

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

**Philip E. Castle, PhD, MPH
ASC Companion Meeting
USCAP 2008**

**Division of Cancer Epidemiology and Genetics, National Cancer Institute (USA),
Bethesda, MD, USA**

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 (\geq 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 \geq 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 \geq CIN3. A viable strategy for managing HPV-positive women, specifically, identifying the subset at risk of \geq 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 (\geq 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 \geq 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 p16^{INK4A}(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 p16^{INK4A} 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, \geq 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:

1. Castle PE, Sideri M, Jeronimo J, Solomon D, and Schiffman M. Risk assessment to guide the prevention of cervical cancer. *Am J Obstet Gynecol* 2007; 197:356.
2. Cuzick J, Clavel C, Petry KU et al. Overview of the European and North American studies on HPV testing in primary cervical cancer screening. *Int J Cancer* 2006; 119:1095-101.
3. Cuzick J, Mayrand MH, Ronco G, Snijders P, and Wardle J. Chapter 10: New dimensions in cervical cancer screening. *Vaccine* 2006; 24 Suppl 3:S90-7. Epub; %2006 Jun 23.:S90-S97.
4. Kurman RJ, Henson DE, Herbst AL, Noller KL, and Schiffman MH. Interim guidelines for management of abnormal cervical cytology. The 1992 National Cancer Institute Workshop. *JAMA* 1994; 271:1866-9.
5. Arbyn M, Sasieni P, Meijer CJ, Clavel C, Koliopoulos G, and Dillner J. Chapter 9: Clinical applications of HPV testing: A summary of meta-analyses. *Vaccine* 2006; 24 Suppl 3:S78-89.:S78-S89.
6. Mayrand MH, Duarte-Franco E, Rodrigues I et al. Human papillomavirus DNA versus Papanicolaou screening tests for cervical cancer. *N Engl J Med* 2007; 357:1579-88.
7. Naucler P, Ryd W, Tornberg S et al. Human papillomavirus and Papanicolaou tests to screen for cervical cancer. *N Engl J Med* 2007; 357:1589-97.
8. Bulkmand N, Berkhof J, Rozendaal L et al. Human papillomavirus DNA testing for the detection of cervical intraepithelial neoplasia grade 3 and cancer: 5-year follow-up of a randomised controlled implementation trial. *Lancet* 2007; ..
9. Castle PE, Wheeler CM, Solomon D, Schiffman M, and Peyton CL. Interlaboratory reliability of Hybrid Capture 2. *Am J Clin Pathol* 2004; 122:238-45.
10. Carozzi FM, Del Mistro A, Confortini M et al. Reproducibility of HPV DNA Testing by Hybrid Capture 2 in a Screening Setting. *Am J Clin Pathol* 2005; 124:716-21.
11. Wright TC, Jr., Massad LS, Dunton CJ, Spitzer M, Wilkinson EJ, and Solomon D. 2006 consensus guidelines for the management of women with abnormal cervical cancer screening tests. *Am J Obstet Gynecol* 2007; 197:346-55.
12. Sherman ME, Lorincz AT, Scott DR et al. Baseline cytology, human papillomavirus testing, and risk for cervical neoplasia: a 10-year cohort analysis. *J Natl Cancer Inst* 2003; 95:46-52.

13. Kjaer S, Hogdall E, Frederiksen K et al. The absolute risk of cervical abnormalities in high-risk human papillomavirus-positive, cytologically normal women over a 10-year period. *Cancer Res* 2006; 66:10630-6.
14. Goldie SJ, Kim JJ, and Wright TC. Cost-effectiveness of human papillomavirus DNA testing for cervical cancer screening in women aged 30 years or more. *Obstet Gynecol* 2004; 103:619-31.
15. Smith JS, Lindsay L, Hoots B et al. Human papillomavirus type distribution in invasive cervical cancer and high-grade cervical lesions: a meta-analysis update. *Int J Cancer* 2007; 121:621-32.
16. Munoz N, Bosch FX, de Sanjose S et al. Epidemiologic classification of human papillomavirus types associated with cervical cancer. *N Engl J Med* 2003; 348:518-27.
17. Khan MJ, Castle PE, Lorincz AT et al. The elevated 10-year risk of cervical precancer and cancer in women with human papillomavirus (HPV) type 16 or 18 and the possible utility of type-specific HPV testing in clinical practice. *J Natl Cancer Inst* 2005; 20:97:1072-9.
18. Kjaer SK, van den Brule AJ, Paull G et al. Type specific persistence of high risk human papillomavirus (HPV) as indicator of high grade cervical squamous intraepithelial lesions in young women: population based prospective follow up study. *BMJ* 2002; 325:572.
19. Schiffman M, Herrero R, Desalle R et al. The carcinogenicity of human papillomavirus types reflects viral evolution. *Virology* 2005; 20:337:76-84.
20. Molden T, Kraus I, Karlsen F, Skomedal H, and Hagmar B. Human papillomavirus E6/E7 mRNA expression in women younger than 30 years of age. *Gynecol Oncol* 2006; 100:95-100.
21. Molden T, Kraus I, Karlsen F, Skomedal H, Nygard JF, and Hagmar B. Comparison of human papillomavirus messenger RNA and DNA detection: a cross-sectional study of 4,136 women >30 years of age with a 2-year follow-up of high-grade squamous intraepithelial lesion. *Cancer Epidemiol Biomarkers Prev* 2005; 14:367-72.
22. Molden T, Nygard JF, Kraus I et al. Predicting CIN2+ when detecting HPV mRNA and DNA by PreTect HPV-proofer and consensus PCR: A 2-year follow-up of women with ASCUS or LSIL Pap smear. *Int J Cancer* 2005; 114:973-6.
23. Klaes R, Woerner SM, Ridder R et al. Detection of high-risk cervical intraepithelial neoplasia and cervical cancer by amplification of transcripts derived from integrated papillomavirus oncogenes. *Cancer Res* 1999; 59:6132-6.

24. Wentzensen N, Hampl M, Herkert M et al. Identification of high-grade cervical dysplasia by the detection of p16INK4a in cell lysates obtained from cervical samples. *Cancer* 2006; 107:2307-13.
25. Mao C, Balasubramanian A, Yu M et al. Evaluation of a new p16(INK4A) ELISA test and a high-risk HPV DNA test for cervical cancer screening: results from proof-of-concept study. *Int J Cancer* 2007; 120:2435-8.
26. Heselmeyer-Haddad K, Janz V, Castle PE et al. Detection of genomic amplification of the human telomerase gene (TERC) in cytologic specimens as a genetic test for the diagnosis of cervical dysplasia. *Am J Pathol* 2003; 163:1405-16.
27. Heselmeyer-Haddad K, Sommerfeld K, White NM et al. Genomic amplification of the human telomerase gene (TERC) in pap smears predicts the development of cervical cancer. *Am J Pathol* 2005; 166:1229-38.
28. Kelly D, Kincaid E, Fansler Z, Rosenthal DL, and Clark DP. Detection of cervical high-grade squamous intraepithelial lesions from cytologic samples using a novel immunocytochemical assay (ProEx C). *Cancer* 2006; 108:494-500.
29. Shi J, Liu H, Wilkerson M et al. Evaluation of p16INK4a, minichromosome maintenance protein 2, DNA topoisomerase IIalpha, ProEX C, and p16INK4a/ProEX C in cervical squamous intraepithelial lesions. *Hum Pathol* 2007; 38:1335-44.
30. Stoler MH, Castle PE, Solomon D, and Schiffman M. The Expanded Use of HPV Testing in Gynecologic Practice per ASCCP-Guided Management Requires the Use of Well-Validated Assays. *Am J Clin Pathol* 2007; 127:1-3.
31. Castle PE, Stoler MH, Solomon D, and Schiffman M. The Relationship of Community Biopsy-Diagnosed Cervical Intraepithelial Neoplasia Grade 2 to the Quality Control Pathology-Reviewed Diagnoses:An ALTS Report. *Am J Clin Pathol* 2007; 127:805-15.

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 rigorously 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.