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

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Running Title: Cervista® HPV 16/18 Genotyping Across Age Strata
Key words: HPV, genotyping, ASC-US, cervical cancer, age stratification
Abstract

Background: The objective of this study was to evaluate the clinical performance of the Cervista® HPV 16/18 genotyping test for detection of 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% confidence intervals [CI]: 54.6-81.9) and 66.7% (95% CI: 46.7-82.0) in women <30 and ≥30 years, respectively. 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) compared to 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.
**Introduction**

High-risk (HR) human papillomavirus (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; Marlborough, MA) is also approved by the US 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).

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 demonstrates 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. Histological 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 performed 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
testing, DNA was isolated from 2 mL using the Genfind™ DNA Extraction kit (Hologic, Inc)
(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 formation, and cleavage by the Cleavase®
enzyme (Hologic, Inc) (12). These reactions detect HPV 16 and HPV 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 no-target 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 cut-off value of 2.13. If the FOZ values are below this cutoff, then the samples are considered negative. To demonstrate that the testing procedure has been properly performed 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 based on 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 ≥CIN 2 in subjects with positive CERVISTA HPV HR results and positive for HPV 16 and/or 18 was 6.6% (40 of 611) for women <30 years of age and 4.1% (29 of 701) for women ≥30 years (Table 1). The sensitivity of the HPV 16/18 genotyping test for predicting ≥CIN 2 was 70.0% (95% confidence intervals [CI]: 54.6–81.9) and 66.7% (95% CI: 46.7–82.0) in women <30 and ≥30 years, respectively. The NPV for ≥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 ≥30 years. As expected, specificity of the test was higher in women ≥30 years (79.9%; 95% CI: 74.9–84.2) compared to 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.

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 ≥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 ≥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 ≥30 years. Similar to women
diagnosed with ≥CIN 2, specificity of the test was higher in women ≥30 years (77.9%; 95% CI:
72.9–82.3) compared to 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 ≥30 years.

Risk of ≥CIN 2 for Different Outcomes of CERVISTA HPV HR and CERVISTA HPV 16/18
Genotyping Tests

Absolute risk and likelihood ratios for ≥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 ≥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 ≥CIN 2 was greater among women positive
for both the HPV HR and HPV 16/18 genotyping tests when compared to 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 >4-fold increase (17.1% vs 4%) in risk of ≥CIN
2 for subjects with ASC-US cytology who were positive for HPV types 16 and/or 18 when
compared to 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 ≥CIN 2 in the present study
demonstrated similar age-related trends to previously reported data; increased specificity with increasing age (5).

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 demonstrated that CERVISTA HPV HR provides 92.8% clinical sensitivity and 44.2% specificity for ≥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 HPV HR-positive results can increase clinical specificity for ≥CIN 2 to 61.9% for women <30 years of age and to 79.9% for women ≥30 years of age. This corresponds to an increase in clinical specificity for ≥CIN 2 of 36%-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 ≥CIN 2 imparted by the detection of HPV types 16 and/or 18 compared to detection of the presence of 12 other HR HPV types (Table 3). Similar increases in absolute risk for ≥CIN 2 has been studied using other tests for HR HPV and HPV types 16 and 18 (16). The risk of ≥CIN 2 in women with ASC-US cytology who are also HPV HR-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.
Disclosures

Dr. Einstein has participated in scientific advisory boards for Hologic, Inc, Qiagen, and Roche but does not receive an honorarium. In specific cases, his hospital, Montefiore Medical Center has received payment for his time. Dr. Garcia has participated in an advisory board for Hologic, Inc but does not receive an honorarium. Dr. Day is an employee of Hologic, Inc. Dr. Mitchell has nothing to disclose.
References


Table 1. Age-stratified clinical performance of the CERVISTA HPV 16/18 Genotyping Test compared with colposcopy/central histology results (≥CIN 2) among women with ASC-US cytology and positive CERVISTA HPV HR results

<table>
<thead>
<tr>
<th>Age Strata</th>
<th>Disease Prevalence (subjects)</th>
<th>Sensitivity (subjects; 95% CI)</th>
<th>Specificity (subjects; 95% CI)</th>
<th>NPV (subjects; 95% CI)</th>
<th>PPV (subjects; 95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;30</td>
<td>6.6% (40/611)</td>
<td>70.0% (28/40; 54.6–81.9)</td>
<td>61.9% (329/531; 57.1–66.4)</td>
<td>95.5% (253/265; 93.4–97.6)</td>
<td>15.2% (28/184; 12.7–19.1)</td>
</tr>
<tr>
<td>≥30</td>
<td>4.1% (29/701)</td>
<td>66.7% (19/29; 46.7–82.0)</td>
<td>79.9% (514/643; 74.9–84.2)</td>
<td>96.6% (227/235; 94.8–98.5)</td>
<td>21.9% (16/73; 17.0–30.7)</td>
</tr>
</tbody>
</table>

HPV = human papillomavirus; CIN = cervical intraepithelial neoplasia; ASC-US = atypical squamous cells of undetermined significance; CI = confidence interval; NPV = negative predictive value; PPV = positive predictive value
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

<table>
<thead>
<tr>
<th>Age Strata</th>
<th>Disease Prevalence (subjects)</th>
<th>Sensitivity (subjects; 95% CI)</th>
<th>Specificity (subjects; 95% CI)</th>
<th>NPV (subjects; 95% CI)</th>
<th>PPV (subjects; 95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;30</td>
<td>2.1% (13/611)</td>
<td>76.9% (10/13; 49.7–91.8)</td>
<td>60.1% (352/585; 55.4–64.6)</td>
<td>98.9% (262/265; 97.8–100.0)</td>
<td>5.4% (10/184; 4.3–7.8)</td>
</tr>
<tr>
<td>≥30</td>
<td>1.3% (9/701)</td>
<td>77.8% (7/9; 45.3–93.7)</td>
<td>77.9% (532/683; 72.9–82.3)</td>
<td>99.1% (233/235; 98.1–100.0)</td>
<td>9.6% (7/73; 7.0–15.2)</td>
</tr>
</tbody>
</table>

HPV = human papillomavirus; CIN = cervical intraepithelial neoplasia; ASC-US = atypical squamous cells of undetermined significance; CI = confidence interval; NPV = negative predictive value; PPV = positive predictive value
Table 3: Risk of ≥CIN 2 for different outcomes of CERVISTA HPV HR and CERVISTA HPV 16/18 genotyping tests

<table>
<thead>
<tr>
<th>HR HPV Result</th>
<th>HPV 16/18 Result</th>
<th>Absolute Risk (subjects)</th>
<th>95% CI</th>
<th>Likelihood Ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HR HPV Positive</strong></td>
<td><strong>HPV 16 and/or 18 Positive</strong></td>
<td>17.1% (44/257)</td>
<td>13.0</td>
<td>22.2</td>
<td>3.72</td>
</tr>
<tr>
<td></td>
<td><strong>HPV 16/18 Negative</strong></td>
<td>4.0% (20/500)</td>
<td>2.6</td>
<td>6.1</td>
<td>0.75</td>
</tr>
<tr>
<td><strong>HR HPV Negative</strong></td>
<td><strong>HPV 16/18 Negative and/or Positive</strong></td>
<td>0.9% (5/555)</td>
<td>0.4</td>
<td>2.1</td>
<td>0.17</td>
</tr>
</tbody>
</table>

Prevalence of ≥CIN 2 = 5.3%

CIN = cervical intraepithelial neoplasia; HPV = human papillomavirus; HR = high-risk; CI = confidence interval
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