Postcolposcopy management strategies for women referred with low-grade squamous intraepithelial lesions or human papillomavirus DNA–positive atypical squamous cells of undetermined significance: A two-year prospective study

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OBJECTIVE: This study was undertaken to compare postcolposcopy management strategies for women referred for low-grade squamous intraepithelial lesions (LSIL) or oncogenic human papillomavirus (HPV) DNA–positive atypical squamous cells of undetermined significance (ASCUS), with cervical intraepithelial neoplasia (CIN) grade 1 or less found at initial colposcopy.

STUDY DESIGN: A 2-year prospective follow-up of 1539 women was designed to assess the percentage sensitivity of different postcolposcopy management strategies to detect subsequent CIN grade 2 or 3 and percentage referral to repeat colposcopy.

RESULTS: HPV testing at 12 months was sensitive (92.2%) for detection of CIN grade 2 or 3 with a referral rate to repeat colposcopy of 55.0%. Repeat semiannual cytology with referral to colposcopy at an ASCUS threshold demonstrated similar sensitivity (88.0%) but with a higher rate of referral to colposcopy (63.6%). Combining cytology and HPV testing did not increase sensitivity and hurt specificity. Baseline viral load and colposcopic impression were not helpful.

CONCLUSION: The most efficient test for identifying women with CIN grade 2 or 3 after colposcopy might be an HPV test alone at 12 months. (Am J Obstet Gynecol 2003;188:1401-5.)

Key words: Cervical intraepithelial neoplasia grade 1, cervix, clinical management, colposcopy, cytology, human papillomavirus

The ASCUS-LSIL Triage Study (ALTS) was designed to address the initial management of mildly abnormal Papanicolaou (Pap) tests. The results demonstrate that Pap tests showing atypical squamous cells of undetermined significance (ASCUS) can be safely triaged using human papillomavirus (HPV) DNA testing, with approximately half of women who are HPV negative not requiring colposcopy.[1,2] Low-grade squamous intraepithelial lesion (LSIL) Pap tests cannot be efficiently triaged because too few are HPV negative to make testing worthwhile.[3,4]

On the basis of these results and other studies,[5] experts participating in a multidisciplinary consensus conference sponsored by the American Society for Colposcopy and Cervical Pathology recommended that an HPV triage test is the preferred management for women with ASCUS when it can be performed conveniently with cytology, whereas women with LSIL should be referred for colposcopy.[6] If the recommendations are adopted throughout the United States, the resultant number of colposcopy examinations will be evidence-based but remain predictably high. About half of the 2.5 million patients with ASCUS Pap tests and all 1 million women with LSIL will be referred for colposcopy, resulting in more than 2 million colposcopy examinations per year. LSIL and HPV positive ASCUS can be viewed as clinically

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**A complete list of ALTS investigators begins on page 1411.*

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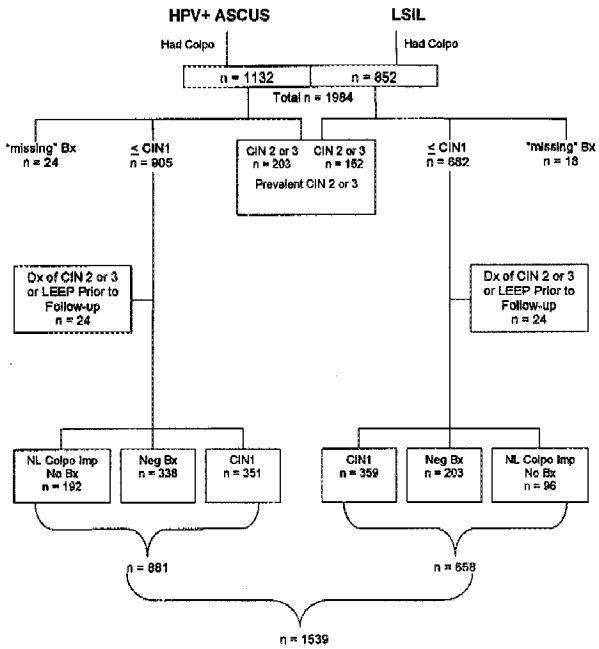

Figure. CONSORT diagram showing composition of study population. Women with LSIL or HPV+ ASCUS who were randomly assigned to the IC or HPV triage study arms and had colposcopy at enrollment are shown in the top boxes. (Women in the CM arm are not included in this analysis because of underreferral to colposcopy at enrollment.) Women with obviously prevalent CIN grade 2 or 3 detected at colposcopy and those missing a biopsy result when the colposcopic impression was considered abnormal are excluded. Women with CIN grade 1 or less diagnosed at the enrollment colposcopy, who were not censored before follow-up visits, constitute the postcolposcopy study population.

equivalent, based on a similar risk of cervical intraepithelial neoplasia (CIN) grade 2 or 3 diagnosed within 2 years (approximately 25%).[7]

Among those women who are referred, approximately 15% to 20% will have *immediately* evident histologically confirmed CIN grade 2 or 3 and will be treated in most instances.[7] Because of imperfect sensitivity, the initial colposcopy and directed biopsy will not detect a sizable fraction of cases of CIN grade 2 or 3. Among the remaining 80% to 85% of women who were categorized initially as having CIN grade 1 or less, about 10% will have histologically confirmed CIN grade 2 or 3 *subsequently* diagnosed within 2 years of follow-up. Moreover, within this group initially categorized as having ≤ CIN grade 1, women with a normal initial colposcopy or a benign histologic diagnosis are at approximately the same risk as women with a CIN grade 1 histologic diagnosis.[7] Cervical histology at initial colposcopy therefore does not provide a clinically useful distinction on which to base further management. Overall, the great majority of women referred to colposcopy still do not develop CIN grade 2 or 3 and very few will develop cancer. It will be critical to optimize postcolposcopy management to detect initially missed CIN grade 2 or 3 while avoiding overtreatment of the many women referred. This article provides estimates of the usefulness of various management options after colposcopy using ALTS data.

Material and methods

Subjects. The ALTS trial and its population are described more completely elsewhere.[2,8] Only details specific to this analysis are presented here.

To simulate a triage strategy of referring women with ASCUS cytology who are HPV positive and all women with LSIL cytology to colposcopy, a subset of the ALTS trial population was included as outlined in the Figure. In total, 1132 women with ASCUS who were oncogenic HPV DNA positive (HPV+ ASCUS) and 852 women referred for LSIL in the immediate colposcopy (IC) and HPV triage study arms underwent enrollment colposcopy. (The CM arm was excluded because of substantial underdetection of disease at enrollment.) Of these 1984 women, 42 (24 HPV+ ASCUS and 18 LSIL) had an abnormal colposcopic impression but did not undergo biopsy and were excluded. We also excluded 355 women (203 HPV+ ASCUS and 152 LSIL) with prevalent histologic CIN grade 2 or 3, diagnosed by the clinical center pathologists. Of the remaining 905 HPV+ ASCUS and 682 LSIL women who received a diagnosis of CIN grade 1 or less on the first enrollment colposcopy, 48 were excluded because either (1) CIN grade 2 or 3 was diagnosed *subsequent* to the first colposcopically directed biopsy but *before* the first follow-up visit or (2) loop electrosurgical excision procedure (LEEP) was performed before the first follow-up visit. The study population for this analysis therefore consists of 881 women with HPV+ ASCUS and 658 with LSIL, all found to have CIN grade 1 or less at initial colposcopy (total n = 1539).

The population described underwent routine follow-up examinations at 6, 12, and 18 months. At the follow-up visits, participants had a pelvic examination similar to that at enrollment, a cervical cell collection for the preparation of a ThinPrep cytology (Cytyc, Boxborough, Mass) masked HPV testing (HC 2, Digene Corporation, Gaithersburg, Md), and two replicate masked Cervigrams (National Testing Laboratories Worldwide, Fenton, Mo). A cytology result of high-grade squamous intraepithelial lesion (HSIL) triggered re-referral to colposcopy during follow-up. All participants in the trial underwent an exit visit at the 24-month period that included colposcopy. At this visit alone, all the available clinical center and Pathology Quality Control (QC) group cytology and histology, HPV results from the previous visits, as well as the last Cervigram (in the form of a photograph) were available to the clinician performing the colposcopy. Details of patient management and test procedures are found elsewhere.[8]

A Pathology QC group reviewed cytology and histology specimens for purposes of disease definition and to pro-

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Table I. Distribution of enrollment colposcopy and directed biopsy results by referral cytology group for study population and risk for subsequently diagnosed CIN grade 2 or 3

Enrollment colposcopy and directed biopsy result	*ASCUS*	*LSIL*	*All*	*Risk of subsequently diagnosed CIN grade 2 or 3 (No. [%])**
Normal colposcopic impression, no biopsy	192	96	288	30 (10.4%)
Negative biopsy	338	203	541	53 (9.8%)
CIN grade 1	351	359	710	80 (11.3%)
Total	881	658	1539	163 (10.6%)

$P = .70$ comparing percentage diagnosed with CIN grade 2 or 3 in three groups.

*On the basis of the initial enrollment colposcopy and biopsy result, risk for subsequently diagnosed CIN grade 2 or 3 (detected either during the 2-year follow-up or at exit colposcopy) is shown.

vide a safety net for study participants.[2] However, unless there was a safety net trigger, clinical management was based on the reading by the clinical center pathologist. The quality control of HPV testing and colposcopy in ALTS is detailed elsewhere.[2,8]

Histologic end points. As in the companion article,[7] the clinical end point used to define the performance of the various potential postcolposcopy tests was a clinical center histologic diagnosis of CIN grade 2 or 3 (including only one case of invasive cancer). Similar conclusions were obtained with the use of a diagnosis of CIN grade 3 by the Pathology QC group; however, those results are not shown for simplicity of presentation.

Statistics. We used standard contingency table methods for simple comparisons, such as the sensitivity and percentage referral of different possible postcolposcopy management strategies for detection of CIN grade 2 or 3. To adjust the denominators of sensitivity and referral percentages, women were censored at the visit yielding a histologic diagnosis of CIN grade 2 or 3 or a LEEP. We accounted for repeated cytology testing and censoring, including missing values because of missed appointments, by standard adjustment for joint probabilities using Kaplan-Meier methods.

Results

The 1539 patients who comprised the study population had a mean age of 25.2 years (SD = 6.8, median = 23, range = 18-71). The self-described racial/ethnic composition of this population was 57.0% white, 36.5% African American, and 6.5% other groups. In a separate question, 4.6% of the population described themselves as Hispanic. The women included in this analysis were mainly HPV positive but without CIN grade 2 or 3 at initial colposcopy. As a correlate of having a sexually transmitted infection, they tended to be younger than women in ALTS who were excluded from the analysis. They also were slightly more likely to have used oral contraceptives and to be African American, but reported slightly fewer live births and smoked less than the excluded women.

Table I summarizes the study population that excludes those with a diagnosis of CIN grade 2 or 3 at the initial

Table II. Performance of baseline HPV viral load assessment in postcolposcopy management of 881 women with CIN grade 1 or less*

Viral load threshold (pg/mL)	*Sensitivity of detection of subsequent CIN grade 2 or 3 (%) (n = 90)*	*Women who would be positive (%)*
1000	21.1	13.2
100	57.8	48.0
10	80.0	74.7
5	85.6	81.5
1	100.0	100.0

*Restricted to 881 women referred for HPV DNA-positive ASCUS because of recommendation against HPV testing of women with cytologic LSIL.

Table III. Performance of baseline colposcopic assessment in postcolposcopy management of 1535* women with CIN grade 1 or less

Enrollment clinical center colposcopic assessment	*Sensitivity of detection of subsequent ≥CIN grade 2 (%) (n = 163)*	*Women who would be positive (%)*
High grade or cancer	12.3	7.2
Low grade	74.2	70.2

*Excludes four women with inadequate or missing colposcopic assessments.

Table IV. Performance of repeat cytology in postcolposcopy management of women with CIN grade 1 or less

Management strategy	*Sensitivity of detection of subsequent CIN grade 2 or 3 (% [95% CI])*	*Women who would be positive (% [95% CI])*
Repeat cytology at HSIL threshold		
1	23.9 (17.4-30.5)	4.7 (3.6-5.8)
2	37.5 (29.9-45.1)	7.2 (5.8-8.5)
3	44.9 (36.9-52.8)	8.3 (6.8-9.8)
Repeat cytology at LSIL threshold		
1	49.1 (41.4-56.8)	25.2 (22.9-27.4)
2	70.5 (63.3-77.7)	34.6 (32.1-37.1)
3	77.2 (70.4-83.9)	38.3 (35.7-40.9)
Repeat cytology at ASCUS threshold		
1	76.7 (70.2-83.2)	51.7 (49.1-54.3)
2	88.0 (82.9-93.1)	63.6 (61.1-66.1)
3	95.1 (91.6-98.6)	70.0 (67.5-72.5)

Each cytology threshold reflects the finding of a cytologic abnormality greater than or equal to the cut point when cytology is performed one, two, or three times at approximately 6-month intervals.

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Table V. Performance of HPV DNA testing and cytology at 6 months or 12 months in postcolposcopy management of women with CIN grade 1 or less

Management strategy	*Sensitivity of detection of subsequent CIN grade 2 or 3 (% [95% CI])*	*Women who would be positive (% [95% CI])*
At 6 mo		
HPV DNA testing	90.9 (85.0-95.1)	62.4 (59.6-65.1)
HPV DNA testing and cytology at ASCUS threshold	93.7 (88.4-97.1)	72.4 (69.8-74.9)
At 12 mo		
HPV DNA testing	92.2 (85.7-96.4)	55.0 (51.9-57.9)
HPV DNA testing and cytology at ASCUS threshold	94.8 (89.0-98.1)	64.1 (61.2-67.0)

biopsy. Overall, 829 (53.9%) of the women had either a normal colposcopic impression with no biopsy or had a negative biopsy result, whereas 710 (46.1%) had a histologic result of CIN grade 1. The risk of subsequent detection of CIN grade 2 or 3 over the follow-up period was similar between those with less than CIN grade 1 and those with CIN grade 1 at initial colposcopy (10.0% vs 11.3%, respectively).

The ability to determine risk for CIN grade 2 or 3 over a 2-year period with a single diagnostic test at the time of initial patient contact would have clinical value. We evaluated the potential role of HPV viral load as an indicator for risk. Women with LSIL cytology were not included in the analysis because HPV testing is not recommended in this population of patients. Table II summarizes the sensitivity and percentage of HPV-positive women at various viral load levels at enrollment in ALTS. To have a high sensitivity for the detection of subsequent disease (80%), a low viral load threshold is required, 10 pg/mL, which would classify 74.7% of the study group as positive. Increasing the viral load threshold to 100 pg/mL resulted in a parallel reduction in referral percentage and sensitivity.

Table III summarizes the performance characteristics of the colposcopic impression at enrollment in identifying a group at especially elevated risk for CIN grade 2 or 3. Like viral load, colposcopic impression did not discriminate women at particularly high risk. To use enrollment colposcopic impression for patient management would require a very low threshold (low-grade impression) to have a sensitivity of 74.2% of detecting subsequent CIN grade 2 or 3 while identifying 70.2% of patients as requiring special attention.

Table IV details the performance of repeated cytologic evaluations by using HSIL, LSIL, and ASCUS cytology thresholds. Each threshold reflects the finding of a cytologic abnormality equal to or greater than the threshold at any visit during follow-up when the test was performed one, two, or three times at approximately 6-month intervals. Repeated cytology screening with an ASCUS threshold was possibly useful, with sensitivities of 88.0% and 95.1% for two or three tests, respectively, and rates of referral of 63.6% and 70.0%. Table V summarizes the performance of HPV testing alone, and in combination with cytologic evaluation at the ASCUS threshold. The clinical sensitivity of HPV testing alone at either 6 or 12 months (90.9% and 92.2%, respectively) was higher than a single cytology. Adding cytology to HPV testing, at either 6 or 12 months, only marginally and nonsignificantly improved the sensitivity of the testing while significantly increasing the percentage of referrals to colposcopy. A single HPV test performed at 12 months had high sensitivity while referring significantly fewer patients back to colposcopy (55.0%) than any comparably sensitive strategy.

Comment

Results from ALTS and other studies have demonstrated that we now know how to identify many women with ASCUS who do not need colposcopy. However, the number of women with oncogenic HPV DNA+ ASCUS or with LSIL presents a sizable management challenge. In addition, initial colposcopy and directed biopsy are not as sensitive as we had previously assumed,[2,4,7] corroborating the findings of other recent investigations.[9] Therefore, strategies to follow women after colposcopy, to identify missed prevalent disease, are needed. By using the ALTS longitudinal data, we analyzed the clinical utility of a variety of follow-up strategies for women referred with LSIL and HPV+ ASCUS and found to have CIN grade 1 or less at initial colposcopy.

Test strategies, including HPV DNA testing, cytology at various thresholds, and initial colposcopic impression, were evaluated for their sensitivity for the detection of subsequently diagnosed CIN grade 2 or 3 and referral rate for repeat colposcopic examination. Although it would be of great clinical benefit to identify a single diagnostic test performed at the initial patient contact that could predict those individuals destined to be found with CIN grade 2 or 3, none performed optimally. For postcolposcopy management, reliance on initial viral load or initial colposcopic impression required an excessively high rate of repeated colposcopic examinations to achieve adequate sensitivity.

An HPV test at 12 months was the single test with the highest sensitivity and lowest referral to repeat col-

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poscopy. Although HPV testing at the 6-month follow-up examination was equally sensitive, it resulted in 13% more patients re-referred to colposcopy (62.4% vs 55.0%). This result is not surprising considering the known regression rate of HPV infection over time.[10]

Combining HPV and cytology testing was also considered. The addition of a cytologic examination at the time of the HPV test only marginally increased the sensitivity for the detection of CIN grade 2 or 3 with a statistically significant increase in the percent referral for repeat colposcopy. Three repeat cytologic examinations (without HPV testing) also provided only a marginal, nonsignificant increase in sensitivity (95%) while referring a much higher percentage of patients to colposcopy, and requiring multiple office visits.

These results suggest that either a single HPV test at 12 months, or repeat cytologic examinations at a threshold of ASCUS would represent reasonable alternative follow-up strategies for women with CIN grade 1 or less at colposcopy and directed biopsy. The issue of patient preference, convenience, quality of life, and cost for each one of these follow-up strategies is still to be determined. Clinicians who choose to use HPV testing at 12 months should consider whether to include concomitant cytology. Our data clearly demonstrate that combining cytology and HPV testing at 12 months would not significantly increase the sensitivity of detecting of CIN grade 2 or 3 compared with HPV testing alone, although greatly increasing the number of repeat colposcopic examinations. Performing an HPV test alone, without cytology, would represent a significant shift in clinical practice.

The strength of these supporting data is based on the size and prospective nature of ALTS. However, ALTS was not specifically designed to address postcolposcopy triage strategies. Limitations of this analysis include the exclusion of a relatively small number of women found to have CIN grade 2 or 3 based on QC intervention and/or reevaluation by the clinical centers, subsequent to the initial colposcopy but before follow-up examinations. This additional censoring resulted in a cohort of women CIN grade 1 or less at slightly less risk of subsequent CIN grade 2 or 3 compared with such women outside the realm of a clinical trial with QC safety reviews. Only a randomized prospective comparison of the most favorable follow-up techniques can optimally define the strategy with the best cost and quality-of-life profile.

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