Controversy exists in the United States regarding the proper evaluation and management of low-grade squamous intraepithelial lesion (LSIL) and equivocal (atypical squamous cells of undetermined significance [ASC-US]) cervical cytologic interpretations. To address this issue, the National Cancer Institute initiated the ASCUS-LSIL Triage Study (ALTS). ALTS is a multicenter, randomized clinical trial designed to evaluate 3 alternative methods of management, namely, immediate colposcopy, cytologic follow-up, and triage by human papillomavirus (HPV) DNA testing. This article summarizes the major findings of ALTS that have been published to date. Patients with ASCUS (n = 3488) or LSIL (n = 1572) were randomly assigned to research arms between November 1996 and December 1998, and were monitored for 2 years. The disease outcome was histologic cervical intraepithelial neoplasia (CIN) 3/cancer. The prevalence of oncogenic HPV was too high to permit effective triage of LSIL using HPV DNA testing by Hybrid Capture 2. However, for the women referred with a cytologic interpretation of ASCUS, HPV triage proved useful, with sensitivity immediately equal to direct colposcopy and a halving of colposcopic referrals. Among older women with ASCUS, HPV testing remained sensitive for detecting CIN 3 and cancer, but the referral percentage was dramatically lower compared to younger women. ALTS yielded insight into the performance of cytology and histopathology; experienced pathologists differed significantly in their interpretations of cervical abnormalities, especially histologic CIN 1 and cytologic ASCUS. Nonetheless, it was possible to distinguish a relatively uncommon type of ASCUS, equivocal for high-grade squamous intraepithelial lesion, that has a high positive predictive value for identifying women with underlying high-grade CIN. Many additional analyses are underway.

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Controversy exists in the United States regarding the proper evaluation and management of low-grade squamous intraepithelial lesion (LSIL) and equivocal (atypical squamous cells of undetermined significance [ASC-US] now ASC-US) cervical cytologic diagnoses. A series of small studies and 1 large observational project indicated that testing for HPV might be useful in the triage of ASCUS, LSIL, or both. To address this issue, the National Cancer Institute initiated the ASCUS-LSIL Triage Study (ALTS). ALTS is a multicenter, randomized clinical trial designed to evaluate 3 alternative methods of management, namely, immediate colposcopy, cytologic follow-up, and triage by human papillomavirus (HPV) DNA testing. This article reiterates the major findings of ALTS that have been published to date.

Globally, cervical cancer is the second most common malignancy of women. In the United States, there are about 12,800 cases of carcinoma resulting in about 4,600 deaths annually. Approximately 0.6% or 330,000 of the 55 million Papanicolaou (Pap) tests estimated to be performed each year are diagnosed as high-grade squamous intraepithelial lesion (HSIL). In comparison, about 2% to 3% are diagnosed as LSIL. The prevalence of LSIL is a function of the prevalence of acute, sexually transmitted HPV infection that, in turn, is highly dependent on the age and related numbers of new sexual partners of the population being screened. The prevalence of ASCUS is more arbitrary, because it represents a poorly defined fraction of the previous expansive “benign atypia” or “Pap Class 2” classification. In the United States, more than 2 million Pap tests per year are interpreted as ASCUS.

Virtually all US health care providers agree that women with cytologic HSIL require colposcopic examination and that those with colposcopic evidence of a significant lesion require cervical biopsy. If the histologic diagnosis is cervical intraepithelial neoplasia (CIN) 2 or more severe, ablative or excisional treatment now relies most commonly on electrosurgical loop excision procedure, cryosurgery, or more rarely, cold-knife conization. No universal agreement exists for managing LSIL or ASCUS. Most low-grade lesions will regress spontaneously, and many equivocal lesions will be shown to be benign. However, management of ASCUS/LSIL is potentially of concern, given that a small but important minority may have CIN 2 or 3, or even carcinoma on colposcopy and biopsy. As a result, many clinicians are not willing to follow ASCUS/LSIL for possible regression, out of concern for missing underlying malignancy.
The need for clear management guidelines for women with ASCUS and LSIL, specifically for more cost-effective approaches to triaging women to colposcopy, was discussed at the 1991 Bethesda Workshop. This discussion was followed by a National Cancer Institute–sponsored workshop to discuss the feasibility of conducting a randomized clinical trial, and to outline the viable management strategies to be evaluated. Although the participants disagreed strongly as to the proper management of ASCUS and LSIL, they agreed as to the main possible choices that merited consideration, namely, immediate colposcopy, cytologic follow-up, and triage using HPV DNA testing.

Advances in HPV DNA testing have produced the first truly accurate and reproducible HPV assay systems. Accurate HPV DNA testing could be helpful to the management of ASCUS/LSIL in 2 ways. First, the type of HPV (low-risk or cancer-associated) is associated with the severity of squamous intraepithelial lesions and their natural history. Secondly, the presence or absence of cancer-associated types of HPV can help predict the accuracy of the original cytologic diagnosis of equivocal and low-grade lesions, in that HPV-negative patients are more likely to have false-positive cytologic diagnoses. It is currently not possible to achieve interpretative consensus using traditional teaching practices, further arguing for the use of an ancillary technique.

With a mandate from a community of clinicians and researchers, the National Cancer Institute began ALTS. Nonpregnant women, 18 years of age and older, with ASCUS or LSIL and with no prior hysterectomy or ablative therapy to the cervix, were referred to 1 of 4 clinical centers around the United States. Eligible and consenting participants were administered a risk factor questionnaire and underwent a pelvic examination, collection of cervical specimens for ThinPrep liquid-based cytology and HPV testing by Hybrid Capture 2 (HC 2), and cervicography.

The choices of ThinPrep and HC 2 were made by formal reviews approved by the ALTS Steering Committee. Both techniques were quite new at the time of their inclusion, but were judged to be at least as good or better than other options. Moreover, they could be done using a single specimen and were already partly standardized.

Patients with ASCUS (n = 3488) (Figure 1) or LSIL (n = 1572) (Figure 2) were randomized between November 1996 and December 1998 to 1 of 3 research arms: (1) immediate referral to colposcopy at enrollment; (2) enrollment testing for HPV DNA (and cytology at an HSIL threshold) to triage to colposcopy; and (3) follow-up with cytology only, using a referral threshold of HSIL. All women were followed up every 6 months for 2 years with pelvic examinations, cytology, masked HPV testing, and masked cervicography. Digital cervical images and cytology and histology slides were externally reviewed to maximize patient safety.

An independent Data and Safety Monitoring Board monitored ALTS. Early in the trial, the Committee voted to close the HPV triage arm for women referred with a cytologic interpretation of LSIL. The prevalence of onco-
genic HPV was too high to permit effective triage of LSIL using HC 2.9

However, for the 3488 women referred with a cytologic interpretation of ASCUS, HPV triage proved useful.10 The underlying prevalence of histologically confirmed CIN 3+ at enrollment was 5%, as expected. Testing for cancer-associated HPV DNA identified 96% (95% confidence interval [CI] = 92%–99%) of women with CIN 3+, while referring 56% of the population to colposcopy. A single repeat cytology using a triage threshold of HSIL identified 44% (95% CI = 36%–53%) of women with CIN 3+, while referring 8%. A lower cytology triage threshold of ASCUS identified 85% (95% CI = 78%–91%), while referring 58%.

We concluded that HC 2 testing for cancer-associated HPV DNA was a viable option in the management of women with ASCUS, demonstrating greater sensitivity for detection of CIN 3+ and comparable specificity compared to a single repeat ASCUS or worse cytology. A strategy of multiple repeat cytology evaluations would be expected to increase sensitivity, but with consequent problems of loss to follow-up, costs of additional visits, and increased referral to colposcopy. More detailed analyses11 and a cost analysis from another group12 have supported this finding. The ALTS cost-utility analysis is pending.

We further analyzed whether considering age, viral load, or other factors would improve the performance of HPV testing relative to repeat thin-layer cytology for women enrolled in ALTS.13 We determined the theoretical sensitivity of HPV testing and repeat thin-layer cytology for detecting CIN 3 and cancer, as well as the percentage of women referred for colposcopy (referrals) in 2198 women with ASCUS and 848 with LSIL. We restricted the analyses to women in the immediate colposcopy or HPV triage arms, in which there was comparably complete determination of CIN 3 and cancer (excluding the relatively insensitive conservative management arm). We analyzed results by age (in tertiles) and by low (1.0 pg/mL) and high (10.0 pg/mL) thresholds of HPV load. Among women with positive HPV tests, we compared median HPV DNA content with histopathologic diagnoses.

Among women with ASCUS aged 29 years or older, HPV testing at 1.0 pg/mL detects 94% (95% CI = 86%–100%) of CIN 3 lesions or cancers and would have resulted in the referral of 31% (95% CI = 28%–34%) of women for colposcopy. Human papillomavirus testing in younger women with ASCUS would have resulted in referral of more than 65% of women. By contrast, among women with ASCUS aged 29 years or older, repeat cytology had a sensitivity of 91% (95% CI = 81%–100%) and would have resulted in the referral of 50% (95% CI = 47%–54%) of women in this age group. Ignoring age, HPV testing performed using a 10.0 pg/mL threshold for referral would have decreased sensitivity 5% and referred 12 fewer women. Among women with LSIL, more than 63% would have been referred using any cytologic or virologic strategy that detected 90% of CIN 3 lesions and cancers. Higher median viral loads were significantly associated with histopathologically confirmed CIN, but load tended to decrease with increasing grade and overlapped considerably among diagnoses. Therefore, viral load was not useful for predicting severity of CIN.

We concluded that among older women with ASCUS, HPV testing remained highly sensitive for detecting CIN 3 and cancer, but the theoretical percentage of women referred for colposcopy was dramatically lower compared with younger women. This result may have implications for developing cost-effective management protocols. Neither a single HPV test nor repeat cytology provides useful triage for women with LSIL.

We also evaluated the sensitivity of cervicography to detect potential cervical cancer precursor lesions in women participating in ALTS based on original and new cervigram evaluation strategies.14 Cervicography functioned moderately well at detecting CIN 2 or 3 in women with ASCUS and LSIL Pap test results. Maximum utility was realized at the original positive cervigram threshold of atypical or higher. Test performance was better in younger women. The sensitivity of cervicography was not enhanced by use of a new method of interpretation using a team of evaluators.

One important product of ALTS has been insight into the performance of cytology and histopathology. Experts differ significantly in their diagnoses of cervical abnormalities. We compared different pathologists’ diagnoses of 4915 thin-layer Pap tests and 5140 biopsy specimens that were collected when women enrolled in ALTS.15 In each case, the Pap tests and biopsies were evaluated by an experienced pathologist at the clinical center where the patient had enrolled and by another expert pathologist at an independent quality control center.

There were substantial differences between the pathologists in interpreting both thin-layer Pap tests and biopsies; agreement (reproducibility) was only moderate. Overall, agreement on the biopsy specimens was not substantially greater than agreement on the thin-layer Pap test specimens. Agreement was about the same for tissue specimens whether they were obtained through punch biopsies or loop electrocautery excision procedures. Cytologic ASCUS and histologic CIN 1 were particularly nonreproducible. We concluded, as we did in another international study,16 that variability among experts in interpreting Pap tests and biopsies should be taken into consideration when using these interpretations and when developing standards of practice.

Recently, we addressed subdivisions of ASCUS using ALTS materials.17 The 1991 Bethesda System recommended that pathologists qualify cervical cytology interpretations of ASCUS as “favor reactive” or “favor a squamous intraepithelial lesion (SIL).” However, the current focus of cervical cancer screening on detection and treatment of high-grade disease (rather than all SIL), suggests that separating “equivocal HSIL” from other types of ASCUS may have greater clinical utility. (This study, performed prior to the 2001 Bethesda System Workshop, used the terms ASCUS-L and ASCUS-H, but these should be considered comparable to the current 2001 Bethesda terminology of ASC-US and ASC-H, respectively.)18 We compared findings associated with cytologic interpretations of ASCUS-L, ASCUS-H, and HSIL using data from ALTS. The frequency of oncogenic HPV detection and underlying lesions of CIN 2 or higher and CIN 3 or higher in women with ASCUS-H was intermediate between that of ASCUS-L and HSIL. Oncogenic HPV DNA was detected in 86% of women with ThinPreps classified as ASCUS-H and 70% of women with ASCUS-H conventional smears. Histopathologic lesions graded CIN 2 or higher were detected in 40% of women with ASCUS-H ThinPreps and 27% of those with ASCUS-H smears; most of these lesions were CIN 3. Nonetheless, more high-grade lesions were preceded by ASCUS-L than by ASCUS-H, because ASCUS-L in-
terpretations were much more common. We conclude that ASCUS-H is a relatively uncommon type of ASCUS that has a high positive predictive value for identifying women with underlying high-grade CIN.

The final prospective data regarding ASCUS/LSIL management were presented to the American Society for Colposcopy and Cervical Pathology Workshop last September and have contributed to newly published guidelines\textsuperscript{19} that would lead to clarification of equivocal cytologic interpretations and optimal use of colposcopic resources.

Although ALTS data, including the final prospective data, suggest that women with ASCUS who are HPV DNA negative do not need colposcopy,\textsuperscript{20} the remaining half of women with ASCUS who are HPV DNA positive, along with women with LSIL will be referred for colposcopy.\textsuperscript{21} As a result, it is important to understand how to manage the approximate 2 million women annually who would undergo colposcopy based on new management guidelines.

A colpobiotherapy histologic diagnosis of CIN 2 or 3 generally triggers treatment. However, the optimal clinical management of women with histologic CIN 1 or less at colpobiosis is not established. Recently completed ALTS analyses consider this question by assessing the risk of CIN 3 during 2 years of follow-up among all women in the Immediate Colposcopy and HPV Triage randomization arms. It appears that once colposcopy has excluded evident CIN 2 or 3, all women with less than CIN 2 at first colposcopy should be managed similarly thereafter based on comparable risk for subsequent CIN 3, whether the initial colposcopy and directed biopsy result was negative or CIN 1.\textsuperscript{22} According to ALTS follow-up data, either cytology or HPV DNA testing might be used for postcolposcopy management.\textsuperscript{23}

More than 30 additional analyses have already been designed from the ALTS database, addressing many aspects of the natural history and clinical management of mild cervical abnormalities.

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