Reflex High-Risk Human Papillomavirus Testing for Women With Atypical Squamous Cells of Undetermined Significance in Cytologic Smears: Effects Since Implementation in a Large Clinical Practice

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Abstract

Reflex high-risk human papillomavirus (HPV) testing often is used in the management of women with atypical squamous cells of undetermined significance identified in cervicovaginal screening. Following implementation of reflex testing, our laboratory processed 8,022 specimens during a 20-month period; sufficient material was available for testing in 7,334 specimens. High-risk HPV was detected in 34.10% of these specimens. Detection rates varied with age, with positive rates as high as 58.46% in women 20 years old or younger, decreasing to 14.58% in women older than 35 years. The detection rate, categorized in 5-year age increments, showed a significant decrease until after 35 years, when the rate remained fairly constant (P < .0001). The detection rate decreased over the time of the study. These results demonstrate that high-risk HPV detection might vary according to the age mix of the population tested and the interval after implementation of testing.

The management of women with atypical squamous cells of undetermined significance (ASCUS) identified in cytologic screening has gone through significant evolution during recent years, primarily owing to the availability of testing for the presence of high-risk human papillomavirus (HPV). Several studies have demonstrated this testing to be highly sensitive for the detection of cervical intraepithelial neoplasia grades 2 and 3 in women with an ASCUS diagnosis from a cytology smear, and the large Atypical Squamous Cell of Undetermined Significance/Low-Grade Squamous Intraepithelial Lesion Study (ALTS) showed that in women with ASCUS identified in smears, high-risk HPV testing had greater sensitivity than and specificity comparable to repeated cytologic smears in the detection of cervical intraepithelial neoplasia 3 or higher.1-5 Based on these studies, the 2001 consensus guidelines published by the American Society for Colposcopy and Cervical Pathology listed high-risk HPV testing as the “preferred” option for the triage of woman with ASCUS identified in smears.6

Although these reports described the usefulness of viral testing in the triage of women with ASCUS identified in smears, little has been reported about the use of high-risk HPV testing on a large scale in clinical practice, particularly in relation to considerations such as patient age and trends in testing results over time, with implementation of widespread reflex testing. Our purpose was to evaluate our experience with reflex high-risk HPV testing in women with ASCUS identified in smears in a large clinical practice, to ascertain whether rates of test positivity were affected by patient age and whether there were changes in positivity rates over time after the implementation of reflex testing.
Materials and Methods

Wilford Hall Medical Center (Lackland Air Force Base, San Antonio, TX) is a tertiary care US Air Force medical treatment facility that processes approximately 70,000 cytology specimens annually. Our laboratory began conversion from conventional cervicovaginal smears to liquid-based cytology (LBC) (ThinPrep Pap Test, Cytyc, Boxborough, MA) in early 2002 and began performing in-house reflex testing on residual LBC specimens of all ASCUS identified in smears in June 2002. Reflex testing on residual LBC samples submitted from several smaller US Department of Defense cytology laboratories began in September 2002; these referral samples constituted a minority (<10%) of the HPV tests performed.

High-risk HPV testing was performed using the Hybrid Capture II (Digene, Gaithersburg, MD) system, using residual PreservCyt (Cytyc) samples that had been processed previously for the cytologic smears. In accordance with manufacturer’s instructions, specimens with less then 4 mL of residual solution were reported as “quantity not sufficient” (QNS), and high-risk HPV testing was not performed. Specimens with sufficient luminescence (relative light unit/cutoff value ratios ≥1.0, which represents detection of approximately 1 pg/mL of HPV DNA) were reported as “detected” (positive); those with lower values were reported as “not detected” (negative).

All reports of high-risk HPV testing performed at our institution from June 2002 through January 2004 were reviewed. Information extracted from the reports included the date of birth and date of specimen accession (from which age was calculated) and the HPV test result. Overall results for all tests performed during this period are reported; the testing results also were stratified by age using 5-year increments and compared according to these age categories. Results of testing also were evaluated for trends over time. In addition, the analysis of test result trends over time was controlled for age, to account for possible changes in age distribution during any particular period after implementation of reflex testing.

Descriptive statistics are reported for the overall group. For the analysis according to age, χ² was used to compare adjacent 5-year increments. Analysis of trends over time was performed using logistic regression. SPSS for Windows (release 11.5.0, SPSS, Chicago, IL) was used for statistical analysis. Statistical significance was set at a P value of .05 or less.

Results

From June 2002 through January 2004, the overall abnormal rate for all cytologic specimens processed by our laboratory was 14.15%, with an ASCUS rate of 9.27%. The rate of a squamous intraepithelial lesion (SIL) diagnosis was 4.88%, and the ASCUS/SIL ratio was 1.9.

A total of 8,022 high-risk HPV test results were available for review. The mean ± SD age of the women overall was 32.2 ± 12.1 years. Insufficient residual specimen for testing (QNS) occurred in 7.29% of the group, and 1.28% of the specimens were rejected because the specimen exceeded the accepted storage time. Therefore, testing was performed on 7,334 specimens. The mean ± SD age of women for whom results were available was 32.3 ± 12.1 years. For the overall group, high-risk HPV testing was positive in 34.10%.

HPV test results stratified by age are summarized in Table 1; positive results are depicted graphically in Figure 1. The results, when stratified by age, demonstrated a significant decrease in the percentage of positive HPV tests in women with ASCUS identified in smears until after 35 years (P < .0001; χ²), after which the positive rate remained fairly constant. Logistic regression was not applied to the analysis of overall results stratified by age owing to the leveling of the rates after 35 years, as depicted in Figure 1. Based on the nonlinear curve, logistic regression would oversimplify the trend and provide a less meaningful comparison between age groups. No differences were noted between age groups for QNS or specimen rejection rates.

Table 1
Summary of Results of High-Risk Human Papillomavirus Testing by Age

<table>
<thead>
<tr>
<th>Age Range (y)</th>
<th>No. Detected</th>
<th>Total No. Tested</th>
<th>Positive Rate (%)</th>
<th>Percentage of All Tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤20</td>
<td>622</td>
<td>1,064</td>
<td>58.46</td>
<td>14.51</td>
</tr>
<tr>
<td>21-25</td>
<td>909</td>
<td>1,800</td>
<td>50.50</td>
<td>24.54</td>
</tr>
<tr>
<td>26-30</td>
<td>355</td>
<td>1,049</td>
<td>36.70</td>
<td>14.30</td>
</tr>
<tr>
<td>31-35</td>
<td>207</td>
<td>828</td>
<td>25.0</td>
<td>11.29</td>
</tr>
<tr>
<td>36-40</td>
<td>138</td>
<td>847</td>
<td>16.3</td>
<td>11.55</td>
</tr>
<tr>
<td>41-45</td>
<td>84</td>
<td>646</td>
<td>13.0</td>
<td>8.81</td>
</tr>
<tr>
<td>46-50</td>
<td>62</td>
<td>462</td>
<td>13.4</td>
<td>6.30</td>
</tr>
<tr>
<td>51-55</td>
<td>40</td>
<td>252</td>
<td>15.9</td>
<td>3.44</td>
</tr>
<tr>
<td>56-60</td>
<td>20</td>
<td>169</td>
<td>11.8</td>
<td>2.30</td>
</tr>
<tr>
<td>&gt;60</td>
<td>34</td>
<td>217</td>
<td>15.7</td>
<td>2.96</td>
</tr>
<tr>
<td>Total</td>
<td>2,501</td>
<td>7,334</td>
<td>34.10</td>
<td>100.00</td>
</tr>
</tbody>
</table>
Trends across age and time after implementation of testing were estimated using a logistic regression with the test result as the dependent variable and age and date of test, along with their interaction, as independent variables. The results suggested that the odds of a positive test decreased significantly with age and time and that the decrease with age was greater as more time elapsed after implementation of testing (the interaction term was statistically significant: $\beta = -4.7 \times 10^{-5}$; $Z = 7.569$; $P = .006$).

Because of the interaction, it is impossible to estimate a single odds ratio describing the relationship between age and test result. At the beginning of the study, the odds of a positive test were 3 times higher in patients 35 years or younger compared with women 36 years or older (odds ratio [OR], 3.076; 95% confidence interval [CI], 2.299-4.116; $P < .001$). The difference between younger and older patients increased during the study. The odds of a positive test declined by 3% per month among women 35 years or younger (OR, 0.979; 95% CI, 0.968–0.990; $P < .001$), whereas the odds of a positive test declined by 6% per month among patients 36 years or older (OR, 0.943; 95% CI, 0.924–0.962; $P < .001$). The rate of decline differed significantly between the 2 age groups (OR, 1.038; 95% CI, 1.015–1.063; $P = .001$). The overall trend over time, categorized according to age ($\leq 35$ years and $\geq 36$ years), is shown in Figure 2.

**Discussion**

Several studies have demonstrated the usefulness of high-risk HPV testing as a triage tool for patients with ASCUS identified in smears; our study reports a large laboratory experience following implementation of this method in clinical practice. In the 2 largest series of US women with ASCUS identified in smears, HPV positivity rates were 39.5% in a study of 995 women enrolled in a Kaiser Permanente Medical Care program and 50.6% in the cross-sectional report of 3,488 women in the ALTS trial.1,3 In more than 7,000 tests performed in women with ASCUS identified in smears, our overall positive rate of 34.10% is lower than the rate reported in either of these studies, although our positive test rate more closely approximates that from the Kaiser Permanente study than the ALTS trial. Several possible explanations might account for this difference in rates. First, the Kaiser and the ALTS studies were designed to analyze follow-up information for women with abnormal smears; our study was designed to evaluate testing results once reflex testing was implemented in clinical practice. In addition, in the Kaiser and ALTS studies, test positivity was based on the number of women with ASCUS identified in smears, whereas in our study, the rate was based on the total number of HPV tests performed.

Another difference in the Kaiser and ALTS studies compared with ours relates to age distribution. The mean ages of the women included in the ALTS trial and the Kaiser Permanente study were 29 and 37 years, respectively; our mean age was between the ages reported in these studies.3,7 In the ALTS trial, only 22.5% of the included participants were 35 years or older; in our study, 35.36% of the women were older than 35 years.

Further consideration of the effect of patient age on HPV test results is provided in a follow-up study of 2,198 women.
from the ALTS study group, in which test sensitivity and colposcopy referral rates, stratified by age, were reported. In this analysis, increasing age was related inversely to HPV test positivity rates. We identified a similarly declining positivity rate with age, but our test positivity rates in the younger age ranges still were lower than reported for similar age categories in the ALTS follow-up study. In the ALTS follow-up study, a large decline in colposcopy referrals was identified in women 29 years or older; we identified a similar large decline in positive test results, but only after age 35 years. Therefore, the inclusion of a greater percentage of women 35 years or older in our study compared with the ALTS trial might account for the overall lower HPV positivity rate in our study. Although our study included a greater percentage of women older than 35 years, 39.05% of our results were from women 25 years or younger; in the ALTS trial, women younger than 24 years accounted for 38.7% of the women included for analysis. Thus, a similar percentage of younger women, in whom positive HPV test rates are higher, were included in the ALTS trial and in our study. The lower overall rate in the Kaiser Permanente study also might be due to the inclusion of women in higher age categories as evidenced by the higher mean age of the women in the study, although the age distribution of the participants was not provided.

There are additional differences between our study and the ALTS trial that might help explain the different test positivity rates. In the ALTS trial, a referral ASCUS cytologic smear was the initial requirement for enrollment; repeated LBC was performed after enrollment, with HPV testing performed on this subsequent LBC residual specimen. Referral cytology included conventionally prepared cytologic smears and LBC, and follow-up cytology smears (prepared from the same specimen as the HPV test) were negative in 41.9% and a repeated ASCUS diagnosis in 32.5% of the patients. In contrast, our study involved simultaneous cytologic smear preparation with reflex testing performed on the residual solution, and all were performed using LBC. In the ALTS trial, the mean time between the initial ASCUS cytology and trial enrollment was 2 months, with a median of 52 days. Therefore, it is possible that the HPV test results might have been influenced by the delay from enrollment to viral testing in the ALTS trial compared with reflex testing in our study.

An interesting finding in the present study that has not been reported previously is the trend of positive test results over time. The reason for this trend is unclear. One possibility is that changes in the percentage of women in different age categories during the study period might affect test positivity rates because positive test rates varied according to age. However, as shown, the trend in test results over time still was observed even in younger study participants and still was significant even when accounting for age distribution. Another possibility might be that early in the study, more tests might have been performed on women with ASCUS previously identified in smears (based on the clinical practice of repeating cytologic smears in women with ASCUS), whereas fewer repeated cytologic smears were performed as the interval after implementation of reflex testing progressed. However, based on the data available, we are unable to determine whether this theory about trends in the performance of repeated cytologic smears is true. Finally, our population is a heterogeneous group, and a significant proportion of the population is transient. Therefore, it is possible that the actual prevalence of HPV in our population at any given time is variable.

A possible concern in our study is the overall ASCUS rate of 9.27% in our cytology laboratory. A 1997 survey published in 2000 by the College of American Pathologists (CAP) demonstrated that for conventional cytology smears, the median ASCUS rate was 4.5%, with rates of 7.0% and 9.9% representing the 75th and 90th percentiles, respectively. A similar study conducted by the CAP in 2003 surveyed laboratories after widespread implementation of liquid-based cytology. This study showed overall 50th, 75th, and 90th percentile ASCUS rates of 3.9%, 5.9%, and 8.7%, respectively, while those percentiles reported for ThinPrep specimens were 4.0%, 5.8%, and 8.9%, respectively. The participating cytology laboratories in the Kaiser study reported an ASCUS rate of 3.5% (range, 2.7%-4.9% from 4 institutions). To our knowledge, no such rate for the participating centers in the ALTS trial has been reported. A high ASCUS rate, possibly representing false-positive cytologic diagnoses, could be expected to lead to a lower rate of disease identified at follow-up. However, ASCUS rates are often dependent on the population, and the ASCUS/SIL ratio of 1.9 for our cytology laboratory was well within the range reported in the earlier CAP survey (median, 2.0) and the ALTS trial (1.0 to 3.5, ASCUS/SIL ratio) and lower than the ratios in the Kaiser study (2.9). This would suggest that our lower rate of HPV positivity, compared to both the Kaiser and ALTS studies, is likely not related to overcalls of ASCUS within our tested population. Of note, however, a later CAP report showed lower overall ASCUS/SIL ratios than the earlier study, with 50th, 75th, and 90th percentile ASCUS/SIL ratios for all specimens of 1.43, 2.08, and 2.86, respectively. For ThinPrep specimens, ratios at the same percentiles were 1.31, 1.87, and 2.69, respectively. The rate in our cytology laboratory over the time studied is above the 50th percentile according to this survey, which is noteworthy if attempting to make comparisons with testing results from other laboratories.

Previous data have shown that high-risk HPV testing is a valuable triage tool for women with ASCUS identified in smears. When implemented in a large clinical practice, we have shown that the overall HPV positive test rate was lower than that in 2 major prospective clinical trials and that test positivity rates vary significantly with age.
In addition, the positive test rates declined after implementa-
tion of reflex testing, and this was not due to variances in age
distribution of the population or to significant variation in
ASCUS or SIL cytologic diagnoses. Providers should be
aware that a small percentage of specimens will be unsuitable
for reflex testing, and, thus, alternative management options
might be required.

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