

Age-Stratified Performance of the Cervista HPV 16/18 Genotyping Test in Women with ASC-US Cytology

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Abstract

Background: The objective of this study was to evaluate the clinical performance of the Cervista HPV 16/18 genotyping test for detection of human papilloma virus (HPV) 16 and 18 in cervical cytology specimens in women stratified by age.

Methods: In a multicenter, prospective clinical study, ThinPrep specimens were tested for the presence of HPV 16 and 18 using the HPV 16/18 genotyping test. Genotyping results from women with atypical squamous cells of undetermined significance or greater cytology were compared with local colposcopy and/or histology results. Sensitivity, specificity, and negative and positive predictive values (NPV and PPV) were determined.

Results: The prevalence of cervical intraepithelial neoplasia (CIN) 2⁺ in subjects positive for HPV 16/18 was 6.6% and 4.1% for women <30 and ≥30 years of age, respectively. The sensitivity of the test was 70.0% (95% CI: 54.6–81.9) and 66.7% (95% CI: 46.7–82.0) in women <30 and ≥30 years, respectively. The NPV was 95.5% (95% CI: 93.4–97.6) in women <30 years and 96.6% (95% CI: 94.8–98.5) in women ≥30 years. Specificity was higher in women ≥30 years (79.9%; 95% CI: 74.9–84.2) than women <30 years (61.9%; 95% CI: 57.1–66.4). The PPV was 15.2% (95% CI: 12.7–19.1) in women <30 years and 21.9% (95% CI: 17.0–30.7) in women ≥30 years.

Conclusions: The performance of the Cervista HPV 16/18 genotyping test for predicting ≥CIN 2 is what would be expected across the key ≥CIN 2 age strata.

Impact: HPV 16/18 genotyping may help further stratify women with a greater potential to develop cervical cancer. *Cancer Epidemiol Biomarkers Prev*; 20(6); 1185–9. ©2011 AACR.

Introduction

High-risk (HR) human papilloma virus (HPV) has been shown to be necessary for the development of cervical cancer (1, 2). HPV 16 and 18, the 2 most common HR types, are responsible for approximately 77% of all cervical cancers in the United States (3). Persistence of these 2 types has also proven to be strongly associated with the development of precancerous lesions [≥CIN (cervical intraepithelial neoplasia) 2], with an even greater association with malignant transformation than caused by other non-16/18 oncogenic HPV types (2, 3). Cervical precancerous lesions are most commonly detected by cervical cytology testing. However, for certain clinically relevant cervical abnormalities, HPV genotyping may be an important test to determine which HR HPV-positive

women will require more aggressive follow-up and management.

Currently, the American Society for Colposcopy and Cervical Pathology (ASCCP) recommends the use of HPV DNA testing to screen patients with atypical squamous cells of undetermined significance (ASC-US) cervical cytology (4). Equivocal cytology results such as ASC-US may not progress to higher grade lesions, but the presence of HR HPV DNA in conjunction with ASC-US cytology indicate an increased risk for developing CIN 2 or CIN 3 (4–6). Consequently, testing for the presence or absence of a specific HR HPV type may identify women that require more aggressive management. Among women ≥30 years with a cytology result of no intraepithelial lesion or malignancy (NILM) who test positive for HR HPV, HPV 16/18 genotyping is recommended as an alternative strategy for primary screening and double-negative patients can be triaged to a lengthened follow-up of 3 years (7). HPV 16/18 testing with the Cervista HPV 16/18 genotyping test (Hologic, Inc) is also approved by the U.S. Food and Drug Administration (FDA) for use in patients with ASC-US cytology to assess the presence or absence of these specific HR HPV types. However, current clinical management guidelines do not recommend such use due to the lack of prospective studies for this testing as a screening tool (7).

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We have previously shown the performance of the HPV 16/18 genotyping test in combination with cervical cytology for use in the triage of women with ASC-US cytology (8). The HPV 16/18 genotyping test shows a high degree of sensitivity, specificity, within-laboratory precision and repeatability, and between-laboratory reproducibility (9). In the present analysis, we evaluate the age-stratified clinical performance of the Cervista HPV 16/18 genotyping test for detection of HPV 16 and 18 in cervical cytology specimens of women with ASC-US cytology.

Materials and Methods

Study design

In a multicenter, prospective clinical study, residual ThinPrep (Hologic, Inc.) liquid-based cytologic specimens of 3,966 women from 89 recruitment sites across the United States were tested for the presence of HR HPV using the Cervista HPV HR test (Hologic, Inc.) and for HPV 16 and 18 using the Cervista HPV 16/18 genotyping test. All subjects with ASC-US or worse cytology were required to undergo a colposcopy; biopsy samples were collected at the discretion of the colposcopist, based on colposcopic findings. Histologic analysis of biopsy specimens was conducted locally at clinical centers or reference pathology laboratories and subsequently reported to the subject's physician per local clinical standards of care. All biopsy slides were then histologically reviewed by a central histology review panel. All ASC-US subjects with a Cervista HPV HR test, Cervista HPV 16/18 genotyping test, and colposcopy or histology results were included in the analysis.

Samples

Cervista HPV 16/18 genotyping test was conducted on cervical specimens collected in PreservCyt (Hologic, Inc.) solution, the ThinPrep Pap Test preservation system. All cervical samples (prospective collection and residual/remnant samples) were collected under protocols reviewed and approved by Institutional Review Boards from each participating site. For all HPV DNA testing, DNA samples were isolated from the initial 2 mL cervical specimen using the Genfind DNA Extraction Kit (Hologic, Inc.; ref. 10). Residual DNA extracted as part of the Cervista HPV HR test was used for the Cervista HPV 16/18 genotyping test.

Cervista HPV 16/18 genotyping test

The Cervista HPV 16/18 genotyping test is a qualitative, *in vitro* diagnostic test for the detection of DNA from HPV 16 and 18. The test uses the Invader Chemistry (Hologic, Inc.), a signal amplification method for detecting specific nucleic acid sequences (11). As described previously, this method utilizes a primary reaction that occurs on the targeted DNA sequence and a secondary reaction that produces a fluorescent signal. Both types of reactions rely on oligonucleotide hybridization, invasive structure for-

mation, and cleavage by the Cleavase enzyme (Hologic, Inc.; ref. 12). These reactions detect HPV 16 and 18 DNA targets as well as human histone 2, H2be (*HIST2H2BE*) DNA that serves as an internal control for detection of cellular DNA. A signal to noise value (sample fluorescence signal divided by the fluorescence signal from a notarget control) is referred to as fold over zero (FOZ). A positive result for HPV 16, HPV 18, or HPV 16 and 18 occurs when the FOZ value is at or above a clinically derived cutoff value of 2.13. If the FOZ values are below this cutoff point, then the samples are considered negative. To show that the testing procedure has been properly conducted and sample genomic DNA (gDNA) was present in sufficient quantity, the *HIST2H2BE* FOZ values (or average gDNA FOZ) must lie at or above a clinically derived cutoff value of 1.5. Samples that generate average gDNA FOZ values below 1.5 are considered indeterminate in the absence of a positive HPV signal.

The clinical performance of the Cervista HPV 16/18 genotyping test was measured against colposcopy and histology results. Non- \geq CIN 2 results were defined by colposcopy-directed biopsy result of "no CIN or CIN 1" by the central histology review panel. Genotyping results were compared with local colposcopy and/or histology results from the central pathology review panel. The sensitivity, specificity, negative, and positive predictive values (NPV and PPV) of the HPV 16/18 genotyping test were determined on the basis of comparisons with colposcopy and histology for the detection of CIN 2 or worse.

Results

Study subjects

A total of 2,086 subjects with ASC-US or greater cytology results participated in the study (8). Of those who consented, 1,940 women were enrolled after they met the inclusion/exclusion criteria of the study. A total of 1,936 subjects had adequate Papanicolaou (Pap) test samples—1,514 of these subjects had ASC-US cytology results. HPV 16/18 results were obtained from 1,398 samples and 116 HPV 16/18 results were not reported due to insufficient volume for testing or due to a protocol deviation such as pipetting error and incomplete extraction of ThinPrep samples. Colposcopy was completed for 1,476 of the ASC-US subjects; 417 (28.3%) had negative colposcopy results and/or no biopsy. Biopsies were obtained from 1,059 subjects with central histology results available for 1,017 of these subjects. Biopsy slides for 42 subjects could not be obtained from the local pathology laboratory. There were 1,312 ASC-US subjects with known disease status (central histology or negative colposcopy) and Cervista HPV HR and Cervista HPV 16/18 results.

Age-stratified clinical performance of the Cervista HPV 16/18 genotyping test for women with positive Cervista HPV HR results

The prevalence of \geq CIN 2 in subjects with positive Cervista HPV HR results and positive for HPV 16

Table 1. Age-stratified clinical performance of the Cervista HPV 16/18 genotyping test compared with colposcopy/central histology results (\geq CIN 2) among women with ASC-US cytology and positive Cervista HPV HR results

Age strata, y	Disease prevalence (subjects)	Sensitivity (subjects; 95% CI)	Specificity (subjects; 95% CI)	NPV (subjects; 95% CI)	PPV (subjects; 95% CI)
<30	6.6% (40/611)	70.0% (28/40; 54.6–81.9)	61.9% (329/531; 57.1–66.4)	95.5% (253/265; 93.4–97.6)	15.2% (28/184; 12.7–19.1)
\geq 30	4.1% (29/701)	66.7% (19/29; 46.7–82.0)	79.9% (514/643; 74.9–84.2)	96.6% (227/235; 94.8–98.5)	21.9% (16/73; 17.0–30.7)

and/or 18 was 6.6% (40 of 611) for women <30 years of age and 4.1% (29 of 701) for women \geq 30 years (Table 1). The sensitivity of the HPV 16/18 genotyping test for predicting \geq CIN 2 was 70.0% (95% CI: 54.6–81.9) and 66.7% (95% CI: 46.7–82.0) in women <30 and \geq 30 years, respectively. The NPV for \geq CIN 2 was 95.5% (95% CI: 93.4–97.6) in women <30 years and 96.6% (95% CI: 94.8–98.5) in women \geq 30 years. As expected, specificity of the test was higher in women \geq 30 years (79.9%; 95% CI: 74.9–84.2) than women <30 years (61.9%; 95% CI: 57.1–66.4). The PPV was 15.2% (95% CI: 12.7–19.1) in women <30 years and 21.9% (95% CI: 17.0–30.7) in women \geq 30 years.

The prevalence of CIN 3 in subjects with positive Cervista HPV HR results and positive for HPV 16 and/or 18 was 2.1% (13 of 611) for women <30 years of age and 1.3% (9 of 701) for women \geq 30 years (Table 2). The sensitivity of the HPV 16/18 genotyping test was 76.9% (95% CI: 49.7–91.8) and 77.8% (95% CI: 45.3–93.7) in women <30 and \geq 30 years, respectively, for predicting CIN 3. The NPV for CIN 3 was 98.9% (95% CI: 97.8–100.0) in women <30 years and 99.1% (95% CI: 98.1–100.0) in women \geq 30 years. Similar to women diagnosed with \geq CIN 2, specificity of the test was higher in women \geq 30 years (77.9%; 95% CI: 72.9–82.3) than in women <30 years (60.1%; 95% CI: 55.4–64.6). The PPV was 5.4% (95% CI: 4.25–7.8) in women <30 years and 9.6% (95% CI: 7.0–15.2) in women \geq 30 years.

Risk of \geq CIN 2 for different outcomes of Cervista HPV HR and Cervista HPV 16/18 genotyping tests

Absolute risk and likelihood ratios for \geq CIN 2 are presented in Table 3. For HR HPV-positive women who were positive for HPV types 16 and/or 18, the absolute risk of having \geq CIN 2 was 17.1% compared with 4% for HR HPV-positive women who were negative for HPV types 16 and 18 ($P < 0.001$). Of note, the absolute risk of \geq CIN 2 was greater among women positive for both the HR HPV and HPV 16/18 genotyping tests when compared with women in which both tests were found to be negative (17.1% vs. 0.9%; $P < 0.001$).

Discussion

This study represents the first multisite analysis and clinical performance of the Cervista HPV 16/18 genotyping test in women across a broad age range of women who are being screened for cervical cancer. The observed greater than 4-fold increase (17.1% vs. 4%) in risk of \geq CIN 2 for subjects with ASC-US cytology who were positive for HPV types 16 and/or 18 when compared with non-16/18 HR HPV types (12 other HR types combined) is consistent with prior studies showing increased carcinogenicity of HPV 16 and, to a lesser degree, HPV 18 (13, 14). In addition, specificity of the genotyping test in detecting \geq CIN 2 in the present study showed similar age-related trends to previously reported data; increased specificity with increasing age (5).

Table 2. Age-stratified clinical performance of the Cervista HPV 16/18 Genotyping Test compared with colposcopy/central histology results (CIN 3) among women with ASC-US cytology and positive Cervista HPV HR results

Age strata, y	Disease prevalence (subjects)	Sensitivity (subjects; 95% CI)	Specificity (subjects; 95% CI)	NPV (subjects; 95% CI)	PPV (subjects; 95% CI)
<30	2.1% (13/611)	76.9% (10/13; 49.7–91.8)	60.1% (352/585; 55.4–64.6)	98.9% (262/265; 97.8–100.0)	5.4% (10/184; 4.3–7.8)
\geq 30	1.3% (9/701)	77.8% (7/9; 45.3–93.7)	77.9% (532/683; 72.9–82.3)	99.1% (233/235; 98.1–100.0)	9.6% (7/73; 7.0–15.2)

Table 3. Risk of \geq CIN 2 for different outcomes of Cervista HPV HR and Cervista HPV 16/18 genotyping tests

HR HPV result	HPV 16/18 result	\geq CIN 2					
		Absolute risk (subjects)	95% CI		Likelihood ratio	95% CI	
HR HPV positive	HPV 16 and/or 18 positive	17.1% (44/257)	13.0	22.2	3.72	2.93	4.54
	HPV 16/18 negative	4.0% (20/500)	2.6	6.1	0.75	0.51	1.06
HR HPV negative	HPV 16/18 negative and/or positive	0.9% (5/555)	0.4	2.1	0.17	0.07	0.36

NOTE: Prevalence of \geq CIN 2 = 5.3%.

Several studies have suggested that the inclusion of adjunctive genotyping for HPV types 16 and 18 in clinical screening algorithms that also include cervical cytology and HR HPV testing may stratify the risk of cervical disease (15, 16). The present study assessed such stratification utilizing the Cervista HPV 16/18 genotyping test. This test may add benefit to screening algorithms for cervical cancer by improving clinical specificity while identifying increased absolute risk of high-grade cervical disease imparted by infection with HPV types 16 and/or 18.

Data from the Cervista clinical trial showed that Cervista HPV HR provides 92.8% clinical sensitivity and 44.2% specificity for \geq CIN 2 (8). Using these data in conjunction with results from the present study illustrates that inclusion of the HPV 16/18 genotyping test in an algorithm for women with ASC-US cytology and HR HPV-positive results can increase clinical specificity for \geq CIN 2 to 61.9% for women <30 years of age and to 79.9% for women \geq 30 years of age. This corresponds to an increase in clinical specificity for \geq CIN 2 of 36% to 81% for a testing algorithm of women with ASC-US cytology who are positive for HR HPV and HPV 16 and/or 18 genotyping results. This finding was not unexpected considering the 4-fold increase in absolute risk for \geq CIN 2 imparted by the detection of HPV types 16 and/or 18 compared with detection of the presence of 12 other HR HPV types (Table 3). Similar increases in absolute risk for \geq CIN 2 has been studied using other tests for HR HPV and HPV types 16 and 18 (16). The risk of \geq CIN 2 in women with ASC-US cytology who are also HR HPV positive is clearly high enough to warrant referral for colposcopy. However, a single colposcopy with biopsy provides poor sensitivity (16). This, coupled with the findings of the present study, suggests that algorithms incorporating the use of HPV 16/18 genotyping for the management of ASC-US cytology may identify those women for whom more

aggressive disease ascertainment and follow-up should be considered if their initial biopsy is not found to have \geq CIN 2.

The clinical performance of the HPV 16/18 genotyping test, as well as evidence from additional studies (4, 14, 15, 17–20), suggest a potential role for genotyping HPV types 16 and 18 primarily as a follow-up to an HR HPV screening test in women \geq 30 years. Additional long-term follow-up studies will be required to determine the most appropriate role for HPV genotyping in women with ASC-US or other abnormal cytology results. These results should be taken into account when considering future triaging strategies for cervical abnormalities as delineated by the scientific and medical communities.

Disclosure of Potential Conflicts of Interest

M.H. Einstein has participated in educational speaking activities and scientific advisory boards for Hologic, Inc., Qiagen, and Roche but did not receive an honorarium from any company. In specific cases, his hospital, Montefiore Medical Center has received payment for his time for these activities from companies involved in cervical cancer diagnostics including Hologic, Roche, and Neodiagnostix. F.A.R. Garcia has participated in an advisory board for Hologic, Inc. but does not receive an honorarium. S.P. Day is an employee of Hologic, Inc. A.L. Mitchell has nothing to disclose.

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