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Editorial

The Orderly Incorporation of Continuing Technologic Advances Into Cervical Cancer Screening

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Human papillomavirus (HPV) testing is replacing cytology for cervical screening due to greater sensitivity and longer reassurance against cervical cancer (1-3). Moreover, self-sampled vaginal specimens can be used for HPV testing with results equivalent to clinician cervical sampling if a sensitive target-amplification DNA assay is used (4,5). The current need for social distancing and avoidance of unneeded clinic visits as part of COVID-19 prevention increases the value of HPV testing of self-samples (6).

In many places, cervical cancer screening is a multistep process involving screening, secondary triage, colposcopic biopsy to confirm precancers, and ultimately treatment. Although HPV testing is sensitive, infections with the dozen causal carcinogenic HPV types are commonly found and typically benign (7). Colposcopic biopsies are used to guide treatment decisions, but colposcopy is a burden to women and health-care systems. Triage tests are used to reduce colposcopy referral and to decide between immediate colposcopy and surveillance (eg, repeat testing in 12 months) (8). Ideally, a screening program should reassure most women of very low risk of cancer, identifying the few that need immediate treatment while minimizing the number of women requiring repeated surveillance visits.

In this issue of the Journal, Rossi and colleagues (1) have evaluated 2 alternatives to cervical cytology for triage of HPV DNA-positive women in the first round of the large Italian NTCC2 trial. The p16-Ki67 dual-stain assay, recently approved as a triage test by the US Food and Drug Administration (FDA) (9), was more accurate than morphologic cytology (Pap test) in diagnosing precancer among HPV-positive women. The other assay, E6/E7 mRNA, is also FDA approved but as a screening assay in combination with cytology ("cotesting") (10). As expected (11), E6/E7 mRNA testing performed not like a triage test but more like an alternative to HPV DNA screening (ie, it was almost as frequently positive and nearly as sensitive). The results lead to the following considerations, primarily from the US perspective.

The E6/E7 mRNA assay was confirmed to be slightly less sensitive than HPV DNA but considerably less likely to be positive in the absence of precancer. At the transition from infection to precancer, viral gene expression switches from productive infection to transforming infection, with increasing E6 and E7 mRNA expression (7). It is not clear how much the difference in assay performance between DNA and E6/E7 mRNA reflects this change in viral transcriptional activity or simply assay analytic sensitivity. Specifically, for HPV16-related HPV types, a higher DNA "load" is associated with greater risk of precancer (12). It would be worth establishing how E6/E7 mRNA testing compares with more stringent restriction of DNA positives to higher viral load.

The long-term negative predictive value of HPV DNA testing has been established by extensive long-term (15+ years) follow-up studies (13,14). More limited prospective data establishing reassurance following E6/E7 mRNA negativity suggest slightly lower negative predictive value after approximately 6 years, though E6/E7 mRNA still provides substantially greater reassurance than negative cytology (11). At present, HPV DNA negativity using signal amplification remains the reference standard for extended screening intervals reliant solely on HPV testing of cervical specimens and, by extension, of self-samples.

Management of positive results is the function of triage. The ideal triage test would retain the sensitivity of HPV DNA screening, refer all and only women at very high risk of precancer, and could be applied to self-samples. HPV genotyping can be considered as a kind of triage; rather than requiring a reflex second test, the useful risk stratification from typing can be incorporated into the initial (self-sampled) screening step. US consensus guidelines already recommend more aggressive management of HPV16 and HPV18, the 2 highest risk types (3,15). Looking forward to possible indications, HPV16, HPV18, and HPV45 cause the overwhelming majority of difficult-to-detect adenocarcinomas. Extended genotyping identifies a subset of types (HPV39, HPV51, HPV56, HPV59, and HPV68) that are at lower risk, possibly warranting less aggressive management than the intermediate HPV16-related group [HPV31, HPV33, HPV52, and HPV58 (16) as well as HPV35 among women of African genetic heritage] (17). Inclusion of HPV66 is a common

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Table 1. Options for cervical screening, per US current practice, pending changes and longer-term considerations[a]

Part of program	Current practice	Near-term considerations?	Future considerations?
Population screening	Cytology at 3 y, HPV DNA test q 5 y with some genotyping, HPV or cytology cotest q 5 y	Extended genotyping for risk stratification Self-sample acceptable for screening using HPV DNA test	E6/E7 mRNA acceptable alone for HPV screening at what interval? Methylation status measurable at screen including self-sample?
Triage of HPV positive	Cytology	Cytology or dual-stain	Automated dual-stain? DNA methylation as reflex test? Deep learning–assisted visual triage?
Colposcopy	Common referral Low yield of precancers per exam. Multiple biopsies of acetowhite lesions.	Less common referral Higher yield of precancers per exam. Increased emphasis on multiple biopsies of acetowhite lesions.	Risk prediction by screening or triage tests decreases reliance solely on colposcopic biopsy?

[a]Current practice defined by US Food and Drug Administration (FDA) clearance and ASCCP Risk-Based Management Consensus Guidelines. Near-term considerations defined by FDA clearance and anticipated consideration for inclusion in guidelines (personal prediction by authors). Future considerations are strictly personal predictions of authors. HPV = human papilloma virus.

mistake in HPV assays; it is so rarely carcinogenic that it should be removed (18). Guidelines regarding HPV typing are needed given recent US FDA approval of an extended genotyping HPV DNA assay (19).

Most available triage tests require a clinician-obtained cervical specimen. Although the current standard for triage is Pap test, the data presented by Rossi et al. support greater accuracy of dual-stain (p16/Ki67) for detection of precancer. Dual-staining indicates disruption of the Rb pathway and also proliferation; together, the 2 stains mark high risk of precancer (20). Finding 1+ dual-stained cell per slide is the threshold for positivity, comparable with a non-normal Pap result. The usefulness of dual-stain when applied to self-samples is not known. To determine if it is worth switching from Pap testing for triage of HPV-positive results to dual-stain, a proprietary assay is under consideration by different organizations. At minimum, before morphologic cytology is fully replaced, the need to distinguish glandular from squamous abnormalities will need to be addressed.

Dual-stain cytology can be automated, with deep learning–based training for more accurate recognition and quantification of dual-stain positive cells (21). Large numbers of dual-stain cells are linked to high risk of precancer, comparable with the finding on cytology of a high-grade squamous intraepithelial lesion. Perhaps the deep learning component of automated dual-stain could also distinguish glandular abnormalities requiring altered management.

We now know a considerable amount about HPV natural history and risk of finding cervical precancer following screening and triage tests (22). The introduction of these and other increasingly accurate and precise biomarkers for cervical precancer will require frequent updating of guidelines. Anticipation of continued technical advancement underlies the recently ratified American Society for Colposcopy and Cervical Pathology risk-based management consensus guidelines (3) (Table 1). To permit a risk-based management approach, newer tests like E6/E7 mRNA and dual-stain cytology must be evaluated in cross-sectional and prospective studies. Based on the posttest risks found, the guidelines for use for management decisions can be decided quickly based on the principle of "equal management of equal risk" consistent with older tests. Results from prospective follow-up of NTCC2 and other high-quality studies will be very useful, particularly in deciding the interval for follow-up of the HPV-positive but triage test-negative woman.

Notes

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