ENTERING THE MOLECULAR AGE OF CERVICAL CANCER PREVENTION: 
WHAT'S NEW AND HOW DO WE BRING ORDER TO THE CHAOS?

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The goal of any cervical cancer screening test, and even diagnostic procedures, is to identify the subset of women at risk for cervical cancer and reassure others against having cervical cancer i.e., risk stratification (1). Historically, cervical cytology/Pap smears have been the primary screening test. Cervical cytology is proven to be one of the great public health interventions, reducing the burden of cervical cancer in developed countries that have established effective programs by 70% or more. However, while effective, cervical cytology is not sensitive for the detection of cervical precancer and cancer (2,3) and therefore its use in cervical cancer screening is not particularly efficient. In the U.S., the cytology-based screening program costs at least $6 billion annually (4). Thus, there is impetus to develop more accurate screening programs.

Based on the central role of persistent, carcinogenic human papillomavirus (HPV) in cervical carcinogenesis, HPV testing has recently been introduced into cervical cancer screening. With proven, greater sensitivity than cytology for detection of cervical precancer (cervical intraepithelial neoplasia grade 3 [CIN3]) and cancer (≥CIN3) (2,5-8) and greater reliability (9,10), HPV testing is now commonly used in the U.S. to triage equivocal cytology for colposcopic referral. HPV testing with cytology is also approved for primary screening of women aged 30 years and older (11), who are past the peak of self-limited infections. Therefore, in women 30 and older, the positive predictive value (PPV) for ≥CIN3 is higher than in younger women. Women aged 30 years and older who test HPV and cytology negative are at an extremely low risk for incipient precancer and cancer over 10 years or more (12,13), and therefore the screening intervals in these women can be extended to 3 years in the United States to make co-testing cost-effective (14). In fact, concurrently-performed cytology adds little to the sensitivity and negative predictive value of HPV testing. On this basis, it is only a matter of time before HPV testing is widely accepted as an alternative to routine cytology as the primary screening test for secondary cervical cancer prevention.

However, the enthusiasm for using HPV testing in primary screening has been tempered by its relatively poor positive predictive value (PPV). Even at older ages, the prevalence of self-limited infections can reach 10%, with only a minority of these women at risk of ≥CIN3. A viable strategy for managing HPV-positive women, specifically, identifying the subset at risk of ≥CIN3 would accelerate adoption of HPV testing into primary testing. New biomarkers, including those that measure the interaction of host and virus, are being considered to either as a stand-alone molecular assay or in conjunction with
cytology or carcinogenic HPV DNA testing to improve its sensitivity or specificity, respectively.

There is already considerable evidence that the absolute risk of cervical precancer (cervical intraepithelial neoplasia grade 3 [CIN3]) and cancer (≥CIN3) varies considerably between specific HPV genotypes (15,16) and that detection of HPV16 and HPV18 may have clinical utility especially among carcinogenic HPV-positive, cytologically negative women (17). Detection of persistent carcinogenic HPV is strongly associated with ≥CIN3 and predicts its development, and might be used to monitor the outcome of HPV infections (18,19), provided that clinicians and patients do not overreact to the initial HPV result and patients are not lost to follow-up.

Progression of HPV infections to a precancerous state is accompanied by dysregulation of carcinogenic HPV oncoproteins E6 and E7 expression and therefore may be a very specific marker of precancerous lesions. Two biomarkers of these events are the transcripts of E6 and E7, i.e., carcinogenic HPV E6/E7 mRNA(20-22), and p16INK4A(23-25) antigen, which is over-expressed in response to inactivation of retinoblastoma by carcinogenic HPV E7 and concomitant cell proliferation. Both biomarkers are correlated with increasing severity of lesions, and are being developed into screening tests (e.g., a p16INK4A ELISA screening test has been recently developed (24,25)). Finally, cytogenetic changes, specifically 3q amplification (26,27), appear to be very specific markers of the epigenetic and genetic changes incurred as the result of HPV-related carcinogenesis. Other promising biomarkers (e.g., ProEx C, which detects MCM 2 and TOP2A proteins by immunostaining of cytologic preparations (28,29)) are almost certainly in the development pipeline.

Although this next generation of biomarkers is promising, there are a number of important issues that warrant consideration before any can be used in screening. First, there must be demonstrated clinical reliable performance in population samples versus a rigorous endpoint, ≥CIN3 (30). Importantly, use of unvalidated tests such as analyte-specific reagents and “home-brews” must be discouraged for patient safety. Also, CIN 2 is not a true biologic entity but an equivocal diagnosis of precancer, representing an admixture of HPV infections by carcinogenic and non-carcinogenic HPV genotypes and precancer (31). Its use as a clinical threshold for treatment provides a margin of safety while leading to significant over-treatment. A useful biomarker will also distinguish CIN2 that is precancer from CIN2 that is nothing more than HPV infection. Second, these assays must user-friendly i.e., high-throughput and automated and the results are easily interpretable. Finally, a risk model should be adopted to guide clinical management now and in the future (1). The model would use thresholds of increasing risk for cervical precancer and treatable cancer to guide clinical decision-making for screening intensity, diagnostic evaluation, or treatment. Experts would decide on these risk thresholds and stratum based on the patient risk-to-benefit, independent of current and future methods of measuring risk.
Selected Reading:


A. Summary:

- Cytology screening, while effective, is inefficient because of poor sensitivity for cervical precancer and treatable cancer.
- Carcinogenic HPV DNA testing has proven to be highly sensitive for cervical precancer and treatable cancer but has low positive predictive value.
- Several biomarkers (e.g., HPV genotypes, carcinogenic HPV E6/E7, p16^{INK4a}, and 3q amplification) may potentially be used to increase the accuracy of cervical cancer screening.
- Assays for new biomarkers must be validated and be user-friendly.
- A risk model should be adopted to guide clinical management now and in the future.

B. Take Home Messages:

- New biomarkers must be rigorous evaluated before being considered for cervical cancer screening. Assays must demonstrate reliable, clinical performance before being used in clinical practice. There are no short cuts to validation.
- The clinical response to a positive and negative test result should be standardized to the associated risk irrespective of the test or biomarker used to determine the risk.