

From Papanicolaou to Papillomaviruses: Evolving Challenges in Cervical Cancer Screening in the Era of Human Papillomavirus Vaccination

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February 2012 marked the 50th anniversary of the death of George Papanicolaou, the inventor of the Pap test for cervical cancer screening. Pap test screening has contributed to sharp reductions in cervical cancer incidence and mortality throughout the developed world (1–5). Despite the success of the Pap test, recognition that it suffers from inadequate single-test sensitivity and frequent equivocal results has prompted searches for newer screening methods.

Demonstration that carcinogenic human papillomavirus (HPV) infections are the cause of nearly all cervical cancer has led to the use of HPV DNA testing for screening. A negative HPV DNA test provides strong reassurance that cervical cancer risk is low and will remain so for many years (6). However, the clinical significance of a positive test is less clear, especially at young ages when most HPV infections are extremely common and self-limited. In this issue of the Journal, Ikenberg and colleagues present results from the Primary, ASC-US, LSIL Marker Study (PALMS), a multicenter investigation of the performance of a dual label p16/Ki-67 immunocytology assay (7). The dual stain assesses molecular changes downstream of HPV infection by assessing coexpression of p16, a marker of transforming HPV infection, and Ki-67, a marker of proliferation, within the same epithelial cell, independent of morphological assessment.

PALMS was a cross-sectional screening study in which 27 349 women were evaluated with a Pap test (conventional or liquid-based), p16/Ki-67 dual-label assay, and HPV DNA testing (Hybrid Capture 2; Qiagen, Germantown, MD). Women with at least one abnormal test (except those younger than 30 years that were only HPV DNA positive) were referred for colposcopy and biopsy to assess test performance in detecting potential histological cervical cancer precursors (cervical intraepithelial neoplasia 2 or worse; ie CIN2+). The p16/Ki-67 dual-label assay achieved statistically significantly higher sensitivity in detecting CIN2+ than Pap cytology among all women (86.7% and 68.5%, respectively), with similar specificity (95.2% and 95.4%, respectively). Among women aged 30 to 65 years, p16/Ki-67 yielded lower sensitivity than carcinogenic HPV testing for CIN2+ (84.7% vs 93.3%, respectively), but with substantially fewer positive tests (4.2% for p16/Ki67 vs 7.5% for HPV testing) resulting in fewer referrals. The negative predictive values of all three tests exceeded 99.5%. Similar results were reported for CIN3+, the most severe cancer precursor.

If validated and cost effective, the p16/Ki-67 assay could have a role in primary screening, especially among younger women, a group in which transient HPV infection is common, resulting in low positive predictive values for detecting cervical cancer precursors. Alternatively, the dual-label assay could serve as a triage for a positive HPV test. The data from Ikenberg et al. show that

detection of CIN2+ among HPV-negative individuals was rare (0.8%; 7 of 912), even in the context of p16/Ki-67 positive test. Detection of CIN2+ was highest among HPV-positive and p16/Ki-67-positive individuals (15.3%; 152 of 996) and low among HPV-positive but p16/Ki-67-negative individuals (1.3%; 22 of 1760). Thus, combined testing could identify CIN2+ and dramatically decrease referrals. A limitation of this analysis is that women aged less than 30 years who were only HPV DNA positive were not further evaluated, so the sensitivity of p16/Ki67 in this group is unknown. Future analyses evaluating p16/Ki-67 as a triage for positive HPV or cytology tests are anticipated.

Clinical implementation of p16/Ki67 testing would require resolution of several issues, including the feasibility of performing the stain routinely, the need for training and certification of cytologists, and clinical validation. Among older women, the high sensitivity of HPV DNA testing and the lower prevalence of transient infections have led to a recommendation in the United States to lengthen the screening interval for individuals aged 30 years and older who are both cytology and HPV DNA negative. The long-term protection of a negative p16/Ki-67 test is unknown. Most recently the Lower Anogenital Squamous Terminology Standardization Project recommended using a positive p16 (single stain) result to define CIN2 lesions as high grade (8,9). Use of p16 staining alone showed that detection of CIN2+ among HPV-positive and p16-negative women was 5.2%, or 30 of 644, over a 3-year period (10). Additional prospective comparisons of clinical effectiveness and cost effectiveness of p16/Ki-67 with other screening modalities, such as high-risk HPV testing, remain of interest.

Cervical cancer claims approximately 275 000 lives annually worldwide, with most deaths occurring in poorer nations (11) that cannot afford screening technologies. Advances in prophylactic HPV vaccination offer hope in these settings.

Vaccination with two US Food and Drug Administration–approved prophylactic vaccines is highly efficacious in preventing HPV16 and -18 infections, which collectively account for 70% of cervical cancers. However, vaccines are unaffordable in poorer nations and current protocols require three doses and refrigerated storage, posing logistical challenges. Retrospective analyses suggest that two or even one vaccine dose might be effective (12), a finding that is supported by studies showing robust serological responses with fewer doses (13). Establishing the long-term efficacy of less than three doses and defining strategies to cover additional carcinogenic HPV types could enable expanded protection prevention where it is most needed.

Currently, HPV vaccination will not eliminate the need for screening for several reasons: 1) many women are beyond the

recommended age for vaccination; 2) vaccines do not protect women infected before vaccination; and 3) vaccines do not protect against all carcinogenic HPV types. By reducing the frequency of the most virulent HPV types, vaccination should decrease the frequency of screen-detected cytologic abnormalities and lower cancer risk related to positive screens. Thus, assessment of new screening methods in vaccinated populations will be crucial.

Uncertainties about the long-term durability of vaccine protection and difficulties in ascertaining women's vaccine histories may pose future challenges for optimizing screening. As more vaccinated women undergo screening, the performance of testing may change, and re-evaluation of these approaches may be required. Nonetheless, developing simple effective screening algorithms will be important.

With the 50th anniversary of Papanicolaou's death, it would seem appropriate to reflect on how technological progress and biological advances have coevolved in the field of cervical cancer prevention. Yet, despite progress, cervical cancer persists as a leading cause of cancer death in most parts of the developing world.

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