Qualification of ASCUS

A Comparison of Equivocal LSIL and Equivocal HSIL Cervical Cytology in the ASCUS LSIL Triage Study

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Abstract

Cytologic detection of high-grade squamous intraepithelial lesions (HSILs) is critical to cervical cancer prevention. Therefore, identifying "equivocal HSIL" (ASCUS [atypical squamous cells of undetermined significance]-H) may be useful. Accordingly, we compared findings associated with "equivocal low-grade SIL" (ASCUS-L), ASCUS-H, and HSIL using data from the ASCUS LSIL (low-grade squamous intraepithelial lesion) Triage Study. The frequency of oncogenic human papillomavirus (HPV) DNA detection and underlying lesions cervical intraepithelial neoplasia (CIN) 2 or worse or CIN 3 or worse in women with ASCUS-H was intermediate between that of ASCUS-L and HSIL. Oncogenic HPV DNA was associated with 85.6% of ASCUS-H ThinPreps and 69.8% of ASCUS-H smears. Histopathologic lesions CIN 2 or worse were associated with 40.5% of ASCUS-H ThinPreps and 27.2% of ASCUS-H smears (mostly CIN 3). Nevertheless, numerically more lesions CIN 2 or worse were preceded by ASCUS-L than by ASCUS-H because ASCUS-L was more common. ASCUS-H is an uncommon interpretation that derives clinical usefulness from its high positive predictive value for lesions CIN 2 or worse.

The term atypical squamous cells of undetermined significance (ASCUS) was introduced in the Bethesda System (TBS) to designate equivocal cytologic changes that may reflect a squamous intraepithelial lesion (SIL).[1] TBS recommends that pathologists qualify ASCUS as "favor reactive" or "favor SIL" to facilitate optimal patient management. Several studies have demonstrated that women with ASCUS, favor SIL, are more likely to have an underlying cervical intraepithelial neoplasia (CIN) than women with ASCUS, favor reactive.[2-8]

TBS recommendations for qualifying ASCUS published in 1992 were predicated on the view that all grades of SIL represent closely related precursors requiring immediate colposcopy and treatment. However, this view has been modified because natural history studies demonstrate that the human papillomavirus (HPV) infections that produce low-grade SIL (LSIL) usually regress spontaneously, especially in young women.[9] Accordingly, current approaches increasingly emphasize early cytologic detection of high-grade SIL (HSIL) and treatment of histopathologic lesions graded as CIN 2 or worse. Given this shift in thinking, a modified subclassification that separates "equivocal HSIL" (ASCUS-H) from "equivocal LSIL" (ASCUS-L) may be more clinically useful.

Small studies have suggested that the cytologic interpretation of ASCUS-H reflects an underlying CIN more often than other types of ASCUS.[8,10-13] In addition, oncogenic HPV DNA was detected more frequently in women with ASCUS-H than in women with other forms of ASCUS, but less frequently than in HSIL in 1 study.[12] However, the significance of ASCUS-H interpretations has not been evaluated systematically in a large multicenter study.

We compared the ages, HPV status, and histopathologic findings among women with thin-layer slides classified as

ASCUS-H to those with ASCUS-L and HSIL. Although we focused the investigation on thin-layer slides, we conducted a parallel, ancillary analysis of conventional smears from the same study population to strengthen previous findings. This report uses enrollment data from the ASCUS LSIL Triage Study (ALTS), a large, prospective, multicenter, randomized trial sponsored by the National Cancer Institute that compares 3 management strategies for women with ASCUS or LSIL.[14,15] Our goal was to determine whether ASCUS-H represents a distinct cytologic category that is associated with a different level of risk compared with ASCUS-L and HSIL.

Materials and Methods

Study Population

ALTS enrolled 3,488 eligible, consenting women with cervical smears interpreted as ASCUS and 1,572 as LSIL from referral areas surrounding the 4 US clinical trial centers. The study was approved by the institutional review boards of the National Cancer Institute and all centers. Details of the ALTS design are presented elsewhere.[14] Briefly, subjects completed an epidemiologic questionnaire assessing risk factors for cervical neoplasia and underwent a pelvic examination followed by repeated cervical cytologic sampling using a Papette broom (Wallach Surgical Devices, Orange, CT) at the clinical centers. Cervical cellular samples were collected in liquid medium (PreservCyt, Cytyc, Boxborough, MA) that was used to prepare Papanicolaou-stained ThinPreps (TPs) (Cytyc) for cytologic interpretation and for HPV DNA testing.[16,17] HPV testing for the presence of 13 oncogenic types at a threshold of 1.0 pg/mL was performed using Hybrid Capture 2 (Digene, Gaithersburg, MD) on 4-mL aliquots of residual volume remaining after preparation of the enrollment TP as previously described. Patients were assigned randomly to 1 of 3 possible colposcopy triage strategies: (1) immediate colposcopy, (2) colposcopy for an enrollment TP classified as HSIL or worse at the clinical centers, and (3) referral for detection of oncogenic HPV DNA or an enrollment TP interpretation of HSIL or worse. Rarely, women were referred for colposcopy for safety triggers based on reviews of pathology, cervicography, or other findings by external quality control (QC) groups. The study population of women with smear or TP interpretations of ASCUS-H was defined using the review interpretations of a 4-member pathology QC panel (see next section).

Pathology Review

The conventional smears that were used to determine eligibility for the trial (termed "referral smears") were originally reported in community laboratories using nonstandardized terminology for qualifying ASCUS. Enrollment cervical samples used to prepare TPs were collected a mean of 2 months after the referral smear. TPs were prepared, screened, and interpreted at the 4 clinical centers. QC cytotechnologists rescreened all available smears and TPs and recorded a provisional opinion. Then, the slides bearing both sets of screening dots and the QC cytotechnologist's opinion were passed to 1 of the 4 QC pathologists for masked, independent review using modified TBS terminology and standardized data collection sheets. ASCUS interpretations were subcategorized as ASCUS-L or ASCUS-H. The QC pathologists did not establish a priori criteria for the interpretation of ASCUS-H; each reviewer used his or her own criteria.

The final QC interpretation for cytology slides was determined using a standardized algorithm. If the first QC reviewer's interpretation matched the interpretation in the community (referral smears) or in the clinical center (TPs), this result was finalized unless the interpretation was HSIL. QC panelists working in pairs confirmed all HSILs at an unmasked, joint review conducted monthly at a multiheaded microscope. If the first QC review and the original interpretation disagreed, a second independent, masked QC review was performed, and the majority opinion became the final interpretation (unless any of the interpretations was HSIL, ASCUS-H, or "unsatisfactory," in which case the slide was examined again at a multiheaded microscope by 2 QC panel members to determine the final interpretation). The panel classified 0.4% of smears and 0.1% of TPs as ASCUS associated with atrophy or thick fragments and 0.1% of both preparations as ASCUS-H plus LSIL; because these interpretations include HSIL in the differential diagnosis, these specimens were combined with ASCUS-H in this analysis.

The QC diagnosis for histologic specimens was derived similarly to cytology except that QC reviews at a multiheaded microscope were conducted when the first QC panelist and the original diagnosis differed (for any set of slides cut from a single block). Patients with clinical center histologic diagnoses of CIN 2 or worse were referred for definitive treatment, usually by loop electrosurgical excision procedure or, rarely, cold knife cone. The most severe diagnosis for any histologic sample obtained within 1 year, as part of the continued enrollment workup of a patient, was considered the final enrollment diagnosis. After the formal QC enrollment review was completed, 2 pathologists (M.E.S., D.S.) reexamined all TPs classified as ASCUS-H for descriptive purposes. Referral smears were returned on a rolling basis to the community laboratories after QC review and, therefore, were not available for descriptive study or photography.

Analysis

The pathology QC interpretations were used to compare cytologic categories and to define histologic outcomes. Results for smears and TPs were analyzed separately.

Women with missing HPV, cytology, or histopathology results or unsatisfactory pathology specimens were excluded from relevant analyses. Data from the immediate colposcopy and HPV testing arms were combined in analyses using histologic outcomes because the detection of lesions CIN 2 or worse was complete in these arms.[15] Detection of histopathologic lesions CIN 2 or worse was incomplete in the conservative management arm, and, therefore, these results were omitted in histopathologic analyses. Patients who were not referred for colposcopy (negative HPV test result and cytology findings less than HSIL in the HPV triage arm), those who had normal colposcopic examinations and had not undergone biopsy, and patients with histologic atypia insufficient for a definitive diagnosis of CIN were combined in the group with women who had benign (negative) histopathology in relevant analyses.

We compared findings in TPs classified as ASCUS-L, ASCUS-H, and HSIL to evaluate whether ASCUS-H represents a distinct cytologic category. First, patient ages associated with these categories were compared. Then, cytologic features of TPs classified as ASCUS-H by the QC panel were described and illustrated. We also compared the percentage of specimens associated with detection of oncogenic HPV DNA (all study arms) and with histopathologic outcomes of CIN 2-only, CIN 3 or worse, and CIN 2 or worse (immediate colposcopy and HPV testing arms) for the 3 cytologic categories. Finally, we calculated the frequency with which each cytologic category was used and compared these data with the percentage of lesions CIN 3 or worse and CIN 2 or worse with which each cytologic category was associated (ie, attributable risk) in the immediate colposcopy and HPV triage arms. Selected analyses were repeated for referral smears.

Results

Age Associated With TP Interpretations

The median age of the 193 women with ASCUS-H was slightly younger (24 years) than that of the 1,211 women with ASCUS-L (25 years) but was similar to that of the 330 women with HSIL.

Cytomorphologic Features of TPs Classified as ASCUS-H

The majority of TPs classified as ASCUS-H contained between 10 and 100 atypical cells, but 25% contained fewer than 10 questionable cells. The atypical cells contained nuclei that were similar in size to those of normal intermediate squamous cells or about 2 to 3 times that of neutrophil nuclei. Although the preservation and appearance of the nuclei varied among specimens, the interpretation of ASCUS-H seemed to have been triggered primarily by difficulties distinguishing HSIL from squamous metaplastic or reserve cells with reactive, reparative, or degenerative nuclei. In about half the specimens, the ratio of nuclear to cytoplasmic area was approximately 50% (resembling CIN 2), whereas in the remainder it was higher, sometimes approaching or exceeding 90% (resembling CIN 3). In the majority of slides, the nuclear chromatin pattern was even and finely granular. Predominance of smudgy hyperchromatic nuclei or macronucleoli in the atypical cells was identified infrequently and tended to predict normal colposcopic findings, negative histology, and negative HPV test results. Most ASCUS-H demonstrated at least focal nuclear notching, grooving, or irregularity, and, when prominent, this feature was strongly associated with underlying lesions CIN 2 or worse **Image 1**. On retrospective review, we identified individual cells considered adequate for a definitive interpretation of HSIL in some of these TPs, but these initially were classified as ASCUS-H, seemingly owing to the low number of atypical cells, because the cell type or preservation was in question or because the background changes were confusing (eg, endometrial cells also present). In some TPs, it was difficult to distinguish nuclear irregularity from binucleation, especially if the cells were partly obscured or degenerated.

Association Between TP Categories and Detection of Oncogenic HPV DNA

The relationship between TP categories and detection of oncogenic HPV DNA showed a striking trend. Oncogenic HPV DNA was detected in 63.2% of women with ASCUS-L, 85.6% with ASCUS-H, and 98.7% with HSIL ($P < .001$ for trend).

Association Between TP Categories and Histopathologically Confirmed High-Grade CIN (Positive Predictive Value)

There was a significant trend toward increased proportion and severity of underlying lesions CIN 2 or worse for TPs classified as ASCUS-L, ASCUS-H, and HSIL **Table 1** ($P = .001$ for trend). The percentages of women with histologic findings of CIN 2 or worse were 11.6% for ASCUS-L, 40.5% for ASCUS-H, and 59.2% for HSIL. Among high-grade histologic lesions associated with ASCUS-H and HSIL cytology, significantly more CIN 3 lesions and carcinomas than CIN 2 lesions were found (chi-square, $P < .01$), whereas the reverse was true for ASCUS-L.

Use of TP Cytologic Categories and Percentage Contribution to the Detection of Histopathologically Confirmed High-Grade Lesions in ALTS

ASCUS-H accounted for only 3.7% of TP interpretations, but was associated with 12.4% of histopathologic findings of CIN 3 or worse and 10.5% of CIN 2 or worse. ASCUS-L, which was more than 6 times as frequent as

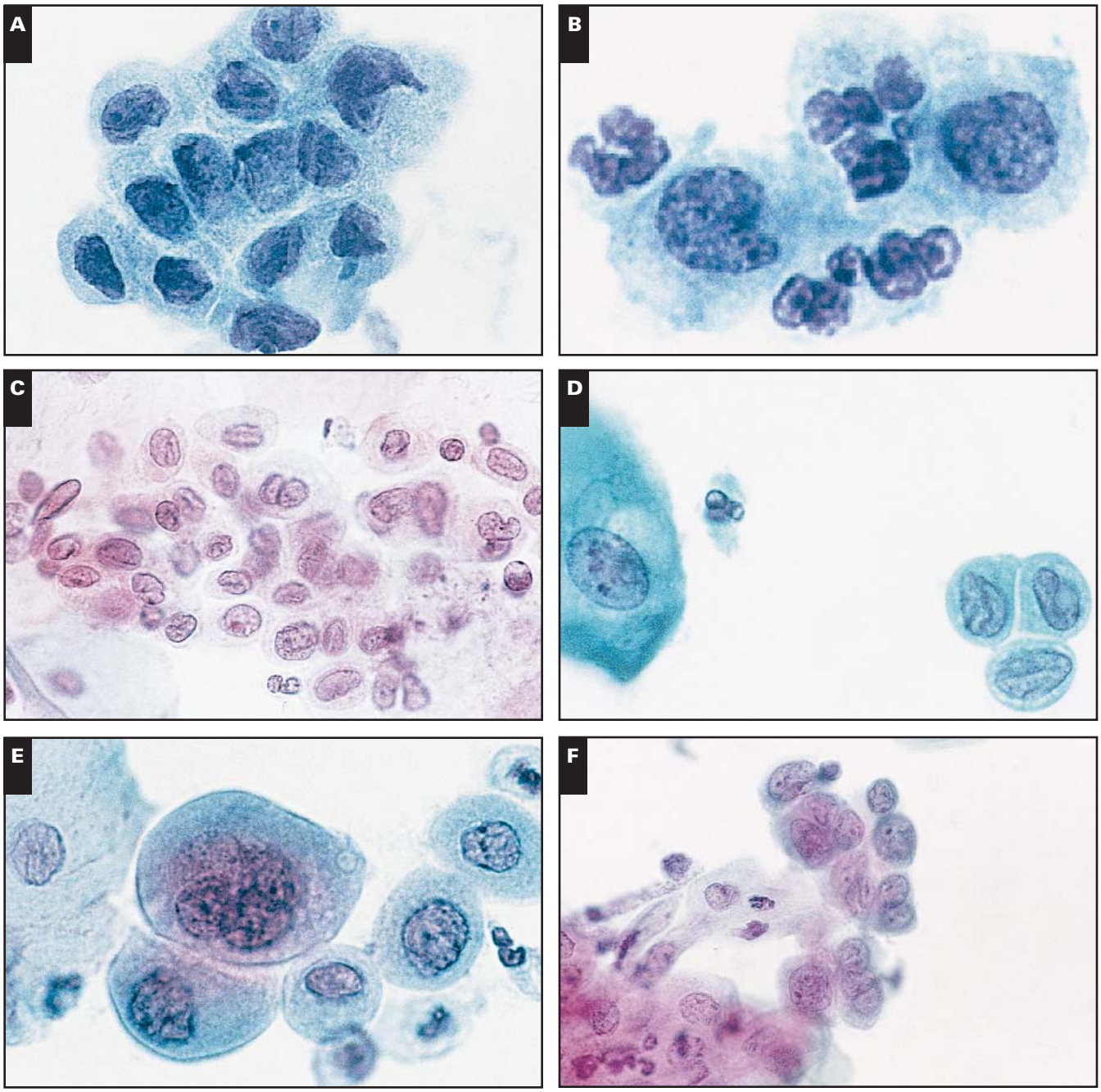

Image 1 ThinPreps (TPs; Cytyc, Boxborough, MA) classified as ASCUS-H (atypical squamous cells of undetermined significance, equivocal for high-grade squamous intraepithelial lesion [HSIL]) by the quality control panel. **A**, Approximately 10 cells with a metaplastic appearance displaying a high nuclear/cytoplasmic (N/C) ratio and hyperchromatic, smudgy chromatin. Initial human papillomavirus (HPV) testing was negative, and colposcopy was not performed. Repeated cytology performed 7 months later was negative. **B**, Cells with hyperchromatic nuclei measuring about twice the size of neutrophil nuclei showing nuclear and cytoplasmic vacuolization, presumably reflecting degeneration. Initial HPV testing and biopsies were negative. HSIL was not detected after 2 years of follow-up, including 4 repeated TPs and negative repeated histologic studies. **C**, Loosely cohesive sheet of cells with pale staining nuclei demonstrating grooving and occasional nucleoli. Initial HPV test was positive, but biopsies were negative. Four repeated cytologic studies and HPV tests were negative, as were 2 repeated histologic samplings. **D**, Three cells with nuclear grooving and notching and a high N/C ratio. The baseline HPV test result was positive; biopsies demonstrated cervical intraepithelial neoplasia (CIN) 2. **E**, Small aggregate of cells with metaplastic-appearing cytoplasm and variable N/C ratios. The largest cell contains multiple nuclei suggestive of low-grade squamous intraepithelial lesion. The remaining uninucleate cells display higher N/C ratios and nuclear irregularities. The baseline HPV test result was positive, and biopsies demonstrated CIN 3. **F**, Loosely cohesive epithelial cells possessing nuclei with delicate chromatin, a high N/C ratio, and small nucleoli associated with neutrophils. The initial HPV test result and histopathologic specimens were negative. HSIL was not detected on follow-up TPs. (Papanicolaou)

Table 1
Histopathologic Outcomes Associated With ThinPrep Interpretations of ASCUS-L, ASCUS-H, and HSIL*

	Histopathologic Lesions		
ThinPrep Category	**CIN 2 Only**	**CIN 3 or Worse**	**CIN 2 or Worse**
ASCUS-L (n = 764)	6.9	4.7	11.6
ASCUS-H (n = 116)	16.4	24.1	40.5
HSIL (n = 213)	21.6	37.6	59.2

ASCUS-H, atypical squamous cells of undetermined significance, equivocal for HSIL; ASCUS-L, atypical squamous cells of undetermined significance, equivocal for low-grade squamous intraepithelial lesion; CIN, cervical intraepithelial neoplasia; HSIL, high-grade squamous intraepithelial lesion.
* Data are given as percentages. Results are from immediate colposcopy and human papillomavirus triage arms. Excludes results for 3 ASCUS-L and 1 HSIL ThinPreps with unsatisfactory histologic specimens. Cytologic interpretations reflect results of quality control panel review. ThinPrep, Cytyc, Boxborough, MA.

ASCUS-H, was associated with 16.0% of CIN 3 and cancers and 19.8% of lesions CIN 2 and worse **Figure 1**. In the pathology QC review, only 6.8% of TPs were classified as HSIL, but these were associated with 35.6% of lesions CIN 3 or worse.

Analysis of Smears

Pathology QC review of referral smears yielded 41.3% ASCUS-L, 3.9% ASCUS-H, and 4.6% HSIL interpretations. The ASCUS-H category was used with similar frequency in the referral smear and TP even though the 2 specimen types from individual women were infrequently concordant (kappa = 0.19; 95% confidence interval = 0.17-0.20), similar to the reproducibility of other categories, except for HSIL, which showed better agreement. Women with ASCUS-H smears tended to be older than those with ASCUS-H TPs. Oncogenic HPV types were detected in 69.8% of patients with ASCUS-H smears, which was intermediate in frequency between ASCUS-L and HSIL smears ($P < .001$ for trend), although detection was less frequent than for ASCUS-H TPs (85.6%; P <001). Interpretations of ASCUS-H on smears or TPs showed similar trends in disease associations. Among women with smears classified as ASCUS-H, 16.8% had histologic findings of CIN 3 or worse, and 27.2% had lesions CIN 2 or worse **Table 2**.

Discussion

In the present study, both TP and referral smear interpretations of ASCUS-H identified women who shared important clinical characteristics. ASCUS-H was more strongly associated with oncogenic HPV DNA detection and underlying histopathologic lesions CIN 2 or worse than ASCUS-L but represented less risk for these findings than HSIL. TP interpretations of ASCUS-H were associated with HPV DNA detection in 85.6% of women and an underlying lesion of CIN 2 or worse in 40.5%. HPV DNA was detected in 69.8% of women with ASCUS-H smears, and 27.2% had an underlying lesion of CIN 2 or worse. Furthermore, most histopathologic outcomes of high-grade CIN associated with ASCUS-H and HSIL were CIN 3, whereas with ASCUS-L, CIN 2 predominated.

ASCUS-H may be conceptually defined as changes suggestive of HSIL but lacking criteria for a definitive interpretation. Most ASCUS-H represents either poorly sampled high-grade CIN or reactive, degenerative, or artifactual changes that mimic HSIL and may be transient or technique-dependent. Therefore, it is not surprising that women in ALTS were unlikely to receive serial ASCUS-H interpretations on their referral smears and enrollment TPs because ASCUS-H does not represent a stable biologic entity. Furthermore, a previous effort to define criteria that would increase the reproducibility of the ASCUS-H interpretation in smears and narrow its range of disease associations failed.[12] In another study of 20 TPs classified as ASCUS-H, intraobserver reproducibility was only 50% with poor interobserver agreement (kappa = 0.11).[13] Despite the lack of well-defined criteria and poor reproducibility, ASCUS-H has been reportedly associated with CIN 2 or 3 in approximately 24% to 96% of patients in different studies.[8,10-13] Performing routine cytologic-histologic correlations, monitoring reporting rates, and tracking outcomes associated with ASCUS-H may help individual laboratories optimize the use of this interpretation, but reproducibility will likely remain imperfect. In summary, ASCUS-H does not represent a unique biologic entity or a highly reproducible cytologic interpretation because it reflects each cytopathologist's personal uncertainty and diagnostic thresholds. Nevertheless, ASCUS-H has clinical usefulness because of its consistently high positive predictive value for detecting lesions CIN 2 or worse compared with ASCUS-L.

The morphologic spectrum of HSIL in smears and TPs differs, probably reflecting differences in fixation, slide preparation, and staining between the methods. Compared with smears, TPs classified as HSIL may contain more isolated SIL cells, and the area of these cells and their nuclei tend to be smaller with higher nuclear to cytoplasmic ratios.[18] Nuclear hyperchromasia tends to be subtle, but nuclear irregularities often are easily detected.

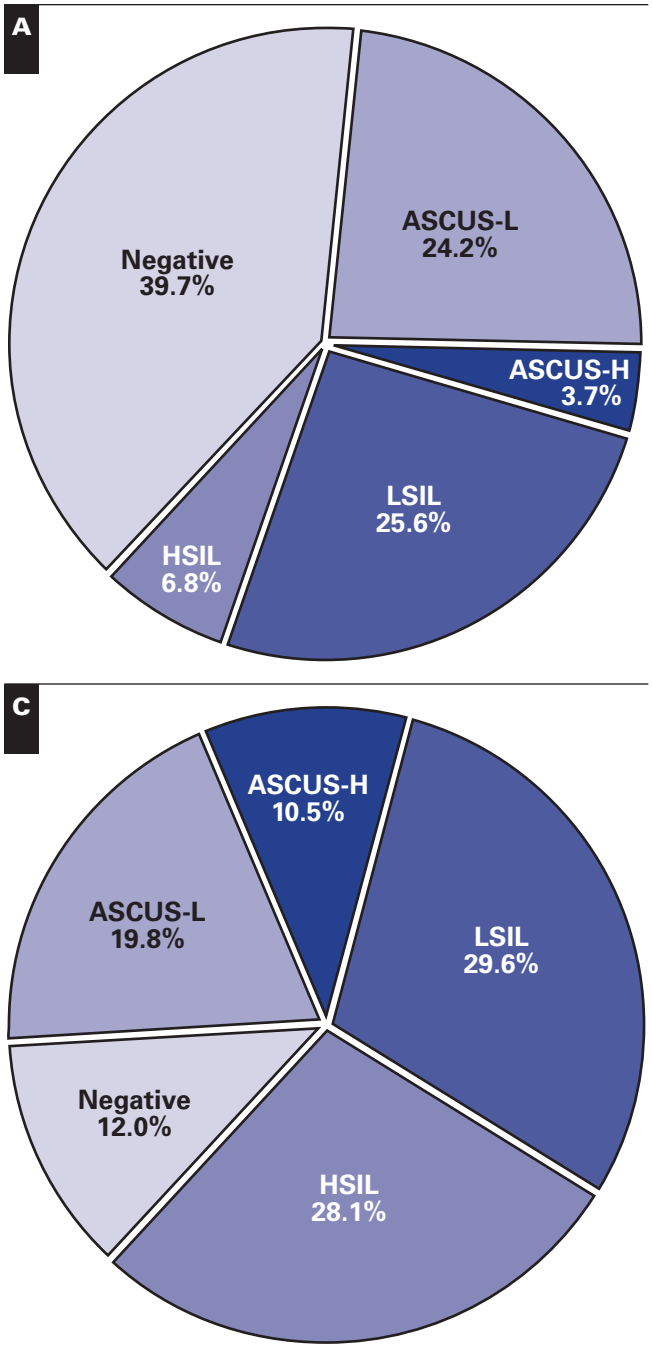

Figure 1 Frequency of utilization of cytologic categories for ThinPreps (Cytyc, Boxborough, MA) by the Pathology Quality Control Group in the ALTS (**A**) compared with the percentage of histopathologic lesions CIN 3 or worse (**B**) and CIN 2 or worse (**C**) preceded by these cytologic interpretations. Unsatisfactory ThinPreps and histologic specimens were excluded. ALTS, ASCUS LSIL Triage Study; ASCUS, atypical squamous cells of undetermined significance; ASCUS-H, ASCUS, equivocal for HSIL; ASCUS-L, ASCUS, equivocal for LSIL; CIN, cervical intraepithelial neoplasia; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion.

Similarly, the present study demonstrates that the appearance of ASCUS-H in smears and TPs also may differ. Smears classified as ASCUS-H may show a variety of findings, including thick sheets of crowded cells, nuclear atypia associated with metaplasia, repair, atrophy, or parakeratosis and equivocal changes resulting from poor preservation or obscuring blood or inflammation.[11,12] In contrast, ASCUS-H TPs displayed a narrower range of changes because poor preservation and obscuring factors were reduced compared with smears. Most TPs classified as ASCUS-H by the pathology panel (without the development of consensus criteria) displayed small aggregates of metaplastic-appearing cells that demonstrated increased ratios of nuclear to cytoplasmic area associated with finely granular chromatin and nuclear irregularities. Some examples resemble the atypical metaplastic cells described previously in smears.[19,20] Our review suggests that when atypical cells show nuclear degeneration or nucleoli, a definitive interpretation of HSIL should be viewed cautiously because these features were not usually associated with lesions CIN 2 or worse. In addition, normal endocervical and endometrial cells and even macrophages may rarely mimic HSIL on TPs depending on the orientation

Table 2
Histopathologic Outcomes Associated With Smear Interpretations of ASCUS-L, ASCUS-H, and HSIL*

	Histopathologic Lesions		
Referral Smear Category	**CIN 2 Only**	**CIN 3 or Worse**	**CIN 2 or Worse**
ASCUS-L (n = 1,347)	5.9	5.5	11.4
ASCUS-H (n = 125)	10.4	16.8	27.2
HSIL (n = 145)	15.9	29.0	44.8

ASCUS-H, atypical squamous cells of undetermined significance, equivocal for HSIL; ASCUS-L, atypical squamous cells of undetermined significance, equivocal for low-grade squamous intraepithelial lesion; CIN, cervical intraepithelial neoplasia; HSIL, high-grade squamous intraepithelial lesion.
* Data are given as percentages. Results are from immediate colposcopy and human papillomavirus triage arms. Excludes results for 2 ASCUS-L and 2 HSIL smears with unsatisfactory histologic specimens. Cytologic interpretations reflect results of quality control panel review.

and preservation of the cells. Conversely, pathologists may learn to recognize HSIL based on minimal qualitative or quantitative evidence (rare cells as illustrated in Images 1D and 1E), but inevitably, this increase in sensitivity will tend to decrease interpretive specificity.

The comfort level of different pathologists with the interpretation of HSIL based on limited evidence varies, and a more conservative interpretation of ASCUS-H may be favored in some young patients in whom a definite cytologic interpretation of HSIL could result in unnecessary ablative therapy. Notably, only 44.8% of women with smears reclassified as HSIL and 59.2% of women with TPs reclassified as HSIL by the QC panel proved to harbor histologic lesions CIN 2 or worse. The unexpectedly low percentage of histopathologically confirmed high-grade lesions in these women compared with screening cytologic interpretations of HSIL generally[21] probably reflects the fact that the ALTS enrolled women with community smears interpreted as ASCUS or LSIL, and, therefore, most clear-cut cases of HSIL were excluded. These findings also suggest that cytologic misclassification is a frequent occurrence even among experts when the quantity, preservation, or visualization of atypical cells is limited, as was the case in many of the smears and TPs reviewed in ALTS. These data cast doubt on the validity of masked or unmasked expert reviews of slides originally interpreted as ASCUS in medicolegal cases.[22]

Current management options for ASCUS-H include referral for colposcopy or repeated cytology. As previously reported, repeated cytology following an initial ASCUS smear fails to identify all women with underlying lesions CIN 2 or worse, even at a threshold of repeated ASCUS for colposcopy referral.[15] Therefore, management of ASCUS-H with repeated cytology might be considered risky, given the strong association with lesions CIN 2 or worse and the possibility of noncompliance with follow-up.

Given the very high sensitivity of HPV testing for detecting lesions CIN 3 or worse in ALTS,[15] HPV testing would seem to permit safe colposcopy triage of women with ASCUS-H. However, the high frequency of oncogenic HPV detection associated with both smears and TPs classified as ASCUS-H undermines the potential usefulness of HPV triage and favors direct colposcopy referral. Furthermore, a positive HPV test result alone is insufficient to upgrade an interpretation of ASCUS-H to definitive HSIL, as fewer than 50% of women with ASCUS-H and oncogenic HPV DNA had histopathologic findings of CIN 2 or worse.

Although ALTS provides a unique opportunity to understand the relationship among cytologic categories, HPV detection, and histologic findings, some limitations are noted. First, the enrollment TPs included in this analysis all were obtained as short-interval repeated samples (mean, approximately 2 months) after an initial smear classified as ASCUS or LSIL in the community rather than as initial cytologic screens. Second, the pathology, colposcopy, HPV testing, and other aspects of this study were standardized through expert reviews, and, therefore, results in community practice may differ. Finally, the vast majority of women in ALTS are premenopausal, limiting the translation of these findings to older women. Nevertheless, the large prospective design of ALTS, the standardized data collection, and the unbiased and nearly complete follow-up are unique features of this study.

The transient nature of most HPV infections and their morphologic correlates, LSIL or CIN 1, suggest that detection of HSIL or histopathologic lesions CIN 2 or worse is central to cervical cancer prevention. Accordingly, using the term ASCUS-H to flag a small number of "suspicious," albeit equivocal, cytologic results for more aggressive follow-up would seem appropriate. The burden of direct colposcopic referral would be relatively minor because ASCUS-H is an uncommon screening cytology interpretation.[8,10-13] Nevertheless, ASCUS-H is a tentative interpretation that may permit gynecologists to spare young women unnecessary treatment if cytologic-histologic correlation and colposcopic findings do not support the diagnosis of a high-grade lesion. Although an individual's risk of harboring CIN 2 or CIN 3 is considerably higher for ASCUS-H compared with ASCUS-L, more lesions CIN 2 or worse are preceded by ASCUS-L because of its numeric predominance in both smears and TPs. Accordingly, effective colposcopy triage of ASCUS-L

remains central to cervical cancer prevention. Data from ALTS related to alternative methods for colposcopy triage of ASCUS and LSIL are given elsewhere[15,23] (M.E. Sherman, MD, M. Schiffman, MD, and J.T. Cox, MD, unpublished data) and are the subject of ongoing analyses.

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References

1. Broder S. From the National Institutes of Health. Rapid communication: the Bethesda System for reporting cervical/vaginal cytologic diagnoses—report of the 1991 Bethesda Workshop. *JAMA*. 1992;267:1892.
2. Collins LC, Wang HH, Abu-Jawdeh GM. Qualifiers of atypical squamous cells of undetermined significance help in patient management. *Mod Pathol*. 1996;9:677-681.
3. Eltabbakh GH, Lipman JN, Mount SL, et al. Significance of atypical squamous cells of undetermined significance on ThinPrep Papanicolaou smears. *Gynecol Oncol*. 2000;79:44-49.
4. Ettler HC, Joseph MG, Downing PA, et al. Atypical squamous cells of undetermined significance: a cytohistological study in a colposcopy clinic. *Diagn Cytopathol*. 1999;21:211-216.
5. Gonzalez D, Hernandez E, Anderson L, et al. Clinical significance of a cervical cytologic diagnosis of atypical squamous cells of undetermined significance favoring a reactive process or low grade squamous intraepithelial lesion. *J Reprod Med*. 1996;41:719-723.
6. Kline MJ, Davey DD. Atypical squamous cells of undetermined significance qualified: a follow-up study. *Diagn Cytopathol*. 1996;14:380-384.
7. Lachman MF, Cavallo-Calvanese C. Qualification of atypical squamous cells of undetermined significance in an independent laboratory: is it useful or significant? *Am J Obstet Gynecol*. 1998;179:421-429.
8. Malik SN, Wilkinson EJ, Drew PA, et al. Do qualifiers of ASCUS distinguish between low- and high-risk patients? *Acta Cytol*. 1999;43:376-380.
9. Ho GY, Bierman R, Beardsley L, et al. Natural history of cervicovaginal papillomavirus infection in young women. *N Engl J Med*. 1998;338:423-428.
10. Genest DR, Dean B, Lee KR, et al. Qualifying the cytologic diagnosis of "atypical squamous cells of undetermined significance" affects the predictive value of a squamous intraepithelial lesion on subsequent biopsy. *Arch Pathol Lab Med*. 1998;122:338-341.
11. Schoolland M, Sterrett GF, Knowles SAS, et al. The "inconclusive–possible high grade epithelial abnormality" category in Papanicolaou smear reporting. *Cancer*. 1998;84:208-217.
12. Sherman ME, Tabbara SO, Scott DR, et al. "ASCUS rule out HSIL": cytologic features, histologic correlates and human papillomavirus detection. *Mod Pathol*. 1999;12:335-342.
13. Quddus MR, Sung CJ, Steinhoff MM, et al. Atypical squamous metaplastic cells: reproducibility, outcome, and diagnostic features on ThinPrep Pap test. *Cancer*. 2001;93:16-22.
14. Schiffman M, Adrianza ME, for the ALTS Group. ASCUS-LSIL Triage Study: design, methods and characteristics of trial participants. *Acta Cytol*. 2000;44:726-742.

15. Solomon D, Schiffman M, Tarone B, for the ALTS Group. The sensitivity of HPV testing and repeat cytology compared to colposcopy for management of ASCUS. *J Natl Cancer Inst*. 2001;93:293-299.
16. Sherman ME, Schiffman MH, Lorincz AT, et al. Cervical specimens collected in liquid buffer are suitable for both cytologic screening and ancillary human papillomavirus testing. *Cancer*. 1997;81:89-97.
17. Ferenczy A, Franco E, Arseneau J, et al. Diagnostic performance of Hybrid Capture human papillomavirus deoxyribonucleic acid assay combined with liquid-based cytologic study. *Am J Obstet Gynecol*. 1996;175(3 pt 1):651-656.
18. Wilbur DC, Dubeshter B, Angel C, et al. Use of thin-layer preparations for gynecologic smears with emphasis on the cytomorphology of high-grade intraepithelial lesions and carcinomas. *Diagn Cytopathol*. 1996;14:201-211.
19. Sheils LA, Wilbur DC. Atypical squamous cells of undetermined significance: stratification of the risk of association with, or progression to, squamous intraepithelial lesions based on morphologic subcategorization. *Acta Cytol*. 1997;41:1065-1072.
20. Sherman ME, Kelly D. High-grade squamous intraepithelial lesions and invasive carcinoma following the report of three negative Papanicolaou smears: screening failures or rapid progression? *Mod Pathol*. 1992;5:337-342.
21. Jones BA, Novis DA. Cervical biopsy-cytology correlation: a College of American Pathologists Q-Probes study of 22,439 correlations in 348 laboratories. *Arch Pathol Lab Med*. 1996;120:523-531.
22. Stoler MH, Schiffman M, for the ALTS Group. Interobserver reproducibility of cervical cytologic and histologic interpretations: realistic estimates from the ASCUS-LSIL Triage Study. *JAMA*. 2001;285:1500-1505.
23. The Atypical Squamous Cells of Undetermined Significance/Low-Grade Squamous Intraepithelial Lesions Triage Study (ALTS) Group. Human papillomavirus testing for triage of women with cytologic evidence of low-grade squamous intraepithelial lesions: baseline data from a randomized trial. *J Natl Cancer Inst*. 2000;92:397-402.