

Have We Resolved How To Triage Equivocal Cervical Cytology?

Diane Solomon, Mark Schiffman

Virtually all cases of cervical cancer are caused by one of the 15 or more oncogenic types of human papillomavirus (HPV) infection. With the development and refinement of assays to detect HPV DNA, the question becomes how best to integrate HPV testing into cervical cancer screening, which for more than half a century has relied almost exclusively on the Papanicolaou cervical cytology test. One approach has been to consider HPV testing as a second triage test for the relatively large number of women with borderline, equivocal cytology findings of atypical squamous cells of undetermined significance (ASCUS).

ASCUS encompasses both reactive changes that mimic, but are unrelated to, HPV and HPV-associated cell abnormalities that fall below the morphologic threshold for a definitive diagnosis. Expert pathologists cannot reliably distinguish between the two conditions (1–3). Given this heterogeneity in interpretation, it is not surprising that women with cytology results of ASCUS have variable clinical outcomes; most do not have clinically significant disease, but some have precancerous lesions or even cancer found on further evaluation. The role of triage is to identify which women with ASCUS are at risk and require colposcopy and which women can be spared the anxiety and costs associated with intensified follow-up.

In this issue of the *Journal*, Arbyn et al. (4) report a meta-analysis comparing the performance of two triage strategies following an ASCUS result: repeating a cytology test and HPV DNA testing. This article is instructive on two levels: 1) the analytic approach highlights some of the problems inherent to meta-analyses of rapidly evolving topics in general, and studies of cervical neoplasia in particular; and 2) despite methodologic concerns, the results are nonetheless helpful in solidifying our understanding of HPV DNA testing and repeat cytology in the triage of equivocal Pap tests and in directing the course of future research.

The usual objective of meta-analyses is to increase statistical power and precision for estimating risks by combining similar studies of good quality. In a meta-analysis of ASCUS triage, several conditions should be met: 1) the term ASCUS should have a reasonably reproducible meaning (both as the cytologic category defining the population to receive triage and when repeated as a triage test), 2) the defined disease outcome (e.g., cervical intraepithelial neoplasia grade 3 [CIN3]) should be a trustworthy surrogate for cancer risk, 3) the HPV tests used in different studies should be reasonably equivalent in performance, and 4) enough studies should be found to merit meta-analysis. In the study of ASCUS triage, each of these conditions is problematic.

First, ASCUS and related equivocal categories (e.g., borderline dyskaryosis) have low interpretive reproducibility, particularly from an international standpoint (5). Moreover, because the same terms may be applied differently, the prevalence of ASCUS in a screening series can vary from 1% (6) to 10% (7). The specificity and predictive values of cytologic triage and

HPV testing vary profoundly with how the ASCUS population is defined.

Second, although many clinicians treat CIN2 as a safety measure, CIN2 is more heterogeneous and has increased rates of regression compared with CIN3. We believe that, in the absence of studying invasive cancer itself, the surrogate outcome should be histologic CIN3, which provides a firmer disease endpoint. In addition, complete ascertainment of underlying CIN3 and/or cancer requires a period of clinical follow-up with repeat colposcopic assessment, because a single colposcopy with directed biopsy is too insensitive to serve as a reference standard (8,9). The meta-analysis by Arbyn et al. (4) relies on cross-sectional data only and includes the less rigorous outcome of CIN2 as well as CIN3.

Third, an inherent problem of meta-analyses of rapidly evolving fields is that much of the published literature may be based on technologies and assays that are no longer current. In diagnostics and molecular epidemiology, misclassification of exposure and disease are as important as sample size for power and precision. Meta-analyses based on literature reviews do not weight on quality of measurement but on size of study. Arbyn et al. (4) considered several generations of HPV DNA test assays with documented differences in performance (10,11). The older tests are no longer used. As would be predicted from published direct methodologic comparisons (12), Arbyn et al. (4) show that a pooled estimate of HPV DNA testing of ASCUS is improper because of excessive assay heterogeneity, and that only studies using the Hybrid Capture II assay are currently relevant until new tests are validated. Of the four studies that evaluated both the Hybrid Capture II assay and repeat cytology at an ASCUS threshold, the ASCUS-LSIL Triage Study (ALTS) alone represented 62% of the included women. Such representation is problematic because a single very large study can dominate a meta-analysis. It was reassuring to learn that a meta-analysis of the three studies that excluded ALTS data showed results for the Hybrid Capture II assay similar to the results derived when including ALTS.

Despite the methodologic concerns, the meta-analysis by Arbyn et al. (4) does demonstrate the higher triage sensitivity (with similar specificity) of the Hybrid Capture II assay compared with repeat cervical cytology for the detection of CIN2 or CIN3 among women with initial ASCUS cytology. Recently

Affiliations of authors: Breast and Gynecologic Cancer Research Group, Division of Cancer Prevention (DS), and Division of Cancer Epidemiology and Genetics (MS), National Cancer Institute, National Institutes of Health, Rockville, MD.

Correspondence to: Diane Solomon, MD, Breast and Gynecologic Cancer Research Group, Division of Cancer Prevention, National Cancer Institute, National Institutes of Health, 6130 Executive Blvd., Rm. 2130, Rockville, MD 20852 (e-mail: ds87v@nih.gov).

DOI: 10.1093/jnci/djh063

Journal of the National Cancer Institute, Vol. 96, No. 4, © Oxford University Press 2004, all rights reserved.

published longitudinal ALTS results reached the same conclusion (8).

In agreement with recent, evidence-based consensus guidelines (13), we believe that the question of triage test performance in the context of equivocal ASCUS cytology has been answered. Large studies of different populations to compare international variation in the performance of HPV testing to triage equivocal cytology are unnecessary. Instead, different geographic regions will need to explore the meaning of local cytologic terminology and its correlation with HPV positivity to determine which equivocal cytology findings can be clarified by triage. Ideally, pathologists from any locale should be able to calibrate their own interpretations against a pool of referenced HPV-tested cases. As one resource, an online library of cytology images from ALTS (linked to HPV and histopathology results) is under development.

Additional diagnostics research should evaluate combinations of cytology and HPV testing for screening the general population. HPV infection is highly prevalent, but only persistent infections with oncogenic HPV types pose a risk of neoplastic progression. Strategies that focus on identifying HPV persistence rather than prevalent infection may provide greater specificity without compromising sensitivity. Eventually, we hope to identify and validate markers of cancer risk that are even more accurate than either cytology or HPV DNA testing.

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