Effects of Age and Human Papilloma Viral Load on Colposcopy Triage: Data From the Randomized Atypical Squamous Cells of Undetermined Significance/Low-Grade Squamous Intraepithelial Lesion Triage Study (ALTS)

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For the Atypical Squamous Cells of Undetermined Significance/Low-Grade Squamous Intraepithelial Lesion Triage Study Group

Background: Testing for oncogenic human papillomavirus (HPV) DNA at a 1.0-pg/mL threshold represents a promising approach for colposcopy triage of atypical squamous cells of undetermined significance (ASCUS), but not for low-grade squamous intraepithelial lesions (LSIL). Considering age or viral load could improve colposcopy triage. Methods: We determined the sensitivity for detecting Cervical Intraepithelial Neoplasia 3 (CIN3) and cancer and the percentage of referrals for colposcopy using HPV testing and repeat thin-layer cytology in 2198 women with ASCUS and in 848 women with LSIL enrolled in ALTS from November 1996 through December 1998. We analyzed results by age and at two thresholds for HPV load and repeat cytology. Results: For ASCUS, the overall sensitivity of HPV testing at 1.0 pg/mL was 96.1% (95% confidence interval [CI] = 92.8 to 99.5%) and varied minimally with age (range, 93.9% to 97.8%). HPV testing at this threshold would refer 31.2% (95% CI = 28.0% to 34.3%) of women aged 29 years or older as compared with more than 65% of younger women. Among women aged 29 years or older with ASCUS, referral for repeat cytology of ASCUS had a sensitivity of 90.9% (95% CI = 81.1% to 100.0%) and would refer 50.1% (95% CI = 46.7 to 53.5%). Among all ASCUS, HPV testing using a 10.0-pg/mL threshold decreased sensitivity to 91.5% and referrals to 41.7%. More than 63% of LSIL would have been referred using any strategy achieving 90% sensitivity. Conclusion: For women with ASCUS, HPV testing was highly sensitive for detecting CIN3 and cancer with dramatically fewer referrals of older women. Neither a single HPV test nor repeat cytology provides useful triage for women with LSIL. [J Natl Cancer Inst 2002;94:102–7]

Each year, more than 2,000,000 women in the United States receive cervical smear reports of either atypical squamous cells of undetermined significance (ASCUS) or low-grade squamous intraepithelial lesions (LSIL) (1). However, only about 5% of ASCUS and 10% of LSIL reflect an underlying cervical intraepithelial neoplasia 3 (CIN3), an immediate cancer precursor (2). The majority of ASCUS and LSIL represent reactive conditions and self-limited human papillomavirus (HPV) infections that are predominantly associated with HPV types known to cause cancer (“oncogenic” types). Although referring all women with these cytopathologic results for colposcopy might provide the safest management, applying this approach would be costly, impractical, and anxiety provoking for healthy women in many regions of the world. Accordingly, alternative approaches for managing these women, such as repeat cytopathology and HPV DNA testing with optimized techniques, have been proposed.

The U.S. National Cancer Institute, Bethesda, MD, launched a randomized, multicenter trial, the Atypical Squamous Cells of Undetermined Significance/Low-Grade Squamous Intraepithelial Lesion Triage Study (ALTS) to clarify the management of equivocal and mildly abnormal cervical cytology (3). ALTS compares the usefulness of three management strategies: 1) referral for immediate colposcopy, 2) colposcopy referral for repeat cytology interpreted as a high-grade squamous intraepithelial lesion (HSIL) or cancer, and 3) referral for detection of oncogenic HPV DNA at a threshold of 1.0 pg/mL or repeat cytology interpreted as HSIL or cancer. Preliminary enrollment data from ALTS demonstrated that 82.9% of women with LSIL tested positive for oncogenic HPV DNA, suggesting that the high prevalence of HPV infection in these women does not selectively reduce referrals, despite the sensitivity of this approach (4). By contrast, HPV testing for colposcopy triage of women with ASCUS detected 96.3% of the women with CIN3 or cancer and resulted in the referral of only 56.1% of women, which equated to improved sensitivity and a comparable number of referrals compared with a single repeat cytologic test (5).

The clinical usefulness of HPV testing for colposcopy triage depends on multiple factors, including the sensitivity, percentage of women referred, and cost-effectiveness of the procedure compared with the available alternatives. Accordingly, we evaluated whether using age-restricted testing, a threshold for referral higher than 1.0 pg/mL of oncogenic HPV DNA in positive samples (viral load), or considering sample or patient characteristics could reduce referrals without substantially lowering sensitivity. The current study uses enrollment data from ALTS. A cost-effectiveness analysis using 2-years of follow-up data is planned.

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See “Appendix” for affiliations of Atypical Squamous Cells of Undetermined Significance/ Low-Grade Squamous Intraepithelial Lesion Study Group.

See “Notes” following “References.”

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SUBJECTS AND METHODS

Study Population

The study design of ALTS and the characteristics of trial participants are presented elsewhere (3). In total, 3488 eligible women with ASCUS (mean age, 29 years) and 1572 eligible women with LSIL (mean age, 25 years) reported by community laboratories were enrolled. National Cancer Institute, Bethesda, MD, and local institutional review boards approved the study.

Enrollment Procedures

After informed written consent was obtained, a clinician performed a pelvic examination and collected a cervical sample with a Papette™ broom (Wallach Surgical Devices, Inc., Orange, CT) that was rinsed into PreservCyt (Cytyc Corporation, Boxborough, MA) for use in preparing a thin-layer slide and for HPV DNA testing (6,7). Additional cells were collected with a cervical brush from women with cervical stenosis and added to the same PreservCyt vial (Cytyc Corporation). Also, two cervigrams (high-resolution photographs; National Testing Laboratories, Fenton, MO) were obtained (8). Repeat specimens were obtained for cytopathology as rapidly as possible from those patients with unsatisfactory slides who had not already been referred for colposcopy.

HPV Testing

Testing for the DNA of oncogenic HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68 was performed on 4-mL aliquots of the PreservCyt samples using the Hybrid Capture 2 assay (Digene Corporation, Gaithersburg, MD) as described elsewhere (9,10). The results of the HPV test were expressed as relative light units (RLU), which represented the ratio of light emission from a sample to the average of three concurrently tested positive control specimens containing 1 pg/mL of HPV16 DNA. An RLU greater than or equal to 1.0, corresponding to greater than or equal to 5000 HPV DNA copies per test well, was considered to be positive. There was a linear relationship between RLU values for positive specimens and viral copy numbers. Recalculations of RLUs based on HPV16-positive controls of other concentrations did not alter the conclusions and are not presented. Testing was performed at the clinical centers and was monitored by a quality control group, which confirmed the high reliability of the assay. Missing HPV results due to inadequate specimen volume were considered to be positive for referral purposes (5).

Procedures for Colposcopy, Biopsy, and Treatment

Gynecologists biopsied all lesions colposcopically suspicious for CIN or cancer and performed endocervical curettages when indicated. Rarely, patients with repeat thin-layer cytopathology interpreted as HSIL or suspicious colposcopy were referred for repeat colposcopy if histopathologic diagnoses were negative or CIN1. Data from such repeat procedures performed within 1 year of enrollment were considered to reflect prevalent disease and, therefore, were included in this cross-sectional analysis. After colposcopy, women who had received a histopathologic diagnosis of CIN2 or a higher grade lesion or adenocarcinoma in situ were referred for definitive treatment, usually by Loop Electrosurgical Excision Procedure. The most severe histopathologic diagnosis per patient was used for the analysis.

Quality-Control Procedures

Pathology quality-control reviews of cytopathology and histopathology were performed to ensure subject safety and to provide a gold standard for interpretation. Final cytopathologic and histopathologic interpretations of CIN3 and cancer were rapidly communicated to the appropriate clinical centers when indicated to avoid delays in treatment. Safety notifications were also issued for women with pelvic examinations, cervigrams, or digital colposcopic images (Denvu, Tucson, AZ) suspicious for cancer.

Analysis and Statistical Methods

Data for women with ASCUS and LSIL were analyzed separately. Analyses were restricted to the immediate colposcopy and HPV triage arms, in which detection of CIN3 and cancer was similar. We excluded 153 women with missing HPV results, 18 with missing cytopathology results, and four with both results missing, which left 2198 women with ASCUS and 848 with LSIL for analysis. Thus, the analytic dataset differs slightly from that of previous reports (3,5).

This analysis examines the theoretical performance of repeat thin-layer cytopathology and HPV testing (performed at the clinical centers) using two definitions of disease based on histopathologic diagnoses rendered by the pathology quality-control group: 1) CIN3 or cancer and 2) CIN2, CIN3, or cancer. The performance of cytopathology was evaluated at two thresholds for colposcopy referral: 1) ASCUS or a more severe interpretation and 2) LSIL or a more severe interpretation. HPV testing was also assessed at two thresholds for a positive result: 1) low viral load (≥1.0 pg/mL) and high viral load (≥10.0 pg/mL). The theoretical sensitivity for detecting disease and the percentage of women referred for colposcopy based on repeat thin-layer cytopathology and HPV testing were each analyzed at their two respective thresholds after stratifying by age in tertiles. We analyzed viral load separately for women entered into the ASCUS and LSIL arms of the trial. Finally, we explored whether colposcopy triage using repeat cytopathology or HPV testing was affected by oral contraceptive use (within 2 years), smoking status (never, past, or current), history of a sexually transmitted disease other than HPV, lifetime number of sex partners (0 or 1 versus at least 2), history of abnormal cervical smear(s), parity (0, 1, or at least 2), time interval from referral smear to collection of cells for HPV testing (0–41, 42–64, or ≥65 days), and volume of PreservCyt solution required to make the thin-layer cytology slide, which was an indicator of specimen cellularity.

We used standard methods to test for statistical significance, i.e., unpaired chi-square tests for contingency tables and analysis of variance (ANOVA) for differences of multiple means after log normalization by use of SAS version 8 (SAS Institute, Inc., Cary, NC). All statistical tests were two-sided and considered to be statistically significant at P<.05.

RESULTS

Colposcopy Triage of ASCUS Using HPV Testing or Repeat Cytopathology: Performance by Age and Threshold for Referral

The sensitivity of oncogenic HPV DNA testing at 1.0 pg/mL for detecting CIN3 or cancer varied minimally from 93.9% to
97.8% among women of different ages (Table 1). Among women aged 29 years and older, 31.2% (95% confidence interval [CI] = 28.0% to 34.3%) would have been referred for colposcopy, a difference of more than 30% compared with women aged 23–28 years (65.2%; 95% CI = 61.6% to 68.9%) or women aged 18–22 years (71.0%; 95% CI = 67.7% to 74.4%). Among women aged 40 years and older, who comprised 14.5% of all patients with ASCUS, HPV testing at 1.0 pg/mL would have referred 19.5% (95% CI = 15.1% to 23.9%) of patients for colposcopy and detected six of seven CIN3 lesions or cancers. HPV testing at a threshold of 10.0 pg/mL would have decreased the sensitivity for the detection of CIN3 or cancer by 3%–6.5% among women of different age ranges and reduced the percentage of women referred for colposcopy by 8.8%–15.3% (Table 1). Among women aged 40 years and older, who comprised 14.5% of all patients with ASCUS, HPV testing at a higher threshold of 10.0 pg/mL would have been less sensitive but still referred 62.7%–77.6% of women in different age ranges. Colposcopy referral based on a repeat cytopathologic interpretation of ASCUS or worse would have detected 89.2%–91.7% of women with CIN3 or cancer. The percentage of projected referrals based on a repeat cytopathologic interpretation of ASCUS or worse was greater than 77.0% for each age group and 80.4% (95% CI = 77.8% to 83.1%) for all women. The sensitivity for detecting CIN3 or cancer based on referral for a repeat cytopathologic interpretation of LSIL or worse ranged from 60.0% to 72.7%, with the percentage of women referred ranging from 17.4% to 33.2% in different age groups.

Results for repeat cytology and HPV triage in identifying women with underlying CIN2 or higher grade lesions were similar to those for detecting CIN3 or cancer (data not shown).

### Colposcopy Triage of LSIL Using HPV Testing or Repeat Cytopathology: Performance by Age and Threshold for Referral

Overall, HPV testing of women with LSIL at a cutpoint of 1.0 pg/mL would have identified 96.7% (95% CI = 93.0% to 100.0%) of women with underlying CIN3 or cancer but required referral of 84.8% (95% CI = 82.4% to 87.2%) of patients. Referrals would have only declined slightly among the oldest women (Table 2). Colposcopy referral based on HPV testing at a higher threshold of 10.0 pg/mL would have been less sensitive but still referred 62.7%–77.6% of women in different age ranges.

### Table 1. Performance of oncogenic HPV DNA testing and repeat thin-layer cytology in colposcopy triage of ASCUS stratified by age*

<table>
<thead>
<tr>
<th>Triage strategy</th>
<th>Sensitivity for detection of ≥CIN3, % (95% CI)</th>
<th>Women referred for colposcopy, % (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age 18–22 y (46 CIN3s)† HPV testing: 1.0 pg/mL</td>
<td>97.8 (93.6 to 100.0)</td>
<td>71.0 (67.7 to 74.4)</td>
</tr>
<tr>
<td>HPV testing: 10.0 pg/mL</td>
<td>91.3 (83.2 to 99.4)</td>
<td>57.3 (53.7 to 61.0)</td>
</tr>
<tr>
<td>Repeat cytology: ASCUS</td>
<td>80.4 (69.0 to 91.9)</td>
<td>65.6 (62.1 to 69.1)</td>
</tr>
<tr>
<td>Repeat cytology: LSIL</td>
<td>60.9 (46.8 to 75.0)</td>
<td>33.2 (29.8 to 36.7)</td>
</tr>
<tr>
<td>Age 23–28 y (50 CIN3s) HPV testing: 1.0 pg/mL</td>
<td>96.0 (90.6 to 100.0)</td>
<td>65.2 (61.6 to 68.9)</td>
</tr>
<tr>
<td>HPV testing: 10.0 pg/mL</td>
<td>92.0 (84.5 to 99.5)</td>
<td>49.9 (46.1 to 53.8)</td>
</tr>
<tr>
<td>Repeat cytology: ASCUS</td>
<td>88.0 (79.0 to 97.0)</td>
<td>63.9 (60.2 to 67.5)</td>
</tr>
<tr>
<td>Repeat cytology: LSIL</td>
<td>60.0 (46.4 to 73.6)</td>
<td>30.0 (26.5 to 33.5)</td>
</tr>
<tr>
<td>Age ≥29 y (33 CIN3s) HPV testing: 1.0 pg/mL</td>
<td>93.9 (85.8 to 100.0)</td>
<td>31.2 (28.0 to 34.3)</td>
</tr>
<tr>
<td>HPV testing: 10.0 pg/mL</td>
<td>90.9 (81.1 to 100.0)</td>
<td>22.4 (19.6 to 25.2)</td>
</tr>
<tr>
<td>Repeat cytology: ASCUS</td>
<td>90.9 (81.1 to 100.0)</td>
<td>50.1 (46.7 to 53.5)</td>
</tr>
<tr>
<td>Repeat cytology: LSIL</td>
<td>72.7 (57.5 to 87.9)</td>
<td>17.4 (14.9 to 20.0)</td>
</tr>
<tr>
<td>All ages (129 CIN3s) HPV testing: 1.0 pg/mL</td>
<td>96.1 (92.8 to 99.5)</td>
<td>54.0 (51.9 to 56.1)</td>
</tr>
<tr>
<td>HPV testing: 10.0 pg/mL</td>
<td>91.5 (86.7 to 96.3)</td>
<td>41.7 (39.7 to 43.8)</td>
</tr>
<tr>
<td>Repeat cytology: ASCUS</td>
<td>86.0 (80.1 to 92.0)</td>
<td>59.1 (57.1 to 61.2)</td>
</tr>
<tr>
<td>Repeat cytology: LSIL</td>
<td>63.6 (55.3 to 71.9)</td>
<td>26.2 (24.4 to 28.0)</td>
</tr>
</tbody>
</table>

*HPV = human papillomavirus; ASCUS = atypical squamous cells of undetermined significance; CIN = cervical intraepithelial neoplasia; ≥CIN3 = CIN3, adenocarcinoma in situ, or invasive carcinoma; CI = confidence interval; LSIL = low-grade squamous intraepithelial lesion; >ASCUS = ASCUS or a more severe interpretation; >LSIL = LSIL or a more severe interpretation.†Number of women with a histopathologic diagnosis of CIN3 or cancer is shown.

### Table 2. Performance of oncogenic HPV DNA testing and repeat thin-layer cytology in colposcopy triage of LSIL stratified by age*

<table>
<thead>
<tr>
<th>Triage strategy</th>
<th>Sensitivity for detection of ≥CIN3, % (95% CI)</th>
<th>Women referred for colposcopy, % (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age 18–22 y (37 CIN3s) HPV testing: 1.0 pg/mL</td>
<td>100.0 (100.0 to 100.0)</td>
<td>86.7 (83.3 to 90.1)</td>
</tr>
<tr>
<td>HPV testing: 10.0 pg/mL</td>
<td>89.2 (79.2 to 99.2)</td>
<td>74.9 (70.6 to 79.3)</td>
</tr>
<tr>
<td>Repeat cytology: ASCUS</td>
<td>89.2 (79.2 to 99.2)</td>
<td>81.2 (77.3 to 85.1)</td>
</tr>
<tr>
<td>Repeat cytology: LSIL</td>
<td>68.9 (49.8 to 88.2)</td>
<td>57.7 (52.8 to 62.7)</td>
</tr>
<tr>
<td>Age 23–28 y (42 CIN3s) HPV testing: 1.0 pg/mL</td>
<td>97.6 (93.0 to 100.0)</td>
<td>88.0 (84.3 to 91.6)</td>
</tr>
<tr>
<td>HPV testing: 10.0 pg/mL</td>
<td>92.9 (85.1 to 100.0)</td>
<td>77.6 (72.9 to 82.3)</td>
</tr>
<tr>
<td>Repeat cytology: ASCUS</td>
<td>90.5 (81.6 to 99.4)</td>
<td>80.9 (76.5 to 85.4)</td>
</tr>
<tr>
<td>Repeat cytology: LSIL</td>
<td>90.5 (81.6 to 99.4)</td>
<td>63.2 (57.7 to 68.7)</td>
</tr>
<tr>
<td>Age ≥29 y (12 CIN3s) HPV testing: 1.0 pg/mL</td>
<td>83.3 (62.2 to 100.0)</td>
<td>74.7 (68.1 to 91.3)</td>
</tr>
<tr>
<td>HPV testing: 10.0 pg/mL</td>
<td>58.3 (30.4 to 86.2)</td>
<td>62.7 (55.3 to 70.0)</td>
</tr>
<tr>
<td>Repeat cytology: ASCUS</td>
<td>91.7 (76.0 to 100.0)</td>
<td>77.7 (71.4 to 84.0)</td>
</tr>
<tr>
<td>Repeat cytology: LSIL</td>
<td>83.3 (62.2 to 100.0)</td>
<td>49.4 (41.8 to 57.0)</td>
</tr>
<tr>
<td>All ages (91 CIN3s) HPV testing: 1.0 pg/mL</td>
<td>96.7 (93.0 to 100.0)</td>
<td>84.8 (82.4 to 87.2)</td>
</tr>
<tr>
<td>HPV testing: 10.0 pg/mL</td>
<td>86.8 (79.9 to 93.8)</td>
<td>73.5 (70.5 to 76.4)</td>
</tr>
<tr>
<td>Repeat cytology: ASCUS</td>
<td>90.1 (84.0 to 96.2)</td>
<td>80.4 (77.8 to 83.1)</td>
</tr>
<tr>
<td>Repeat cytology: LSIL</td>
<td>79.1 (70.8 to 87.5)</td>
<td>58.0 (54.7 to 61.3)</td>
</tr>
</tbody>
</table>

*HPV = human papillomavirus; ASCUS = atypical squamous cells of undetermined significance; CIN = cervical intraepithelial neoplasia; ≥CIN3 = CIN3, adenocarcinoma in situ, or invasive carcinoma; CI = confidence interval; LSIL = low-grade squamous intraepithelial lesion; >ASCUS = ASCUS or a more severe interpretation; >LSIL = LSIL or a more severe interpretation.†Number of women with a histopathologic diagnosis of CIN3 or cancer is shown.
detecting CIN2 or higher grade lesions was similar to that for CIN3 or cancer (data not shown). Among women aged 29 years or older with LSIL, HPV testing performed at a cutoff of 1.0 pg/mL would have detected 93.8% of CIN2 or higher grade lesions and HPV testing at 10.0 pg/mL would have detected 75.0% of CIN2 or higher grade lesions (data not shown). Therefore, higher sensitivity was achieved for this disease definition than for the small set of CIN3 or cancers in this group.

Viral Load by Enrollment Status and Histopathologic Diagnoses

Women with histopathologically confirmed CIN had higher viral loads than women with negative histopathology or colposcopy (ANOVA, P<.001), but load did not increase with the grade of CIN (Fig. 1). In fact, among women with histopathologically confirmed CIN, the viral load tended to decline with the severity of the final diagnosis for women referred with ASCUS (P = .11) or LSIL (P = .02). The conclusions were not changed by age stratification.

Effect of Patient and Sample Characteristics on HPV Testing

Among women with ASCUS, HPV testing performed using a cutoff of 1.0 pg/mL was positive in 59.0% (95% CI = 55.4% to 62.6%) of patients when the test was performed on a sample collected within 41 days of the cytologic smear, in 53.2% (95% CI = 49.6% to 56.8%) when the test was performed on a sample collected between 42 and 64 days, and in 49.9% (95% CI = 46.3% to 53.5%) when the test was performed on a sample collected after 64 days (P for trend <.001). Among women with LSIL, the differences in HPV detection varied minimally for longer intervals between the time of collection for the initial smear and that of the HPV test sample. There was no effect of any of the other patient characteristics on the performance of viral load measurements after age stratification.

Discussion

Colposcopy triage of women with ASCUS using oncogenic HPV DNA testing at a threshold of 1.0 pg/mL was associated with a dramatic reduction in referrals among women aged 29 years and older compared with younger women, without a substantial reduction in sensitivity. Among women aged 29 years and older, HPV testing at a threshold of 1.0 pg/mL was positive in 93.9% of women with CIN3 or cancer and would have led to the referral of only 31.2% of these patients for colposcopy. Thus, the absolute number of women who would have been referred for colposcopy was reduced by more than 30% compared with HPV testing in younger women. Among women aged 29 years and older in the ASCUS arm of the trial, the sensitivity of repeat cytology of ASCUS or worse for detecting CIN3 or cancer was 90.9%, with a projected referral of 50.1% of these women for colposcopy. Thus, the findings for repeat cytology demonstrated a parallel but less dramatic decrease in the percentage of referrals with increasing age as was seen for HPV testing. HPV testing was statistically significantly more sensitive than cytology in women overall and especially among the youngest women. Among women with ASCUS, the sensitivity of HPV testing for detecting CIN2 or worse was nearly identical to that for CIN3 and cancer. Similarly, the sensitivity of repeat cytology did not vary substantially for the two disease definitions.

The striking reduction in referrals for oncogenic HPV DNA testing among older women with ASCUS is consistent with previous studies (11–13), demonstrating that the prevalence of HPV infection declines with age. The reduced referrals with HPV testing in older women may be important in assessing the cost-effectiveness of HPV triage and developing management recommendations (14,15). In addition, the increase in the frequency of persistent HPV infections with age (16) suggests that additional follow-up of older women who test positive for HPV but do not have prevalent CIN2 or CIN3 may be more cost-effective than in younger women.

Compared with testing at a threshold of 1.0 pg/mL, colposcopy triage of ASCUS using HPV testing at a threshold of 10.0 pg/mL reduced the percentage of referrals a total of 12.3% and detected 91.5% of CIN3 lesions or cancers. However, the slight decrease in sensitivity compared with HPV testing at 1.0 pg/mL might be unacceptable if maximal detection of CIN3 is paramount. The repeat cytology of LSIL or worse for colposcopy triage of ASCUS was insensitive, especially in the youngest women.

In contrast with ASCUS, we did not identify a promising strategy for colposcopy triage of LSIL in any age range. Approaches that achieved a sensitivity of 90% required a referral of approximately 63% of women or more. In contrast with previous results (17), the percentage of women with positive HPV tests did not decline dramatically with age among subjects enrolled with LSIL. The improved accuracy of this cytopathologic inter-
preparation limited the potential role of HPV testing for colposcopy triage.

Studies using a wide range of designs, laboratory assays, and analytical methods have suggested that higher viral loads, especially for HPV16, may be associated with prevalent CIN2 or CIN3 (18–25) or with the progression of HPV infection to CIN3 over time (26,27). Nonetheless, the clinical usefulness of measuring viral load has been debated. In ALTS, higher viral load values were associated with histopathologically confirmed disease, but the values overlapped considerably among grades of CIN and did not increase with severity of disease. We specifically restricted our analysis to women with a positive HPV test because our goal was to determine whether higher viral load would separate HPV-positive women with ASCUS harboring an underlying CIN2 or CIN3 from those women with CIN1 or lower grade lesions. Most analyses of viral load, including ours, did not adjust for specimen cellularity, number of atypical cells, multiple infections with different HPV types, or numerous other factors that could affect the result. In an analysis that corrected load measurements of HPV types 16, 18, 31, and 45 for cellular DNA (25), only the HPV16 load was associated with disease severity, and the authors concluded that the wide range of values limited clinical utility. Our results suggest that viral load as measured in this study may actually decrease with the lesion grade, but the great overlap would rule out clinical use.

Features related to sample quality or patient history produced minor effects on viral load and did not seem to improve the performance of HPV testing for colposcopy triage. The percentage of HPV-positive samples was approximately 9% lower for women with the longest time interval between the initial cytopathologic interpretation of ASCUS and collection of the viral test samples as compared with women with the shortest time interval. Therefore, our results may have differed slightly had testing been performed with specimens collected on the same day that the cytology was performed. Moreover, enrollment in ALTS was based on cytopathologic interpretations of smears rendered in the community, not thin-layer cytology. Data suggest that thin-layer cytology may be more sensitive in detecting CIN2 or higher grade lesions (28,29).

ALTS provides unique data related to colposcopy triage because of its large size, broad representation of the U.S. population, and quality-control components. Accordingly, findings in ALTS should apply to most U.S. screening populations in which cytopathology is performed with standard diagnostic criteria. Women in ALTS are followed for 2 years, with full evaluation for CIN at study exit. Cost-effectiveness analyses of triage methods in ALTS are planned.

APPENDIX

The affiliations of the ALTS (i.e., the Atypical Squamous Cells of Undetermined Significance/Low-Grade Squamous Intraepithelial Lesions Triage Study) Group are as follows:

National Cancer Institute, Bethesda, MD: D. Solomon, Project Officer; M. Schiffman, Co-Project Officer; and R. Tarone, Statistician.

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Clinical Center, University of Oklahoma, Oklahoma City: J. L. Walker, Principal Investigator; G. A. Johnson, Co-Principal Investigator; and A. Yadack, Study Manager.

Clinical Center, Magee-Womens Hospital of the University of Pittsburgh Medical Center Health System, PA: R. S. Guido, Principal Investigator; K. McIntyre-Seltman, Co-Principal Investigator; R. P. Edwards, Investigator; and J. Gruss, Study Manager.

Clinical Center, University of Washington, Seattle: N. B. Kiviat, Co-Principal Investigator; L. Koutsky, Co-Principal Investigator; C. Mao, Investigator; and J. M. Haag, Study Manager.

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HPV Quality Control Group: C. M. Wheeler, Principal Investigator, University of New Mexico Health Sciences Center, Albuquerque; C. Peyton-Goodall, Laboratory Manager, University of New Mexico Health Sciences Center; and M. M. Manos, Co-Investigator, Kaiser Permanente, Oakland, CA.

Pathology Quality Control Group: R. J. Kurman, Principal Investigator, The Johns Hopkins Hospital, Baltimore, MD; D. L. Rosenthal, Co-Investigator; The Johns Hopkins Hospital; M. E. Sherman, Co-Investigator, National Cancer Institute; and M. H. Stoler, Co-Investigator, University of Virginia Health Science Center, Charlottesville.

Cost Utility Analysis Group: D. M. Harper, Investigator, Dartmouth Hitchcock Medical Center, Lebanon, NH.

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NOTES

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