Cervical-Cancer Screening with Human Papillomavirus and Cytologic Cotesting

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A healthy 35-year-old woman wants to discuss cervical-cancer screening. She reports no symptoms and has a negative screening history except for an “abnormal Pap” about 10 years previously that did not require treatment. She has two children, is currently taking oral contraceptives, and does not smoke. She is interested in human papillomavirus (HPV) testing because of an article she read in a magazine, which suggested that “testing with HPV and Pap is better than just Pap alone.” What would you advise?

Cervical cancer is the third leading type of cancer in women worldwide. There are approximately 530,000 new cases and 275,000 associated deaths annually.1 The disease burden is highest in resource-poor countries, where more than 80% of cases occur.1 In the United States and other countries that have developed widespread programs for cervical cytologic screening (Papanicolaou [Pap] tests), morbidity and mortality from cervical cancer have been greatly reduced. Nevertheless, approximately 4000 women die from cervical cancer each year in the United States.2

A single Pap test has limited sensitivity for the detection of cervical cancer and its immediate precursors. Strategies routinely used in the past to compensate for the limited sensitivity included repeat screening at short intervals and a low cytologic threshold for additional follow-up. However, this approach is costly and generates many unnecessary follow-up tests, such as colposcopic examinations. Expenditures in the United States total $6 billion annually for more than 50 million screening tests and for the clinical care of women with mainly minor screening abnormalities.3

Virtually all cases of cervical cancer are caused by persistent infection with one of about a dozen carcinogenic HPV genotypes — specifically, HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68.4,5 The carcinogenic HPV types are evolutionarily related and belong to the alphapapillomavirus genus; they are distantly related to the viruses associated with common skin warts.6-8 HPV-16 is the most carcinogenic type, accounting for half of cervical-cancer cases.9 HPV-18, which is implicated in many cases of endocervical adenocarcinoma,10 is the second most carcinogenic type, accounting for approximately 15% of all cervical cancers.11

An understanding of the central role of HPV in the development of cervical cancer has led to the following two effective preventive strategies, in addition to Pap tests: primary prevention by vaccination,12-14 and enhanced secondary prevention (screening) with the use of HPV-based molecular assays that detect viral DNA
or RNA.\textsuperscript{15} Owing to incomplete coverage of the HPV types and partial population uptake (approximately one third of adolescent girls at the target ages have completed the three-dose vaccination regimen), the advent of vaccination has not yet altered screening recommendations in the United States.\textsuperscript{16}

Molecular testing for the pool of carcinogenic types of HPV was initially introduced as a secondary test (so-called reflex HPV testing, used as a triage tool) for clarifying whether women with equivocal lesions (atypical squamous cells of undetermined significance [ASC-US]) on cytologic screening require colposcopy. More recently, the number of assays for HPV that have been approved by the Food and Drug Administration has increased,\textsuperscript{15,17-19} and the use of testing for carcinogenic HPV types in women 30 years of age or older has been expanded to include HPV testing in combination with cytologic evaluation (HPV cotesting).\textsuperscript{16,20,21} Stand-alone HPV screening\textsuperscript{22} is not currently recommended in the United States, although it has been adopted elsewhere. This article discusses the rationale for HPV cotesting, as well as the benefits and challenges of cotesting.

**Strategies and Evidence**

The many cytologic and histologic terms used in cervical screening to describe the natural history of cervical cancer have recently been consolidated in the United States (Table 1 in the Supplementary Appendix, available with the full text of this article at NEJM.org). For squamous disease, accounting for the great majority of cancer cases, the stages consist of low-grade squamous intraepithelial lesions (LSIL), indicating microscopical evidence of acute HPV infection; high-grade squamous intraepithelial lesions (HSIL), indicating precancer; and cancer.\textsuperscript{9} Histologically, HSIL correlates with cervical intraepithelial neoplasia grade 3 (CIN 3) and most cases of cervical intraepithelial neoplasia grade 2 (CIN 2).\textsuperscript{23}

The introduction of HPV testing into the screening protocol for women 30 to 65 years of age was prompted by prospective observational studies showing the high sensitivity of HPV testing, as well as the corollary reassurance that a negative test confers a very low risk of cancer. Several long-term, prospective studies (lasting more than 10 years) have shown that the risk of CIN 3 or cancer is approximately 1\% among women with a negative test for HPV, as compared with 5 to 10\% among women with a positive test.\textsuperscript{24-27} Randomized trials comparing HPV testing alone with cytologic testing alone\textsuperscript{28-32} have shown that more cases of CIN 3 or cancer are detected with the use of HPV testing than with the use of cytologic testing in the first round of screening, with a commensurate decrease in the number of cases at the subsequent screening. A large trial of HPV testing, which was conducted in India among women who had never before been screened, convincingly showed during an 8-year follow-up period that deaths from cervical cancer were extremely rare among women who had a single negative HPV screening test.\textsuperscript{33}
Proper use of HPV testing is guided by the typical time course (Fig. 1) and age at diagnosis (Fig. 2) for each step in cervical carcinogenesis. Proper use of HPV testing is guided by the typical time course (Fig. 1) and age at diagnosis (Fig. 2) for each step in cervical carcinogenesis.9,15 Cervical HPV is transmitted by direct sexual contact; transmission does not require penetrative sexual intercourse. Peak transmission is in adolescence and young adulthood.9 Most infections, including the minority that cause microscopically evident equivocal abnormalities (ASC-US) or definite abnormalities (LSIL), clear (i.e., become undetectable on sensitive molecular assays) in 1 to 2 years. Those infections that remain detectable are considered to be persistent. Only persistently infected cervical cells have a substantial likelihood of gradually growing into diagnosable HSIL. Cohort studies have shown that most diagnoses of HSIL are made 5 to 10 years after infection.9 The long time frame, typically at least one decade and possibly more than three decades,35 between the development of HSIL and the development of invasive cancer explains why screening is usually effective — treatment of HSIL at any time during this interval can prevent cancer.

Since adolescents are at extremely low risk for cervical cancer but have high rates of transient HPV infections, current guidelines (Table 1) recommend that cervical-cancer screening start at the age of 21 years, regardless of the age at first intercourse.16,20,21 For women who are 21 to 29 years of age, the prevalence of HPV remains very high, but the risk of cancer increases only slightly; as a result, cytologic screening alone every 3 years is recommended. Decision modeling suggests that starting at 21 years of age,
screening at a 3-year interval as compared with annual screening results in a small difference in the predicted lifetime risk of cervical cancer (8.5 cases per 1000 women vs. 2.5 cases per 1000 women) and a substantial reduction in the number of lifetime colposcopic examinations (approximately 760 per 1000 women vs. 1930 per 1000 women). Cotesting is not used for primary screening in this age group, since the positive predictive value of the HPV test is low. However, if the cytologic result is ASC-US, then reflex HPV testing is used as a triage test; among women 25 years of age or older, reflex HPV testing is preferred over repeat cytologic testing or immediate colposcopy.

For women 30 to 65 years of age, the current strategy in the United States is cotesting every 5 years or cytologic testing alone every 3 years. Either conventional or liquid-based cytologic tests are acceptable, given their similar results when they are well performed. A major advantage of cotesting in women 30 years of age or older (i.e., past the age at which the prevalence of HPV infection peaks) is that both cytologic testing and HPV testing will be negative in the great majority of women, indicating reassuringly low risk and permitting lengthened screening intervals. A large-scale study at Kaiser Permanente Northern California, which adopted cotesting in 2003, has shown a low risk of cervical cancer among women who have had negative results of HPV and cytologic cotesting. Among approximately 300,000 women in the Kaiser Permanente database who were 30 years of age or older and had negative cotests, the 5-year cumulative incidence of CIN 3 or cancer was less than 2 cases per 1000 women, and the 5-year incidence of cancer was less than 2 cases per 10,000 women—less than half the cancer risk associated with negative results of cytologic testing alone. The improved negative predictive value of cotesting over that of cytologic testing alone is the basis for the current recommendation to extend the screening interval from every 3 years (if cytologic screening alone is negative) to every 5 years (if cotesting is negative).

In populations with a low risk of cervical cancer, screening is not recommended. These populations include women older than 65 years of age who have had an adequate number of negative screening results previously and women who have had a hysterectomy with removal of the cervix and have no history of HSIL.

**Table 1. Cervical-Cancer Screening Guidelines.**

<table>
<thead>
<tr>
<th>Age group</th>
<th>Screening Recommendation</th>
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<tbody>
<tr>
<td>&lt;21 yr</td>
<td>Do not screen.</td>
</tr>
<tr>
<td>21–29 yr</td>
<td>Perform cytologic testing alone every 3 years.</td>
</tr>
<tr>
<td>30–65 yr</td>
<td>Perform cytologic and HPV cotesting every 5 years (preferred), or perform cytologic testing alone every 3 years (acceptable).</td>
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<tr>
<td>&gt;65 yr</td>
<td>Discontinue screening if there has been an adequate number of negative screening results previously (3 consecutive negative cytologic tests or 2 consecutive negative cotests in the past 10 years, with the most recent test in the past 5 years) and if there is no history of HSIL, adenocarcinoma in situ, or cancer.</td>
</tr>
<tr>
<td>Women who have undergone hysterectomy</td>
<td>Discontinue screening if the patient has undergone a total hysterectomy with removal of cervix and if there is no history of HSIL, adenocarcinoma in situ, or cancer.</td>
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Among women with HPV infection, the risk of cervical cancer is increased in association with multiparity, prolonged use of oral contraceptives, and smoking, but the effect of these cofactors is not strong enough to affect clinical management. Immunosuppression, in contrast, is associated with a risk of HSIL that is high enough to warrant distinct screening guidelines, although the care of women who have abnormal screening results is not altered.

**Areas of Uncertainty**

**Cost-effectiveness of Various Screening Strategies**

There has been insufficient comparison of the cost-effectiveness of various screening options (in particular, cytologic and HPV cotesting vs. HPV testing alone). The addition of cytologic testing to HPV testing is much more expensive than HPV testing alone and provides limited ad-
ditional reassurance that cancer is neither present nor about to develop. Among the women involved in the Kaiser Permanente Northern California study, the 5-year risk of CIN 3 or cancer after negative cotests was estimated to be 0.16%, which was not meaningfully lower than the risk after a negative HPV test alone (0.17%).

It is unclear whether U.S. clinicians and women who are accustomed to more frequent Pap tests will accept the extended screening interval warranted by more sensitive screening. If cotesting were performed at more frequent intervals, new tests with positive results in women with previously negative test results would most likely represent incident HPV infections, with a low associated risk of HSIL or cancer; thus, the identification of HPV infections would be likely to increase costs and downstream testing without improving outcomes.

**MANAGEMENT OF POSITIVE SCREENING COTESTS**

Less than 10% of women will have a positive screening result on cotesting. The various combinations of positive cytologic categories and HPV test results are associated with different risks of HSIL or cancer. Management options include continued regular screening, increased surveillance (shorter screening intervals), or colposcopic assessment, depending on the risk.

For high-risk combinations (e.g., cytologic HSIL, regardless of the HPV test result), immediate colposcopy is warranted. However, for some very common combinations associated with lower risk, the best methods of management are uncertain. The incidence of various combinations of cotest results, the associated 5-year risks of histologic HSIL (approximated by data for CIN 2 and CIN 3) and cancer and the currently recommended management are shown in Table 2. Risk-based management guidelines were developed to ensure that women at equal risk for cancer receive similar care according to existing and accepted practice. However, randomized trials of various management options have not been conducted, and many recommendations are based on observational data or expert opinion.

The most common combination of positive screening cotests is a positive HPV test and a negative cytologic test; approximately 4% of women 30 years of age or older who undergo cotesting have this combination of results.

Among such women, the 5-year risk of HSIL or cancer is approximately 10%, which is not quite high enough to justify immediate referral for colposcopy; moreover, referral would triple the number of colposcopic examinations. Intensified surveillance is recommended, but the best timing for repeat testing is uncertain; longer intervals allow a higher proportion of infections to clear but result in a greater, though still small, risk of cancer. The most recent guidelines recommend repeat cotesting in 1 year, with referral for colposcopy if cytologic testing is positive or if HPV testing remains positive, suggesting persistent infection.

An alternative is genotyping DNA or RNA specifically for HPV-16 and HPV-18, with immediate colposcopy if either of these highest-risk types is found and repeat cotesting in 1 year if neither type is found. This is currently the only recommended use of genotyping to determine individual HPV types. Biomarkers have also been proposed to help identify women who are at particularly high risk for HSIL and cervical cancer and require colposcopy. One proposed biomarker measured in a cytologic specimen is p16, which if positive indicates an increased risk of HSIL or cancer. Further evaluation of the p16 assay is needed, as are definitive comparisons of alternative options for women who have positive HPV test results and negative cytologic test results.

Data are also lacking to guide the care of the very small percentage of HPV-positive women who have persistently positive HPV tests for years without evident progression to HSIL. The question is whether to choose indefinitely heightened surveillance with repeated testing and perhaps colposcopy or presumptive treatment with loop electrosurgical excision.

Another combination of screening cotest results, affecting approximately 1 million women a year in the United States, is a negative HPV test with a cytologic test showing ASC-US. ASC-US is by far the most common abnormal cytologic finding (representing approximately 5% of cytologic test results) but is an equivocal or borderline result. At least half of women with a cytologic test result of ASC-US have a negative test for carcinogenic HPV, and theoretically, such women should not be at increased risk for cervical cancer; however, in the Kaiser Permanente
Northern California study, the 5-year risk of cancer among these women (0.05%) was meaningfully higher than the risk among women with “dual negative” cotests (0.01%). Owing to this ambiguity, current guidelines provide different recommendations for the timing of repeat cotesting; the recommended interval is currently 3 years in one set of guidelines and 5 years in another. There is a delicate balance between tailoring follow-up screening intervals to gradations of demonstrated risk and issuing overly complex guidelines that are impractical for clinical implementation.

By identifying women without definite abnormalities on cytologic tests but with positive HPV tests, cotesting will lead to colposcopy in many women in whom HPV infection would be likely to clear. Other women will have very small HSIL that are difficult to visualize colposcopically and that are probably more likely to regress spontaneously than are larger HSIL identified on Pap testing. Obtaining more than one biopsy specimen, targeting even faintly visible aceto-white lesions (lesions that turn white with the application of acetic acid), substantially increases the sensitivity of colposcopy for the detection of small HSIL.
GUIDELINES

In 2011, the American Cancer Society, together with two dozen other organizations, developed evidence-based screening guidelines for the prevention and early detection of cervical cancer. Separately, the American College of Obstetricians and Gynecologists and the U.S. Preventive Services Task Force convened different sets of experts to develop guidelines. To minimize the potential for confusion associated with multiple guidelines, the groups agreed to harmonize wording when their conclusions were concordant. The recommendations issued by the three groups are largely identical (Table 1). The central points are that cytologic screening at 3-year intervals is accepted as the standard of care in the United States, and cotesting at 5-year intervals is judged to result in equivalent or even greater reductions in the incidence of cervical cancer and in mortality associated with cervical cancer.

In 2013, a multisociety group led by the American Society for Colposcopy and Cervical Pathology (ASCCP) published updated guidelines for managing abnormal cervical findings. To the extent practicable, similar management strategies are recommended for women at similar risk for HSIL and cancer, regardless of the particular findings used to determine the level of risk (Table 2).

CONCLUSIONS AND RECOMMENDATIONS

For women 30 years of age or older, such as the woman described in the vignette, we would recommend HPV and cytologic cotesting over cytologic testing alone (although cytologic testing alone is acceptable). Cotesting is associated with greater sensitivity, greater reassurance if both tests are negative, and longer screening intervals. Our recommendation is not changed by the woman’s history of apparently minor abnormalities on a Pap test in the distant past. The great majority of women who undergo cotesting will have normal results and can reasonably wait 5 years for repeat screening. For the 5 to 10% of women with positive cotest results, management guidelines are tailored to the level of risk associated with the specific combination of cytologic and HPV test results.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

REFERENCES


