screening tool for this population.

4. List and describe current and new findings of the protein biomarkers that are used in cervical cancer screening and discuss the benefit of these findings for developing countries.

For CEU credit, complete the test on page 15 .

continued from page 8

fined, including approximately 40 that have can be detected in the reproductive tract, with 14 strains that are categorized as high-risk types.[11] Persistent infections with high-risk strains of HPV, including types 16 and 18, cause 70 percent of all cervical (and other genital) cancers in the U.S. and in many but not all parts of the world.

HPV-DNA testing has been widely deployed throughout the U.S. and many other industrialized nations to improve Pap test sensitivity for the detection of underlying high grade and malignant lesions of the cervical mucosa. Because HPV infection is present in virtually all cases of HSIL and cervical cancer, HPV-DNA-negative test results provide high negative predictive value to exclude clinically significant lesions in patients with normal or ASC-US cytology results. Conversely, however, HPV-positive test results have limited specificity for clinically significant lesions because the vast majority of HPV infections in young women are transient and do not lead to clinically significant disease. This limitation can be mitigated to some extent by limiting HPV testing to low-risk populations, but positive test results still result in a dilemma regarding the clinical management of patients with normal or minimally abnormal cytology findings. Incremental improvement in test specificity may also be achieved by a focus on detection of E6 and E7 oncogene expression, but the underlying problem of poor positive predictive value of positive test results has not been resolved. Thus, the clinical management of infections by an oncogenic virus (high-risk HPV) of undetermined origin (for a given patient) that cannot be effectively treated and usually resolves without treatment, but can cause HSIL or cancer, drives increased healthcare expenditures and resource utilization for the great majority of patients who are unlikely to benefit from clinical intervention.

In April 2014, the FDA approved the first frontline screen with an HPV-DNA test that could be used in place of the Pap test in women age 25 and older. The agency's approval came despite objections from several parties, including a coalition of 17 patient and women's health groups that expressed "grave concerns" that using the HPV test as a primary screen lacked support from evidence-based guidelines and may result in more invasive procedures for clinically insignificant infections. FDA approval required that a positive test for HPV types 16 or 18 be directly referred for colposcopy even though the HPV test does not reliably distinguish between transient versus transforming infections and does not necessarily indicate the presence of disease in multivariate modeling.[12] The FDA approval states that women who test positive for one or more of the other high-risk HPV types should have a Pap test to determine the need for a colposcopy. In following this approach, clinicians would essentially replace the Pap test as a highly effective screening device with a more expensive cervical cancer screening assay (the HPV test) that will increase the cost of cervical screening, in the absence of clear evidence of enhanced overall accuracy.

In the U.S., approximately 79 million individuals are currently infected with HPV and approximately 14 million additional individuals are newly infected each year.[13] By contrast, the prevalence of cervical cancer in the U.S. is approximately 12,900 cases/year.[14] Thus, only about 1.6 in 10,000 women with HPV infection actually have cervical cancer (and only about 0.7 percent of women with HPV infection have underlying HSIL).[15] Thus the vast majority of women with positive HPV test results do not have underlying lesions that require therapeutic intervention. (This general conclusion holds true even when factoring in women with high grade glandular lesions of the cervical mucosa which are also usually caused by HPV infection.) The extraordinarily high prevalence of HPV infection compared to the relatively low prevalence of cervical cancer and HSIL suggests that most cases of HPV infection have limited clinical significance. This is because HPV infection in the vast majority of cases will spontaneously resolve without treatment within two years, and most patients with positive HPV test results do not have lesions that require clinical intervention.

HPV testing can also be limited by false-negative test results. Although there are numerous anecdotal reports of false-negative testing, the largest study of its kind, the Quest Diagnostics Health Trends study (based on tests of more than 250,000 women), found that nearly five percent of women with a CIN3+ (severely abnormal cells and cancer cells) Pap test had a negative HPV test.[16] Within five years, nearly 25 percent of these women were found to have cervical cancer.[17] This finding could be due to several factors including transient infection that has cleared after initiation of the oncogenic processes or a false-negative HPV test result. In either case, this result confirms the risk of using HPV as the sole testing in the frontline screening of cervical cancer.

Need for cervical cancer screening in developing countries

In a seminal study, Gakidou et al.[20] investigated cervical cancer screening in 57 developing countries and found that the percentage of women being screened, on average, was 19 percent, compared to 63 percent in developed countries, and ranged from one percent in Bangladesh to 73 percent in Brazil. Effective coverage varied widely across countries, from more than 80 percent in Austria and Luxembourg to one percent or less in Bangladesh, Ethiopia, and Myanmar (Burma). In many countries, a large proportion of women have had pelvic examinations, but the exam was not accompanied by laboratory tests or was not done in the three years preceding the survey. In the nation of Georgia, for example, 67 percent of women have had a pelvic exam, but only 11 percent had had one in the preceding three years, accompanied by a Pap smear; likewise in China, crude coverage (i.e., any past pelvic exam) is 70 percent but effective screening coverage (e.g., a pelvic exam with Pap test sometime in the preceding three years) is only 23 percent.

The study analysis points to an acute shortage of cervical cancer prevention services across much of the developing world and striking inequalities in access to these services, highlighting the need for new prevention and treatment strategies. This study also identifies a number of countries where the vast majority of women have never had a pelvic exam. In such settings, where the health system is unable to provide even low levels of crude coverage of this basic intervention, improved screening is urgent, especially for women older than 35.

The burden on healthcare availability and delivery will be exacerbated as world population grows. Significantly, the world population will be aging faster by 2050 than in the U.S., according to a 2014 Pew Research Center analysis of a recent United Nations study: *World Population Prospects 2012*. By 2050, the report notes, the number of people 65 and older is projected to triple, from 531 million in 2010 to 1.5 billion in 2050. In the U.S., the number of people 65 and older is expected to slightly more than double, from 41 to 86 million.[18]

In early 2015, the WHO estimated that the number of new cancer cases is expected to rise by about 70 percent over the next two decades, from 14 million in 2012 to 22 million new cases.[19] The report notes that cancer-causing viral infections such as HBV/HCV and HPV are responsible for up to 20 percent of cancer deaths in low- and middle-income countries and that more than 60 percent of the world's total new annual cases occur in Africa, Asia and Central and South America. These regions account for 70 percent of the world's cancer deaths.

Clearly, there is a pressing medical need for highly accurate [1]

continued on page 13

continued from page 13

Protein biomarkers in cervical cancer testing

Failure of the immune system to clear HPV may enable persistent infection and increase the risk of HPV viral genome integration into the genome of cervical mucosa basal cells.[5]

HPV viral integration, and possibly other pathways that lead to an HPV-transforming event, disrupt normal cell cycle regulatory mechanisms and result in the degradation of the key cell cycle regulatory proteins p53 and Rb, enabling cells to continue to divide, even in the face of genomic DNA damage. These mechanisms may explain, at least in part, how a persistent infection can ultimately lead to malignant transformation of the cervical mucosa, reflected by changes in the expression of the range of proteins that both have roles in the pathogenesis of cervical cancer and also could serve as biomarkers of premalignant and malignant lesions.

Altered protein expression can be measured using quantitative assays for biomarkers that increase with the disease progression. Although HPV testing generally provides information only on the presence or absence of viral infection, tests based on cervical cancer biomarkers may identify patients that are most likely to benefit from clinical intervention while reducing the number of unnecessary biopsies. Several host and viral molecules are being investigated for their use in the detection of HSIL.[22] One study involved testing of 302 biopsy samples against a panel of 13 different potential biomarkers.[6,7]

Multivariate modeling completed in this study, as well as additional modeling, strongly demonstrate that a panel of three to five markers can be used to confidently grade cervical intraepithelial lesions. Another study showed that measurement of HPV E6 protein in cervical cytology samples could be used to distinguish HSIL from cytologically normal specimens, improving diagnostic accuracy compared to what can be achieved by traditional HPV DNA tests.[23]

Most recently, a team of investigators at Stony Brook University Medical Center led by one of the authors of this article, Dr. Kenneth Shroyer, conducted research leading to the identification of unique protein biomarkers that have both the potential to play a role as diagnostic biomarkers and to be prognostic for patient survival. In this lab, mass spectrometry of laser capture-microdissected cervical biopsy specimens enabled the discovery of more than 2,000 proteins that were differentially expressed in cervical cancer and HSIL compared to normal mucosa and productive HPV infections.[24] In silico analysis and immunohistochemical evaluation subsequently identified keratin 17 (K17) as a powerful prognostic biomarker for survival of patients with SCC that was independent of tumor grade, stage, or HPV status.[24,25] Patients with elevated K17 expression have a very poor prognosis for survival, even in cases that appeared to be confined to the cervix at the time of diagnosis, while patients with advanced-stage disease but low K17 status have excellent long-term survival.[24,25] Further investigation led to the surprising discovery that K17 is able to enter the nucleus of cancer cells, where it binds to and ultimately causes the degradation of p27KIP1, an important regulator of early (G1) cell cycle arrest. Thereby, the disruption of normal cell cycle regulatory mechanisms explains, at least in part, why cervical SCCs with high K17 are much more likely be fatal than SCCs that express only a low level of K17.

These findings support a premise that the measurement of protein biomarkers in cervical cytology samples is a valid approach that not only can provide clinically useful information in identifying high-grade cervical lesions and cervical cancer in developed nations, but could potentially be utilized in developing countries where there are obstacles to deployment of the Pap test for cervical cancer screening. Furthermore, multiplexing of the relevant cervical cancer biomarkers has the potential to both establish disease status and predict cervical cancer progression, providing a valuable diagnostic tool for early detection to enable more focused treatment of high-grade cervical lesions in low-resource settings where colposcopy and histological analyses are less readily available.

Summary

Cancer rates worldwide are expected to increase disproportionally in coming decades relative to the projected increase in population, especially in the developing world. The general unavailability of the Pap test and the cost of the HPV test in the developing world have precluded the deployment of effective cervical cancer screening programs in many developing countries. Recent improvements in testing technology arise from a need to overcome the significant limitations of the Pap test and HPV test, but results require first-world technology and validation.

Developing countries, where cervical cancer remains one of the most important causes of cancer death, have the greatest need for an affordable, easy-to-use, and highly reliable cancer screening method that can return a diagnosis through efficient laboratory analysis or, more easily, at a woman's point of care.

While research, testing, and vaccine improvements in recent years continue to lower the incidence of cervical cancer in some developed countries such as the U.S., HPV testing research needs to do more than test for the presence of virus. The tests must determine the presence and progression of cervical disease. Tests should be more sensitive and specific than Pap tests and Pap-related tests, and should be accurate in more than 90 percent of cases. Tests also need to be low-cost, objective, and easy to perform so screening programs can be widely implemented in developing countries where the need for a better cervical cancer screening test is highest. Such tests may be available through the recent advances in specific biomarkers of cervical cancer and multiplex detection technologies. Development of the next generation of cervical cancer tests that are more specific, sensitive, and informative than the traditional Pap or HPV test will make a significant impact on the reduction of cervical cancer worldwide.

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