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Accuracy of cervical specimens obtained for biomarker studies in women with CIN3

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Abstract

Objective—We developed a protocol to collect representative cervical specimens based on colposcopic evaluation from women treated with loop electrosurgical excision procedure (LEEP).

Methods—We analyzed the histology of biopsies targeting the worst and a normal area on the cervical surface in 74 women referred for LEEP because of cervical intraepithelial neoplasia grade 3 (CIN3) detected in a previous biopsy. Lesions and normal tissue were identified in colposcopy, marked, and removed after LEEP. Cervical cytology specimens collected at the same time were analyzed using Pap cytology and human papillomavirus (HPV) genotyping.

Results—All but two women had an abnormal colposcopic impression with 59 of 68 (87%) showing an impression of CIN2 or greater. In 19 of 58 (33%) women, the histology result of the frozen specimen targeting the worst lesion was $\leq$ CIN1. In 18 of 46 (40%) women, the histology of the frozen specimen targeting normal tissue was CIN2+. A concordant histology result in specimens targeting the worst lesion was associated with a greater extension of the CIN3 in the LEEP (p Trend=0.002) and a HSIL cytology result (p Trend=0.02).

Conclusion—It is challenging to sample representative cervical tissue. Even in women with confirmed CIN3, colposcopy performance to identify the worst lesion on the cervix was limited. Correctly identified CIN3s were more likely to be larger lesions that may have a higher risk of progression to cancer.

Keywords

cervical cancer; HPV; SUCCEED; epidemiology; molecular; colposcopy; biopsy

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Conflict of interest statement

All authors declare that there are no conflicts of interest.

Introduction

Cytological screening has contributed to reducing cervical cancer mortality in industrialized nations by allowing the detection and eradication of precursors prior to the development of invasion, but cytology is very subjective and has limited single-test sensitivity to detect cancer precursors (1). Testing exfoliative cervical cells for human papillomavirus (HPV) DNA, the causative agent of cervical cancer, is becoming increasingly important (2–4). However, the identification of additional molecular markers that predict persistence of HPV and progression to a proximate cancer precursor could further improve screening, and efforts are underway to identify such markers.

Although technological advances are increasing the range of molecular assays that can be performed on fixed tissue, snap frozen tissue is currently the most flexible specimen for large scale molecular profiling. However, collecting tissues of cervical cancer precursors is limited by difficulties in identifying cancer precursors in the fresh state and immediately freezing tissues without delays required for histologic examination. One attempt to address this challenge is to carefully coordinate efforts between colposcopists examining magnified images of the cervix *in vivo* and those of on-site tissue collection teams. In our Study to Understand Cervical Cancer Early Endpoints and Determinants (SUCCEED), we attempted to collect snap frozen fresh tissues of cervical intraepithelial neoplasia grade 3 (CIN3) and normal cervical epithelium from women referred for loop electrosurgical excision procedure (LEEP) to treat CIN3. This report details the procedures employed and the success in obtaining specimens anticipated to represent CIN3 or normal epithelium.

Given that gynecologists typically examine magnified images of the cervix with a colposcope prior to biopsy, one might conclude that coordination between colposcopists and tissue procurement staff might overcome some limitations related to the similar gross appearances of cancer precursors and normal cervix. However, colposcopy itself has only limited accuracy for tissue assessment. In the ASCUS-LSIL triage study (ALTS), up to 50% of CIN3 lesions diagnosed within two years of follow-up were not found at the initial colposcopy, suggesting that many such lesions were unrecognized (5). Regardless of the experience of the colposcopist, sensitivity of detection of CIN3 was improved by increasing the number of biopsies taken (6). Similarly, in the Shanxi Province Cervical Cancer Screening Studies (SPOCCS), random biopsies of visually normal areas significantly increased CIN3 detection (7).

Like clinical practice, tissue analysis in molecular research is limited by the accuracy of colposcopy and biopsy placement. The data from ALTS and SPOCCS show that reliance on a single biopsy for diagnosis may lead to substantial under-estimation of disease severity. In turn, this misclassification might lead to conclusions that a biomarker is non-specific. Cytological specimens, which widely sample the surface epithelium may yield different results than the typical 2–3 mm biopsy of full thickness cervical epithelium. For example, data suggest that detection of HPV DNA in biopsy tissues and cytology collections are discordant about 25% of the time (8). Data also suggest that cytologic interpretations of severe lesions are highly specific and that discordant biopsy results often reflect sampling error (9). Likewise, molecular profiles from biopsy specimens may differ substantially depending on the most severe lesion in the patient.

Methods

SUCCEED study population

Enrollment into SUCCEED started in November 2003 and ended in September 2007. We enrolled women referred to colposcopy at the University of Oklahoma Health Sciences Center (OUHSC) Dysplasia Clinic following an abnormal Pap smear result or a biopsy diagnosis of

CIN. Details of study design, inclusion criteria, HPV DNA genotyping, histology, and cytology procedures have been described elsewhere (9;10). Briefly, women who were less than 18 years-of-age, pregnant at the time of their visit, previously treated with chemotherapy or radiation for any cancer, or women scheduled for vaginal colposcopy were excluded from participation. Written informed consent was obtained from all women enrolled into the study and Institutional Review Board approval was provided by OUHSC and the U.S. National Cancer Institute.

Colposcopy, specimen collection, and LEEP

Per standard practice, all lesions diagnosed as CIN2 or above (CIN2+) were treated by LEEP of the transformation zone. Before LEEP, colposcopic examination was conducted by an experienced gynecologic oncology attending or a fellow under supervision according to routine practice at OUHSC. Quality of colposcopy performance was continuously monitored by weekly meetings to review colposcopy drawings and impression, referral Pap result, and pathology results. Prior to colposcopic examination, cervical cell samples were collected and rinsed directly into PreservCyt™ solution (Hologic, Boxborough, MA) as described previously (11). The cytology specimen was used for ThinPrep™ (Hologic, Boxborough, MA) cytology and for HPV genotyping using the Linear Array (LA) HPV Genotyping Test (Roche Molecular Diagnostics, Branchburg, NJ). First, acetic acid was applied to analyze the overall visual impression. Cervical assessment was based on the categories margin, color, and vessels of the Reid colposcopic index. Based on these categories, the colposcopist documented the overall visual impression of the cervix as normal, CIN1, CIN2, CIN3, or cancer. When required, iodine was applied to identify transformation zone boundaries.

Before performing the LEEP, the colposcopist identified and marked the worst appearing area and a normal appearing area with ink. Of note, in seven of the 77 women included in this analysis, identification of the worst and normal region was based on visual inspection in vivo without the use of a colposcope. Exclusion of these women from the analysis did not alter the results. After performing the LEEP and orienting the specimen according to the markings in colposcopy, 3mm wedges were taken from both areas using a sterile forceps and snap-frozen in liquid nitrogen immediately. Subsequently, 4um-thick sections were cut from the frozen specimens, stained with hematoxylin and eosin and evaluated by a gynecological pathologist (MES).

The LEEP tissue was fixed in formalin and embedded in paraffin. Per standard protocol, every LEEP specimen was separated into 12 blocks and analyzed by the SUCCEED pathologist (REZ or RZ). Lesion size was defined by the number of LEEP blocks showing CIN3 (from a total of 12 blocks analyzed for each LEEP). Frozen tissue was embedded in OCT, stored at -80 C, and sectioned on a cryostat according to standard procedures. Personnel rendering cytology and histologic diagnoses were masked to each other and to HPV genotyping data. The gynecologist performing the LEEP was not aware of the location of the previous biopsy that showed CIN3.

Cases included in the analysis

This analysis includes 77 women scheduled for LEEP for a previously detected CIN3 from whom biopsies at the time of LEEP were taken and evaluated. During histological workup, seven of the LEEPs showed early invasion. These cases were upgraded to cancers and managed accordingly. Of the 77 frozen tissues identified as representing the most severe cervical lesion based on colposcopic impression 13 were not evaluable due to technical reasons or lacked epithelium. In 12 of the 77 women, the colposcopist did not identify a region of normal epithelium and none was collected as frozen tissue for research. In addition, 14 biopsies designated as normal could not be evaluated histologically because of technical reasons or did not display epithelium. After these exclusions, 74 of the 77 women were available for analysis,

with 64 frozen biopsies from regions designated as the worst lesion and 51 frozen biopsies taken from regions designated as normal. Paired biopsies of both the worst lesion and the normal epithelium were available from 41 women. To analyze whether the CIN3 cases included in this study were representative of all CIN3 in SUCCEED, we compared age, cytology result, and HPV16 positivity between the 67 CIN3 cases included in this analysis and the remaining 203 CIN3 LEEPs and did not find any significant differences (data not shown). Six cases were excluded from the comparison of visual diagnosis and histology results due to the lack of visual data (cases included in analysis: 68). Seven cases with early invasive cancer were excluded from the cross-tabulation of worst and normal biopsy results (cases included in analysis: 67). Four cases did not have cytology information (cases included in analysis: 70) and one case with a biopsy targeting the normal tissue did not have information on lesion size (cases included in analysis: 50).

Statistical analyses

First, we compared LEEP and colposcopic impression using histological categories. Next we compared histology results of the biopsies from the worst lesion to those of normal tissue using histological categories. To analyze predictors of discrepant results, we used dichotomous disease groups, $\leq$ CIN1 and of CIN2+. A discrepancy was defined as an interpretation of the frozen tissue as normal/CIN1 when the tissue removed was anticipated to represent CIN3 or greater in the LEEP. Conversely, a biopsy targeting normal tissue was called discrepant when the result was CIN2 or greater.

We analyzed determinants of discrepancies by comparing age, cytology, HPV genotypes and size of the lesion in the LEEP specimens between women with and without discrepancies. To analyze differences of median age and lesion size between women who had a discrepant biopsy result and those who did not, we used the non-parametric Mann Whitney U test. We analyzed percent correctly identified CIN3, percent correctly identified normal tissue, and percent HSIL by lesion size. The Cochran-Armitage Trend test was used to analyze trends of CIN3, normal tissue, and HSIL detection with increasing lesion size. Multivariate logistic regression including age, lesion size, visual impression, HPV16 positivity, and HSIL positivity as independent variables and discrepancy between biopsy and LEEP result as dependent variable was performed to determine predictors of discrepancy. All p-values are two-sided and p-values less than 0.05 were considered significant. All analyses were performed using SAS 9.1 (SAS Institute, Cary, NC) and Stata 10 (Stata Corporation, College Station, TX).

Results

LEEP results and visual impression

Sixty seven of 74 LEEPs included in this analysis had an overall diagnosis of CIN3 and seven showed early invasive cancer by histological examination of paraffin-embedded LEEP tissues. Of the 64 LEEPs from which tissue of the worst lesion was frozen for research, 58 were diagnosed as CIN3 and six as cancer. Of the 51 LEEPs from which a frozen tissue sample of a colposcopically normal appearing region was removed for research, 46 were diagnosed as CIN3 and five as cancer. Among the 41 women with biopsies from both worst appearing and normal appearing regions, 37 had CIN3 and four had cancer.

Colposcopic impression of the most severe lesion was recorded for 68 of the 74 cases. Two of the 61 CIN3 cases (3.3%) had a normal colposcopy impression, seven (11.5%) had a low grade colposcopic impression, while all seven cancer cases had a high grade colposcopic impression. Notably, the most frequent visual impression was CIN2 (40 of 68, 59%) (Table 1).

Discrepancies between frozen biopsy result and LEEP diagnosis

We evaluated the histological diagnoses of the frozen biopsies targeting the worst lesion and normal tissue in cases with a CIN3 in the LEEP. Of the 58 frozen tissue samples targeting the worst lesion, 27 were diagnosed as CIN3 (47%), 12 as CIN2 (21%), ten as CIN1 (17%), and nine as normal (16%), indicating a discrepancy of 33% at the threshold of CIN2+ (Table 2). Five of seven cases with an early cancer identified in LEEP had a cancer biopsy result, the other two were CIN3.

Of the 46 frozen specimens targeting normal tissue in CIN3 cases, 8 were diagnosed as CIN3 (17%), 10 as CIN2 (22%), 6 as CIN1 (13%), and 22 as normal (48%), demonstrating a discrepancy between expected (normal) and observed histology result in 40% of the cases (Table 2). All five frozen specimens targeting normal tissue in cancer cases had a histological diagnosis of CIN3 or cancer.

We compared histological results of frozen specimens targeting the worst lesion with specimens targeting normal tissue in 37 women from whom both biopsies were collected and who had CIN3 in LEEP (central non-shaded area in Table 2). Only 14 of 37 cases (38%) had CIN2+ in the specimen targeting the worst lesion and a $\leq$ CIN1 result in the specimen targeting normal cervix, i.e. observed and expected results agreed. Eleven cases (30%) had a CIN2+ in both specimens, seven cases (19%) had a $\leq$ CIN1 result in both specimens, and in five cases (14%) the lesion was missed in the specimen derived from the worst lesion, but detected in the specimen targeting normal cervix.

Determinants of discrepant histology between biopsy and LEEP

We compared age, cytology, HPV status, visual impression, and lesion size of 19 cases that had a $\leq$ CIN1 result in the biopsy targeting the worst lesion with the 45 cases that had a CIN2+. Women with $\leq$ CIN1 in the specimen targeting the worst lesion had fewer HSIL cytology results (69% vs. 89%, $p=0.062$). The mean number of blocks from LEEPs in which CIN2+ was identified was 6.2, while it was 4.1 in women with a $\leq$ CIN1 result ($p=0.003$). Similarly, we compared age, cytology, HPV status, colposcopic impression, and lesion size of 28 cases that showed a $\leq$ CIN1 result in the specimen from the visually normal region with 23 cases that had high grade histology result. Women with a less than $\leq$ CIN1 result in the visually normal specimen had fewer HSIL cytology results (68% vs. 95%, $p=0.02$). The mean size of the lesions in LEEP was 4.8 for women with a $\leq$ CIN1 result and 5.8 for women with a CIN2+ ($p=0.10$). There was no association between age, HPV16 status, and visual impression with either missed lesion or missed normal tissue in crude or multivariate analyses. Only lesion size was independently predictive of a discrepant biopsy result.

Table 3 shows the association of lesion size with frozen specimen results and cytology results. With increasing number of LEEP segments positive for CIN3 or early cancer, a frozen specimen was more likely to correspond to the worst lesion (p Trend=0.002). Similarly, a larger lesion was more likely to be associated with an HSIL result (p Trend=0.02).

Discussion

Visual signs of precancer have low specificity and are not well reproduced. Therefore, colposcopic impression is frequently inaccurate and biopsy sites may not target the worst lesion on the cervix (12;13). Missing the worst lesion in colposcopically directed biopsy affects cervical cancer screening performance, evaluation of new screening assays, and tissue-based etiologic research.

We analyzed the accuracy of biomarker specimen sampling in a series of LEEPs from women with CIN3 and cancer. In a cross-sectional study of women referred to colposcopy and LEEP,

snap-frozen specimens from cervical lesions and normal tissues were obtained during the LEEP visit. The frozen specimen result was compared to the worst histology result in the LEEP specimen. We identified substantial discrepancies between the expected (i.e. the worst LEEP result) and observed histology: 30% of the expected CIN3 lesions were only CIN1 or normal, and 40% of the expected normal specimens were CIN2 or higher.

Several previous studies have compared cervical biopsies with subsequent LEEP results (14–16). There are two main limitations of such studies: It has been demonstrated that small dysplasias may regress or may be completely removed following the biopsy procedure. Thus, the biopsy result cannot be confirmed in the subsequent LEEP. Even more important, referral to LEEP is usually based on the biopsy result. Thus, false negative biopsies cannot be identified due to referral bias. In a retrospective review, Barker et al. showed 84% agreement between cervical biopsy and subsequent LEEP, but in this setting, the biopsy result determined whether LEEP was performed, indicating referral bias and inflated agreement measures (14). Massad et al. (1996) described a series of women with low grade biopsies that decided to undergo LEEP and showed that 50% of the women with CIN1 biopsies had high-grade lesions in their LEEP.

In our study, LEEP was performed at the same time at which the frozen biopsy was taken, allowing to study biopsy placement with complete disease ascertainment. However, we note several differences between our study procedures and regular colposcopy in women with abnormal screening results. Most importantly, in all women included in this study, a CIN3 was confirmed in a previous biopsy. The gynecologist performing the LEEP was not aware of the location of the previous biopsy although we cannot exclude that a healing wound was still visible at colposcopy. In contrast to regular colposcopy, the task was not to determine the presence of cervical lesions, but to localize them on the cervix. In addition, the frozen specimen was not taken directly under colposcopic view, but marked during colposcopy and excised after the LEEP was performed. It is conceivable that errors were introduced during orientation of the LEEP specimens that would not occur in regular colposcopy. Furthermore, excised tissue may contract leading to distorted appearance and altered spatial relationships. The histological assessment of the LEEP and the frozen specimen was performed in different tissue preparations (paraffin embedded tissue versus snap frozen tissue) and by different pathologists. We do not consider this an important limitation since we did not aim at analyzing subtle histological graduations, but wanted to identify the presence of either CIN3 or normal tissue (with the less reproducible categories CIN1 and CIN2 used as buffer between normal and CIN3).

We analyzed the determinants of discrepancies between observed and expected histology result of frozen specimens. Two variables were associated with a discrepant result, HSIL cytology and lesion size. Smaller lesions and a lower HSIL frequency were associated with cases in which prevalent CIN3 was missed. Conversely, cases in which the specimen intended to sample a normal area, but picked up CIN2+, had larger lesions and a higher HSIL frequency.

Our results demonstrate that even in a previously confirmed CIN3, it can be challenging to identify the worst lesion on the cervix. We can assume that women included in this analysis had large CIN3s, since they were referred to LEEP because of CIN2+ detected in previous biopsies or because they repeatedly showed HSIL cytology.

The goal of collecting frozen biopsies in our study was to analyze molecular determinants of cervical precancer. We are currently conducting mRNA expression analyses of the frozen tissue specimens to identify molecular changes in the transitions from HPV infection to CIN3 and from CIN3 to cancer. Our findings underline the importance of thorough histological assessment of samples collected for research on cervical cancer precursors. Although collected from an undisputed CIN3 or cancer case, almost a third of the samples had $\leq$ CIN1 histology. Analysis of these samples without histological review under the assumption that they represent

a CIN3 results in substantial misclassification. Conversely, a low grade lesion isolated from a cervix that has a concurrent CIN3 or cancer may have different molecular features than a solitary low grade lesion. Our data show that exclusion of specimens not representative of the worst lesion lead to an enrichment of larger, more advanced CIN3s. Therefore it is most difficult to study molecular changes in small, early cancer precursors.

Uncertainty of visual impression during colposcopy results in low sensitivity of current colposcopy-biopsy protocols. Lesion size was an important determinant of correct identification of CIN3. The most promising strategy to improve the detection of small CIN3 during colposcopy is to increase the number of biopsies, as demonstrated in ALTS and SPOCCS (6;7). In the Biopsy Study, an extension of SUCCEED, we are analyzing the effect of collecting more biopsies on cervical disease ascertainment. Extensive digital documentation of colposcopic impression will allow better analyses of the relation between visual and histological findings and may improve current colposcopy-directed biopsy procedures. At the same time, we will be able to improve our gold standard and achieve better precision for etiologic and biomarker studies.

References

1. Schiffman M, Castle PE, Jeronimo J, Rodriguez AC, Wacholder S. Human papillomavirus and cervical cancer. *Lancet* 2007 Sep 8;370(9590):890–907. [PubMed: 17826171]
2. Naucle P, Ryd W, Tornberg S, Strand A, Wadell G, Elfgrén K, et al. Efficacy of HPV DNA testing with cytology triage and/or repeat HPV DNA testing in primary cervical cancer screening. *J Natl Cancer Inst* 2009 Jan 21;101(2):88–99. [PubMed: 19141778]
3. Sankaranarayanan R, Nene BM, Shastri SS, Jayant K, Muwonge R, Budukh AM, et al. HPV screening for cervical cancer in rural India. *N Engl J Med* 2009 Apr 2;360(14):1385–1394. [PubMed: 19339719]
4. Schiffman M, Solomon D. Findings to date from the ASCUS-LSIL Triage Study (ALTS). *Arch Pathol Lab Med* 2003 Aug;127(8):946–949. [PubMed: 12873166]
5. Cox JT, Schiffman M, Solomon D. Prospective follow-up suggests similar risk of subsequent cervical intraepithelial neoplasia grade 2 or 3 among women with cervical intraepithelial neoplasia grade 1 or negative colposcopy and directed biopsy. *Am J Obstet Gynecol* 2003 Jun;188(6):1406–1412. [PubMed: 12824970]
6. Gage JC, Hanson VW, Abbey K, Dippery S, Gardner S, Kubota J, et al. Number of cervical biopsies and sensitivity of colposcopy. *Obstet Gynecol* 2006 Aug;108(2):264–272. [PubMed: 16880294]
7. Pretorius RG, Zhang WH, Belinson JL, Huang MN, Wu LY, Zhang X, et al. Colposcopically directed biopsy, random cervical biopsy, and endocervical curettage in the diagnosis of cervical intraepithelial neoplasia II or worse. *Am J Obstet Gynecol* 2004 Aug;191(2):430–434. [PubMed: 15343217]
8. Gravitt PE, Van Doorn LJ, Quint W, Schiffman M, Hildesheim A, Glass AG, et al. Human papillomavirus (HPV) genotyping using paired exfoliated cervicovaginal cells and paraffin-embedded tissues to highlight difficulties in attributing HPV types to specific lesions. *J Clin Microbiol* 2007 Oct;45(10):3245–3250. [PubMed: 17699644]
9. Wentzensen N, Schiffman M, Dunn ST, Zuna RE, Walker J, Allen RA, et al. Grading the severity of cervical neoplasia based on combined histopathology, cytopathology, and HPV genotype distribution among 1,700 women referred to colposcopy in Oklahoma. *Int J Cancer* 2009 Feb 15;124(4):964–969. [PubMed: 19030188]
10. Wang SS, Zuna RE, Wentzensen N, Dunn ST, Sherman ME, Gold MA, et al. Human papillomavirus cofactors by disease progression and human papillomavirus types in the study to understand cervical cancer early endpoints and determinants. *Cancer Epidemiol Biomarkers Prev* 2009 Jan;18(1):113–120. [PubMed: 19124488]
11. Schiffman M, Adriánza ME. ASCUS-LSIL Triage Study. Design, methods and characteristics of trial participants. *Acta Cytol* 2000 Sep;44(5):726–742. [PubMed: 11015972]
12. Jeronimo J, Massad LS, Castle PE, Wacholder S, Schiffman M. Interobserver agreement in the evaluation of digitized cervical images. *Obstet Gynecol* 2007 Oct;110(4):833–840. [PubMed: 17906017]

13. Massad LS, Jeronimo J, Schiffman M. Interobserver agreement in the assessment of components of colposcopic grading. *Obstet Gynecol* 2008 Jun;111(6):1279–1284. [PubMed: 18515509]
14. Barker B, Garcia F, Lozevski J, Warner J, Hatch K. The correlation between colposcopically directed cervical biopsy and loop electrosurgical excision procedure pathology and the effect of time on that agreement. *Gynecol Oncol* 2001 Jul;82(1):22–26. [PubMed: 11426957]
15. Costa S, Nuzzo MD, Rubino A, Rambelli V, Marinelli M, Santini D, et al. Independent determinants of inaccuracy of colposcopically directed punch biopsy of the cervix. *Gynecol Oncol* 2003 Jul;90(1): 57–63. [PubMed: 12821342]
16. Massad LS, Halperin CJ, Bitterman P. Correlation between colposcopically directed biopsy and cervical loop excision. *Gynecol Oncol* 1996 Mar;60(3):400–403. [PubMed: 8774646]

Table 1

Correlation of visual impression and LEEP diagnosis

		LEEP diagnosis		
		CIN3	CA	Total
Visual impression	NL	2	0	2
	CIN1	7	0	7
	CIN2	38	2	40
	CIN3	14	3	17
	Ca	0	2	2
	Total	61	7	68

NL: Normal; CA: Cancer; NA: Not analyzed

Table 2

Comparison of histology results between targeted normal and targeted lesion in women with CIN3 in LEEP

	Biopsy targeting CIN3				
	NL	CIN1	CIN2	CIN3	NA
Biopsy targeting	6	1	3	9	3
normal	0	0	2	0	4
cervix	2	1	3	4	0
	1	1	1	3	2
	1	6	3	11	0
Total	10	9	12	27	9

NL: Normal; CA: Cancer; NA: Not analyzed

Table 3

Association of lesion size with frozen biopsy result and cytology

LEEP segments with lesion	Frozen biopsy result		HSIL positive n=70
	Worst lesion identified n=64	Normal epithelium* identified n=50	
1	6/14 (43%)	8/11 (73%)	12/18 (67%)
2	6/9 (67%)	3/7 (43%)	8/10 (80%)
3	8/14 (57%)	7/11 (64%)	11/13 (85%)
4	4/5 (80%)	2/3 (67%)	4/5 (80%)
5	8/8 (100%)	5/9 (56%)	9/10 (90%)
6	6/7 (87%)	1/3 (33%)	7/7 (100%)
7	1/1 (100%)	0/2 (0%)	1/1 (100%)
8	1/1 (100%)	0/1 (0%)	1/1 (100%)
9	1/1 (100%)	1/1 (100%)	1/1 (100%)
10	2/2 (100%)	NA	2/2 (100%)
11	2/2 (100%)	1/2 (50%)	2/2 (100%)
p Trend	0.002	0.24	0.02

NA: Not analyzed

* One case is missing lesion size information