Histopathologic Extent of Cervical Intraepithelial Neoplasia 3 Lesions in the Atypical Squamous Cells of Undetermined Significance Low-grade Squamous Intraepithelial Lesion Triage Study: Implications for Subject Safety and Lead-time Bias

Mark E. Sherman, Sophia S. Wang, Robert Tarone, Laurie Rich, and Mark Schiffman

The National Cancer Institute, Division of Cancer Epidemiology and Genetics, Bethesda, Maryland 20892 [M. E. S., S. S. W., R. T., M. S.], and Information Management Services, Silver Spring, Maryland [L. R.]

Abstract

Cervical intraepithelial neoplasia 3 (CIN3) is the precursor of most squamous carcinomas and serves as a surrogate end point. However, small CIN3 lesions are rarely associated with concurrent invasion. We hypothesized that aggressive follow-up for cytology of atypical squamous cells of undetermined significance (ASCUS) or low-grade squamous intraepithelial lesion (LSIL) leads predominantly to detection of smaller CIN3 lesions than those usually associated with cancer. We assessed this hypothesis in a masked histopathologic review of 330 CIN3 lesions in the ASCUS LSIL Triage Study, focusing on ASCUS referrals. ASCUS referrals underwent randomized management [colposcopy for repeat cytology of high-grade squamous intraepithelial lesion (HSIL), colposcopy for oncogenic human papillomavirus (HPV) detection or repeat HSIL, or immediate colposcopy]; then all were followed with repeat cytology for 2 years, followed by colposcopy and aggressive treatment. We assessed all CIN3 lesions qualitatively and measured 39 of them. CIN3 lesions were overwhelmingly small. Compared with enrollment, lesions found at follow-up or exit involved fewer tissue fragments (P < 0.01) and showed less diffuse gland involvement (P = 0.03). CIN3 lesions found postenrollment after HPV testing involved the fewest tissue fragments [versus immediate colposcopy (P = 0.04) or repeat cytology of HSIL (P = 0.02)], and none showed diffuse gland involvement. The median distal-proximal length was 6.5 mm (median replacement of total epithelium = 5%) in the 39 measured cases. We conclude that CIN3 lesions underlying ASCUS or LSIL generally lack features associated with invasion, particularly if managed using HPV testing, suggesting that aggressive management leads to early detection of CIN3 but probably prevents relatively few cancers in screened populations.

Introduction

The goal of cervical cancer prevention programs is to detect and treat all committed cancer precursors before invasion develops. Although it cannot be determined whether any individual precursor lesion will progress to cancer, data indicate that higher grades of CIN3 are more likely to persist and progress than lower grade lesions (1–4). Specifically, most CIN1 lesions, and a smaller, but substantial proportion of CIN2 lesions, regress spontaneously, and the HPV infections that cause these lesions become undetectable using currently available methods. In contrast, untreated CIN3 lesions pose a substantially greater cumulative cancer risk over time, which makes CIN3 an attractive surrogate end point for cancer in cervical screening studies.

The true natural history of CIN3 is unknown because it would be unethical to follow women prospectively with untreated CIN3 for prolonged periods. However, the fact that CIN3 lesions are detected ~10 years earlier than microinvasive carcinoma (5) implies that most CIN3 lesions persist for lengthy periods before becoming invasive. In a study that examined outcomes after incomplete treatment of cervical carcinoma in situ, 22% of 131 women developed invasion, and 69% had persistent cervical carcinoma in situ during follow-up of 5–19 years (6). More recently, Nobbenhuis et al. (7) carefully followed a group of women until they had developed extensive colposcopic evidence of CIN3 without any patients developing carcinoma, consistent with the hypothesis that invasion usually occurs after years of persistence and intraepithelial expansion of CIN3.

Similarly, pathologic studies have suggested that CIN3 lesions are heterogeneous with respect to immediate invasive potential (8–11). In one study, CIN3 lesions associated with invasion were seven times larger than CIN3 lesions without coexisting invasion (8). Cytoplasmic maturation and necrosis in CIN3 lesions were also associated with invasion (8).

Characterizing the size and appearance of CIN3 lesions detected by different screening modalities can provide insights into the performance of these methods that would be missed by

Received 8/8/02; revised 12/10/02; accepted 1/18/03.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 Supported by The National Cancer Institute and NIH Department of Health and Human Services contracts CN-55153, CN-55154, CN-55155, CN-55156, CN-55157, CN-55158, CN-55159, and CN-55105.

2 To whom requests for reprints should be addressed, at National Cancer Institute, Division of Cancer Epidemiology and Genetics, 6120 Executive Boulevard, Room 7080, Rockville, MD 20892-7374. Phone: (301) 594-7661; Fax: (301) 402-0916; E-mail: shermann@nih.gov.

The abbreviations used are: CIN, cervical intraepithelial neoplasia; HPV, human papillomavirus; ASCUS, atypical squamous cells of undetermined significance; ALTS, atypical squamous cells of undetermined significance low grade squamous intraepithelial lesion triage study; LSIL, low-grade squamous intraepithelial lesion; LEEP, loop electrosurgical excision procedure; HSIL, high-grade squamous intraepithelial lesion; TDS, total dimension score; CI, confidence interval.

3 The abbreviations used are: CIN, cervical intraepithelial neoplasia; HPV, human papillomavirus; ASCUS, atypical squamous cells of undetermined significance; ALTS, atypical squamous cells of undetermined significance low-grade squamous intraepithelial lesion triage study; LSIL, low-grade squamous intraepithelial lesion; LEEP, loop electrosurgical excision procedure; HSIL, high-grade squamous intraepithelial lesion; TDS, total dimension score; CI, confidence interval.
simply comparing the number of lesions detected. Assessing
the size of CIN3 lesions is clinically relevant because extensive
CIN3 lesions are more difficult to treat than smaller ones and
have a higher risk of recurrence (12). However, aggressive
follow-up of women with ASCUS and LSIL cytology that
identifies tiny CIN3 lesions lacking immediate invasive poten-
tial might be considered to represent lead-time bias compared
with routine follow-up if delayed detection would have resulted
in equivalent outcomes.

Analysis of enrollment data from the ALTS has demon-
strated that repeat cytological testing, even using the most
sensitive threshold of repeated ASCUS for colposcopy referral
(rather than HSIL as dictated by the ALTS protocol), was
significantly less sensitive than HPV testing but would have
referred a comparable percentage of women (13). Among
women with LSIL, neither repeated cytology nor HPV testing
would have achieved high sensitivity and reduced referrals
(14). Professional societies, based on results from ALTS and
other studies, have concluded that HPV testing is the “pre-
ferred” method for managing women with ASCUS when viro-
logic testing can be performed concurrently with the cytological
interpretation (15).

Given the rarity of cervical cancer among young women
and association between the extent of CIN3 and risk of inva-
sion, we assessed the size of the CIN3 lesions that were de-
tected in ALTS to understand the overall cancer risk of women
in the trial and determine whether risk varied by management
arm. Given that HPV testing was more sensitive for detecting
CIN3 lesions than repeat cytology, we hypothesized that CIN3
lesions discovered after management with HPV testing would
be smaller than those found among women managed with
repeat cytology.

Materials and Methods

Study Population. ALTS enrolled 3488 eligible volunteers
with community smears reported as ASCUS and 1572 with
LSIL residing in four regions in the United States (13, 14,
16–19). Ethical review boards at the National Cancer Institute
and clinical study centers approved the study. Details of the
study design and analysis of key enrollment findings are pre-
sented elsewhere (13, 14, 16–19).

Enrollment Procedures. At enrollment, clinicians obtained a
cervical sample with a Papanicolaou (Waltach Surgical De-
VICES, Orange, CT) that was rinsed in 20 ml of PreservCyt
(Cytec Corp., Boxborough, MA). The resulting cell sus-
pensions were first used to prepare a ThinPrep slide, and then 4 ml
of residual specimen were used for HPV DNA testing. HPV
testing was performed for oncogenic types was performed using the Hybrid
Capture 2 microplate assay (Digene Corp., Gaithersburg, MD)
at a threshold of 1 pg/ml (~5000 viral copies; Refs. 13, 14, and
16–20). Patients were randomized to one of three initial (en-
rollment) management strategies: (a) conservative management
with referral to colposcopy for repeat cytology of HSIL (CM-
Arm); (b) referral to colposcopy for a positive HPV test or
repeat cytology of HSIL (HPV-Arm); or (c) immediate referral
to colposcopy (IC-Arm). The cytological and histopathologic
interpretations made at the four ALTS clinical centers were
used for patient management. Patients with histopathologic
CIN2 or worse (including endocervical adenocarcinoma in situ)
were treated, almost exclusively by LEEP. Rarely, women
were referred for colposcopy because reviews conducted by pathol-
ogy or colposcopy quality control groups identified previously
unrecognized findings of concern on slides or cervigrams
(National Testing Laboratories, Fenton, MO). In addition, re-
peat colposcopy was performed for an unconfirmed clinical suspi-
on of CIN2 or worse in a small number of women.

Postenrollment (Follow-up and Exit) Procedures. After the
enrollment examination, randomization, and colposcopy if in-
dicated, every patient referred for ASCUS was followed simi-
larly with repeat thin-layer cytology every 6 months for 2 years.
Women were referred to colposcopy for cytological HSIL or
safety net notifications. The Data and Safety Monitoring Board
required two modifications of the follow-up protocol for LSIL
referrals: (a) the HPV-Arm was closed early for women with
LSIL because the high percentage of women who tested posi-
tive for HPV (83%) was not considered to represent an im-
provement over automatic colposcopy referral (14); and (b) an
interim analysis of data among LSIL referrals demonstrated that
the CM-Arm was insensitive in detecting CIN3. For safety,
subjects referred for LSIL who were randomized to the CM-
Arm and remained in follow-up were immediately referred to
colposcopy if this had not been already performed, leading to
slightly earlier detection of some CIN3 lesions.

After 2 years of follow-up, all women remaining in the
trial participated in an exit visit that included colposcopy, and
LEEP was performed for women with CIN2 or worse or per-
sistent LSIL. Therefore, the exit visit identified cases of CIN3
that were not detected by cytology, HPV testing, or colposcopi-
cally directed biopsy. These CIN3 lesions may have been
present at enrollment but were not detected (“missed prev-
lent”) or developed during 2 years of follow-up (bona fide
incident disease).

LEEPs were prepared for histopathologic study in a con-
ventional manner by sectioning the cone-shaped resections from
distal (ectocervix) to proximal (endocervix), creating tissue
fragments lined on one side by squamous and/or endocer-
vascular epithelium, often with underlying endocervical glandular
invaginations (Fig. 1). In well-oriented specimens, serial sec-
tioning created tissue slices circumferentially distributed about
the cervical os in a manner resembling the relationship of the
numbers on the face of a clock to its center. Fragmented or
poorly oriented specimens were sectioned to maximize visual-
ization of the mucosal surfaces. In general, blocks of his-
topathologic specimens had been cut at three levels to prepare
standard H&E-stained sections. Rarely, additional levels had
been cut to search more thoroughly for lesions that were not
identified on initial sections.

A pathology quality control group performed masked re-
views of all referral smears and enrollment thin-layer slides, a
sample of thin-layer slides obtained during follow-up and all
exit cytology. The pathology quality control group also
reviewed all histopathologic specimens obtained throughout the
trial, which provided the basis for the primary trial end point,
histopathologic CIN3. The algorithm used for the panel review
and developing final diagnoses are detailed elsewhere (13, 14,
16–19).

Intensive Descriptive Histopathologic Review of CIN3. At
the end of the field effort, all available histopathologic slides of
specimens obtained from patients that were diagnosed with
CIN3 or cancer by the pathology quality control group were
assembled for three of the four clinical centers. Release of
slides from one center was proscribed by institutional policy.

A single reviewer (M. E. S.) masked to referral cytology
interpretation (ASCUS or LSIL), study arm (CM, HPV, or IC),
and phase of the trial (enrollment, follow-up, or exit) recorded
descriptive features using a standardized, preprinted data col-
lection instrument that listed the original pathology quality
control group diagnosis by block. The review focused on de-
scribing the size and appearance of the CIN3 lesions rather than confirming the diagnosis established previously by the pathology panel.

For each block, the following data were recorded: (a) total number of pieces of tissue; (b) number of pieces of tissue containing CIN3; (c) qualitative assessment of the linear extent of CIN3 for each involved piece (potentially multiple pieces per block); and (d) qualitative assessment of gland involvement. Tissue that was denuded or appeared to have represented a fragment of a larger piece was not counted separately. To qualitatively assess the linear extent of CIN3, we identified the distal (toward the ectocervix) and proximal (toward the endocervix) limits of each focus and then visually compared this linear span to the total length of the epithelium on that piece of tissue. Lesions were classified as focal if <25% of the length of the epithelium was involved, moderate if >25% but <50% was involved, and diffuse if ≥50% was involved. These measurements were combined to create a TDS (see below) for each case. Punch biopsies and small tissue pieces in LEEPs were classified as focal or moderate exclusively. Gland involvement by CIN3 was classified as none, focal if fewer than three spaces were involved or growth was limited to the superficial portions of glands, or diffuse if three distinct spaces were ≥50% filled. The presence of cytoplasmic maturation, necrosis, or distal extension beyond the last endocervical gland was noted for each case.

Quality Control Review. At the completion of the intensive descriptive review, we examined a subset of 40 LEEPs a second time in a masked fashion to determine the reproducibility of assessments and measure (in millimeters) each focus of focal, moderate, and diffuse CIN3 and the sum of these measurements per case. Subsequently, these cases were reviewed a third time to record the total extent of surface epithelium, excluding areas of denudation and endocervical invaginations (favoring an underestimate of the total epithelium and, therefore, an overestimate of the percentage involved with CIN3). This subset of 40 cases was selected as a stratified random sample of those LEEP specimens from each study arm with an original pathology quality control group diagnosis of CIN3. Assessment of the total number of tissue fragments containing CIN3 per case was moderately reproducible (κ = 0.51, 95% CI = 0.34–0.68) as was the subclassification of focal, moderate, or diffuse foci per block (κ = 0.59, 95% CI = 0.44–0.75) and extent of gland involvement (κ = 0.41, 95% CI = 0.17–0.65). Lesions classified as focal, moderate, and diffuse had median lengths of 2, 3.5, and 6 mm, respectively, as determined by microscopic identification of the limits of each lesion and measurement of the span with a conventional ruler in 40 cases.

Analysis. We separately analyzed data for women referred into the trial with community smear interpretations of ASCUS and LSIL. Our analysis focused on women referred with ASCUS because these women were followed according to the study protocol. The size of the CIN3 lesions was based on assessment of the LEEP specimens for cases in which the LEEP showed CIN3. In the remaining cases, in which CIN3 was demonstrated only on biopsies or curettings (the LEEP demonstrated CIN2 or a less severe lesion), the size of the CIN3 lesion was assessed using these specimens. The presence of necrosis, cytoplasmic maturation, and distal extension beyond the last endocervical gland was assessed using all slides that demonstrated CIN3.

To provide a summary statistic for the size of the CIN3 lesions in ALTS (especially those that we did not attempt to measure), we constructed a TDS for each case as follows: TDS equals the number of tissue fragments scored as focal times one plus the number of tissue fragments scored as moderate times two plus the number of tissue fragments scored as diffuse times three. The TDS per case was a function of both the distal-proximal length of each CIN3 focus and number of tissue pieces involved, which reflected the degree of circumferential involvement of the cervix. As would be predicted, the TDS was highly correlated with the total number of pieces involved by CIN3 among the 330 reviewed (Spearman correlation coefficient = 0.93) and total linear measurement in millimeters in the subset of 40 cases (Spearman correlation coefficient = 0.97). The main analysis compared the number of pieces of tissue involved with CIN3, the TDS, and the extent of gland involve-
ment with CIN3 for women referred with ASCUS or LSIL by enrollment arm (CM, HPV, and IC) and phase of the trial. Cases were divided into two groups based on the phase of the trial during which they were discovered: (a) lesions found at enrollment; and (b) all other CIN3 lesions found in the trial either during follow-up or at the exit visit (referred to collectively as “postenrollment”). Lesions detected at enrollment directly reflected the performance of the three different management strategies used in each arm. After enrollment, the protocol for all patients was identical; therefore, CIN3 lesions detected postenrollment were either missed by the initial management strategy used at enrollment (missed prevalent) or developed postenrollment (incident).

In a separate analysis that specifically used exit visit data exclusively (which provided essentially complete ascertain-ment of CIN3 lesions), we compared concurrent cytological interpretations and colposcopic impressions with the mean TDS ± SE, combining results for all arms. We limited this analysis to exit data because ascertainment of CIN3 was incomplete during follow-up (women were referred for colposcopy only for cytology of HSIL or a safety net determination).

Nonparametric analyses of variance for the number of tissue pieces involved with CIN3 and TDS were performed using the Kruskal-Wallis test (three-way comparisons) and Wilcoxon rank-sum test (two-way comparisons). The extent of gland involvement was assessed using the standard χ² test. For ASCUS referrals, the number of pieces containing CIN3 was presented as a box plot and extent of gland involvement as a bar graph, with each bar subdivided into focal and diffuse involvement.

Results
Distribution of Cases by Referral Interpretation, Study Arm, and Phase of the Trial. A total of 539 women in ALTS received a histopathologic diagnosis of CIN3 or worse at some time point during the trial, including 7 women with invasive carcinoma, all of whom were referred to colposcopy at enrollment. Slides contributed by one of four participating centers (177 cases) were unavailable for intensive descriptive review. We excluded 18 cases diagnosed on a biopsy or curettage (evenly distributed by triage arm) because a LEEP was performed but not available for review, precluding an accurate assessment of lesion size. We also excluded 1 case in which carcinoma merged imperceptibly with the CIN3 lesion, precluding accurate evaluation, and 4 cases in which key slides were missing. In addition, we excluded 9 cases in which CIN3 was not confirmed in this review. In 9 CIN3 cases diagnosed by the ALTS quality control group, CIN3 was not found in the post-trial descriptive review; 4 (44%) of the 9 tested negative for HPV at enrollment as compared with 4% of all CIN3 cases detected at enrollment.

Microscopic review was successfully completed for 330 cases, of which 161 had received referral cytological reports of ASCUS and 169 of LSIL. A total of 171 (51.8%) cases was diagnosed at enrollment and 159 (48.2%) postenrollment. The median age of cases was 23 years (mean = 25 years) with 90% of women aged ≤34 years. There were 54 (33.5%) women referred for ASCUS and 61 (36.1%) referred for LSIL in whom histopathologic CIN3 was diagnosed on a biopsy or curettage, which led to the performance of a LEEP that demonstrated only CIN2 or a less severe diagnosis. The diagnoses for these 115 LEEPs were negative in 44 (38.3%), squamous atypia in 6 (5.2%), koilocytic atypia or CIN1 in 21 (18.3%), and CIN2 in 44 (38.3%). The total number of pieces per LEEP (regardless of morphology) was not strongly associated with the extent of CIN3 (data not shown). Neither the TDS nor extent of gland involvement was significantly correlated with patient age (data not shown).

Among ASCUS referrals, the total number of CIN3 lesions detected in the IC-Arm during the trial was highest in the center excluded from this analysis. Consequently, the total number of cases among ASCUS referrals in the IC-Arm is lower than for other arms in this analysis, although these data are similar when data for all four centers are considered. The size of the CIN3 lesions, as assessed by the number of tissue blocks determined to be involved in the original ALTS pathology panel review, was similar for all four centers (data not shown).

ASCUS Referrals: Extent and Characteristics of CIN3. Among ASCUS referrals in this analysis, 58 CIN3 cases were detected in the CM-Arm, 64 in the HPV-Arm, and 39 in the IC-Arm. In the CM-Arm, 34.5% of CIN3 lesions were found at
enrollment as compared with 70.3% in the HPV-Arm and 51.3% in the IC-Arm ($P < 0.001$).

Among ASCUS referrals, the total number of tissue fragments containing CIN3 did not differ significantly between study arms when all phases of the trial were combined or when data for the arms were compared at enrollment. However, differences between arms were found when postenrollment data were analyzed separately ($P = 0.04$; Fig. 2). At enrollment, CIN3 lesions in the HPV-Arm involved more tissue fragments per case than in the other arms, but pair-wise comparisons did not demonstrate statistically significant differences. In contrast, CIN3 lesions detected postenrollment involved significantly fewer tissue fragments in the HPV-Arm as compared with the IC-Arm ($P = 0.04$) or CM-Arm ($P = 0.02$). CIN3 lesions detected postenrollment (all arms combined) involved fewer fragments than at enrollment ($P < 0.01$). A significant difference between enrollment and postenrollment was found in the HPV-Arm ($P = 0.001$) but not in the CM- or IC-Arm.

Among ASCUS referrals, the overall comparisons for TDS were similar to those for the number of pieces involved with CIN3 (data not shown). However, postenrollment, the TDS was only marginally smaller for cases in the HPV-Arm as compared with the CM-Arm ($P = 0.08$) and IC-Arm ($P = 0.07$).

Among ASCUS referrals, involvement of endocervical glands by CIN3 was identified in 89 (55.3%) women, but only 16 (9.9%) had diffuse involvement (Fig. 3). The extent of gland involvement did not differ significantly between arms when enrollment and postenrollment phases of the trial were combined or when analyzed separately, but small numbers limited the latter analyses. Gland involvement during the postenrollment period was significantly less extensive than at enrollment when data for all arms were combined ($P = 0.03$), but within each arm, comparisons were not statistically different based on small numbers. Diffuse gland involvement postenrollment was rare in all arms with only 1 case identified in the CM-Arm, none found in the HPV-Arm, and two detected in the IC-Arm.

**LSIL Referrals: Extent and Characteristics of CIN3.** Analysis of LSIL referral data was limited by modification of the study protocol, especially early closure of the HPV-Arm, which reduced the number of cases available for review. Among LSIL referrals, the number of tissue fragments involved with CIN3 did not differ significantly between study arms when enrollment and postenrollment phases of the trial were combined or when arms were compared at enrollment and postenrollment separately. Postenrollment, marginally fewer tissue fragments were involved in the IC-Arm as compared with the CM-Arm ($P = 0.06$), but none of the other pair-wise comparisons of the number of fragments involved at enrollment or postenrollment approached significance. There were no significant differences in the number of pieces containing CIN3 between enrollment and postenrollment for all arms combined or within individual arms, although in the IC-Arm, lesions tended to be smaller postenrollment as compared with at enrollment ($P = 0.08$).

For LSIL referrals, comparisons of TDS data were similar to those for the number of tissue fragments involved with CIN3. Postenrollment, the TDS of cases in the IC-Arm was smaller than those in the CM-Arm ($P = 0.04$), and the cases in the HPV-Arm were marginally smaller than those in the IC-Arm ($P = 0.08$). In the IC-Arm, the TDS was significantly smaller for postenrollment cases as compared with those detected at enrollment ($P = 0.02$). Gland involvement by CIN3 was identified in 102 (60.4%) women referred for LSIL without significant differences between study arms or phases of the trial.

**Other Comparisons.** The median number of tissue fragments involved with CIN3 was 2 for both ASCUS and LSIL referrals with a mean $\pm$ SE of 2.8 $\pm$ 0.2 for ASCUS and 3.1 $\pm$ 0.2 for LSIL. Similarly, the median TDS was 2 for both ASCUS and LSIL referrals with a mean of 4.2 $\pm$ 0.4 for ASCUS and 4.6 $\pm$ 0.4 for LSIL. Among women with ASCUS referral cytology, cytoplasmic maturation was found in 5 (3.1%) cases, extension of CIN3 to the ectocervix in 8 (5%), and necrosis in 4 (2.5%); among LSIL referrals, the corresponding frequencies of these features were 9 (5.3%), 2 (1.2%), and 6 (3.6%), respectively.

**Total Linear Dimension (in Millimeters) of CIN3 Lesions per Case.** The distal-proximal length of each microscopic focus of CIN3 and total linear extent of all surface epithelium were measured with a ruler (in millimeters) in a random subset of 40 LEEP (Table 1). One case was not analyzed because CIN3 was not identified on review. The median total length of CIN3 per case was 6.5 mm (mean $= 9.5$ mm $\pm 9.1$) with 26 (66.7%) demonstrating a total linear dimension of 0–10 mm. The median total length of intact surface epithelium was 105 mm (mean $= 102.8 \pm 5.5$), resulting in a median percentage of 5.5, resulting in a median
replacement of total epithelium by CIN3 per case of 5.7% (mean = 10.4% ± 1.9%).

**TDS of CIN3 Lesions Detected at Exit Visit Compared with Exit Cytological Interpretations, Colposcopic Impressions, and HPV Testing.** Among 101 cases of histopathologic CIN3 identified at the study exit visit, the clinical centers and pathology control group reported 20 (19.8%) and 21 (20.8%) cases as cytologically negative, respectively (Table 2). As the severity of cytological interpretation reported by both pathology groups increased, the mean TDS for CIN3 cases found at exit also increased progressively. The incremental increase in the size of CIN3 lesions between adjacent cytological categories was small, but the largest increase was found in the comparison of LSIL and HSIL. Colposcopic impressions of HSIL were associated with a mean TDS ± SE of 6.2 ± 1.5, as compared with 2.2 ± 0.3 for women classified as LSIL and 1.8 ± 0.4 for women categorized as atypical squamous metaplasia. There was also 1 case at exit with a normal colposcopic impression (TDS = 7) and 2 cases with miscellaneous benign descriptions (mean TDS = 7.5). Among the women with positive HPV tests at exit, the mean TDS ± SE was 3.6 ± 0.6, as compared with a mean TDS = 2.5 ± 0.9 among the women who tested negative.

**Discussion**

Our analysis demonstrated that the vast majority of CIN3 lesions detected in ALTS was small, with a median number of involved tissue fragments and median TDS of 2 each. The sum of the distal-proximal measurements of all microscopic foci of CIN3 per case was between 0 and 10 mm in 67% of specimens, with only 15% exceeding 20 mm, based on a review of 40 LEEPs originally classified as CIN3. These CIN3 lesions replaced a median of 5.7% of the total surface epithelium. In approximately one-third of women who underwent a LEEP for CIN3 diagnosed on a biopsy or curettage, the resulting LEEP specimen was not interpreted as CIN3, including 38% that were interpreted as negative, suggesting that these CIN3 lesions were extremely small. Cyttoplasmic maturation and necrosis, characteristics that have been associated with CIN3 that has progressed to invasion, were found in <5% of cases. In aggregate, our data demonstrate that the clear majority of CIN3 lesions in ALTS was smaller than CIN3 lesions typically associated with concurrent invasion and did not display other specific histopathologic features associated with incipient carcinoma. Postenrollment, CIN3 lesions found among ASCUS referrals in the HPV-Arm were uniformly small with a median number of tissue fragments containing CIN3 and a TDS of 1, with no cases of diffuse gland involvement. The rarity of invasive carcinoma in ALTS (7 cases among 5060 women) is consistent with the conclusion that the cancer risk for young women with ASCUS and LSIL cytology is generally quite low.

**Table 1** Total measured length of 40 randomly selected CIN3 lesions in ALTS

<table>
<thead>
<tr>
<th>Length (mm)</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–10</td>
<td>26 (66.7)</td>
</tr>
<tr>
<td>&gt;10–20</td>
<td>7 (18.0)</td>
</tr>
<tr>
<td>&gt;20–30</td>
<td>5 (12.8)</td>
</tr>
<tr>
<td>&gt;30–40</td>
<td>1 (2.6)</td>
</tr>
<tr>
<td>Total</td>
<td>39 (100.0)</td>
</tr>
</tbody>
</table>

* One specimen with unconfirmed CIN3 on review was excluded from this analysis.

**Table 2** Comparison of cytologic interpretations and TDS at exit visit in ALTS

<table>
<thead>
<tr>
<th>Clinical center</th>
<th>Quality control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. (%)</td>
<td>Mean TDS ± SE</td>
</tr>
<tr>
<td>Missing</td>
<td>0</td>
</tr>
<tr>
<td>Negative</td>
<td>20 (19.8)</td>
</tr>
<tr>
<td>ASCUS</td>
<td>38 (37.6)</td>
</tr>
<tr>
<td>LSIL</td>
<td>25 (24.8)</td>
</tr>
<tr>
<td>HSIL</td>
<td>18 (17.8)</td>
</tr>
</tbody>
</table>

Tidbury et al. (11) found that the mean total dimension of 39 CIN3 lesions (linear surface involvement plus perimeter of involved endocervical glands) associated with microinvasive carcinoma was 63.5 mm (range 11.4–162.4 mm); only three lesions demonstrated <20 mm of surface involvement. Al-Nafussi et al. (8) reported that replacement of >25% of surface and glandular cervical epithelium with CIN3, mucosal distention with CIN3, necrosis, and cytoplasmic maturation were markers of CIN3 associated with microinvasion. When all of these features were present, 29 of 36 CIN3 lesions demonstrated invasion on deeper levels of blocks or subsequent specimens; when none of these features were present, invasion was not identified in 64 women. Ostor found extensive CIN in all but 9 of 200 meticulously processed cone biopsies containing microinvasive carcinoma (10). Although our size assessments are not strictly comparable with previous studies, our data demonstrate that most CIN3 lesions in ALTS were considerably smaller than CIN3 lesions that have been associated with invasion.

A woman’s risk of having an underlying CIN3 is highly correlated with her cytological screening result (21). When CIN3 is discovered after a less severe cytological reading than HSIL, the CIN3 lesion tends to be small. In a study of 22 women who had mild dyskaryosis on two smears and proved to have an underlying CIN3, Jarmulowicz et al. (22) found that every CIN3 lesion measured <10 mm, which was smaller than every CIN3 associated with microinvasion described by Tidbury et al. (11) The CIN3 lesions found in ALTS were generally small as would be expected for a study enrolling women with ASCUS or LSIL cytology. CIN3 lesions detected at the exit visit that were associated with cytological interpretations of HSIL were larger than those associated with milder cytological interpretations, consistent with the finding reported previously relating increasing severity of cytological interpretation with greater extent of CIN3. The identification of extensive CIN3 in a patient with ASCUS or LSIL is exceptional and should prompt a quality assurance review if practical to determine a possible role for sampling, screening, or interpretive errors. Similarly, colposcopic impressions of high-grade disease were associated with the largest cases, and lesions associated with a negative HPV test at the exit visit were slightly smaller than those that tested positive. Therefore, these data demonstrate that both cytology and colposcopy particularly underestimate the prevalence of small CIN3 lesions, which also have a slightly higher risk of being associated with a false negative HPV test.

Among women in ALTS with a positive HPV test, a similar percentage of women referred with ASCUS or LSIL had an underlying CIN3. Furthermore, these lesions were similar in size for both referral categories, underscoring the comparable clinical significance of these cytological interpretations among women with detectable HPV and justifying their identical management (15).
Analysis of CIN3 lesions among LSIL referrals was limited by changes to the study protocol, whereas among ASCUS referrals, the CIN3 lesions found postenrollment (during follow-up or at exit) reflected a combination of prevalent cases missed at enrollment plus incident cases that had developed during 2 years of follow-up. Among ASCUS referrals randomized to the CM-Arm, most cases were discovered postenrollment, consistent with the insensitivity of the enrollment protocol (colposcopy referral for repeat cytology of HSIL) for detecting small CIN3 lesions. In contrast, in the HPV-Arm, 70% of cases were found at enrollment, reflecting the high sensitivity of this management strategy. Furthermore, the lesions found postenrollment in the HPV-Arm were smaller than in the CM- and IC-Arm, particularly as assessed by the number of pieces involved by CIN3. These data demonstrate that HPV testing sensitively detected even the smallest CIN3 lesions at enrollment; cases found postenrollment in the HPV-Arm either represented lesions present at enrollment that had not enlarged substantially or were truly incident.

The extent of endocervical gland involvement by CIN3 was similar across study arms for both ASCUS and LSIL referrals. In ALTS, as in a previous study, (23) more extensive gland involvement was associated with greater linear extent of disease. Diffuse gland involvement by CIN3 was not identified in any case postenrollment in the HPV-Arm. Extensive gland involvement may represent both a risk factor for invasion (8) and recurrence after a LEEP (12, 24). Other factors predictive of recurrence may include higher grade of CIN, lesion size, and positive margins (12, 24, 25).

Given that older age and larger CIN3 size are associated with increased risk of invasion, one would expect that age and size would also be correlated. However, age and size were not related in this analysis. Possible explanations for this result include the fact that ALTS did not enroll women with HSIL cytology and most subjects were young. Nonetheless, the relationship between age and cancer risk is important for planning screening strategies, especially in light of the increased specificity of both cytology and especially HPV testing as women age (18).

We acknowledge that variations in specimen processing may have affected our results and that our qualitative and semiquantitative techniques of size assessment were approximate. However, we suspect that had we used an ideal three-dimensional reconstruction technique to estimate total lesion volume, our results would have been strengthened, because nondifferential misclassification of lesion size in our study probably led to an underestimate of associations. The number of tissue fragments that were prepared from LEEPs did not seem to confound the analysis of lesion size, an expected result given the predominantly small size of the lesions.

Currently in the United States, detection and eradication of every CIN3 lesion are desirable because the risk of invasion is cumulative over time and unpredictable in a given patient. Women with ASCUS and LSIL cytology represent a large reservoir of mostly young women with CIN3, which has remained occult previously but is now increasingly discovered with more sensitive methods, such as liquid-based cytology (26, 27) and HPV testing (13). Aggressive follow-up of ASCUS in the United States has resulted in lead-time bias with regard to many cases of CIN3, which would not have resulted in interval cancers among routinely screened patients if they had been detected later. However, this approach may have prevented some rapidly developing cervical cancers, reduced risk among noncompliant women, and limited morbidity related to treating large CIN3 lesions. Nonetheless, the cost-effectiveness of detecting tiny CIN3 lesions may be questionable if resources are limited.

Kim et al. (28) developed a computer-generated model to assess the cost-effectiveness of screening compared with no screening when ASCUS is managed using different strategies. The model predicted that compared with no screening, screening with conventional cytology would decrease cancer incidence by 75% and liquid-based cytology by 84% if ASCUS were simply ignored. Using HPV testing to manage ASCUS would reduce cancer incidence by 86% when coupled with conventional cytology and 90% when used with liquid-based cytology. However, this would add about $30,000/year of life gained. Using repeat cytology was considerably more expensive.

In conclusion, aggressive follow-up of ASCUS and LSIL leads mainly to detection of CIN3 lesions that are smaller than those typically associated with invasion, especially when HPV testing is used. Optimally, future molecular studies of precursor lesions should incorporate assessment of epidemiological data and detailed pathologic characterization in order to maximize the knowledge gained about the pathogenesis of cervical cancer.

Acknowledgments
We thank all of the ALTS investigators for their efforts and Dr. Diane Solomon for her critical review of this manuscript. The ALTS Ancillary Studies Committee approved this study.

References


Histopathologic Extent of Cervical Intraepithelial Neoplasia 3 Lesions in the Atypical Squamous Cells of Undetermined Significance Low-grade Squamous Intraepithelial Lesion Triage Study: Implications for Subject Safety and Lead-time Bias

Mark E. Sherman, Sophia S. Wang, Robert Tarone, et al.