A randomized trial on the management of low-grade squamous intraepithelial lesion cytology interpretations

The ASCUS-LSIL Triage Study (ALTS) Group*

OBJECTIVE: This study was undertaken to compare alternative strategies for the initial management of low-grade squamous intraepithelial lesion (LSIL) cytology.

STUDY DESIGN: A total of 1572 women with a community-based LSIL interpretation were randomly assigned to immediate colposcopy, triage based on enrollment HPV DNA testing and liquid-based cytology at a colposcopy referral threshold of high-grade squamous intraepithelial lesion (HSIL), or conservative management based on repeat cytology at a referral threshold of HSIL. All arms included 2 years of semiannual follow-up and colposcopy at exit. Loop electrosurgical excision procedure was offered to women with histologic diagnoses of cervical intraepithelial neoplasia (CIN) grade 2 or 3 at any visit or persistent CIN grade 1 at exit. The main study end point was 2-year cumulative diagnosis of CIN grade 3.

RESULTS: The 2-year cumulative diagnosis of CIN grade 3 was approximately 15% in all study arms. The HPV triage arm was closed early because more than 80% of women were HPV positive, precluding efficient triage. The immediate colposcopy strategy yielded 55.9% sensitivity for cumulative cases of CIN grade 3 diagnosed over 2 years. A conservative management strategy of repeat cytology at the HSIL threshold referred 18.8% of women while detecting 48.4% of cumulative CIN grade 3. At lower cytology thresholds, sensitivity would improve but would ultimately yield unacceptably high referral rates.

CONCLUSION: LSIL cytology is best managed by colposcopy initially, because there was no useful triage strategy identified. Management of these patients, after colposcopy to rule out immediately overt CIN grade 2 or 3, needs to be determined. (Am J Obstet Gynecol 2003;188:1393-1400.)

Key words: Low-grade squamous intraepithelial lesion, human papillomavirus, cervix, clinical management, randomized clinical trial, cytology, colposcopy

The Bethesda System for the reporting of cervical cytology integrates scientific understanding of human papillomavirus (HPV) and its role in cervical cancer with clinically relevant diagnostic terminology. Specifically, the category of low-grade squamous intraepithelial lesion (LSIL) subsumes cytologic features of HPV infection, previously termed "koilocytotic atypia," and mild dysplasia or cervical intraepithelial neoplasia (CIN) grade 1. These cytomorphologic patterns all reflect infection with HPV and further subclassification does not provide useful clinical risk stratification. Management options for LSIL have included immediate colposcopy, cytology follow-up, or triage with HPV DNA testing for cancer-associated HPV.[1]

The ASCUS/LSIL Triage Study (ALTS), a randomized multicenter trial sponsored by the National Cancer Institute (NCI), was designed to compare three management strategies for women with ASCUS or LSIL cervical cytology interpretations: immediate colposcopy (IC) [all women referred to colposcopy], HPV triage (HPV) (referral to colposcopy only if the enrollment HPV DNA test was positive or missing, or the enrollment ThinPrep cytology [Cytyc Corporation, Boxborough, Mass] was high-grade squamous intraepithelial lesion [HSIL]), and conservative management (CM) (a program of repeat cytology follow-up with referral to colposcopy for HSIL).

All women, regardless of randomization assignment and initial management during enrollment, were scheduled for follow-up with cytology at 6-month intervals for 2 years. Women with HSIL were referred (or referred again) to colposcopy, and histologic CIN grade 2 or 3 was treated with loop electrosurgical excision procedure (LEEP). At the 24-month exit visit, colposcopy was performed and the option of LEEP was extended to women with persistent CIN grade 1 to ensure patient safety and to provide complete ascertainment of disease before a

From the ALTS Group.

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woman exited the study. The main study end point was detection of histologically confirmed CIN grade 3, chosen because there is general consensus that this is a high-risk lesion for progression to invasive cancer and requires definitive treatment. A priori, the IC and HPV triage strategies were designed to detect CIN grade 3 at enrollment, based on the initial examination and colposcopic referral. However, the CM strategy relied on repeat cytology; therefore, detection of CIN grade 3 during either the enrollment or follow-up study periods was considered success.

This report compares the relative effectiveness of the three management strategies for the 1572 women who entered the trial with an LSIL community cytology diagnosis. Results for ASCUS are reported separately.[2]

Methods

Recruitment. ALTS involved four clinical centers: University of Alabama (Birmingham, Ala), Magee-Womens Hospital of the University of Pittsburgh Medical Center Health System (Pittsburgh, Pa), the University of Oklahoma (Oklahoma City, Okla), and the University of Washington (Seattle, Wash). The study was approved by the NCI and local institutional review boards.

Randomization arms. A total of 1572 women with community-read LSIL cytology results enrolled in the study from January 1997 to December 1998. Fewer women were randomly assigned to HPV triage as this arm closed early, in November 1997. Routine follow-up and exit visits concluded in January 2001. Demographic characteristics of the enrollees are described more completely elsewhere.[3]

All women in each arm underwent the same enrollment pelvic examination with collection of specimens as outlined below under examination procedures. Referral to colposcopy at enrollment was based on the randomization arm and enrollment test results. (This was the *only* management decision that differed among arms.) Subsequent follow-up was the same for all arms. An exit examination, with colposcopy scheduled for *all* women regardless of arm or prior procedures, was performed at 2 years as described under follow-up and exit management below.

Examination procedures. At each patient visit, nurse-clinicians typically conducted the pelvic examination and collected two cervical specimens. The first cervical specimen was collected with a Papette broom (Wallach Surgical, Orange, Conn) and was rinsed directly into a PreservCyt vial (Cytyc Corporation, Boxborough, Mass). This specimen was used for both the preparation of ThinPrep cytology slides and for HPV testing by using Hybrid Capture 2 (HC 2) high-risk probe set (Digene Corporation, Gaithersburg, Md). A second cervical specimen, collected with a Dacron swab, was obtained for investigational HPV DNA typing; these results were not used for patient management in the trial. After the collection of the cervical specimens, the cervix was rinsed twice with a 5% solution of acetic acid and 2 Cervigrams (National Testing Laboratories Worldwide, Fenton, Mo) were taken.

Patient management at enrollment. Women randomly assigned to the IC arm proceeded immediately to colposcopy or were given an appointment to return for the procedure within 3 weeks if colposcopy could not be performed the same day. Women randomly assigned to the HPV triage arm were called back for colposcopy if the HPV test was positive or not performed (missing), or if there was an ALTS clinical center enrollment cytology diagnosis of HSIL or a glandular abnormality (these interpretations as a group have been termed HSIL). A missing HPV test result was most commonly the result of having less than 4 mL of residual specimen in the PreservCyt vial to test after preparing the ThinPrep, an arbitrary minimum volume. Women in the HPV triage arm with no HPV test results were triaged to colposcopy because it was considered to be an impractical triage strategy to recall women for repeat collection of a specimen for the HPV test alone. In the CM arm, only women with a clinical center cytology diagnosis of HSIL were referred to colposcopy. Unsatisfactory cytology led to recall for repeat specimen collection unless the patient had already been referred for colposcopy on the basis of randomization (IC arm) or HPV test result (HPV triage arm). Very rarely, clinicians referred patients to colposcopy on the basis of visualizing a lesion suspicious for cancer during the pelvic examination. Any safety net notification (see below) issued by a quality control (QC) group also triggered colposcopy. Women with colposcopically directed biopsy results of CIN grade 2 or 3 diagnosed by the clinical center were treated by LEEP.

Patient management at follow-up. Women were scheduled to return for follow-up visits at 6, 12, and 18 months from the date of enrollment regardless of arm and treatment received. Pelvic examinations and specimen collections were conducted as at enrollment. Women were referred (or referred again) to colposcopy for a clinical center cytology diagnosis of HSIL, or a safety net trigger. During follow-up, HPV results were masked in all arms.

Patient management at exit. Exit visits, scheduled for approximately 24 months from the date of enrollment, included colposcopy for all women. To ensure patient safety and to provide complete ascertainment of disease end points before a woman exited the study, all available clinical information was unmasked and provided to the clinician conducting the exit pelvic examination and colposcopy. This included all previous cytology and histopathology reported by the clinical center and the pathology QC group, the most recent cervigram photograph and report, as well as all previous HPV results.

At exit, colposcopy was performed in the same manner as at enrollment and follow-up. However, the threshold

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for treatment was lower at exit; in addition to treating women with CIN grade 2 or 3 on colposcopically directed biopsy, women with persistent low-grade lesions were offered LEEP. A woman was considered to have a persistent low-grade lesion if the colposcopically directed biopsy specimen at exit showed CIN grade 1 *and* cytology results from at least one of the previous two visits showed LSIL or HPV+ ASCUS.

Protocol modification for women in the CM arm. An interim safety analysis of sensitivity of detection of CIN grade 3, conducted for the Data and Safety Monitoring Committee, revealed a deficit in the LSIL CM arm, suggesting this arm was too insensitive in identifying CIN grade 3 to ensure patient safety. Effective July 1999, women in the LSIL CM arm who had not previously been triaged to colposcopy were referred for this procedure at their next follow-up visit. If this happened to be the 18-month follow-up visit and colposcopy was performed, the 18-month visit became the participant's "exit" visit. In such instances, test results and patient management followed exit protocols and were included in the exit period for analytic purposes.

Laboratory processing and interpretation of cervical specimens. Liquid-based, ThinPrep cytology slides were prepared from PreservCyt vial specimens. After the clinical center evaluation, slides were sent to the pathology QC group for rescreening and rereview. After the preparation of the ThinPrep, the PreservCyt vial was forwarded for HPV testing with the HC 2 assay to detect cancer-associated HPV types. An HPV QC group monitored the performance of the HPV assay. All referral slides, ThinPreps, and histology slides were sent to the pathology QC group that was based at Johns Hopkins Hospital for rereview and final case definition following an algorithm detailed elsewhere.[2] However, clinical management was based on the reading by the clinical center pathologist.

Safety notifications. In addition to providing expert interpretation for purposes of disease definition, the pathology QC review was also designed to provide a "safety net" for study participants. For cytologic and histologic specimens, a pathology QC diagnosis of CIN grade 3 (that had been called less than CIN grade 2 at the center) triggered a safety notification sent by fax to the clinical centers. Cervigrams and digital colposcopic images also underwent external review for safety purposes. The threshold for safety notification for cervicography and digital colposcopic images was "suspect cancer."

Statistical analyses. The primary study scientific endpoint was established a priori as a pathology QC histologic diagnosis of CIN grade 3, adenocarcinoma in situ (AIS), or cancer. Because there were so few cases of cancer (n = 5) or AIS (n = 1), we refer to the scientific endpoint for simplicity as CIN grade 3. We also present a clinical endpoint of histologic CIN grade 2 or 3 as diagnosed at the clinical centers because women were treated on the basis of clinical center diagnoses at this threshold. For analyses related to time of diagnosis, we collapsed the periods into enrollment, follow-up, and exit. Additional procedures performed within 1 year of enrollment, as part of the continued diagnostic workup of a patient, are included in the enrollment period.

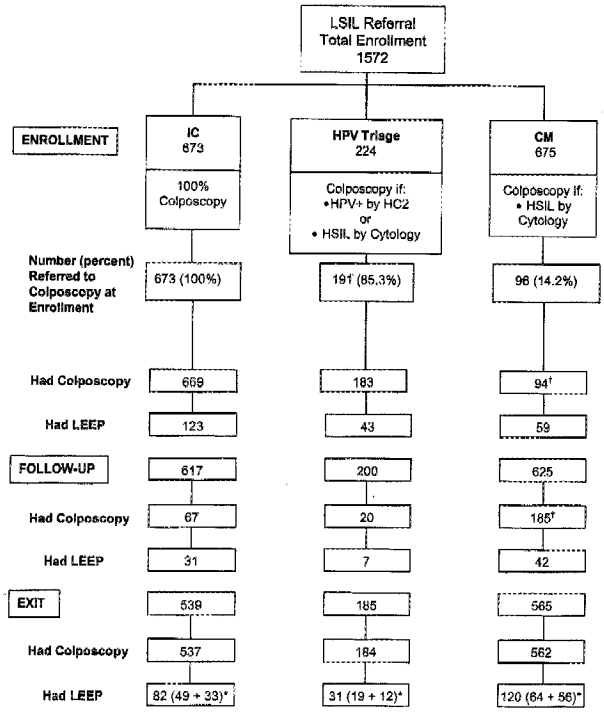

Figure. CONSORT diagram of participants in ALTS referred for LSIL cytology. The number of women enrolled in each arm, the triage strategy for referral to colposcopy at enrollment, and the percent of women referred are shown at *top*. The first row of numbers for the follow-up and exit periods reflect women remaining in the trial at that time. Subsequent rows indicate the number of women who had colposcopy and/or LEEP during the period. *Asterisk*, Note that Exit LEEP numbers are subdivided in parentheses by the indication triggering the procedure: persistent low-grade disease versus presence or suspicion of high-grade disease, respectively; *dagger*, in CM, seven women during enrollment and 136 women during follow-up were sent to colposcopy on the basis of a protocol modification initiated as a safety intervention (see Methods section).

The binomial distribution was used to compute exact confidence intervals for proportions (eg, sensitivity). Pearson χ^2 tests for contingency tables were used to assess the associations between categorical variables (eg, cytology interpretations vs HPV test results). The McNemar test was used to assess the significance of differences in paired data, such as the comparison of the sensitivities of cytology and HPV testing in the same subjects. The χ^2 statistics for trend were calculated to test the significance of data with evident ordering (such as increasing severity of

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Table I. Clinical center enrollment liquid–based cytology diagnoses compared with HPV DNA test results*

	HPV DNA test result			
Cytology	*Negative (row %)*	*Missing (row %)‡*	*Positive (row %)*	*Total (column %)†*
Unsatisfactory or missing	1 (16.7%)	2 (33.3%)	3 (50.0%)	6 (0.4%)
Negative	119 (40.5%)	8 (2.7%)	167 (56.8%)	294 (18.7%)
ASCUS	80 (22.0%)	15 (4.1%)	269 (73.9%)	364 (23.2%)
LSIL	35 (4.9%)	42 (5.9%)	632 (89.1%)	709 (45.1%)
HSIL–CIN grade 2	2 (1.1%)	10 (5.6%)	165 (93.2%)	177 (11.3%)
HSIL–CIN grade 3	0 (0.0%)	2 (9.1%)	20 (90.9%)	22 (1.4%)
Total	237 (15.1%)	79 (5.0%)	1256 (79.9%)§	1572 (100.0%)

*HC 2 includes probes for cancer-associated HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68.
†$P_{trend} < .001$ for association between severity of cytologic abnormality and HPV DNA positivity.
‡5.0% of HC 2 results were missing due to <4 mL residual cytology specimen in Preservcyt collection vial after preparing ThinPrep cytology slides.
§84.1% (1256/1493) of the HC 2 results that could be performed were positive.

Table II. Clinical center histologic diagnosis from first colposcopically directed biopsy in IC arm

Clinical center histologic diagnosis	*No.**	*Percentage*
Missing valid biopsy result	11†	1.6
No biopsy taken, normal colposcopic impression	75	11.2
Negative	171	25.6
CIN grade 1	302	45.1
CIN grade 2	76‡	11.4
CIN grade 3	34‡	5.1
Total	669	100.0

*Of the 673 women in the IC study arm, 4 women referred to colposcopy did not attend.
†Of the 669 that attended, 7 had no biopsy specimen taken despite an abnormal colposcopic appearance and 4 had unsatisfactory biopsy specimens (n = 11 missing biopsy results).
‡The numbers of CIN grades 2 and 3 are clinical center diagnoses of the initial colposcopically directed biopsy; these numbers cannot be directly compared with subsequent tables that use pathology QC group diagnoses.

cytology interpretations related to HPV positivity). Life-table methods were used to account for censoring in analyses where such an adjustment was needed. All statistical tests were two sided and were considered significant at $P < .05$.

Results

ALTS enrolled 1572 women with a community Papanicolaou (Pap) smear interpretation of LSIL and randomized 673 to the IC arm, 224 to the HPV triage arm, and 675 to the CM arm (Figure). Fewer women were randomly assigned to HPV triage because this arm closed early, in November 1997, because an interim analysis revealed 83% HPV positivity. The ALTS group concluded that this high percentage would not provide sufficient clinical utility for HPV as a triage test for LSIL.[4]

Throughout the study, 92% of women had at least one follow-up visit and 82% had an exit visit; retention did not differ by study arm. Virtually all women referred to colposcopy did attend and virtually all referred for LEEP received treatment. Therefore, percentage differences in colposcopies or LEEPs performed reflect meaningful differences between study arms and periods, not biased participation rates (Figure). Absolute numbers cannot be compared directly because of the limited randomization to the HPV triage arm.

During follow-up, a higher percentage of women in the CM arm were sent to colposcopy, in part because of the protocol modification instituted because of safety concerns (see Methods).

Approximately one third of women had LEEP over the course of the study; this fraction did not vary by study arm. However, the timing of the LEEP differed among arms as a corollary of the time of detection of disease. About half of the LEEPs in IC and HPV were performed during the enrollment period compared with one fourth in the CM arm. At exit, in addition to treating women with histologic CIN grade 2 or 3, women with persistent low-grade lesions (defined as CIN grade 1 on colposcopically directed biopsy *and* cytology results from at least one of the two immediately preceding visits showing LSIL or HPV+ ASCUS) were offered LEEP. One hundred thirty-two women underwent LEEP triggered for persistent low-grade disease, representing one fourth of all LEEPs performed during the study.

The average time between the LSIL referral smear and the clinical center enrollment examination was 2.0 months (median 1.7, range 0.3-6.0). Table I shows a comparison of the clinical center enrollment ThinPrep interpretation and HPV test results. Approximately half (45.1%) of the ThinPrep results were LSIL, with the remainder mainly ASCUS (23.2%) or negative (18.7%). A smaller number (199 or 12.7%) were HSIL. There was a significant trend of increasing positivity for oncogenic types of HPV DNA with severity of the ThinPrep interpretation, even among this group of women, all referred with

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Table III. Cumulative histologic diagnoses of CIN grade 2 and CIN grade 3* by pathology QC group, stratified by study arm

	IC (column %)	*HPV triage (column %)*	*CM (column %)*	*P value†*	*Total (column %)*
CIN grade 2	90 (13.4%)	24 (10.7%)	51 (7.6%)	.002	165 (10.5%)
CIN grade 3	102 (15.2%)	41 (18.3%)	93 (13.8%)	.26	236 (15.0%)
CIN grades 2 and 3	192 (28.5%)	65 (29.0%)	144 (21.3%)	.004	401 (25.5%)
Total No. women	673 (100.0%)	224 (100.0%)	675 (100.0%)		1572 (100.0%)

*CIN grade 3 includes five cases of invasive cancer (2 each in IC and CM, and 1 in HPV triage) and one case of AIS in the IC.
†*P* values from χ^2 test for comparison between study arms. Direct comparison of CIN grade 2 by study arm was statistically significant for CM vs IC ($P < .001$).

Table IVA. Cumulative histologic diagnoses of CIN grade 3* by pathology QC group, stratified by study arm and period†

	IC	*HPV triage*	*CM*	*Total CIN grade 3*
Enrollment	**64 (62.7%)**	**28 (68.3%)**	**34 (36.6%)**	126 (53.4%)
Follow-up	20 (19.6%)	4 (9.8%)	**25 (26.9%)**	49 (20.8%)
Exit	18 (17.6%)	9 (22.0%)	34 (36.6%)§	61 (25.8%)
Total	102 (100.0%)	41 (100.0%)	93 (100.0%)	236 (100.0%)

*CIN grade 3 includes five cases of invasive cancer (2 each in IC and CM, and 1 in HPV triage) and one case of AIS in the IC.
†The figures in bold areas indicate the a priori–defined period for the strategy to successfully detect CIN grade 3 within the study arm (ie, enrollment for IC and HPV triage, and enrollment plus follow-up periods for CM). These numbers of CIN grade 3 include cases detected through safety interventions; such cases are not counted as successes in the comparison of management strategy performance in Table V (CIN grade 3 detected through safety intervention: IC, n = 7; HPV, n = 1; CM, n = 14).
‡$P < .001$ from χ^2 test for overall comparison of study arm versus time of diagnosis of CIN 3. $P_{trend} = .93$ for IC versus HPV triage. $P_{trend} < .001$ for IC versus CM. $P_{trend} = .005$ for HPV triage versus CM.
§Among the 98 women who finished the CM protocol before the protocol modification (see Methods section), three (33.3%) of the nine cumulative CIN grade 3 cases were diagnosed at exit.

Table IVB. Cumulative histologic diagnoses of CIN grade 2 or 3 by clinical center pathologists, stratified by study arm and period

	IC	*HPV triage*	*CM*	*Total CIN grade 2 or 3*
Enrollment	127 (67.2%)	45 (76.3%)	54 (35.8%)	226 (56.6%)
Follow-up	27 (14.3%)	5 (8.5%)	41 (27.2%)	73 (18.3%)
Exit	35 (18.5%)	9 (15.3%)	56 (37.1%)	100 (25.1%)
Total	189 (100.0%)	59 (100.0%)	151 (100.0%)	399 (100.0%)

$P < .001$ from χ^2 test for overall comparison of study arm versus time of diagnosis of CIN grade 2 or 3. $P_{trend} = .29$ for IC versus HPV triage. $P_{trend} < .001$ for IC versus CM. $P_{trend} < .001$ for HPV triage versus CM.

a community interpretation of LSIL. Of note, virtually all the women (185 of 187 = 98.9%) with HSIL ThinPrep cytology and available HC 2 assay results were positive for oncogenic types of HPV.

Women randomly assigned to the IC arm usually had colposcopy on the same day as enrollment (mean 2.4, median 0.0, mode 0.0). Given universal colposcopy in the IC arm, the clinical center results reflect the distribution of disease detected at initial examination after LSIL cytology (Table II). Approximately half (45.1%) of the women had histologic CIN grade 1. A large percentage of women had either negative biopsy specimens (25.6%) or no biopsy taken because of a normal colposcopic appearance (11.2%). CIN grade 2 or 3 was diagnosed in 16.4%.

Tables III through VI present complementary approaches to the analysis of the longitudinal data. We separately considered (1) the findings in the "study arm," (2) the performance of the "management strategy," and (3) the optimized "triage test performance."

Study arm findings. As the simplest, descriptive comparison of the study arms, Tables III and IVA present all disease endpoints diagnosed by the Pathology QC group during the trial. Table III shows that the cumulative diagnoses of CIN grade 3, the a priori study end point, did not vary significantly by study arm (IC 15.2%, HPV 18.3%, CM 13.8%). However, the cumulative diagnoses of CIN grade 2 were significantly lower in the CM arm (7.6%) compared with the IC arm (13.4%), thought to be a consequence of regression of missed prevalent CIN grade 2 in the CM arm (see Comment). The smaller numbers in the truncated HPV triage arm reduced statistical power of related comparisons.

Table IVA shows the cases of CIN grade 3 in each study arm, stratified by period. Although the total percentage

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Table V. Performance of management strategies for detection of cumulative histologic diagnosis of CIN grade 3* by pathology QC group

Management strategy	*IC*	*HPV triage*	*CM*	*P value*
Sensitivity for CIN grade 3 (%)†	55.9% (45.7-65.7)	65.9% (49.4-79.9)	48.4% (37.9-59.0)	.16
Referral to colposcopy (%)	100.0% (99.4-100.0)	85.3% (79.9-89.6)	18.8% (15.9-22.0)	<.001

*CIN grade 3 includes five cases of invasive cancer (2 each in IC and CM, and 1 in HPV triage) and one case of AIS in the IC.

†The management strategy performance calculations consider as "successes" only those cases of CIN grade 3 detected by the clinical application of the management strategy at the centers within the a priori--defined period for that strategy (ie, enrollment period for IC and HPV triage, and enrollment plus follow-up periods for CM) (see bold areas of Table IV). Cases of CIN grade 3 missed by the strategy but detected by safety net interventions and cases detected after the defined period for that strategy are not included in the numerator for calculating sensitivity.

Table VI. Estimated* triage test performance for detection of cumulative histologic diagnosis of CIN grade 3† by pathology QC group

	Sensitivity for CIN grade 3 (%) (CI)	*Referral (%) (CI)*
Enrollment HPV DNA test	95.2% (91.5-97.6)	84.1% (82.2-85.9)
HSIL cytology threshold‡		
1	36.0% (29.7-42.6)	12.6% (10.9-14.4)
2	55.1% (44.9-65.2)	16.8% (14.0-19.7)
3	65.1% (55.1-75.0)	19.5% (16.4-22.6)
LSIL cytology threshold‡		
1	72.8% (66.5-78.5)	57.4% (54.8-59.9)
2	86.0% (79.0-93.1)	64.9% (61.3-68.5)
3	93.0% (87.7-98.3)	68.6% (65.1-72.2)
ASCUS cytology threshold‡		
1	90.8% (86.3-94.2)	80.8% (78.7-82.8)
2	98.9% (96.8-100)	87.4% (84.9-90.0)
3	100.0% (100-100)	88.9% (86.5-91.4)

*For these estimates, missing test results, missed visits, and the timing of visits were ignored to focus on the performance of the tests according to how many were completed.

†CIN grade 3 includes five cases of invasive cancer and one case of AIS.

‡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. The enrollment HPV test was compared with the first cytology using data from all study arms to maximize statistical power. Because of extensive censoring in the IC and HPV arms, only data from the CM arm were used to estimate the performance of two or three repeat cytology examinations.

of CIN grade 3 diagnosed by the pathology QC group in each arm was equivalent, the timing of diagnosis was significantly heterogeneous ($P < .001$). Of the total CIN grade 3 cases in each arm, those in the HPV triage and IC arms were diagnosed earlier compared with the CM arm, and 36.6% of cases were not diagnosed in CM until the exit period.

It is important to note that seven cases of CIN grade 3 at exit were found only by offering LEEP to 132 women with persistent low-grade lesions (Figure). In terms of contribution to total numbers of CIN grade 3, these seven cases represent 11.5% of the CIN grade 3 diagnosed at exit, and 3.0% of the total number of cases of CIN grade 3 in the study.

Table IVB addresses the clinical end point of CIN grade 2 or 3 diagnosed by the clinical center pathologists. Although the numbers of end points are greater, the percent distribution of time of diagnosis mirrors the findings that were based on the scientific end point.

Management strategy performance. In Table V, the management strategy performance calculations consider as "successes" only those cases of CIN grade 3 detected by the clinical application of the management strategy at the centers within the a priori-defined period for that strategy (ie, enrollment period for IC and HPV triage, and enrollment plus follow-up periods for CM---see bolded figures of Table IVA). Cases of CIN grade 3 missed by the strategy but detected by QC safety net intervention, and cases detected after the defined period for that strategy, are not included in the numerator for calculating sensitivity.

Table V compares the alternative management strategies on the basis of the sensitivity for the detection of CIN grade 3 and the percentage of women requiring colposcopy under that strategy. In IC, only 55.9% of cumulative cases of CIN grade 3 diagnosed over the 2-year study period were detected during the enrollment period. This management strategy required colposcopy in 100% of women, significantly more than the other two strategies. In HPV triage, 65.9% of cumulative cases of CIN grade 3 were detected during enrollment, a sensitivity that was marginally greater ($P = .09$) than for CM. Of note, the HPV triage strategy theoretically depended on either

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HPV positive results or HSIL cytology at enrollment for referral to colposcopy, but none of the cases of CIN grade 3 were referred to colposcopy on the basis of cytology alone. The percentage of women referred to colposcopy at enrollment by HPV triage (85.3%) was significantly less than the universal referral in IC but was much greater than the 18.8% referral (enrollment plus follow-up) for the CM strategy.

Triage test performance. While Table V shows the actual performance of the three alternative management strategies in clinical settings subject to the limitations of colposcopically directed biopsy and loss to follow-up, Table VI gives theoretical estimates of optimal performance for HPV testing and cytology at three thresholds of colposcopic referral. For these estimates, we ignored the imperfect sensitivity of colposcopy and excluded missing test values, to evaluate (1) what percentage of cases of CIN grade 3 would have been referred, based on a positive triage test and threshold (% sensitivity) and (2) how many referrals would have resulted by using each triage test and threshold (% referral). Of the women originally referred to ALTS with LSIL cytology who were ultimately found to have CIN grade 3, enrollment HPV testing would have properly triaged 95.2% (CI = 91.5-97.6) while referring the great majority of women overall to colposcopy (84.1%, CI = 82.2-85.9) (exclusion of missing tests accounts for difference with Table I). Examination of the sensitivities and referral percentages for various thresholds of repeat cytology, determined from the CM arm, demonstrates that repeating cytology three times at a referral threshold of LSIL would provide comparable sensitivity (93.0%, CI = 87.7-98.3) while referring approximately two thirds of women (68.6%, CI = 65.1-72.2). Repeat cytology at a threshold of ASCUS would be sensitive, but would require more than 80% colposcopic referral even with a single repeat cytology.

Comment

ALTS found that a cytology interpretation of LSIL is fairly reproducible and is associated with a 25% risk of histologic CIN grade 2 or 3 within 2 years. However, we did not find a triage strategy that would safely spare many women from colposcopic referral.

ALTS evaluated three management strategies for women with LSIL in a prospective, randomized design: immediate colposcopy, triage that was based on HPV DNA testing and cytology, and repeat cytology at 6-month intervals. Retention and compliance with recommended interventions were excellent, did not differ by arm, and therefore did not influence the outcomes of the study.

Among women enrolled in the trial with a community (referral smear) of LSIL, more than two thirds were again LSIL or ASCUS on the enrollment ThinPrep, and the great majority was positive for oncogenic HPV types. Approximately half of the women who had colposcopy and directed biopsy in the IC arm had histologic CIN grade 1, and 16% had CIN grade 2 or 3 as diagnosed by the clinical centers. Because the cytology interpretation of LSIL is fairly reproducible[5] and most LSIL cases are oncogenic HPV positive, the use of HPV testing for the initial management of LSIL should be discouraged. Pending cost-utility analyses will almost certainly show that its cost cannot be justified, given that very few women would be triaged away from colposcopic referral by such testing. In particular, "confirmatory" HPV testing of LSIL cases should not be routinely performed unless the interpretative accuracy of the cytopathology laboratory is in question.

The overall detection of CIN grade 2 or 3 as diagnosed by the pathology QC group was 25%. There were five invasive cancers and one case of AIS, all of which were HPV positive. The cumulative 2-year rate of detection of CIN grade 3 was approximately 15% and did not vary significantly by study arm. In contrast, the cumulative percentage of CIN grade 2 alone was significantly reduced in the CM arm compared with the IC arm, suggesting substantial regression of missed prevalent CIN grade 2 over the 2 years. (Small numbers precluded comparison with the HPV arm on this issue.) CIN grade 2 is likely to represent a heterogeneous collection of lesions, only some of which are incipient CIN grade 3. Current practice is to treat CIN grade 2 but, to prevent possible overtreatment and its sequelae, particularly among younger women, it would be very useful to be able to identify those lesions destined to regress. In ancillary ALTS investigations, we are joining the search for biomarkers of cancer risk among CIN grade 2 cases.

By design, each arm of the trial represented an alternative management strategy. These can best be evaluated by comparing the percent of CIN grade 3 cases detected by each strategy, without crediting cases of CIN grade 3 that were found by one of multiple safety nets in the trial. We judged the success of the immediate colposcopy and HPV triage strategies by detection of CIN grade 3 during the enrollment period only; however, for CM, cases of CIN grade 3 detected during the enrollment *or* follow-up periods (approximately 18 months) were considered success.

The IC strategy detected only 56% of the cumulative CIN grade 3 found over 2 years. The HPV triage was at least as sensitive as immediate colposcopy, but was not efficient, referring more than 80% of women to colposcopy. The CM strategy of repeat cytology examinations at the high referral threshold of HSIL, detected 48% of cumulative CIN grade 3 during enrollment and follow-up, but referred far fewer patients (19%) to colposcopy. This suggests that repeat cytology at an HSIL threshold, although referring few women and similar in sensitivity to a single colposcopy, is not optimally sensitive for the timely detection of CIN grade 3.

When we estimated the performance of repeat cytology at different referral thresholds (Table VI), a single repeat cytology at the ASCUS threshold referred more than 80%

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of women, too high to justify its use. A program of three sequential cytology examinations with a referral threshold of LSIL demonstrated reasonable sensitivity (93%) and referral (69%). However, high patient retention would be critical to such a strategy. ALTS participants were highly motivated to return to their follow-up appointments by intensive outreach and retention efforts, including modest financial incentives. As a result, only 15% failed to complete the study, a degree of compliance that is unrealistic for most community practices. Follow-up with repeat cytology at a referral threshold of LSIL would require a commitment and partnership on the part of the patient and the physician to obtain quality cervical samples every 6 months. Even so, approximately two thirds of the women would eventually be referred to colposcopy.

If compliance can be achieved, cytology follow-up might be considered in selected patient populations (eg, adolescents who are at high risk of HPV infection and abnormal cervical cytology results but at low risk of invasive cancer).[6] Obviously, in the United States, which is characterized by geographic mobility and often-changing insurance and clinicians, loss of follow-up of patients is a major concern.

The strategy of immediate colposcopy was included in the trial as the reference standard of optimal sensitivity and safety. Although the ALTS clinical center colposcopists were well trained and many could be considered expert, IC was only 56% sensitive for cumulative CIN grade 3 detected during the trial. For CIN grade 3 diagnosed during follow-up or exit, it is impossible to accurately separate missed prevalent from newly incident cases. Therefore, it is possible that some of the CIN grade 3 developed after enrollment and was detected appropriately at follow-up visits. However, our review of complete records for cases of CIN grade 3 diagnosed after enrollment suggests that many cases represented missed prevalent disease falling below the triggers for safety net notifications.

Some of the CIN grade 3 lesions detected during exit resulted from the decision to offer LEEP to women with persistent low-grade lesions. Moreover, the CIN grade 3 lesions detected at exit tended to be very small; many would have been safely detected later or even might have eventually regressed.[7] The long-term prospective fate of CIN grade 3 can no longer be studied ethically. However, if the goal is the timely detection of CIN grade 3, our findings do suggest that colposcopically directed biopsy cannot be considered a gold standard of absolute sensitivity.

ALTS data suggest that there is currently no efficient triage for LSIL, and that in general, the level of risk for CIN grade 2 or 3 warrants colposcopic evaluation. However, the imperfect sensitivity of colposcopy raises the issue of how to manage women, who continue to be at risk of CIN grade 2 or 3, after an initial colposcopy result of CIN grade 1 or less.[8] We address this question in an accompanying analysis.[9]

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