**The Interplay of Age Stratification and HPV Testing on the Predictive Value of ASC-US Cytology**

Results From the ATHENA HPV Study

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**Key Words:** Age stratification; ASC-US; Cervical cancer screening; Cytology; Genotype; Human papillomavirus; HPV DNA testing; Triage

**Abstract**

We have previously shown that human papillomavirus (HPV) genotyping, using the cobas HPV Test (Roche Molecular Systems, Pleasanton, CA), can be used to identify women with atypical squamous cells of undetermined significance (ASC-US) at the highest risk for cervical intraepithelial neoplasia (CIN) grade 2 or worse. We investigated the impact of age stratification on the risk of CIN 2 or worse in women with ASC-US and the performance of HPV genotyping in different age strata.

The sensitivity of the cobas HPV Test was 93.3% in the 21- to 29-year-old age group and 67.7% in the 40 years or older group, most likely owing to pathologic misclassification of CIN 2 or worse in older women. The prevalence of CIN 2 or worse in younger women was nearly 4-fold that detected in older women and was predominantly HPV-16–related.

Age-specific evaluation of ASC-US cytology in conjunction with HPV genotype status enables more effective risk assessment and could be used in clinical management.

Equivocal cervical cytologic abnormalities, referred to as atypical squamous cells of undetermined significance (ASC-US) using the Bethesda System, account for 4% to 5% of all cytologic interpretations in the United States and as such represent the most commonly diagnosed cervical cytologic abnormality.¹² Owing to the nonspecific nature of the cellular changes that lead to this equivocal diagnosis and the variability in how different laboratories diagnose ASC-US, women with ASC-US can have a spectrum of cervical lesions ranging from reactive or inflammatory conditions to invasive cancer. The introduction of high-risk human papillomavirus (HR-HPV) DNA testing for the triage of women with ASC-US cytology provided a valuable tool to distinguish patients with benign changes from patients with high-grade cervical intraepithelial neoplasia (CIN 2 and CIN 3) or cervical cancer.³⁵ However, even when clinically validated HPV DNA tests are used, the high prevalence of HR-HPV DNA positivity relative to CIN 2 or worse in women with ASC-US can limit the usefulness of HPV DNA testing.⁶ This was first suggested by the findings from the National Cancer Institute’s large ASC-US/LSIL [low-grade squamous intraepithelial lesion] Triage Study (ALTS). In ALTS, the prevalence of HR-HPV DNA positivity was 65% in the subset of women with ASC-US who were 22 to 28 years old compared with only 19.5% in women 40 years or older. The reduction in HR-HPV positivity with increasing age was accompanied by a reduction in high-grade cervical neoplasia. Only 1.4% of women 40 years or older with ASC-US in ALTS were found to have CIN 3 or worse at the initial colposcopy.⁷

To improve the management of women with ASC-US, additional information on how best to stratify the risk of CIN 2 or worse is needed. One approach to risk stratification
might be to incorporate HPV genotyping results into management. Previously, we have shown that genotyping for HPV-16 and HPV-18 identifies a subset of women with ASC-US at particularly high risk for having CIN 2 or worse. The prevalence of CIN 2 or worse in women 21 years or older with ASC-US enrolled in the ATHENA trial is particularly high risk for having CIN 2 or worse. The prevalence of CIN 2 or worse in women 21 years or older with ASC-US enrolled in the ATHENA trial is particularly high risk for having CIN 2 or worse.8 The prevalence of CIN 2 or worse in women 21 years or older with ASC-US enrolled in the ATHENA trial was 24.4%. However, the risk of CIN 2 or worse among women who were positive for the 12 other HR-HPV genotypes was 8.6%, which many clinicians may consider high enough to necessitate performing an immediate colposcopy.

Because prior studies have shown age-dependent variations in HR-HPV prevalence and the prevalence of CIN 2 or worse in women with ASC-US, further investigation seems warranted into the impact of age on HPV test performance, risk of CIN 2 or worse, and the effect of specific HPV genotype on risk. In this study, we investigated the interplay between HR-HPV testing with integrated genotyping of HPV-16 and HPV-18 using the cobas HPV Test (Roche Molecular Systems, Pleasanton, CA) and age on the following: (1) test performance, (2) prevalence of cervical disease, and (3) the absolute risk for CIN 2 or worse and CIN 3 or worse in women with ASC-US cytology enrolled in the ATHENA trial.

Materials and Methods

Study Population

The ATHENA study population included 47,208 women 21 years or older who underwent routine cervical screening in 61 clinical centers across the United States between May 2008 and August 2009. All women met the study inclusion/exclusion criteria as previously described. The study protocol was approved by the institutional review boards of all study sites, and written informed consent was given by all women before undergoing study procedures. This analysis focuses on the evaluable ASC-US population, ie, 1,578 (82.1%) of the 1,923 women 21 years or older with ASC-US cytology. All 1,578 women underwent colposcopy and had valid HPV tests and cervical biopsy results.

Study Protocol

Cytology and HPV Testing

Demographic information, medical history, and informed consent were obtained from all women at the enrollment visit. Two liquid-based cervical cytology samples (Thin-Prep, Hologic, Bedford, MA) were obtained from each participant by using a plastic spatula and an endocervical brush, according to the manufacturer’s instructions. The first cervical sample was used for cytology and HPV testing. Cytologic evaluation was carried out at 1 of 4 accredited clinical laboratories in the United States, as previously described, and results were reported using the 2001 Bethesda System nomenclature. HPV testing was carried out using the first-generation AMPLICOR HPV Test (detecting 13 HR-HPV genotypes; Roche Molecular Systems) and LINEAR ARRAY High Risk HPV Genotyping Test (detecting HPV-16 individually, HPV-18 individually, and 12 pooled HR-HPV genotypes), as previously described. The second cervical sample was used for HPV testing with the Hybrid Capture 2 (hc2) HPV test (detecting 13 pooled HR-HPV genotypes; QIAGEN, Gaithersburg, MD), as previously described.

Colposcopy

All women with ASC-US cytology results were referred for colposcopy. Participants and colposcopists were masked to the patients’ cytology and HPV test results. Colposcopy with biopsy and/or endocervical curettage (ECC) were performed within 12 weeks of enrollment according to a standardized protocol that included biopsy of all visible cervical lesions or, in women with a satisfactory colposcopy result but without visible cervical lesions, a random biopsy at the squamocolumnar junction. ECC was performed on all women with an unsatisfactory colposcopy result. Biopsy and ECC specimens were reviewed in a blinded manner by a Central Pathology Review panel of 3 pathologists and diagnosed using standard criteria and the CIN terminology.

p16INK4A Immunohistochemical Staining

p16INK4A is a cyclin-dependent kinase inhibitor that has a crucial regulatory role in cell proliferation. Overexpression of p16INK4A has been demonstrated to be an excellent biomarker of high-grade cervical neoplasia and can readily be detected by immunohistochemical studies. Therefore, p16INK4A immunohistochemical analysis was carried out according to the instructions of the manufacturer on selected cervical biopsy specimens using the CINtec Histology Kit (mtm laboratories, Heidelberg, Germany) that has a primary antibody against p16INK4A. For each staining run, a positive control slide containing tissue sections from a cervical biopsy specimen with known positive immunoreactivity for p16INK4A was used to validate the staining procedure.

Statistical Analysis

HPV test performance characteristics (sensitivity, specificity, negative predictive value, and positive predictive value) for the identification of high-grade cervical disease were determined using standard statistical tests. Absolute
risk of high-grade cervical disease and the respective 95% confidence interval were determined for different categories of cobas HPV Test results: HPV-16 and HPV-18 results were analyzed individually; HPV-16+ results included positive for HPV-16 alone, with or without HPV-18, and with or without 12 other HR-HPV genotypes present. HPV-18+ results included positive for HPV-18 alone, with or without a positive result for 12 other HR-HPV, and negative for HPV-16. Twelve other HR-HPV+ samples were positive only for 1 or more of these high-risk types.

Results

Demographics and Cytologic and Histologic Distribution

The overall prevalence of ASC-US cytology results in the enrollment cervical cytology among women 21 years or older in the ATHENA trial was 4.1%. The demographics of the women with ASC-US cytology (n = 1,578) have been previously reported, but in brief, the mean age ± SD was 37.1 ± 11.3 years, 40.7% of the study participants were 40 years or older, and 23.1% were postmenopausal by self-report.8 After adjusting for age at initiation of screening, the percentage of women who had had a Papanicolaou test within the 5 years before enrollment (90.9%) was comparable across age groups; a lower percentage of women 40 years or older had had colposcopy performed within the previous 5 years than was reported for the younger age groups, and vaccination against HPV was essentially limited to the 21- to 29-year-old age group Table 1. Racial distribution was 80.5% white and 16.5% black or African American, and 19.1% listed their ethnicity as Hispanic or Latino.

As previously reported, the prevalence of ASC-US dropped with increasing age and was 5.4%, 4.1%, and 3.5%, respectively, for participants who were 21 to 29 years old, 30 to 39 years old, and 40 years or older (P = .001; Table 1). Thus, there was a 1.5-fold reduction in ASC-US over the 3 age strata. The overall prevalence of biopsy-confirmed CIN 2 or worse among women with ASC-US was 5.1% and ranged from 8.8% in the 21- to 29-year-old group to 2.3% in the 40 years or older group (P = .001; Table 1). This represents a 3.8-fold reduction in the prevalence of biopsy-confirmed CIN 2 or worse among older women compared with younger women and is more than twice the reductions observed with increasing age for the prevalence of ASC-US cytology among women being screened. Of note is the fact that approximately half of all cases of biopsy-confirmed CIN 2 or worse occurred among women in the youngest age group (21-29 years old).

Performance of HPV Testing in Women With ASC-US Cytology Stratified by Age

The performance of the cobas HPV Test (14 high-risk types) and the hc2 test when stratified by age in women with ASC-US is shown in Table 2. The sensitivity of both tests declined quite dramatically with increasing age, whereas specificity improved. For example, the sensitivity of the cobas HPV Test for CIN 2 or worse in the 21- to 29-year-old group was 93.3%, but it decreased to 66.7% in the 40 years or older group (P = .02). A similar reduction in sensitivity for CIN 2 or worse with increasing age occurred with the hc2 test (P = .06). Nevertheless, the negative predictive values of the cobas HPV Test for CIN 2 or worse were 99% or more in all age groups.

<table>
<thead>
<tr>
<th>Age Group (y)</th>
<th>21-29 (n = 514)</th>
<th>30-39 (n = 422)</th>
<th>≥40 (n = 642)</th>
<th>Overall (N = 1,578)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Papanicolaou test past 5 y</td>
<td>88.7</td>
<td>92.9</td>
<td>91.3</td>
<td>90.9</td>
</tr>
<tr>
<td>HPV test past 5 y</td>
<td>27.6</td>
<td>26.3</td>
<td>28.7</td>
<td>27.7</td>
</tr>
<tr>
<td>Colposcopy past 5 y</td>
<td>22.0</td>
<td>16.8</td>
<td>12.0</td>
<td>16.5</td>
</tr>
<tr>
<td>HPV vaccinated</td>
<td>13.0</td>
<td>0.0</td>
<td>0.2</td>
<td>4.3</td>
</tr>
<tr>
<td>Prevalence of ASC-US cytology†</td>
<td>5.4 (629/11,734)</td>
<td>4.1 (509/12,528)</td>
<td>3.5 (785/22,625)</td>
<td>4.1 (1,923/46,887)</td>
</tr>
<tr>
<td>Final pathologic diagnosis in women with ASC-US cytology‡</td>
<td>77.6</td>
<td>84.4</td>
<td>91.1</td>
<td>84.9</td>
</tr>
<tr>
<td>Negative (n = 1,340)</td>
<td>13.6</td>
<td>10.9</td>
<td>6.5</td>
<td>10.0</td>
</tr>
<tr>
<td>CIN 1 (n = 158)</td>
<td>4.1</td>
<td>2.1</td>
<td>0.6</td>
<td>2.2</td>
</tr>
<tr>
<td>CIN 2 (n = 34)</td>
<td>4.7</td>
<td>2.6</td>
<td>1.7</td>
<td>2.9</td>
</tr>
</tbody>
</table>

ASC-US, atypical squamous cells of undetermined significance; CIN, cervical intraepithelial neoplasia; HPV, human papillomavirus.

* Values are given as percentages unless otherwise indicated.
† Prevalence of ASC-US was based on all ASC-US cases (n = 1,923) among the eligible women. Values are given as percentage (number total).
‡ Prevalence of pathologic diagnoses was based on the data for 1,578 women with ASC-US cytology and a biopsy result.
HR-HPV Prevalence Stratified by Age and Cervical Disease Status

Among women with ASC-US cytology results, the prevalence of HR-HPV declines with age, as reported previously. For example, in the 21- to 29-year-old group, HR-HPV prevalence, as detected by the cobas HPV Test (14 types), is 54.1% but declines to 16.2% in women 40 years or older (P = .001). The impact of age on the prevalence of HPV-16 and HPV-18 in women with ASC-US mirrors the reductions observed for all 14 HR-HPV genotypes combined. The prevalence of HPV-16 detected by the cobas HPV Test declined 5.4-fold from 16.1% in the youngest age group to 3.0% in the oldest group.

### Table 2

<table>
<thead>
<tr>
<th>Performance of the cobas HPV Test and the hc2 Test in Detecting CIN 2 or Worse and CIN 3 or Worse in the ASC-US Population by Age Group*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age Group (y)</td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>Central Pathology Result</td>
</tr>
<tr>
<td>All ASC-US</td>
</tr>
<tr>
<td>HR-HPV+</td>
</tr>
<tr>
<td>HPV-16+</td>
</tr>
<tr>
<td>HPV-18+</td>
</tr>
<tr>
<td>Other 12+</td>
</tr>
<tr>
<td>Negative</td>
</tr>
<tr>
<td>HR-HPV+</td>
</tr>
<tr>
<td>HPV-16+</td>
</tr>
<tr>
<td>HPV-18+</td>
</tr>
<tr>
<td>Other 12+</td>
</tr>
<tr>
<td>CIN 1</td>
</tr>
<tr>
<td>HR-HPV+</td>
</tr>
<tr>
<td>HPV-16+</td>
</tr>
<tr>
<td>HPV-18+</td>
</tr>
<tr>
<td>Other 12+</td>
</tr>
<tr>
<td>CIN 2</td>
</tr>
<tr>
<td>HR-HPV+</td>
</tr>
<tr>
<td>HPV-16+</td>
</tr>
<tr>
<td>HPV-18+</td>
</tr>
<tr>
<td>Other 12+</td>
</tr>
<tr>
<td>CIN 3 or worse</td>
</tr>
<tr>
<td>HR-HPV+</td>
</tr>
<tr>
<td>HPV-16+</td>
</tr>
<tr>
<td>HPV-18+</td>
</tr>
<tr>
<td>Other 12+</td>
</tr>
</tbody>
</table>

ASC-US, atypical squamous cells of undetermined significance; CIN, cervical intraepithelial neoplasia; hc2, Hybrid Capture 2 HPV DNA test; HPV, human papillomavirus; NPV, negative predictive value; PPV, positive predictive value.

* Values are given as percentage (number/total) (95% confidence interval).
† P values for sensitivity in 21-29 vs ≥40 y are .02 for detecting CIN 2 or worse and .03 for detecting CIN 3 or worse.
‡ P values for sensitivity in 21-29 vs ≥40 y are .06 for detecting CIN 2 or worse and .08 for detecting CIN 3 or worse.
The impact of age on the prevalence of HPV genotypes in women stratified by cervical disease status demonstrates that the reductions in HR-HPV observed for women with ASC-US cytology also occur in women with ASC-US who have biopsy-confirmed CIN (Table 3). In particular, HPV-16 accounts for 62% (28/45) of the CIN 2 or worse detected in the youngest age group compared with 20% (3/15) in women 40 years or older. In addition, approximately 80% of CIN 3 or worse cases are found to be genotype HPV-16+ and/or HPV-18+ in the 21- to 29-year-old age group compared with only 27.3% in the 40 years or older group. Conversely with increasing age, there is a trend for the 12 other HR-HPV genotypes to be present in high-grade disease as compared with HPV-16/HPV-18. Of note, among a subset of the youngest age group, women 21 to 24 years old with ASC-US cytology, the prevalence of HR-HPV was 58.7% and disease prevalence values were 4.7% and 4.7% for CIN 2 and CIN 3, respectively. Most notably, all of the women in this age group who were found to have CIN 3 were positive for HPV-16 and/or HPV-18.

Age Stratification of Risk Estimates by cobas HPV Test Result

Interactions of age, disease status, and variations in HPV genotype can best be appreciated by determining the absolute risk of CIN 2 or worse associated with testing positive for a specific HPV genotype(s). In general, the absolute risk for CIN 2 or worse is considerably higher among women with ASC-US who are HPV-16+ compared with women who are positive for HPV-18 or the 12 other high-risk types of HPV

Characterization of cobas HPV Test–Negative CIN 2 or Worse Cases

To investigate whether the decreased sensitivity of HPV testing for the detection of CIN 2 or worse that was observed with increasing age was due to the pathologic misclassification of cervical biopsy specimens, we further analyzed the 8 biopsy specimens that were diagnosed as CIN 2 or worse but that were HR-HPV– by the cobas HPV Test. Of the 3 cases that occurred in women younger than 40 years, 2 were confirmed as being HR-HPV– by the AMPLICOR HPV Test, the LINEAR ARRAY HPV Genotyping Test, and the hc2 test. These 2 cases also stained negatively for p16INK4A. Of the 3 cases, 1 was positive for HPV-82 by the LINEAR ARRAY HPV Genotyping Test, a genotype not considered high risk, nevertheless, this case stained positively for p16INK4A.

Of the 5 such cases that occurred in women 40 years or older, all were confirmed as being HR-HPV– using the AMPLICOR HPV Test, the LINEAR ARRAY HPV Genotyping Test, and the hc2 test. In addition, 3 of the 5 cases occurring in women 40 years or older stained negatively for p16INK4A by immunohistochemical analysis. These findings suggest that a considerable proportion of the apparent decline in sensitivity of HR-HPV testing observed in older women can be attributed to the histologic misclassification of age-related mimics of high-grade CIN.
Discussion

In this analysis, we evaluated the associations of age, HPV genotype, and high-grade cervical neoplasia in women with ASC-US enrolled in the ATHENA trial. ATHENA is a large, multicenter clinical trial that enrolled women undergoing routine cervical cancer screening at 61 clinical centers across the United States. The prevalence of ASC-US declined with increasing age in this screening population; however, this reduction was only modest. Among women 21 to 29 years old, ASC-US was diagnosed in 5.4%, whereas in women 40 years or older, it was diagnosed in 3.5%. More important, the ATHENA results confirm the decrease in prevalence of HR-HPV with increasing age in women with ASC-US that has been observed in other studies conducted in the United States and in Europe.7,9–17 In ATHENA, the prevalence of HR-HPV, detected using the cobas HPV Test, in women 21 to 29 years old with ASC-US was 54.1%, which is similar to the prevalence reported by other recent, large studies that used the hc2 test, including those of Bergeron et al10 from France (53%) and Kendall et al14 and Selvaggi16 from the United States (45% in both). However, it is considerably lower than the 72% prevalence of HR-HPV found by Einstein et al13 when women 21 to 29 years old with ASC-US were tested using the Cervista HPV test. Among women 40 years or older with ASC-US enrolled in ATHENA, the prevalence of HR-HPV showed a dramatic reduction to 16.2%. This is similar to the prevalence of 14% found by Kendall et al,14 who evaluated a similar age group of women with ASC-US using hc2, but again is considerably lower than the 35% prevalence recently reported for Cervista in this age group.13

There are relatively limited data available on how age impacts the prevalence of specific HR-HPV genotypes in women with ASC-US. Therefore, it is of interest that the dramatic reductions observed for overall HR-HPV positivity with increasing age were accompanied by similar reductions in HPV-16 and HPV-18 positivity. The prevalence of HPV-16 dropped from 16.1% in women 21 to 29 years old to 3.0% in women 40 years or older, and the prevalence of HPV-18 dropped from 5.6% to 0.9%. For comparison, Einstein et al13 found, using the Cervista HPV-16 and HPV-18 test, that the prevalence of HPV-16 in women 21 to 29 years old with ASC-US was 24% and was 8% in a group 39 years or older.

As might be expected, the highest prevalence of HR-HPV in women with ASC-US is found in the population that also has the highest prevalence of high-grade cervical disease, and the dramatic decline in HR-HPV prevalence that occurred with age corresponded to a decline of similar magnitude in high-grade cervical disease. In women 21 to 29 years old with ASC-US cytology, the prevalence of CIN 2 or worse was 8.8% and this dropped to 2.3% among women 40 years or older. The 3.8-fold reductions in CIN 2 or worse observed with increasing age in ATHENA are greater than those reported in several other studies. For example, in the study by Einstein et al,13 CIN 2 or worse was found in 6.8% of women 21 to 29 years old with ASC-US compared with 4% in women 39 years or older (1.7-fold reduction). In the study by Bergeron et al,10 CIN 2 or worse was found in 5.2% of women 20 to 29 years old with ASC-US compared with 4.4% of women 41 years or older (1.2-fold reduction). An explanation for why a greater reduction in CIN 2 or worse was observed in the present study than in other studies is not readily apparent but most likely reflects the well-recognized variability in the criteria used by different cytology laboratories and cytopathologists when diagnosing ASC-US.23

The performance of the cobas HPV Test in women with ASC-US has been reported previously and is comparable to the performance of the hc2 test.8 The present analysis demonstrates that the sensitivity of the cobas and hc2 HPV tests declines significantly in women 40 years or older compared with women 21 to 29 years old. This decline in test performance has been suggested in prior studies but has not, to date, been widely recognized by clinicians and laboratories.13,24 Potential explanations for why more HR-HPV– CIN 2 or worse cases are found in women 40 years or older, which results in a reduced sensitivity of HR-HPV DNA testing, include the following: (1) more CIN 2 or worse lesions caused by HPV types not included in the cobas HPV Test or hc2 test in this age group; (2) increased numbers of false-negative HPV results occur; and (3) pathology misclassification of non-CIN lesions as CIN 2 or worse, despite adjudication of the pathology.25

Five women 40 years or older who were negative for HPV DNA by multiple different HPV tests were given a diagnosis by adjudicated pathology of CIN 2 or worse, thereby raising the possibility that these cases may represent non-CIN lesions that were misclassified as CIN 2 or worse. The adjudicated pathology diagnoses were made in a blinded manner by a panel of pathologists who were not aware of a given patient’s age, and it is well recognized that atrophy of the squamous epithelium can histologically mimic dysplastic changes.18 Therefore, all of the HR-HPV– cases were submitted for p16INK4A immunohistochemical staining, a technique shown to distinguish CIN 2 or worse lesions from normal conditions such as atrophy.20,21,26 Of the 5 HR-HPV– CIN 2 or worse cases in women 40 years or older, 3 were p16INK4A negative and, therefore, most likely represented non-CIN lesions that were pathologically misclassified as CIN 2 or worse. However, 2 of the cases stained positively for p16INK4A, indicating that they were most likely CIN 2 or worse lesions and false-negatives for HPV testing. If the 3 apparently misclassified CIN 2 or worse lesions are discounted, the sensitivity of cobas HPV testing in the older age group increases to 83.3%. Moreover, the negative predictive value for CIN 3 or worse remained 99% or more across all
age groups, affirming the safety of using HPV testing to triage older women with ASC-US cytology.

This analysis of associations of age, HPV genotype, and high-grade cervical neoplasia in women with ASC-US demonstrates that the absolute risk of high-grade disease in HPV-16+ women decreases with age, whereas the absolute risk in women positive for the 12 other, non-16/18 HPV genotypes does not. Approximately 1 of 3 HPV-16+ women younger than 40 years with ASC-US had high-grade cervical disease compared with only 15.8% of women 40 years or older. For comparison, the absolute risk of CIN 2 or worse for the 12 other non-16/18 HPV genotypes ranged from 7.8% to 9.8% in the 3 age groups. As a result, the proportion of high-grade cervical disease associated with non–HPV-16 high-risk genotypes increases with age. One possible explanation for the lower risk for HPV-16–associated disease in older women may be that HPV-16–associated lesions develop earlier and are larger than lesions associated with other HR-HPV genotypes, thus making them more likely to have been previously identified and treated.27 It is also well documented that HPV persistence is more likely with HPV-16 than with other HR-HPV genotypes, and it is possible that a longer interval of persistence is necessary to cause significant disease for many high-risk genotypes other than HPV-16.28-31

The younger than 25-year-old population in this study is of particular interest in terms of risk stratification. In this age group, the prevalence of HR-HPV was quite high, 58.7%, and there was a high prevalence of high-grade cervical disease, 9.4%. Among the women in this age group with CIN 3 (no cases of CIN 3 or worse were found in women <25 years), all were positive for HPV-16/HPV-18, thereby emphasizing the high-risk status imparted by HPV-16/HPV-18, even in this young age group. Conversely, infection with non-16/18 HPV would indicate a very low tendency toward having CIN 3 and may support a more conservative approach to management in HR-HPV+ women younger than 25 years who are not HPV-16+/HPV-18+.

The present analysis demonstrates that the prevalence of ASC-US declines with increasing age in a screening population, as do the prevalence of HR-HPV DNA (including HPV genotypes 16 and 18) and the prevalence of high-grade cervical disease. Of note, the prevalence of HR-HPV declines more acutely with age than does the rate of ASC-US cytology and corresponds more closely to the age-dependent decline of disease prevalence. The prevalence of HR-HPV and cervical disease in the 21- to 29-year-old population vs the population 40 years or older declines by a factor of approximately 3, while the rate of ASC-US cytology declines by a factor of approximately 1.5. ASC-US cytology in younger women is more prevalent and more indicative of disease, which is predominantly HPV-16–related. By comparison, in older women, ASC-US cytology is more equivocal because it is less likely to be associated with high-grade cervical disease.

Evaluating ASC-US cytology results in the context of age and HPV genotype status promotes a better refinement of risk assessment and will provide valuable information for clinical management decisions because it allows women with HPV-16 who are at highest risk for high-grade disease to be identified. Age stratification could provide a further refinement of risk for high-grade disease that could be used in future management guidelines based on risk assessment.

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