

# The Relationship of Community Biopsy-Diagnosed Cervical Intraepithelial Neoplasia Grade 2 to the Quality Control Pathology-Reviewed Diagnoses

## An ALTS Report

Philip E. Castle, PhD, MPH,<sup>1</sup> Mark H. Stoler, MD,<sup>2</sup> Diane Solomon, MD,<sup>3</sup>  
and Mark Schiffman, MD, MPH,<sup>1</sup> for the ALTS Group\*

**Key Words:** Cervical intraepithelial neoplasia; CIN; Human papillomavirus; HPV; Loop electrosurgical excision procedure; Biopsy; Cytology; Colposcopy

DOI: 10.1309/PT3PNC1QL2F4D2VL

### Abstract

We examined the predictors (cytologic interpretations, pathology review, human papillomavirus [HPV] testing results, and colposcopic impressions) of precancer among 545 women with clinical center biopsy diagnoses of cervical intraepithelial neoplasia (CIN) 2 in the ASCUS LSIL Triage Study. Among women with a CIN 2 biopsy result, there was an increasing likelihood that the loop electrosurgical excision procedure (LEEP) tissue sample was diagnosed as precancer (CIN 3) with an increasing number of clinical risk factors of cervical precancer (high-grade squamous intraepithelial lesion [HSIL] cytology, high-grade colposcopy, detection of HPV type 16;  $P_{trend} < .0005$ ). In a multivariate model, using a case definition of worst histologic diagnosis made by the quality control pathology review of biopsy and LEEP tissue samples, HPV-16 was positively associated (odds ratio [OR], 4.8; 95% confidence interval [CI], 2.6-8.8) with a CIN 3 diagnosis, whereas testing negative for HPV or positive for noncarcinogenic HPV types was negatively associated (OR, 0.32; 95% CI, 0.14-0.75) with a CIN 3 diagnosis. Although we found clear evidence that HPV-16 detection helped clarify whether a biopsy specimen diagnosed as CIN 2 represented HPV infection or cervical precancer, this relationship was not sufficiently robust to be clinically useful for reducing the overtreatment of women with HPV infection.

Prevention of cervical cancer has primarily relied on cytology-based screening, colposcopic evaluation of the cervix with biopsy of potentially abnormal tissue, and treatment of the lesion by excision or ablation of the cervical transformation zone for a biopsy specimen diagnosed as cervical intraepithelial neoplasia (CIN) grade 2 or worse. There is now strong epidemiologic, clinical, and laboratory evidence demonstrating that cervical infections by approximately 15 cancer-associated (carcinogenic) human papillomavirus (HPV) types cause virtually all cervical cancer and its precursor lesions.<sup>1,2</sup> Based on the fundamental role of carcinogenic HPV as the necessary but not sufficient cause of cervical cancer, a new paradigm of cervical carcinogenesis had been developed based on 4 reliably measured stages: HPV acquisition, HPV persistence (vs clearance), progression to precancer, and invasion.<sup>3</sup> This conceptual model of HPV and cervical carcinogenesis has firm empirical support. It now seems unlikely that cancer develops according to a former morphology-based model of stepwise progression from normal to atypia to CIN 1 to CIN 2 to CIN 3 to cancer.<sup>4</sup>

Nevertheless, histopathologic results remain the foundation of clinical care where screening and colposcopy programs have been established. In the United States and Europe, diagnosis of CIN 2 or worse is the clinical threshold leading to ablative or excisional therapy. However, CIN 2 as a separate diagnostic category remains a clinical enigma, given its poor reproducibility,<sup>5</sup> and there is evidence that CIN 2 is significantly more likely to regress than CIN 3.<sup>6</sup> In its most simple definition, CIN 2 is defined as "...the immature basaloid cells occupy up to two-thirds of the epithelial thickness but do not extend into the upper third of the epithelium. Similarly mitoses are found in the lower two-thirds of the epithelium but

not in the upper third.”<sup>7</sup> On biopsy, however, the orientation of the tissue specimen can make the distinction between CIN 1 and CIN 2 difficult to the point of arbitrary. Moreover, pathologists do not necessarily agree on additional cytomorphologic criteria for histologic diagnoses of CIN 2 or even the conceptual definition of whether a CIN 2 diagnosis should be considered a low-grade or high-grade lesion.

Thus, CIN 2 diagnoses may represent an equivocal diagnosis rather than a separate biologic stage in cancer development. It certainly overlaps with CIN 1, which is synonymous with signs of (usually recently acquired) HPV infection, and CIN 3, which is essentially carcinoma in situ. In its heterogeneity, CIN 2 may be in some ways conceptually analogous to ASCUS (atypical squamous cells of undetermined significance) cytology, which is an equivocally abnormal cytologic interpretation that represents an admixture of HPV-associated cytologic abnormalities and benign reactive changes. For ASCUS, carcinogenic HPV DNA testing clarifies true abnormality from the mimics, ie, carcinogenic HPV+ ASCUS poses a similar risk of precancer as cytologic low-grade squamous intraepithelial lesion (LSIL) and colposcopy is recommended, whereas carcinogenic HPV– ASCUS poses little risk for precancer and colposcopy is not recommended.<sup>8</sup>

We do not know how to subdivide CIN 2 for optimal clinical management. Given the potential negative impact of the loop electrosurgical excision procedure (LEEP) on reproductive outcomes<sup>9</sup> and the frequency of CIN 2 in young women, there is value to increasing our understanding of CIN 2 with the hope of eventually distinguishing CIN 2 diagnoses that represent transient HPV infection from those that represent true precancer, which warrants treatment. As a first step, we sought to characterize the biopsy diagnoses of CIN 2 by the clinical center in the ASCUS and LSIL Triage Study (ALTS) by comparing this initial diagnosis with our reference biopsy diagnosis, the diagnosis of the tissue removed at LEEP treatment, and the worst diagnosis on biopsy or LEEP specimens as made by the Pathology Quality Control (QC) Group (QC Pathology Group).

## Materials and Methods

### Study Design and Population

ALTS was a randomized trial comparing 3 management strategies for 5,060 women with ASCUS ( $n = 3,488$ ) or LSIL ( $n = 1,572$ )<sup>10</sup>: (1) immediate colposcopy (referral to colposcopy regardless of enrollment test results); (2) HPV triage (referral to colposcopy if enrollment HPV result by Hybrid Capture 2 [Digene, Gaithersburg, MD] was positive or missing or if the enrollment cytologic result was high-grade squamous intraepithelial lesion [HSIL]); or (3) conservative management

(referral to colposcopy at enrollment if the cytologic result was HSIL). At enrollment, all women underwent a pelvic examination with collection of 2 cervical specimens, the first specimen in PreservCyt for ThinPrep cytologic examination (Cytyc, Marlborough, MA) and the second in specimen transport medium (STM, Digene). Women in all 3 arms of the study were reevaluated by cytologic examination every 6 months for 2 years and sent to colposcopy if the cytologic result was HSIL. An exit examination with colposcopy was scheduled for all women, regardless of study arm or prior procedures, at the completion of the follow-up. We refer readers to other references for details on randomization, examination procedures, patient management, and laboratory and pathology methods.<sup>8,10-13</sup> The National Cancer Institute, Bethesda, MD, and local institutional review boards approved the study, and all participants provided written informed consent.

### HPV DNA Testing

HPV genotyping was performed using an L1-based polymerase chain reaction (PCR) assay that uses a primer set designated PGMY09/11 and was performed on the STM specimen<sup>14</sup> described in the preceding section, which was obtained at the time that the cytologic specimens were obtained rather than at the time of clinical follow-up when biopsies and LEEP were performed. Amplimers were subjected to reverse-line blot hybridization for detection of 27 individual HPV genotypes (6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51-59, 66, 68, 73 [PAP238a], 82 [W13b], 83 [Pap291], and 84 [PAP155]).<sup>15</sup> We also tested for an additional 11 noncarcinogenic HPV genotypes (61, 62, 64, 67, 69-72, 81, 82 variant [IS39], and 89 [CP6108]) in approximately half of the specimens (58%) at enrollment and in all specimens obtained at the follow-up visits.<sup>16</sup> HPV genotyping did not influence clinical management because the testing was conducted retrospectively after clinical decisions were made.

We considered HPV genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68 as the primary carcinogenic types.<sup>17,18</sup> Women were assigned to an HPV risk group according to a priori established cervical cancer risk: (1) positive for HPV-16, (2) positive for any other carcinogenic HPV types and negative for HPV-16 (carcinogenic HPV [without HPV-16]), (3) positive for any noncarcinogenic HPV types and negative for all carcinogenic types, or (4) PCR negative for HPV. For these analyses, we combined the latter 2 categories because of small numbers.

### Pathology and Treatment

Clinical management was based on the clinical center pathologists' cytologic and histologic diagnoses. There were 1 to 2 staff pathologists per clinical center (clinical center pathologists), who worked independently to review the cytologic and histologic results originating from their center.

ALTS-related materials were not identified or separately reviewed from other clinical materials in routine practice. No training of the clinical center pathologists was conducted, and no diagnostic criteria were provided to clinical center pathologists, ie, they used the same criteria that they used in the daily sign-out. No conferences were held regarding cases. Thus, the clinical center evaluations were independent to ensure “real-world” or representative community diagnoses in ALTS.

In addition, all referral smears, ThinPrep specimens, and histologic slides were sent to the QC Pathology Group based at the Johns Hopkins Hospital, Baltimore, MD, for review and secondary diagnoses. The a priori trial design of ALTS was to use QC Pathology Group–adjudicated interpretations as “truth,” and the conclusions derived from that predetermined decision are partially reflected in the data presented herein.

Initial QC review was randomly assigned to 1 of 4 QC pathologists. The QC review was masked to the clinical center interpretation and all other test results. The QC Pathology Group reviewed biopsy and LEEP specimens independently such that the pathologists had no knowledge of which biopsy specimen was related to which LEEP specimen.

The present analysis is based on the comparison of clinical center with first QC interpretations. When the first QC reviewer disagreed with the original clinical center interpretation, additional reviews were performed,<sup>5,10</sup> including reviews for the tiny fraction of cases in which consensus adjudication was needed and 2 QC pathologists, knowing what the other pathologist diagnosed, performed a joint review at a multi-headed microscope. Although the QC algorithms and panel interpretations were used for ALTS to define disease end points, patients were managed by the original clinical center interpretations unless CIN 3 or cancer was suspected, in which case the final QC opinion was unmasked.

A CIN 2 or worse diagnosis based on the clinical center pathology or a CIN 3 or worse diagnosis based on QC Pathology Group review triggered treatment by LEEP. In addition, women with persistent LSIL or carcinogenic HPV-positive ASCUS at the time of exit from the study were offered LEEP.

### Statistical Methods

Of the 5,060 women enrolled in ALTS, 545 women (10.8%) had a colposcopically directed biopsy specimen diagnosed as CIN 2 by the clinical center pathologist at enrollment or during the 2-year follow-up; for 524 women, this was the worst biopsy diagnosis by the clinical center. Of the 545 women (median age, 23 years; range, 18–52 years) with a clinical center biopsy diagnosis of CIN 2, 497 (91.2%) returned to the participating clinical center and underwent LEEP. We considered the QC Pathology Group diagnosis of the LEEP tissue sample at the follow-up visit as a more accurate measure of disease. LEEP removes the entire cervical transformation zone,

permitting full pathologic evaluation of the susceptible tissue. We recognize, of course, that histologic diagnoses on LEEP specimens are still subject to interpathologist variability, particularly for low-grade disease,<sup>5</sup> and that in some cases, biopsy may have removed the entire area of precancerous lesion.

Therefore, in some analyses, we combined the QC Pathology Group review of biopsy and LEEP specimens into a worst QC Pathology Group diagnosis by using the most severe diagnosis of the two (eg, a woman with a biopsy specimen called CIN 2 and a LEEP specimen called CIN 1 by the QC Pathology Group had a worst QC Pathology Group diagnosis of CIN 2). We used this approach to help overcome misclassification of the disease and the possibility that the biopsy procedure ablated the lesion, which consequently could not be observed in the LEEP tissue specimen. In cases in which only 1 QC diagnosis was available (5 were missing a biopsy diagnosis, 46 were missing a LEEP diagnosis, and 2 were missing both), that diagnosis was used; 543 (99.6%) of 545 cases with a clinical center CIN 2 biopsy diagnosis had at least 1 QC diagnosis, and 492 cases had biopsy and LEEP diagnoses (90.3%). Two cases were excluded because they had no QC diagnosis (0.4%).

We first compared the worst biopsy diagnosis by clinical center for each woman with the QC Pathology Group diagnosis using  $\kappa$  statistics and percentage of agreement, and we used the symmetry  $\chi^2$  test to assess the difference in severity of the diagnoses made by the 2 groups. We compared the paired diagnoses with the percentage positive for any carcinogenic HPV and for HPV-16 (the most carcinogenic HPV type) and with the percentage of LEEP specimens with CIN 2 or worse and CIN 3. Specifically, a nonparametric test for trend in a variable across ordered groups was used to evaluate the relationships between the diagnoses by one pathology group, given a diagnosis of the other group, and the percentage with carcinogenic HPV, HPV-16, and LEEP diagnoses of CIN 2 or worse and CIN 3.

We compared the severity of disease ( $\leq$ CIN 1, CIN 2, and CIN 3) on LEEP among all women who had a biopsy of CIN 2 with the results of QC Pathology Group review of the biopsy specimen ( $\leq$ CIN 1, CIN 2, and CIN 3), cytologic interpretation of the liquid-based cytology (normal, atypical squamous cells [ASC], LSIL, and HSIL), colposcopic impression ( $<$ high-grade, high-grade), age at biopsy (18–21, 22–23, 24–27, and 28–52 years), and HPV risk group, and tested for statistical differences using the Pearson  $\chi^2$ . (NOTE: A small percentage of women had more than 1 specimen diagnosis as CIN 2 or worse, even after treatment by LEEP,<sup>19</sup> and were excluded from these analyses. This explains the discrepancy between the number of women who had any CIN 2 biopsy diagnosis and the number of women whose worst biopsy diagnosis was CIN 2.) The colposcopic impression ( $<$ high-grade, high-grade) at the LEEP procedure was highly concordant with the colposcopic

impression at time of the biopsy (99% agreement), so only the latter was used. We then examined the trends of the absolute risk of CIN 3 on LEEP specimens for combinations of QC Pathology Group review of the biopsy specimen, cytologic interpretation, and colposcopic impression and tested for statistically significant trends.

We used multinomial (polytomous) logistic regression<sup>20</sup> to calculate multivariate (adjusted) odds ratios (ORs) and 95% confidence intervals (CIs) for QC Pathology Group diagnoses of CIN 3 based on LEEP and based on the worst diagnosis of LEEP and biopsy by the QC Pathology Group for the aforementioned covariates and age, with less than CIN 2 as the reference outcome and CIN 2 as a distinct intermediate so as to not combine the CIN 2 with less than CIN 2 or CIN 3. Models also adjusted for the clinical center of the patients.

A *P* value of less than .05 was considered statistically significant. Stata version 8.2 (Stata, College Station, TX) was used for all statistical analyses.

## Results

### Comparison of Clinical Center and QC Pathology Biopsy Diagnoses

**Table 1** shows the crude agreement of the clinical center and QC Pathology Group review of biopsy specimens throughout the 2-year study and the relationship of the paired diagnoses to detection of carcinogenic HPV and HPV-16. The  $\kappa$  for 2 reviews was 0.49 (95% CI, 0.47-0.51), with a percentage of

overall agreement of 64%. This  $\kappa$  was very similar to that previously reported between 2 groups of pathologists evaluating the baseline diagnoses of biopsy specimens, 0.46 (95% CI, 0.43-0.49), although in that analysis, no distinction was made between CIN 2 and CIN 3.<sup>5</sup>

There was an overall tendency for clinical center pathology to make a more severe diagnosis than the QC Pathology Group (*P* < .00005) primarily because the clinical center pathology diagnoses of ASC or CIN 1/LSIL were often called normal by the QC Pathology Group.

Agreement on CIN 2 (median age, 23 years; mean age, 24.9 years; SD, 5.9 years) was poor (data not shown). Of the 523 clinical center biopsy diagnoses of CIN 2 and any QC Pathology Group biopsy diagnosis, only 227 (43.4%) were also diagnosed as CIN 2 by the QC Pathology Group, with 153 (29.3%) being called less than CIN 2 and 143 (27.3%) being called CIN 3 by the QC Pathology Group. There was no significant difference in age of patients whose diagnoses were given as less than CIN 2, CIN 2, or CIN 3 by the QC Pathology Group.

### Relationships of Biopsy Diagnoses and Carcinogenic HPV and HPV-16 Detection

There was a strong trend of increasing likelihood of testing positive for carcinogenic HPV (*P*<sub>trend</sub> < .00005) and for HPV-16 (*P*<sub>trend</sub> < .00005) with an increasingly severe diagnosis by the QC Pathology Group for a given diagnosis by clinical center pathology (Table 1). Likewise, there was a strong trend of increasing likelihood of testing positive for carcinogenic HPV (*P*<sub>trend</sub> < .00005) and for HPV-16 (*P*<sub>trend</sub> < .00005)

**Table 1**  
Agreement of CC and QC Pathology Biopsy Diagnoses for the Worst Cervical Biopsy Results for Each Woman Participating in ALTS\*

CC Pathology Biopsy Diagnosis	QC Pathology Biopsy Diagnosis					Total (n = 1,134)
	Normal (n = 1,105)	ASC (n = 27)	CIN 1/LSIL (n = 628)	CIN 2 (n = 227)	CIN 3+ (n = 203)	
Normal (%)	1,105 (32)	6 (0)	10 (0)	9 (0)	4 (0)	1,134
Carc HPV+ (%)	37	50	56	75	50	
HPV-16+ (%)	7	0	11	13	25	
ASC (%)	249 (7)	27 (1)	28 (1)	5 (0)	3 (0)	312
Carc HPV+ (%)	49	54	59	80	33	
HPV-16+ (%)	11	0	15	0	0	
CIN 1/LSIL (%)	463 (13)	15 (0)	628 (18)	83 (2)	18 (1)	1,207
Carc HPV+ (%)	54	67	76	84	95	
HPV-16+ (%)	12	13	16	14	47	
CIN 2 (%)	34 (1)	14 (0)	105 (3)	227 (7)	143 (4)	523
Carc HPV+ (%)	67	57	88	88	95	
HPV-16+ (%)	15	14	32	45	58	
CIN 3+ (%)	7 (0)	4 (0)	6 (0)	59 (2)	203 (6)	279
Carc HPV+ (%)	67	50	100	93	95	
HPV-16+ (%)	33	25	60	50	65	
Total	1,858	66	777	383	371	3,455

ALTS, ASCUS [Atypical Squamous Cells of Undetermined Significance] and LSIL Triage Study; ASC, atypical squamous cells; CC, clinical center; CIN, cervical intraepithelial neoplasia; HPV, human papillomavirus; LSIL, low-grade squamous intraepithelial lesion; QC, quality control.

\* The relationship of the paired diagnoses to the percentage of women with positive results for carcinogenic HPV (Carc HPV+) or HPV-16 (HPV-16+) using the exfoliative cervical specimen obtained at the visit leading to colposcopy is given.  $\kappa$  = 0.49 (95% confidence interval, 0.47-0.51); agreement, 64%; *P* < .00005, McNemar  $\chi^2$ .

with an increasingly severe diagnosis by the clinical center pathology group for a given diagnosis by the QC Pathology Group. Nearly 100% of the cases in which both pathology groups called the biopsy CIN 3 or worse were carcinogenic HPV+, and 65% were positive for HPV-16.

Of note, clinical center pathology biopsy diagnoses of CIN 2 that were called CIN 3 by the QC Pathology Group (n = 143 [27.3%]) were nonsignificantly more likely to test positive for HPV-16 than clinical center pathology biopsy diagnoses of CIN 3 that were called CIN 2 by the QC Pathology Group (n = 59 [15.4%]) (58% vs 50%).

### Relationships of Clinical Center Biopsy Diagnoses and QC LEEP Diagnoses

Of the 497 women who had a clinical center biopsy diagnosis of CIN 2 and a QC Pathology Group–reviewed LEEP specimen, only about 25% had a QC LEEP diagnosis of CIN 2, with most being given a diagnosis of CIN 1 or less severe (~48%) or CIN 3 (~25%) (data not shown). The severity of QC Pathology Group review of the clinical center–diagnosed CIN 2 biopsy samples ( $P < .0005$ ), interpretation of the liquid-based cytology (<HSIL or HSIL) from the immediately

preceding clinical visit ( $P < .0005$ ), the clinical center colposcopic impression (<high-grade or high-grade) ( $P < .0005$ ), and HPV risk group ( $P < .0005$ ) were strongly and positively associated with increasing severity of the QC Pathology Group LEEP diagnosis (<CIN 2 vs CIN 2 vs CIN 3) **Table 2**. Age at biopsy was only weakly associated with diagnosis based on the QC Pathology Group review of the LEEP tissue samples ( $P = .03$ ). Time between biopsy and LEEP was not associated with the QC Pathology Group LEEP diagnosis ( $P = 0.2$ ; nonparametric test of trend).

When cytologic interpretation, colposcopic impression, and QC Pathology Group review of the biopsy sample were combined, a gradient of increasing risk of a QC Pathology Group LEEP diagnosis of CIN 3 could be constructed ( $P_{\text{trend}} < .0005$ ) **Figure 1**. At the extremes, 9.2% of women with less than HSIL cytologic findings, less than high-grade colposcopic findings, and a clinical center CIN 2 biopsy diagnosis down-graded by QC to less than CIN 2 had a QC Pathology Group LEEP diagnosis of CIN 3, whereas 55.3% of women with an HSIL cytologic diagnosis, high-grade colposcopic findings, and a CIN 2 biopsy diagnosis up-graded to CIN 3 had a QC Pathology Group LEEP diagnosis of CIN 3.

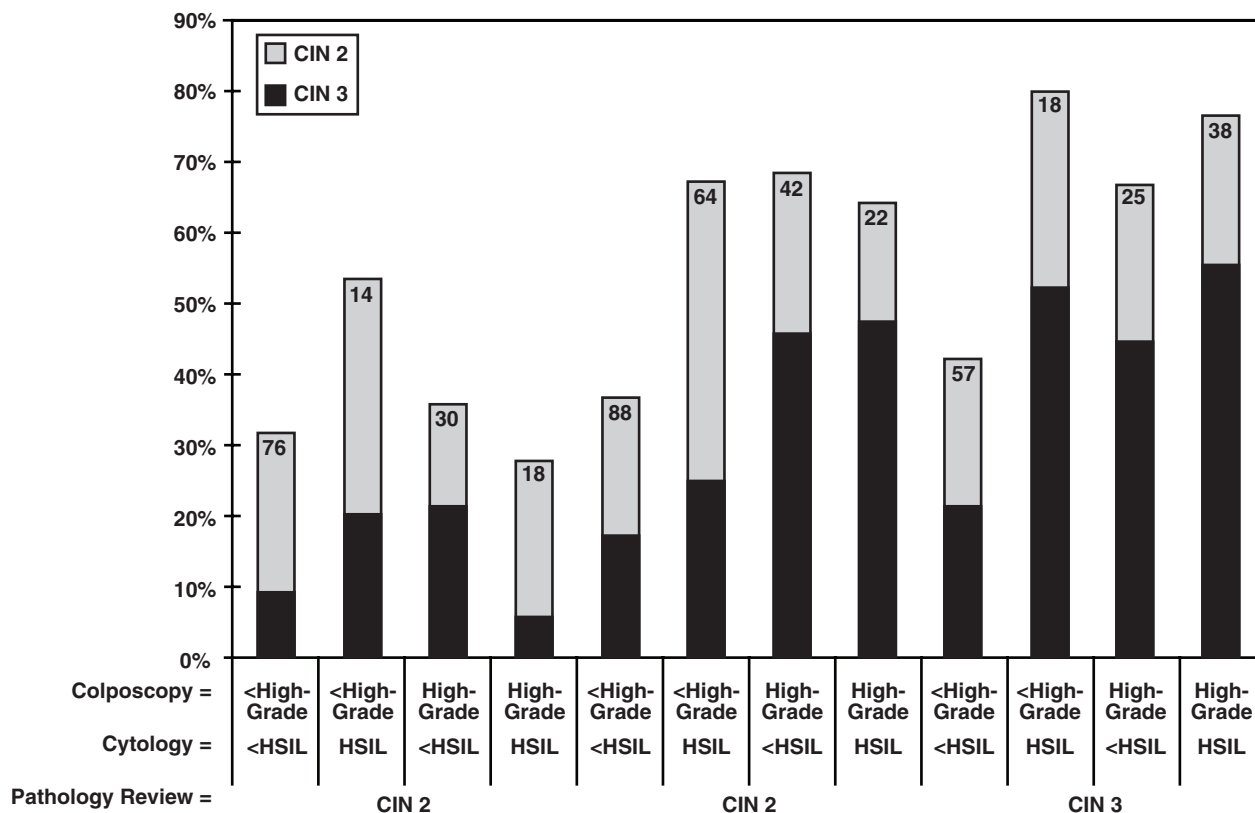
**Table 2**  
Crude Association of QC Pathology Review Diagnosis, Cytologic Interpretation by the CC of Liquid-Based Cytology, Colposcopic Impression, Age at Biopsy, and HPV Risk Group Based on PCR Testing With the QC Pathology Review Diagnosis of the LEEP Tissue Specimen Among Women With a CIN 2 Biopsy Result Diagnosed by CC Pathologists\*

	QC Review of LEEP Tissue Samples			Total	P
	<CIN 2	CIN 2	CIN 3		
QC review of biopsy specimen					<.0005
<CIN 2	88 (36.7)	33 (27.5)	17 (12.9)	138	
CIN 2	99 (41.3)	56 (46.7)	61 (46.2)	216	
CIN 3	53 (22.1)	31 (25.8)	54 (40.9)	138	
Total	240	120	132	492	
CC liquid-based cytology					<.0005
Normal	27 (11.2)	13 (11.0)	13 (9.8)	53	
ASC	53 (22.0)	20 (16.3)	19 (14.3)	92	
LSIL	84 (34.9)	26 (21.1)	24 (18.0)	134	
HSIL	77 (32.0)	64 (52.0)	77 (57.9)	218	
Total	241	123	133	497	
Colposcopic impression at biopsy					<.0005
<High-grade	182 (75.5)	93 (75.6)	70 (52.6)	345	
High-grade	59 (24.5)	30 (24.3)	63 (47.4)	152	
Total	241	123	133	497	
Age at biopsy (y)					.03
18-21	81 (33.6)	50 (40.7)	30 (22.6)	161	
22-23	47 (19.5)	15 (12.2)	30 (22.6)	92	
24-27	63 (26.1)	33 (26.8)	35 (26.3)	131	
28-52	50 (20.7)	25 (20.3)	38 (28.6)	113	
Total	241	123	133	497	
HPV status by PCR					<.0005
Negative	18 (7.5)	3 (2.6)	2 (1.5)	23	
Noncarcinogenic	24 (10.0)	6 (5.1)	3 (2.3)	33	
Carcinogenic	123 (51.3)	51 (43.6)	46 (34.8)	220	
HPV-16	75 (31.3)	57 (48.7)	81 (61.4)	213	
Total	240	117	132	489	

ASC, atypical squamous cells; CC, clinical center; CIN, cervical intraepithelial neoplasia; HPV, human papillomavirus; HSIL, high-grade squamous intraepithelial lesion; LEEP, loop electrosurgical excision procedure; LSIL, low-grade squamous intraepithelial lesion; PCR, polymerase chain reaction; QC, quality control.

\* Data are given as number (percentage).





**Figure 1** The absolute risk of a quality control pathology loop electrosurgical excision procedure diagnosis of cervical intraepithelial neoplasia (CIN) 2 (gray) and CIN 3 (black) among women with a clinical center pathology biopsy diagnosis of CIN 2 with increasing clinical indications of a precancerous lesion. The number within each category is shown at the top of the bar. HSIL, high-grade squamous intraepithelial lesion.

By using a multivariate model, we examined among women with a clinical center–diagnosed CIN 2 biopsy specimen the associations of the aforementioned factors with having a subsequent QC Pathology Group LEEP diagnosis of less than CIN 2 or CIN 3 (Table 3). With less than CIN 2 as the reference end point, there was a 2- to 3-fold greater likelihood of having a QC Pathology Group LEEP diagnosis of CIN 3 if the cytologic interpretation was HSIL (vs <HSIL), colposcopic impression was high-grade (vs <high-grade), or PCR was positive for HPV-16 (vs carcinogenic HPV without HPV-16). By comparison, there was a 2- to 3-fold reduced likelihood of having a QC Pathology Group LEEP diagnosis of CIN 3 if a CIN 2–diagnosed biopsy was called less than CIN 2 on review by the QC Pathology Group (vs CIN 2) or if the PCR was negative or positive for noncarcinogenic HPV types (vs carcinogenic HPV without HPV-16). The youngest women (18-21 years) were less likely (OR, 0.48; 95% CI, 0.24-0.97) and the oldest women (28-52 years) were more likely (OR, 1.5; 95% CI, 0.73-3.0) to have a QC Pathology Group LEEP diagnosis of CIN 3 than women 22 or 23 years old, although the

latter finding was not significant; however, by using the youngest group of women as the referent group, there was a significant trend of increasing age and increasing likelihood of a QC Pathology Group LEEP diagnosis of CIN 3 ( $P_{\text{trend}} = .004$ ).

#### Relationships of Clinical Center Biopsy Diagnoses and Worst QC Diagnoses

Table 4 shows the relationship of the paired QC Pathology Group diagnoses of biopsy and LEEP specimens, the combination of which was used to create a worst QC Pathology Group diagnosis, and the percentage of women in each combination who tested positive for carcinogenic HPV and for HPV-16. Of the women with any CIN 3 diagnosis (biopsy or LEEP) and for whom the LEEP tissue samples were reviewed (regardless of the diagnosis of the other tissue), 94% or greater tested positive for carcinogenic HPV and 50% or greater tested positive for HPV-16. Similar percentages of women tested positive for carcinogenic HPV and HPV-16 if both the biopsy and LEEP specimens were called CIN 2. Women who were given a diagnosis based on

biopsy and LEEP specimens as less than CIN 2 were the least likely to test positive for carcinogenic HPV (74%) and for HPV-16 (18%). When QC Pathology Group diagnoses of biopsy and LEEP were combined into a worst QC Pathology Group diagnosis, diagnoses of CIN 3 were 95% positive for carcinogenic HPV and 60% positive for HPV-16.

Finally, we used a worst QC Pathology Group diagnosis as our end point (Table 5). Testing positive for HPV-16 was strongly associated with a diagnosis of CIN 3 (OR, 4.8; 95% CI, 2.6-8.8), whereas testing negative or positive for noncarcinogenic HPV types was negatively associated (OR, 0.32; 95% CI, 0.14-0.75) with a diagnosis of CIN 3. Having an HSIL cytologic interpretation by the clinical center pathologist was associated with a CIN 3 diagnosis (OR, 2.1; 95% CI, 1.2-3.7). Having a colposcopic impression of high-grade was marginally associated with having a CIN 3 diagnosis (OR, 1.7; 95% CI, 0.91-3.1).

## Discussion

We demonstrated in ALTS, a randomized clinical trial with rigorous pathology review, that the majority of women with a CIN 2 diagnosis on biopsy by the clinical center pathologists were not subsequently given a diagnosis of CIN 2 based on the review of the LEEP tissue samples by the QC Pathology Group. We demonstrated that some covariates were correlated with our best diagnosis of disease, ie, women

**Table 3**  
Multivariate Model to Estimate the Association of Select Covariates With a QC Pathology LEEP Diagnosis of CIN 3 (vs <CIN 2) Following a CC Pathology Biopsy Diagnosis of CIN 2\*

	Odds Ratio (95% Confidence Interval)	P
QC review of biopsy specimen		
<CIN 2	0.45 (0.23-0.89)	.02
CIN 2 <sup>†</sup>	1.0	
CIN 3	1.3 (0.70-2.2)	.5
HPV risk group by PCR		
Negative or noncarcinogenic	0.38 (0.13-1.1)	.07
Carcinogenic (without HPV-16) <sup>†</sup>	1.0	
HPV-16	2.7 (1.6-4.5)	<.0005
CC liquid-based cytology		
<HSIL <sup>†</sup>	1.0	
HSIL	2.8 (1.7-4.8)	<.0005
Colposcopic impression at biopsy		
<High-grade <sup>†</sup>	1.0	
High-grade	1.9 (1.1-3.2)	.014
Age at biopsy (y)		
18-21	0.48 (0.24-0.97)	.04
22-23 <sup>†</sup>	1.0	
24-27	0.84 (0.42-1.7)	.6
28-52	1.5 (0.73-3.0)	.3

CC, clinical center; CIN, cervical intraepithelial neoplasia; HPV, human papillomavirus; HSIL, high-grade squamous intraepithelial lesion; LEEP, loop electrosurgical excision procedure; PCR, polymerase chain reaction; QC, quality control.

\* The model was adjusted for CC of the patient.

<sup>†</sup> Reference group.

who had riskier HPV types were more likely to have a diagnosis of CIN 3 by the QC Pathology Group review of biopsy or LEEP specimens or the worst of the two.

**Table 4**  
Comparison of QC Biopsy and LEEP Diagnoses for 541 of 543 Patients With One or the Other Diagnosis\*

QC Biopsy Diagnosis	QC LEEP Diagnosis					Total
	<CIN 2	CIN 2	CIN 3	Missing		
<CIN 2						
No. of cases (%)	88 (16)	33 (6)	17 (3)	19 (4)	157 (29)	
Carc HPV+ (%)	74	91	100	84	82	
HPV-16+ (%)	18	36	50	28	26	
CIN 2						
No. of cases (5)	99 (18)	56 (10)	61 (11)	19 (4)	235 (43)	
Carc HPV+ (%)	83	93	95	84	89	
HPV-16+ (%)	29	53	67	33	45	
CIN 3						
No. of cases (%)	53 (10)	31 (6)	54 (10)	8 (1)	146 (27)	
Carc HPV+ (%)	96	94	96	75	95	
HPV-16+ (%)	58	53	59	63	58	
Missing						
No. of cases (%)	1 (0)	3 (1)	1 (0)		5 (1)	
Carc HPV+ (%)	100	100	100		100	
HPV-16+ (%)	100	67	100		80	
Total						
No. of cases (%)	241 (45)	123 (23)	133 (25)	46 (9)	541 (100)	
Carc HPV+ (%)	83	93	96	83	88	
HPV-16+ (%)	31	49	61	37	42	

CIN, cervical intraepithelial neoplasia; HPV, human papillomavirus; LEEP, loop electrosurgical excision procedure; QC, quality control.

\* The relationship of the paired diagnoses to the percentage of women with positive results for carcinogenic HPV (Carc HPV) or HPV-16 (HPV-16) using the exfoliative cervical specimen obtained at the visit leading to colposcopy is given.

**Table 5**  
**Multivariate Model to Estimate the Association of Select Covariates With a Worst (LEEP or Biopsy) QC Pathology Diagnosis of CIN 3 (vs <CIN 2) Following a CC Pathology Biopsy Diagnosis of CIN 2\***

	Odds Ratio (95% Confidence Interval)	P
HPV risk group by PCR		
Negative or noncarcinogenic	0.32 (0.14-0.75)	.009
Carcinogenic (without HPV-16) <sup>†</sup>	1.0	
HPV-16	4.8 (2.6-8.8)	<.0005
CC liquid-based cytology		
<HSIL <sup>†</sup>	1.0	
HSIL	2.1 (1.2-3.7)	.014
Colposcopic impression at biopsy		
<High-grade <sup>†</sup>	1.0	
High-grade	1.7 (0.91-3.1)	.1
Age at biopsy (y)		
18-21	0.64 (0.30-1.3)	.2
22-23 <sup>†</sup>	1.0	
24-27	1.3 (0.60-3.0)	.5

CC, clinical center; CIN, cervical intraepithelial neoplasia; HPV, human papillomavirus; HSIL, high-grade squamous intraepithelial lesion; LEEP, loop electrosurgical excision procedure; PCR, polymerase chain reaction; QC, quality control.

\* The model was adjusted for the clinical center of the patient.

<sup>†</sup> Reference group.

### Comparison of Clinical Center and QC Pathology Biopsy Diagnoses and HPV Detection

We observed that a biopsy diagnosis of CIN 2 by either pathology group was the least likely to be confirmed by the other. Compared with concordant diagnoses, there was a consistent general trend for the percentage of women testing positive for HPV-16 to track with the severity of the second diagnosis. We infer that HPV risk group may be a useful marker for clarifying disease severity, given the recognized uncertainty of histopathologic diagnoses.<sup>5</sup>

The κ values reported herein for the interrater agreement for diagnoses of cervical biopsy specimens were within the wide range of previously reported values in other studies (reviewed by Malpica et al<sup>21</sup>). In another comparably large study of interrater agreement on cervical biopsy specimens,<sup>21</sup> the authors observed high interpathologist and intrapathologist agreement but low interinstitutional agreement, the latter of which had κ values similar to those observed and may be closer in analytic design to the design used for the present study. Thus, the level of agreement in this analysis was not exceptional compared with other studies that have examined this issue.

### Relationships of Clinical Center Biopsy Diagnoses and QC LEEP Diagnoses

We found that about 75% of women with a clinical center biopsy diagnosis of CIN 2 had no worse than CIN 1 or an HPV infection (<CIN 2; ~48%) or had precancer (CIN 3; ~27%)

based on QC Pathology Group diagnoses of LEEP specimens. That is, a clinical center biopsy diagnosis of CIN 2 had only a 52% predictive value for a CIN 2 LEEP or more severe diagnosis and 27% for a CIN 3 LEEP diagnosis. When the clinical center pathologists and QC pathologists diagnosed CIN 2 or worse on a biopsy specimen, it was much more likely that the subsequent LEEP specimen was diagnosed as CIN 2 or worse or CIN 3 or worse, and this outcome was strongly correlated with a greater fraction of women testing positive for HPV-16.

We also documented that several factors, such as cytologic interpretation of HSIL and colposcopic impression of high-grade disease, were associated with a QC Pathology Group LEEP diagnosis of CIN 3. When cytologic, colposcopic, and biopsy review results were combined as a possible surrogate for lesion size, there was an increasing probability of a QC Pathology Group LEEP diagnosis of CIN 3 with an increasing number of clinical indicators of precancerous lesions. By contrast, a QC Pathology Group LEEP diagnosis of CIN 2 was largely independent of these risk factors ( $P_{\text{trend}} = .7$ ; data not shown). That is, although the likelihood of a CIN 3 or CIN 1 LEEP diagnosis was directly related to other correlative measures of a high- or low-grade lesion, the proportion of CIN 2 LEEP diagnoses was relatively constant in all subsets of women with a CIN 2 biopsy result (eg, 22% of women with a QC Pathology Group review of a biopsy diagnosis of <CIN 2, QC cytologic findings of <HSIL, and colposcopic impression of <HSIL had a CIN 2 LEEP diagnosis vs 21% of women with a QC Pathology Group review biopsy diagnosis of CIN 3, QC cytologic findings of HSIL, and colposcopic impression of high-grade had a CIN 2 LEEP diagnosis). This strongly suggests that **CIN 2 diagnosed on a LEEP sample is not a real disease state but a misclassification of biologic CIN 3 or CIN 1 (HPV infection) that** is independent of other clinical markers of precancer.

### Relationships of Clinical Center Biopsy Diagnoses and Worst QC Diagnoses

One limitation to our analysis comparing biopsy with LEEP diagnoses is the possibility that some biopsy procedures may have resulted in near or complete removal of the lesion. However, the documented inaccuracies of colposcopy<sup>22</sup> would argue against the notion that most lesions were eradicated by the biopsy because colposcopy often misses the most severe pathology. Also, the clinical indications that correlated with the severity of QC Pathology Group LEEP diagnosis were essentially determined before the biopsy, eg, the specimens for cytology and HPV testing were obtained at the screening visit immediately preceding the colposcopic examination during which the colposcopic impression led to a CIN 2 biopsy result. If biopsy eliminated the lesion completely, we would anticipate no associations between these factors



and the observed diagnosis on the LEEP specimen. Complete excision of the lesion by the biopsy, as is possible, would lead to an underdiagnosis of disease, using LEEP as a standard and, therefore, an underestimate of underlying disease associated with a CIN 2 biopsy result. In the context of ALTS, a clinical trial of young women with small precancerous lesions,<sup>23</sup> the likelihood of a well-placed biopsy removing the entire lesion is juxtaposed with the uncertainty of a good representation of the lesion by colposcopically directed biopsy when it is not well placed.

To correct for possible misclassification and to avoid biasing against the smallest lesions that might be most impacted by biopsy, we combined the QC Pathology Group diagnosis of the biopsy and LEEP specimens into a worst histologic categorization. We still found that about 20% were reclassified as no worse than CIN 1 or HPV infection (<CIN 2) and about 41% were reclassified as CIN 3. Of the 188 called CIN 2 on biopsy or LEEP samples, more than half (~63%) represented less than CIN 2–CIN 2 combinations, ie, the biopsy or LEEP specimen was called less than CIN 2 and the other was called CIN 2. Notably, these cases were less likely to test positive for carcinogenic HPV or for HPV-16 than when the biopsy and LEEP specimens were called CIN 2 or at least 1 tissue sample was called CIN 3. In the latter case, women were more likely to test positive for carcinogenic HPV or for HPV-16 than when both diagnoses were called less than CIN 2, suggesting that many of these cases were not precancer. Thus, combining diagnoses led to better identification of CIN 3 but also to some misclassification of disease by diagnosing some cases of HPV infection as CIN 2.

Of note, when QC Pathology Group diagnoses of the biopsy and LEEP specimens were combined to obtain the worst histologic diagnosis, the HPV risk group association strengthened, with HPV-16 being strongly associated with having CIN 3 and testing PCR negative or positive for noncarcinogenic HPV types being negatively associated with CIN 3 (or associated with <CIN 2). Although these patterns are unlikely to be useful in making clinical decisions about who gets treated, the greater prevalence of HPV-16 in exfoliative cervical cells preceding biopsies and LEEPs with results of CIN 2 or worse or CIN 3 suggests a possible role of HPV-16 testing in the quality assurance of histopathologic diagnosis, analogous to the use of carcinogenic HPV testing to monitor cytologic interpretations,<sup>24</sup> particularly of ASCUS. Given the high absolute risk of cervical precancer and cancer attributable to HPV-16 infections,<sup>25,26</sup> even among women with normal cytologic findings, it seems likely that the next generation of commercially available, validated HPV tests will at least include type-specific detection of HPV-16 and HPV-18. Monitoring the correlation of HPV-16 detection with severity of histopathologic diagnosis could reduce systematic overcall and thereby reduce unnecessary treatment.

## Conclusions

Our data are more consistent with a newer model of cervical carcinogenesis,<sup>3</sup> HPV acquisition, HPV persistence, progression to precancer (CIN 3), and invasive cancer than a morphology-based model of incremental progression from normal to atypia to CIN 1 to CIN 2 to CIN 3 to cancer. In particular, we provide evidence that **CIN 2 is not a true biologic entity but an equivocal diagnosis of precancer, representing an admixture of HPV infection and precancer.** The existence of CIN 2 biopsy results as a clinical entity may be the consequence of the inaccuracies of colposcopy and colposcopically directed biopsy, which could result in less-than-perfect representation of the underlying disease state. That CIN 2 is the least reproducible of all histopathologic diagnoses may in part reflect sampling error,<sup>5</sup> ie, the biopsy procedure could make a CIN 1 or HPV infection appear worse by sampling the lesional area diagonally and, thereby, make the lesion appear thicker and could make a CIN 3 lesion appear less severe by only partial sampling the precancerous lesion.

We acknowledge an important limitation of this analysis. The median age of all women with diagnoses of CIN 2 and CIN 3 in ALTS was 23 years, which may be a younger median age than found in routine clinical practice. This younger age may be attributed to the aggressive management leading to early detection of smaller lesions.<sup>23</sup> Thus, the conclusions of this study may not generalize to all populations. Yet, ALTS was designed to be demographically representative of the broader US population, and it is difficult to envision how the analysis presented herein could be biased by a younger median age given that diagnostic criteria for CIN are generally not age-biased.

It is perhaps apparent but important to reemphasize that CIN 2 is a clinical end point of potential worth to ensure safety of treatment protocols, but it has severe limitations when included with CIN 3 as a surrogate end point for etiologic studies of cervical cancer. This may partially account for the greater clearance rates observed for CIN 2 vs CIN 3 in some studies,<sup>27-29</sup> especially when diagnostic criteria are varied, just as they are for cytologic interpretations.<sup>30</sup> In ALTS, we observed indirect but convincing evidence of CIN 2 regression because there were 40% fewer cases of cumulative CIN 2 during 2 years in the conservative management arm because of less sensitive detection of CIN 2 at baseline than in the other 2 arms of the study; by comparison, we observed no difference in the number of cumulative CIN 3 cases by trial arm.<sup>12,13</sup> Studies evaluating the prevention or treatment of CIN 2 and CIN 3, such as therapeutic vaccines, need to take into account that many of the women with a CIN 2 biopsy diagnosis have an infection destined to clear even in the absence of an intervention. Some interventions may hasten the clearance of a subset of CIN 2 cases that are infections rather than precancer. For example, in light of our data, the observed increase

of clearance of CIN 2 in women younger than 25 years vaccinated with a therapeutic vaccine (vs placebo)<sup>31</sup> is probably due to increased clearance of HPV infection rather than precancer.

In ALTS, it is interesting to note that despite the clinical relevance of a CIN 2 biopsy result, it was no better at detecting or predicting a precancerous lesion, QC Pathology Group diagnosed CIN 3, than HPV-16 detection or HSIL cytologic findings. That is, the 2-year absolute risk of CIN 3 or worse, as diagnosed on a LEEP or biopsy specimen by the QC Pathology Group, was similar for a biopsy sample diagnosed as CIN 2 by clinical center pathologists (~41%), HPV-16 detection (~35%),<sup>25</sup> and HSIL cytologic interpretation by the clinical center (~44%).<sup>32</sup>

Although several factors, like HPV-16 detection, were statistically significantly associated with our best measurements of underlying disease, it is important to recognize that none were sufficiently strong indicators for use in clinical practice, which requires a strength of association (OR) of about 25 to be clinically useful.<sup>33</sup> For example, even among women with either PCR negative results or who tested positive for noncarcinogenic HPV types, about 9% had a QC Pathology Group LEEP diagnosis of CIN 3, most likely a function of sampling error. Alternatively, these cases certainly could be the result of misclassification of the QC diagnosis given the observed interrater variability of pathology<sup>5</sup> rather than truly missed CIN 3 diagnoses.

The use of colposcopy-directed CIN 2 biopsy as the clinical threshold of treatment provides a clear margin of safety against "missed" CIN 3 (called CIN 2) but likely results in significant overtreatment of misclassified benign conditions (<CIN 2 called CIN 2). Distinguishing these 2 states of cervical cancer risk, HPV infection vs precancer, is an unresolved but important clinical issue. Excisional treatment based on CIN 2 warrants further consideration, especially in younger women, given the impact of LEEP on pregnancy outcomes.<sup>9</sup> Effective nonsurgical treatments, such as cryotherapy, and the development of effective topical chemopreventives and therapeutic vaccines for women given diagnoses of CIN 2 on biopsy could directly benefit women by reducing the unnecessary surgical treatment of benign conditions. Elimination of the CIN 2 diagnosis by improving the distinction between HPV infection and its manifestations and precancer by using stricter criteria or molecular markers for distinguishing the 2 conditions would also accomplish the same goal. We continue to explore both approaches to improving biopsy-based diagnosis. However, given the inherent limitations of the biopsied tissue itself owing to the de facto errors of colposcopy<sup>22</sup> and biopsy, it is unclear whether clinically useful greater diagnostic accuracy using biopsied tissue samples is achievable. Given our inability to accurately distinguish between HPV infection and precancer among women with a biopsy diagnosis of CIN 2, destructive treatment of such lesions remains warranted to maximize safety.

From the <sup>1</sup>Division of Cancer Epidemiology and Genetics and <sup>3</sup>Division of Cancer Prevention, National Cancer Institute, National Institutes of Health, Department of Health and Human Services, Bethesda, MD; and the <sup>2</sup>Robert E. Fechner Laboratory of Surgical Pathology University of Virginia Health System, Charlottesville.

Supported by contract Nos. CN-55153, CN-55154, CN-55155, CN-55156, CN-55157, CN-55158, CN-55159, and CN-55105 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

Address reprint requests to Dr Castle: Division of Cancer Epidemiology and Genetics, National Cancer Institute, 6120 Executive Blvd, Room 7074, EPS MSC 7234, Bethesda, MD 20892-7234.

Acknowledgments: Some of the equipment and supplies were donated or provided at a reduced cost by Digene, Gaithersburg, MD; Roche Molecular Systems, Alameda, CA; Cytyc, Fenton, MO; Denvu, Tucson, AZ; and TriPath Imaging, Burlington, NC.

\*Affiliations of the ALTS Group: National Cancer Institute, Bethesda, MD: D. Solomon, project officer; and M. Schiffman, coproject officer; Clinical centers: University of Alabama at Birmingham: E.E. Partridge, principal investigator; L. Kilgore, coprincipal investigator; and S. Hester, study manager; University of Oklahoma, Oklahoma City: J.L. Walker, principal investigator; G.A. Johnson, coprincipal investigator; and A. Yadack, study manager; Magee-Womens Hospital of the University of Pittsburgh Medical Center Health System, Pittsburgh, PA: R.S. Guido, principal investigator; K. McIntyre-Seltman, coprincipal investigator; R.P. Edwards, investigator; and J. Gruss, study manager; University of Washington, Seattle: N.B. Kiviat, coprincipal investigator; L. Koutsky, coprincipal investigator; and C. Mao, investigator; Colposcopy Quality Control Group: D. Ferris, principal investigator, Medical College of Georgia, Augusta; J.T. Cox, coinvestigator, University of California at Santa Barbara; and L. Burke, coinvestigator, Beth Israel Deaconess Medical Center Hospital, Boston, MA; HPV Quality Control Group: C.M. Wheeler, principal investigator, and C. Peyton-Goodall, Lab Manager, University of New Mexico Health Sciences Center, Albuquerque; and M.M. Manos, coinvestigator, Kaiser Permanente, Oakland, CA; Pathology Quality Control Group: R.J. Kurman, principal investigator, D.L. Rosenthal, coinvestigator, and M.E. Sherman, coinvestigator, Johns Hopkins Hospital, Baltimore, MD; and M.H. Stoler, coinvestigator, University of Virginia Health Science Center, Charlottesville; Quality of Life Group: D.M. Harper, investigator, Dartmouth Hitchcock Medical Center, Lebanon, NH; Westat, Coordinating Unit, Rockville, MD: J. Rosenthal, project director; M. Dunn, data management team leader; J. Quarantillo, senior systems analyst; and D. Robinson, clinical center coordinator; and Digene, Gaithersburg, MD: A. Lorincz, senior scientific officer.

## References

- Walboomers JM, Jacobs MV, Manos MM, et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. *J Pathol.* 1999;189:12-19.
- Munoz N, Bosch FX, de Sanjose S, et al. Epidemiologic classification of human papillomavirus types associated with cervical cancer. *N Engl J Med.* 2003;348:518-527.
- Wright TC Jr, Schiffman M. Adding a test for human papillomavirus DNA to cervical-cancer screening. *N Engl J Med.* 2003;348:489-490.

4. Richart RM, Barron BA. A follow-up study of patients with cervical dysplasia. *Am J Obstet Gynecol.* 1969;105:386-393.
5. Stoler MH, Schiffman M. Interobserver reproducibility of cervical cytologic and histologic interpretations: realistic estimates from the ASCUS-LSIL Triage Study. *JAMA.* 2001;285:1500-1505.
6. Solomon D, Castle PE. Findings from ALTS: impact on cervical cytology screening, triage, and patient management. *Pathol Case Rev.* 2005;10:128-137.
7. Wright TC, Kurman RJ, Ferenczy A. Precancerous lesions of the cervix. In: Kurman RJ, ed. *Blaustein's Pathology of the Female Genital Tract.* New York, NY: Springer-Verlag; 2002.
8. Solomon D, Schiffman M, Tarone R. Comparison of three management strategies for patients with atypical squamous cells of undetermined significance: baseline results from a randomized trial. *J Natl Cancer Inst.* 2001;93:293-299.
9. Kyrgiou M, Koliopoulos G, Martin-Hirsch P, et al. Obstetric outcomes after conservative treatment for intraepithelial or early invasive cervical lesions: systematic review and meta-analysis. *Lancet.* 2006;367:489-498.
10. Schiffman M, Adriaan ME. ASCUS-LSIL Triage Study: design, methods and characteristics of trial participants. *Acta Cytol.* 2000;44:726-742.
11. 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.
12. ASCUS-LSIL Triage Study (ALTS) Group. A randomized trial on the management of low-grade squamous intraepithelial lesion cytology interpretations. *Am J Obstet Gynecol.* 2003;188:1393-1400.
13. ASCUS-LSIL Triage Study (ALTS) Group. Results of a randomized trial on the management of cytology interpretations of atypical squamous cells of undetermined significance. *Am J Obstet Gynecol.* 2003;188:1383-1392.
14. Gravitt PE, Peyton CL, Alessi TQ, et al. Improved amplification of genital human papillomaviruses. *J Clin Microbiol.* 2000;38:357-361.
15. Gravitt PE, Peyton CL, Apple RJ, et al. Genotyping of 27 human papillomavirus types by using L1 consensus PCR products by a single-hybridization, reverse line blot detection method. *J Clin Microbiol.* 1998;36:3020-3027.
16. Peyton CL, Gravitt PE, Hunt WC, et al. Determinants of genital human papillomavirus detection in a US population. *J Infect Dis.* 2001;183:1554-1564.
17. Bosch FX, Manos MM, Munoz N, et al, for the International Biological Study on Cervical Cancer (IBSCC) Study Group. Prevalence of human papillomavirus in cervical cancer: a worldwide perspective. *J Natl Cancer Inst.* 1995;87:796-802.
18. Cogliano V, Baan R, Straif K, et al. Carcinogenicity of human papillomaviruses. *Lancet Oncol.* 2005;6:204.
19. Kreimer AR, Guido RS, Solomon D, et al. Human papillomavirus testing following loop electrosurgical excision procedure identifies women at risk for posttreatment cervical intraepithelial neoplasia grade 2 or 3 disease. *Cancer Epidemiol Biomarkers Prev.* 2006;15:908-914.
20. Long JS. Nominal outcomes: multinomial logit and related models. In: *Regression Models for Categorical and Limited Dependent Variables.* Thousand Oaks, CA: Sage Publications; 1997:148-186.
21. Malpica A, Maticic JP, Niekirk DV, et al. Kappa statistics to measure interrater and intrarater agreement for 1790 cervical biopsy specimens among twelve pathologists: qualitative histopathologic analysis and methodologic issues. *Gynecol Oncol.* 2005;99(3 suppl 1):S38-S52.
22. Guido R, Schiffman M, Solomon D, et al. Postcolposcopy management strategies for women referred with low-grade squamous intraepithelial lesions or human papillomavirus DNA-positive atypical squamous cells of undetermined significance: a two-year prospective study. *Am J Obstet Gynecol.* 2003;188:1401-1405.
23. Sherman ME, Wang SS, Tarone R, et al. 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. *Cancer Epidemiol Biomarkers Prev.* 2003;12:372-379.
24. Sherman ME, Schiffman MH, Lorincz AT, et al. Toward objective quality assurance in cervical cytopathology: correlation of cytopathologic diagnoses with detection of high-risk human papillomavirus types. *Am J Clin Pathol.* 1994;102:182-187.
25. Castle PE, Solomon D, Schiffman M, et al. Human papillomavirus type 16 infections and 2-year absolute risk of cervical precancer in women with equivocal or mild cytologic abnormalities. *J Natl Cancer Inst.* 2005;97:1066-1071.
26. Khan MJ, Castle PE, Lorincz AT, et al. The elevated 10-year risk of cervical precancer and cancer in women with human papillomavirus (HPV) type 16 or 18 and the possible utility of type-specific HPV testing in clinical practice. *J Natl Cancer Inst.* 2005;97:1072-1079.
27. Meyskens FL Jr, Surwit E, Moon TE, et al. Enhancement of regression of cervical intraepithelial neoplasia II (moderate dysplasia) with topically applied all-trans-retinoic acid: a randomized trial. *J Natl Cancer Inst.* 1994;86:539-543.
28. Follen M, Atkinson EN, Schottenfeld D, et al. A randomized clinical trial of 4-hydroxyphenylretinamide for high-grade squamous intraepithelial lesions of the cervix. *Clin Cancer Res.* 2001;7:3356-3365.
29. Ruffin MT, Bailey JM, Normolle DP, et al. Low-dose topical delivery of all-trans retinoic acid for cervical intraepithelial neoplasia II and III. *Cancer Epidemiol Biomarkers Prev.* 2004;13:2148-2152.
30. Scott DR, Hagmar B, Maddox P, et al. Use of human papillomavirus DNA testing to compare equivocal cervical cytologic interpretations in the United States, Scandinavia, and the United Kingdom. *Cancer.* 2002;96:14-20.
31. Garcia F, Petry KU, Munderspach L, et al. ZYC101a for treatment of high-grade cervical intraepithelial neoplasia: a randomized controlled trial. *Obstet Gynecol.* 2004;103:317-326.
32. Wang SS, Walker JL, Schiffman M, et al. Evaluating the risk of cervical precancer with a combination of cytologic, virologic, and visual methods. *Cancer Epidemiol Biomarkers Prev.* 2005;14:2665-2668.
33. Pepe MS, Janes H, Longton G, et al. Limitations of the odds ratio in gauging the performance of a diagnostic, prognostic, or screening marker. *Am J Epidemiol.* 2004;159:882-890.