HPV Testing

Visible Expectations and Hidden Realities

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In this issue of the Journal, de Cremoux et al1 report a study examining the efficiency of human papillomavirus (HPV) testing using the Hybrid Capture 2 (HC2) in cervical cancer screening. In conducting this study, they focused on several factors that are emerging as important determinants of accuracy in the use of HC2 in any setting, including the following: (1) sensitivity, (2) positive predictive value, (3) false-positive results, and (4) cross-reactivity between the probe set used in HC2 and viral types not included in the probe cocktail. Two groups of subjects were studied, including those referred for colposcopy because of a smear abnormality and consecutively screened women. All subjects underwent colposcopic evaluation.1

HPV testing by HC2 is becoming widely accepted in the United States because of the recent recommendations for its use in managing nondiagnostic squamous atypia (atypical squamous cells of undetermined significance [ASCUS]) and the ease with which patients are triaged into follow-up and intervention (colposcopic triage) categories.2,3 The role of HPV testing in the management of women with no previous Papanicolaou smear abnormality is early in its development, but the notion that testing could replace the Papanicolaou smear in screening women of reproductive age is not far-fetched.4 Obstacles to such a transition include the potential of missing other abnormalities in the smear, an inherent small but definite false-negative rate with a single HPV test for high-grade squamous intraepithelial lesion (HSIL), a high rate of HPV-positive results in women younger than 30 years, and a medical profession that is unprepared to counsel a large number of women likely to have a positive result for a sexually transmitted virus even in the absence of a cytologic abnormality. These issues are likely to remain in the forefront for the next several years as proponents of the “DNA Pap” evaluate the capacity of the medical and lay populations for changes in practice and outlook.

Two issues arise in the study that are important and remain to be sorted out in practice. The first is the sensitivity of the assay. Estimations of sensitivity can be applied to both adjudicated Papanicolaou smear and biopsy diagnoses. Both have some degree of false negativity depending on what is defined as a “gold standard.” It is reasonable to assume that sensitivity will be slightly lower when the end point is a cytologic diagnosis, inasmuch as not every HSIL can be distinguished from immature metaplasia, atypical atrophy, and lower uterine segment on cytologic grounds. In contrast, biopsy specimens are considered more valuable, notwithstanding problems in interobserver reproducibility.

By using histologic results as their outcome variable, Clavel et al5 reported a sensitivity of 100% for HSIL biopsy outcome in screened samples using HC2. This is similar to our own experience with women who have had a previous biopsy result of HSIL and were followed up with cone biopsy (C.P. Crum and R. Urban, unpublished data, 2003). In contrast, in this report the sensitivity of HC2 for biopsy proven HSIL was just 83%.1

The logical question that arises is whether this low sensitivity reflects histologic misclassification, ie, HPV-negative conditions are overclassified as HSIL on the biopsy specimen. Lonky et al6 found that 25% of histologically verified HSILs scored negative by HC2, yet HPV was verified by polymerase chain reaction (PCR) analysis in the histologic material of many of these cases. Moreover, histologic misclassification is highly unlikely to occur in diagnoses of invasive squamous carcinoma.

Thus, the classification of 17% of HSILs and 14% of invasive squamous cell carcinomas as HPV-negative in the
present study is a concern and underscores the fact that an HPV-negative value should not be relied on to guide therapy when the diagnosis is in the higher end of the spectrum. When combined with the low specificity of HPV-positive results relative to cytology, this limitation supports triage strategies that do not rely solely on HPV status for screening and follow-up. This is mirrored in the current recommendations for managing atypical Papanicolaou smears. Although the risk of a false-negative result is low (1%), the potential consequences of ignoring a case with a negative HPV result and a diagnosis of HSIL or cancer are obvious. Thus, as an adjunct to cytologic examination, HPV testing can mandate an increased follow-up interval only when the cytologic smear is normal. If cytologic abnormalities are identified in the absence of HPV detection, the recommended management would be a repeated smear in 12 months in the case of ASCUS and colposcopy if the diagnosis was SIL.

The second important issue is the potential misclassification of HPV status or type category, which can occur via 3 mechanisms. The first is the presence of an HPV that is not within the probe set but nevertheless reacts with the probes used. This is highly dependent on the population being evaluated (ASCUS vs HSIL) and the type and concentration of virus present. In the present study, the authors noted cross-hybridization between the high- and low-risk probe sets in 1.90%, indicating that even HPV type 6 will score positive by HC2 if the amount of virus is increased. In contrast, when low- and medium- or uncertain-risk HPVs are considered, the frequency is higher and may range from 10% to 20%.

The second is the inherent lack of reproducibility of the assay between 0.8 and 1.5 relative light units (RLUs; T. Wright, oral communication, June 2003). Like any test, the threshold of 1.0 RLU is assigned arbitrarily using a series of controls, and, not unexpectedly, a sample scoring 1.0 in one test run might score higher (positive) or lower (negative) in another. In our experience, PCR-analyzed samples in this range often are negative (C.P. Crum and R. Urban, unpublished data, 2003).

The third variable that may contribute to misclassification is one or more forms of sample contamination, which conceivably could occur during automated processing of liquid samples for routine cytologic examination, leading to carry-over of viral DNA from one sample to another. The extent of this risk is unknown but is presumed to be low. A second form of potential “contamination” is in the form of “signal leak,” in which the chemiluminescent emission generated by a strongly positive sample contributes to a slight but significant increase in the luminometer reading of an adjacent negative sample, raising the value above 1.0 RLU. The authors suspected this in 4.31% of their cases.

There are several potential responses to this issue. The first would be to reanalyze all samples scoring between 0.8 and 3.0 RLUs by PCR. The second would be to simply raise the threshold for a positive determination. The authors cite previous studies in which a cutoff value of 4.0 RLUs for HPV positivity still permitted detection of more than 95% of HSILs. However, physicians, laboratory scientists, and patients must be aware that irrespective of the threshold used, a small percentage of patients with HSIL or cancer could have a negative result for HPV. This is an undeniable fact that must be placed in appropriate context in an atmosphere of promise generated by more sophisticated and objective detection methods. It emphasizes the importance of a second evaluation process, be it visually based, image-based, or biochemically based, for all cervix screening and, for detected abnormalities (ASCUS), vigilance in the follow-up of HPV-negative results.

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References


