HPV Testing: Is It Useful in Triage of Minor Pap Abnormalities?

J. Thomas Cox, MD
Santa Barbara, California

Just over 15,000 women develop cervical cancer in the United States annually,[1] and while the majority develop in the segment of the population that remains unscreened, approximately 4500 women develop cervical cancer annually who have had reasonable, if not all perfect, Papanicolaou (Pap) smear screening results. Between 13% and 31% of women who develop cervical cancer have had at least one negative Pap report within the preceding 3 years.[2] The lifetime likelihood that a women never screened will develop cervical cancer is 3748 women per 100,000 (3.7%). Even with annual screening, approximately 305 per 100,000 women (0.3%) will develop cervical cancer during their lifetime,[3,4] a toll that is individually agonizing for both patient and for caregiver. Even though this risk is statistically small, in order to protect our patients and ourselves from the vicissitudes of missed cervical cancer, the medical community has responded by the pursuit of even the most minimally atypical cells. The result has been an excessively expensive screening program fraught with the risk of overdiagnosis, overtreatment, and increased psychological burden.[5] While all who have taken the Hippocratic Oath desire to do anything and everything possible to prevent an untimely loss of life, we must admit that much of our response to minor cytologic abnormalities has developed less out of reason than out of fear of liability.

Cytology is subjective, as much an art as a science. Although artificial categories have been set up to divide a continuum of abnormal cells, nature's paintbrush is not as specific as we would like. While severely abnormal cells are likely to reflect a similar histology, minor atypia and low-grade abnormal cells are confusing and often of less clear origin. Even moderate dysplasia may be very difficult to differentiate from inflammatory effects on immature metaplasia.[6] Subjectivity in both cytology and histology results in significant variability in interpretation between observers, and even with the same observer at different times.[7-9] The result is that one cytopathologist's atypical squamous cells of undermined significance (ASCUS) is another's low-grade squamous intraepithelial lesion (LSIL), or another's reactive and reparative (normal) change.

With so much inter- and intraobserver variability, it is not uncommon for cytologic and histologic findings to be misclassified.[8] Misclassification is particularly problematic in women older than 35 years. In a study by Schiffman et al,[10] cytologic diagnosis of LSIL was prone to misclassification that increased with age. Older women were more likely to have had initial false-positive Pap smear reports of possible dysplasia that, on review, were ruled out by a panel of five expert cytopathologists. In peri- and postmenopausal women, squamous atypia are often mistakenly classified as koilocytotic atypia but when evaluated by polymerase-chain-reaction analysis,[11] these findings have been shown not to be associated with the human papillomavirus (HPV). Furthermore, evaluations of women with higher median age often detect HPV DNA in only 30% to 60% of women with low-grade cervical intraepithelial neoplasia (CIN).[12-14] In contrast, Chesebro and colleagues,[15] reporting on a young population of women, detected high-risk HPV by Hybrid Capture assay (Digene Diagnostics, Silver Spring, Md) in 80% referred with an LSIL Pap smear report, indicating that misclassification of LSIL at this age is rare.

HPV testing should help in evaluating equivocal and CIN 1 histology and low-grade cytology by clarifying confusion created by interobserver variability and misclassification.[16] Women most likely to be normal (HPV negative) could be more safely followed by cytologic evaluations, whereas women at greatest risk for current or incipient

From the Student Health Services, University of California–Santa Barbara. Requests for reprints should be addressed to J. Thomas Cox, MD, Student Health Services, University of California–Santa Barbara, Santa Barbara, CA 93106.

CIN (HPV positive) could be referred for colposcopy. However, commercial HPV tests have been available for 9 years, yet HPV testing in clinically equivocal situations has not been widely utilized. Relatively few evaluations of HPV testing for clinical management have been published, and wide variation in results have not always provided clear endorsement of the technique.

This month's issue of the *Journal* has two articles by Ferris and colleagues that provide important insight into the potential for HPV testing performed in a clinical setting. One article evaluates several management protocols for women with Pap smear reports of ASCUS and LSIL: immediate referral to colposcopy, or triage to colposcopy on the basis of the results of repeat cytology or of the presently commercially available FDA-approved Hybrid Capture tube-based test for HPV DNA.[17] The other article evaluates the performance of this HPV test in comparison with the new generation Hybrid Capture II microplate assay (Digene Diagnostics, Silver Spring, Md) that utilizes samples drawn from the residual liquid-based cervical cytologic transport media (Cytyc Corp, Boxsborough, Mass),[18] as proposed for ASCUS triage in 1995.[19] The findings provide important insight into the relative value of each management protocol.

Ferris found equivocal or low-grade Pap smear triage to colposcopy on the basis of a repeat abnormal smear result of LSIL or higher grade to be "clearly ineffective for detecting high-grade cervical precancerous conditions." Lowering of the threshold for colposcopic referral to also include repeat ASCUS expanded the pool of women referred to colposcopy to 64% (56% of women referred for ASCUS and 76% for LSIL), but continued to miss 17% of the women with CIN grade 2 or 3. These results add to the increasing evidence that cytologic follow-up is not the best option. A recent meta-analysis of Pap smear accuracy found that repeat cytology had a mean sensitivity of 66%, and this was affected little by histologic threshold.[20] Others have documented that the false-negative rate of repeat cytology may be higher than with primary screening,[21-23] and high-grade CIN has been detected in women after two negative follow-up smears.[24] Mayeaux et al[21] found that 73% of 110 women with high-grade CIN had either low-grade or normal follow-up Pap smears, concluding that using follow-up cervical smears to monitor patients with Pap smear reports of LSIL carries unacceptable risks.

HPV testing with the Hybrid Capture (HC) test also performed poorly as a triage option in the Ferris study. For reasons that are not apparent, the sensitivity for detection of CIN 2/3 (50%) was significantly less than reported in other studies of young women. Chesebro detected HPV DNA by HC in 92.3 % of women with high-grade CIN,[15] similar to the 92.5% sensitivity in our study on ASCUS triage,[19] and to the 93% documented by Hall and colleagues.[25] Others have found HPV test results to be intermediate between those documented in these studies and the results by Ferris. Ferenczy et al[13] detected HPV DNA by HC in 77% of CIN 2/3 documented in 364 women referred for evaluation of an abnormal Pap. When both repeat Pap and HPV tests were negative, the chance of missing a high-grade lesion was extremely low, in the order of 3%.

Wright et al[14] outlined management algorithms for the follow-up of low-grade cytologic abnormalities based on the results of a retrospective review of Pap, colposcopy, and HPV results obtained in follow-up to ASCUS and LSIL Pap smear reports. They concluded that HPV testing is now at least as sensitive and specific as repeat cervical cytology in detection of CIN, and that an intermediate triage protocol based on a combination of repeat cytology and HPV testing has a sensitivity similar to colposcopy for the detection of high-grade CIN. The negative predictive value of the combination of a repeat Pap and an HPV test for CIN 2/3 in every one of these studies has been between 95% and 100%. In contrast, Ferris concluded that HPV testing was not of value because the sensitivity for high-grade disease was low, and the combination of repeat cytology and HPV testing did not significantly improve detection.

I would like to argue that the weight of the evidence, to the contrary, supports the accuracy of HC for the detection of high-grade disease, particularly when combined with a repeat Pap. Reasonable concern persists, however, that the sensitivity for detection of HPV DNA by HC (approximately 100,000 copies of HPV DNA per test) may not provide that margin of safety necessary as a stand-alone triage for older women at risk for high-grade CIN and cervical cancer. A cost analysis for ASCUS triage on the basis of two repeat Pap smears and an HC HPV test, how-

ever, has not shown significant cost savings over immediate referral to colposcopy.[14]

For HPV testing to be a cost-effective option, sensitivity for detection of CIN 2/3 must consistently provide a reassurance that allows triage on the basis of an HPV test result without the requirement for a repeat office visit and Pap smear. Reflex testing for HPV of residual Pap smear samples in liquid transport media by HC II microplate, with a 97% negative predictive value, appears to offer the reassurance necessary for a "stand-alone" triage test.[18] The HC II HPV test was set at a positive threshold that is approximately 50- to 100-fold more sensitive (1000 copies of HPV DNA per test) than the HC tube-based test. This increased sensitivity for detection of CIN 2/3 to 90.5% without the addition of a repeat Pap.

Because the HPV test taken directly from the residual Pap smear cells in a liquid transport media identified almost all cases of high-grade CIN, such a triage strategy allows for immediate "reflex" HPV testing of all ASCUS Pap smears directly at the laboratory, without the requirement for a repeat office visit to obtain the HPV test. The Pap could then be reported as "ASCUS–high-risk HPV positive," or as "ASCUS–HPV negative." Women with ASCUS reported as HPV positive would be referred to colposcopy, whereas women with HPV-negative ASCUS could be followed more safely with cytologic follow-up before returning to annual examinations. Such a triage protocol should be substantially cost-effective and reduce patient anxiety by more quickly reassuring women with HPV-negative ASCUS and by providing less uncertainty about the meaning of an ASCUS Pap for those women HPV positive. For this test to be optimally cost-effective, however, the positive threshold may need to be set at different levels for women at different ages, since it is clear that increased sensitivity results in decreased specificity, particularly for younger women.

HPV testing, however, does not appear to be helpful for women with LSIL. When disease prevalence is low (21.9% CIN in our ASCUS study) and the HPV test does not identify too many women who do not have disease (42% were HPV positive with one half having CIN), HPV testing performs very well as an objective and cost-effective triage tool.[19] In contrast, women with LSIL are much more likely to have CIN (67% in the Chesebro study) and most (80%) test positive for high-risk HPV.[15]

Two large studies are currently being conducted that will provide the definitive data on the potential utility of HPV DNA testing in the triage of women with ASCUS and LSIL Pap smear reports. Kaiser Northern California embarked on an ambitious study of 50,000 women in 1995 on ASCUS and LSIL triage by HC II HPV DNA testing from a liquid cytology transport media. Preliminary results have been very similar to those of the Ferris study on HC II despite the much higher average age (37) of this population. The National Cancer Institute is at present conducting a study of 7500 women with low-grade (ASCUS and LSIL) cytologic readings, using the same HPV test and liquid cytology technology as the Kaiser study. The women are randomized into one of three arms: colposcopy at the initial referral visit, HPV testing with colposcopy of only those women positive for oncogenic HPV types, and follow-up by cytology with colposcopy of only women with HSIL Pap smear reports. All patients are being followed for 2 years and only high-grade CIN is being treated. The answers provided by these two studies should settle the question of whether HPV testing is a valuable and cost-effective triage tool for women with low-grade Pap abnormalities.

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