

## Multiple Biopsies and Detection of Cervical Cancer Precursors at Colposcopy

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### A B S T R A C T

#### Purpose

Women with abnormal cervical cancer screening results are referred to colposcopy and biopsy for diagnosis of cervical cancer precursors (high-grade squamous intraepithelial lesions [HSILs]). Colposcopy with a single biopsy can miss identification of HSILs. No systematic study has quantified the improved detection of HSIL by taking multiple lesion-directed biopsies.

#### Methods

The Biopsy Study was an observational study of 690 women referred to colposcopy after abnormal cervical cancer screening results. Up to four directed biopsies were taken from distinct acetowhite lesions and ranked by colposcopic impression. A nondirected biopsy of a normal-appearing area was added if fewer than four directed biopsies were taken. HSIL identified by any biopsy was the reference standard of disease used to evaluate the incremental yield and sensitivity of multiple biopsies.

#### Results

In the overall population, sensitivities for detecting HSIL increased from 60.6% (95% CI, 54.8% to 66.6%) from a single biopsy to 85.6% (95% CI, 80.3% to 90.2%) after two biopsies and to 95.6% (95% CI, 91.3% to 99.2%) after three biopsies. A significant increase in sensitivity of multiple biopsies was observed in all subgroups. The highest increase in yield of HSIL was observed for women with a high-grade colposcopic impression, HSIL cytology, and human papillomavirus (HPV) type 16 positivity. Only 2% of all HSILs diagnosed in the participants were detected by biopsies of normal-appearing transformation zone.

#### Conclusion

Collection of additional lesion-directed biopsies during colposcopy increased detection of histologic HSIL, regardless of patient characteristics. Taking additional biopsies when multiple lesions are present should become the standard practice of colposcopic biopsy.

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### INTRODUCTION

Women with abnormal cervical cancer screening results are referred to colposcopy for diagnosis and management. Each year in the United States, about three million women are evaluated with colposcopic procedures by gynecologic oncologists, gynecologists, family physicians, and nurse practitioners.<sup>1,2</sup> Typically, colposcopy results are characterized by a colposcopic impression and by selection of the worst-appearing site for biopsy.<sup>3</sup> The biopsy result determines whether treatment is required by excision of the transformation zone. In addition, women with persistent abnormalities after colposcopy and women un-

dergoing surveillance after treatment are managed with colposcopy.<sup>4</sup>

Despite its importance in the management of cervical abnormalities, there has been no primary evaluation of colposcopy-biopsy performance in the United States. Secondary analyses from screening and vaccination trials have suggested that colposcopic impression and biopsy placement are poorly reproducible<sup>5,6</sup> and fail to detect 30% to 50% of prevalent high-grade squamous intraepithelial lesions (HSILs).<sup>7,8</sup> These data also suggest that taking more biopsies increases the detection of HSILs. Some investigators have proposed that taking biopsies of normal-appearing cervix may identify up to 30% more HSILs.<sup>9,10</sup>

The considerable variability of colposcopy protocols demonstrates uncertainty about standard practice and performance of colposcopy. Although most commonly a single biopsy from the worst-appearing area on the cervix is taken, some centers have adopted an extended biopsy protocol with four-quadrant biopsies.<sup>10</sup> In clinical trials, multiple-biopsy protocols have been used to maximize disease ascertainment.<sup>8,11</sup> We designed the present investigation to quantify the benefit of taking multiple lesion-directed biopsies and an additional biopsy of normal-appearing cervix in a US colposcopy population.

## METHODS

### Population

Women age 18 years and older with abnormal cervical cancer screening results were enrolled and treated at the University of Oklahoma Health Sciences Center (Oklahoma City, OK) between February 2009 and September 2012. Exclusion criteria included previous surgical treatment for cervical disease, prior chemotherapy or radiation treatment for cervical neoplasia, pregnancy, or known HIV infection. Of 2,270 women with appointments at the colposcopy clinic, 897 were found not eligible for enrollment. A total of 690 (50.3%) of 1,373 eligible women agreed to participate in the study and provided written informed consent at the time of enrollment. Institutional review board approval for the study was provided by University of Oklahoma Health Sciences Center and the National Cancer Institute.

### Colposcopy and Biopsy Protocol

Referral to colposcopy was based on the 2007 American Society for Colposcopy and Cervical Pathology guidelines.<sup>4</sup> Six colposcopists performed between 60 and 179 colposcopic examinations each. Before colposcopy, cervical specimens for cytology and human papillomavirus (HPV) testing were collected using a Wallach broom and transferred to PreservCyt solution (Hologic, Marlborough, MA). An extended biopsy protocol involved digital photographic documentation of colposcopic impression and biopsy sites. Up to four lesion-directed biopsies were obtained from distinct areas of epithelium that turned white on application of 5% acetic acid (acetowhite lesions) in the cervical transformation zone using sharp Tischler or baby Tischler biopsy forceps. When less than four directed biopsies were taken, a biopsy was added targeting normal-appearing cervical transformation zone epithelium. The colposcopist ranked all biopsies by order of severity at the time of colposcopy. For each biopsy, hematoxylin and eosin-stained tissue sections were evaluated by a study pathologist for clinical management. In addition, an adjacent tissue section was stained with p16 (CINtec; Roche mtm Laboratories, Mannheim, Germany) and evaluated to establish biomarker-adjudicated HSIL end points.<sup>12</sup> All colposcopic images from study participants were evaluated by an expert colposcopist blinded to the clinical diagnosis. For a sensitivity analysis of nondirected biopsies, we only considered those biopsies evaluated as normal by both the clinic colposcopist and the external reviewer to be nondirected.

### Cytology and HPV Testing

Referral cytology was community based; results were reported using the Bethesda nomenclature, including the categories normal for intraepithelial lesion or malignancy; atypical squamous cells of undetermined significance (ASCUS); low-grade squamous intraepithelial lesion (LSIL); atypical squamous cells, favor high grade; and HSIL.<sup>13</sup> HPV genotyping was conducted from liquid-based cytology specimens collected at enrollment using the Linear Array HPV Genotyping Test (Roche Molecular Diagnostics, Branchburg, NJ) according to the manufacturer's instructions with slight modifications.<sup>14,15</sup>

### Definition of Histologic End Points

Histologic end points were defined based on the worst result from all biopsies taken at colposcopy. We adopted the Lower Anogenital Squamous Terminology guidelines,<sup>16</sup> which dichotomize histology results into LSIL and

HSIL. All patients with cervical intraepithelial neoplasia (CIN) grade 2 (CIN 2) that stained diffusely positive for p16 were considered as having HSIL, along with all patients with CIN grade 3 (CIN 3) regardless of p16 staining. Additional ancillary analyses were conducted to evaluate CIN 2+ and CIN 3 end points. Patients with histologically confirmed CIN 3 and most patients with CIN 2 were referred for treatment according to clinical guidelines.<sup>4</sup>

### Statistical Analysis

The population was stratified by referral cytology result (normal for intraepithelial lesion or malignancy; ASCUS; LSIL; or HSIL, including atypical squamous cells, favor high grade), colposcopy impression (normal, acetowhitening, low-grade lesion, or high-grade lesion), HPV-16 status (negative or positive), and age group (21 to 24, 25 to 29, or  $\geq 30$  years). We considered the following two related measures to evaluate the incremental benefit of multiple biopsies: relative sensitivity and absolute disease yield. Sensitivities for one, two, three, and all four biopsies are the percentages found to have HSIL among all women eventually diagnosed with HSIL. Yield has the same numerator as sensitivity, but the denominator is all women with one, two, three, or four biopsies. Because the colposcopists ranked the biopsies that were taken in terms of severity, we were able to directly measure the marginal increase in sensitivity and yield for each additional biopsy.

When fewer than four biopsies were taken, we could not directly measure the yield of four biopsies. The major results were confirmed for the biopsies actually taken, but for more detailed analyses, we imputed HSIL outcomes for biopsies that were not performed, bracketing the range of plausible estimates. One model imputed the unobserved yields and sensitivities based on an assumption that additional biopsies would not have detected any additional HSIL (ie, that yield of additional biopsies would have been zero), which is the implicit assumption of current clinical practice that takes only one biopsy. Imputation in the second model was based on the assumption that each additional biopsy would have had the same marginal yield as among women for whom the additional directed biopsies were actually performed. As an intermediate, we estimated that additional biopsies had the same yield of HSIL as additional biopsies from normal-appearing sites. All three reasonable assumptions gave similar answers; for data presentation, we showed the third, intermediate imputation approach when estimating yield and sensitivity of multiple biopsies. We calculated 95% CIs for sensitivity estimates using the bootstrap approach. In brief, 1,000 repeated random samples of the same size equaling the overall study population or individual strata were drawn with replacement from the data. The 95% CIs were based on the range of estimates from the 2.5 to the 97.5 percentiles of the ranked random samples. All analyses were performed with SAS version 9.1 (SAS Institute, Cary, NC).

To calculate adjusted yields, we fit multinomial logistic regression models adjusting for colposcopy impression, cytology, HPV status, and age. We used the computer software package SUDAAN (RTI International, Research Triangle Park, NC) to calculate the predictive margins (adjusted proportions) based on the entire analytic sample. SUDAAN produced predictive margins for each category of each covariate in the model. We multiplied predictive margins by the totals from each category to get the expected counts. This method directly standardizes the crude proportions to the distribution of the covariates for the entire sample of study participants<sup>17</sup> to obtain the adjusted proportions.

## RESULTS

### Study Population and Colposcopy Procedures

The median age of women enrolled onto the study was 26 years (range, 18 to 67 years) (Table 1). All women had at least one biopsy; 54.6% of women had four biopsies, 26.6% of women had three biopsies, and 18.8% of women had less than three biopsies. As expected, the number of biopsies increased with increasingly severe colposcopic impression. The median numbers of biopsies were one, three, and

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Table 1. Clinical Characteristics of Study Population

Characteristic	Total No. of Patients	Histologic Diagnosis							
		< CIN 1		CIN 1		CIN 2		CIN 3+*	
		No. of Patients	%	No. of Patients	%	No. of Patients	%	No. of Patients	%
Visit age, years	677†								
Median		27		26		25		26	
Range		19-67		18-67		19-50		19-66	
Referral result	667†	151	100	244	100	196	100	76	100
NILM	2	2	1.3	0	0	0	0	0	0
ASCUS	159	49	32.5	73	29.9	26	13.3	11	14.5
LSIL	287	66	43.7	126	51.6	77	39.3	18	23.7
HSIL	209	34	22.5	44	18.0	89	45.4	42	55.3
Previous biopsy	10	0	0	1	0.4	4	2.0	5	6.6
HPV status	680†	161	100	247	100	196	100	76	100
HPV negative	57	28	17.4	26	10.5	3	1.5	0	0
Noncarcinogenic HPV	86	25	15.5	51	20.6	9	4.6	1	1.3
Carcinogenic HPV, no HPV-16	348	78	48.4	134	54.3	110	56.1	26	34.2
HPV-16	189	30	18.6	36	14.6	74	37.8	49	64.5
Colposcopic impression	678†	159	100	248	100	197	100	74	100
Normal	48	27	17.0	19	7.7	2	1.0	0	0
Acetowhitening	71	31	19.5	31	12.5	7	3.6	2	2.7
Low-grade lesion	339	76	47.8	142	57.3	97	49.2	24	32.4
High-grade lesion	215	25	15.7	56	22.6	91	46.2	43	58.1
Invasive cancer	5	0	0	0	0	0	0	5	6.8
No. of biopsies	683†								
Median		3		3		4		4	
Range		1-4		1-4		1-4		2-4	
p16 immunohistochemistry	682†	160	100	249	100	197	100	76	100
Negative	282	127	79.4	136	54.6	19	9.6	0	0
Positive	400	33	20.6	113	45.4	178	90.4	76	100

Abbreviations: ASCUS, atypical squamous cells of undetermined significance; CIN, cervical intraepithelial neoplasia; HPV, human papillomavirus; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion; NILM, negative for intraepithelial lesion or malignancy.  
 \*Including four cancers.  
 †Missing values are excluded.

four among women with normal colposcopic impression, acetowhitening, and low-grade or high-grade colposcopic impression, respectively. Among 285 women who had endocervical sampling performed, only four women with CIN 2 and three women with CIN

3 were detected in addition to disease that was found with colposcopic biopsies; we did not consider endocervical sampling further in the analysis. All women with CIN 3 or cancer and 90% of women with CIN 2 were positive for p16 in histology (Table 1). We combined

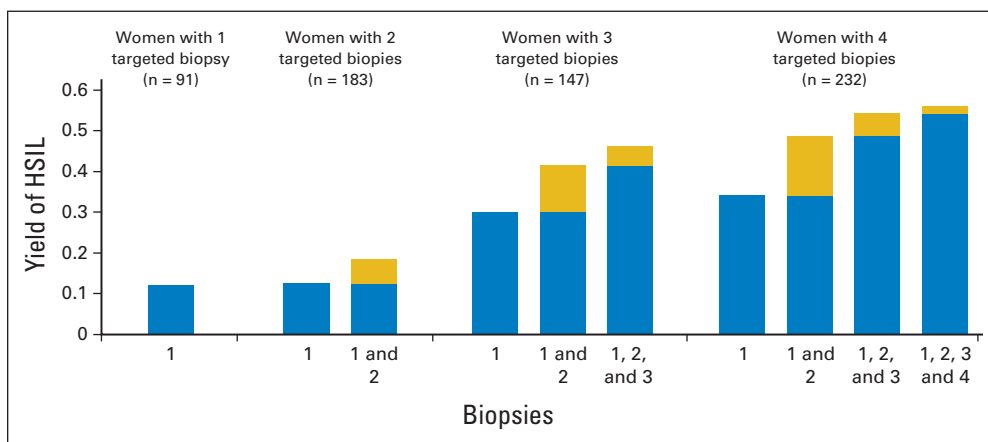


Fig 1. Yield of high-grade squamous intraepithelial lesions (HSILs) by number of biopsies. Bars show the incremental yield of HSILs with increasing numbers of biopsies in women with one, two, three, and four biopsies. All biopsies were targeted at acetowhite or worse-appearing areas in the transformation zone. The gold bars show the incremental yield of each additional biopsy. The blue and gold bars combined show the total yield for the respective number of biopsies.

**Table 2.** Detection of HSIL by Biopsies Targeting an Additional Normal-Appearing Area, by Number of Biopsies Targeting Acetowhite Areas

No. of Biopsies Targeting Acetowhite Areas	No. of Patients	Yield of HSILs Based on Biopsies Targeting Acetowhite Areas		Additional Yield of Biopsy Targeting Normal-Appearing Area	
		No. of HSILs	%	No. of HSILs	%
None	30	NA		1	3.3
1	90	11	12.2	3	3.3
2	181	34	18.8	4	2.2
3	145	68	46.9	2	1.4

Abbreviations: HSIL, high-grade squamous intraepithelial lesion; NA, not applicable.

p16-positive CIN 2 and CIN 3 into an end point of HSIL.<sup>16</sup> Four cancers were included in this group.

### Detection of HSIL Using Lesion-Directed Biopsies

We directly measured the detection of HSIL by increasing numbers of lesion-directed biopsies, separately for women with one, two, three, and four biopsies taken (Fig 1). The yield of HSIL for the first biopsy increased from 12% in women with one directed biopsy to 34% in women with four lesion-directed biopsies, reflecting the increasing severity of the cases. When examining the marginal gain of taking more than one biopsy, the greatest increase in yield was observed for the second biopsy, with decreasing increments for additional biopsies. A second biopsy led to a 6% increase in yield in women with two biopsies and to a 15% increase in yield in women with four biopsies. A third biopsy increased the yield of HSIL by 5% in women with three biopsies and by 6% in women with four biopsies. A fourth biopsy increased the yield of HSIL by 2%.

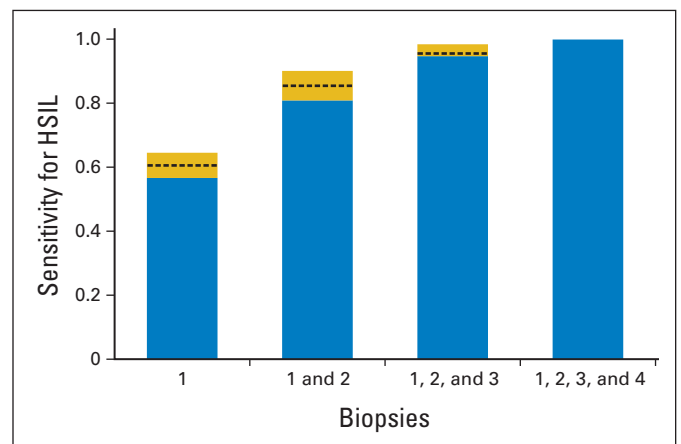
### Detection of HSIL Using Biopsies Targeting Normal-Appearing Areas

In 446 (65%) of 690 women with fewer than four biopsies targeting acetowhite areas, a nondirected biopsy was added. In 30 women, the nondirected biopsy was the only biopsy taken, whereas 90, 181, and 145 women had the nondirected biopsy taken as the second, third, or fourth biopsy, respectively, after lesion-directed biopsies (Table 2). Overall, only 10 additional HSILs were detected by nondirected biopsies (4% of all HSILs in the study). Only one (3%) of 30 nondirected biopsies in women without colposcopic abnormalities yielded HSIL. Among women with one directed biopsy, adding the nondirected biopsy increased the yield of HSIL from 12% to 15%. Among women with two directed biopsies, the yield increased from 19% to 21%, and among women with three directed biopsies, the yield of HSIL increased from 46% to 48%. In models adjusted for colposcopy impression, cytology, and HPV status, we found similar yields of HSIL for additional random biopsies taken (Appendix Table A1, online only). In an ancillary analysis that considered a biopsy as nondirected only if both the clinic review and the external review called the colposcopic impression at the biopsy site normal, only five additional HSILs were detected (2% of all HSIL in the study).

### Cumulative Sensitivity and Yield of HSIL With Increasing Number of Biopsies

Figure 2 shows the incremental sensitivity for HSIL of taking one to four lesion-directed biopsies. We constructed this summary figure using the three imputation approaches, all of which gave similar re-

sults. The sensitivity of the first biopsy was 60.6% (95% CI, 54.8% to 66.6%) and increased to 85.6% (95% CI, 80.3% to 90.2%) for two biopsies and 95.6% (95% CI, 91.3% to 99.2%) for three biopsies (Table 3). The estimated proportion of women who would have an HSIL diagnosis in the overall population increased from 0.24 for taking one biopsy to 0.39 for taking four biopsies. In stratified analyses, the highest yields of HSIL were observed for women with a high-grade colposcopic impression (0.60), HSIL cytology (0.61), and HPV-16 positivity (0.64). In all strata (except for the few women with normal colposcopic impression), the absolute increase in sensitivity from the first to the second biopsy was statistically significant and ranged from 19% to 50%. In women with high-grade colposcopy impression, HSIL cytology, and HPV-16 positivity, we also observed a statistically significant increase of sensitivity from the second to the third biopsy, ranging from 7% to 11%. In models adjusted for colposcopy impression, cytology, HPV status, and age, we found similar marginal yields of HSIL for additional biopsies taken (Appendix Table A2, online only). In sensitivity analyses, results for conventional histologic end points CIN 2 or greater and CIN 3 or greater were similar (Appendix Table A3, online only). We observed similar results for each of the six colposcopists who performed at least 60 colposcopies, and we did not



**Fig 2.** Sensitivity to detect high-grade squamous intraepithelial lesions (HSILs) with increasing numbers of biopsies. Blue bars indicate minimum estimate for sensitivity, with imputation based on the assumption that additional biopsies had the same yield as among women for whom four lesion-directed biopsies were performed. Blue plus gold bars indicate maximum estimate for sensitivity, with imputation based on the assumption that additional biopsies did not detect additional HSILs. Dashed line indicates intermediate estimate for sensitivity, assuming that additional biopsies had the same yield of HSILs as additional biopsies from normal-appearing sites in the transformation zone.

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**Table 3.** Sensitivity for and Yield of HSIL With Increasing Number of Lesion-Directed Biopsies

Biopsy*	Cumulative Sensitivity (%)	95% CI (%)	No. of HSILs Detected	Cumulative HSIL Yield
<b>All (N = 653)</b>				
Biopsy 1	60.6	54.8 to 66.6	157	0.24
Biopsies 1-2	85.6	80.3 to 90.2	222	0.34
Biopsies 1-3	95.6	91.3 to 99.2	246	0.38
Biopsies 1-4	100	100 to 100	252	0.39
<b>Colposcopy impression normal (n = 20)</b>				
Biopsy 1	0	0 to 0	0	0.00
Biopsies 1-2	100	100 to 100	1	0.05
Biopsies 1-3	100	100 to 100	1	0.05
Biopsies 1-4	100	100 to 100	1	0.05
<b>Colposcopy impression acetowhitening (n = 69)</b>				
Biopsy 1	37.5	37.5 to 37.5	3	0.04
Biopsies 1-2	87.5	87.5 to 87.5	7	0.10
Biopsies 1-3	100	100 to 100	8	0.12
Biopsies 1-4	100	100 to 100	8	0.12
<b>Colposcopy impression low grade (n = 339)</b>				
Biopsy 1	58.5	47.65 to 69	67	0.20
Biopsies 1-2	83	74.55 to 91.5	95	0.28
Biopsies 1-3	91.5	83.1 to 99	104	0.31
Biopsies 1-4	100	100 to 100	109	0.32
<b>Colposcopy impression high grade (n = 220)</b>				
Biopsy 1	65	56.8 to 73.5	86	0.39
Biopsies 1-2	88.4	82.7 to 93.8	117	0.53
Biopsies 1-3	99.2	97.5 to 100	131	0.60
Biopsies 1-4	100	100 to 100	132	0.60
<b>Cytology ASCUS (n = 152)</b>				
Biopsy 1	50	31.5 to 67.8	16	0.11
Biopsies 1-2	87.5	75 to 100	28	0.18
Biopsies 1-3	100	100 to 100	32	0.21
Biopsies 1-4	100	100 to 100	32	0.21
<b>Cytology LSIL (n = 269)</b>				
Biopsy 1	54.3	43.1 to 65.4	48	0.18
Biopsies 1-2	83.7	73 to 93.2	74	0.28
Biopsies 1-3	92.8	82.5 to 100	82	0.30
Biopsies 1-4	100	100 to 100	85	0.32
<b>Cytology HSIL (n = 208)</b>				
Biopsy 1	67.8	59.15 to 75.6	86	0.41
Biopsies 1-2	89	83.3 to 94.3	113	0.54
Biopsies 1-3	98.4	96 to 100	124	0.60
Biopsies 1-4	100	100 to 100	126	0.61
<b>HPV-16 negative (n = 465)</b>				
Biopsy 1	50.5	41.8 to 59.25	70	0.15
Biopsies 1-2	80.7	71.85 to 88.9	112	0.24
Biopsies 1-3	92.3	84.1 to 99.1	127	0.27
Biopsies 1-4	100	100 to 100	132	0.28
<b>HPV-16 positive (n = 185)</b>				
Biopsy 1	72.6	63.75 to 80.3	87	0.47
Biopsies 1-2	91.8	85.95 to 96.7	110	0.59
Biopsies 1-3	99.2	97.2 to 100	118	0.64
Biopsies 1-4	100	100 to 100	119	0.64
<b>Age 21-24 years (n = 240)</b>				
Biopsy 1	55.5	45.45 to 65.6	54	0.23
Biopsies 1-2	87.4	78.4 to 94.95	85	0.35
Biopsies 1-3	96.6	89.15 to 100	94	0.39
Biopsies 1-4	100	100 to 100	95	0.40
<b>Age 25-29 years (n = 202)</b>				
Biopsy 1	58.7	47.6 to 69.35	51	0.25
Biopsies 1-2	80.6	70.15 to 90	70	0.35
Biopsies 1-3	93.4	84.05 to 100	80	0.40
Biopsies 1-4	100	100 to 100	83	0.41

(continued on following page)

**Table 3.** Sensitivity for and Yield of HSIL With Increasing Number of Lesion-Directed Biopsies (continued)

Biopsy*	Cumulative Sensitivity (%)	95% CI (%)	No. of HSILs Detected	Cumulative HSIL Yield
Age $\geq$ 30 years (n = 185)				
Biopsy 1	65	53.15 to 77.2	42	0.23
Biopsies 1-2	88.2	79.75 to 96.35	57	0.31
Biopsies 1-3	96.9	92.1 to 100	62	0.34
Biopsies 1-4	100	100 to 100	64	0.35

Abbreviations: ASCUS, atypical squamous cells of undetermined significance; HPV, human papillomavirus; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion.

\*Results include imputed biopsy results. Biopsy 1 = HSIL found with the first biopsy only. Biopsies 1-2 = HSIL found with the first two biopsies. Biopsies 1-3 = HSIL found with the first three biopsies. Biopsies 1-4 = HSIL found with all four biopsies.

observe any temporal changes during the course of the study (Appendix Table A4, online only).

## DISCUSSION

Recent evidence-based updates of cervical cancer screening have led to major changes in screening and management algorithms but have not addressed colposcopy practice.<sup>3,18</sup> The lack of consensus about colposcopic practice in the United States is reflected by a wide variation of colposcopy-biopsy procedures.<sup>10,19</sup>

In the Biopsy Study, we demonstrated that adding a second biopsy, targeting even minimally abnormal-looking areas of the cervical transformation zone, increased the sensitivity to detect a prevalent HSIL significantly from 61% to 86%. Adding a third biopsy increased the sensitivity further to 96%. The incremental benefit of taking multiple biopsies was present regardless of referral cytology, HPV-16 status, and colposcopic impression; for example, even when there was a high-grade colposcopic impression, a single biopsy did not identify a prevalent HSIL in 35% of the women. Importantly, the absolute increase in disease detected by additional biopsies differed across risk strata. In women with increased prior risk of HSIL, related to more severe referral cytology, HPV status, and colposcopic impression, additional biopsies found more disease. Our results were consistent across six well-trained and experienced colposcopists.

Our results extend the evidence from previous secondary analyses from clinical trials suggesting that a single biopsy can fail to detect HSIL in 30% to 50% of patients.<sup>7,8</sup> Unlike the study by Gage et al,<sup>7</sup> we stratified our results by colposcopic impression, which is related both to number of biopsies and severity of disease, and we provide similar estimates of yields when accounting for colposcopic impression, cytology, HPV-16 status, and age. Unlike the study by Stoler et al,<sup>8</sup> which was conducted in young women enrolled onto a vaccination trial, our findings are more generalizable to a US colposcopy population.

Our study identified additional benefit from directed biopsies of visible lesions but limited benefit from additional nondirected biopsies. In studies conducted in China and the United States, multiple nondirected biopsies were taken, and up to 30% of CIN3 lesions were found by these biopsies.<sup>9,10</sup> In our study, multiple lesion-directed biopsies were taken at a lower threshold for abnormality (acetowhitening), possibly explaining the low yield from subsequent nondirected biopsies.

Our study has several other strengths. We used digital documentation of colposcopy impression; all images were re-evaluated by an

experienced colposcopist. Although we used a low threshold of abnormality for targeted biopsies, more than 95% of HSILs were identified in women with at least low-grade colposcopy impression, supporting that most HSILs are found in colposcopically abnormal areas. This is also corroborated by a prospective evaluation that showed a low risk of HSIL in women with normal colposcopic findings when re-examined several years later.<sup>20</sup>

One unavoidable limitation of our study is the lack of a perfect gold standard. Misclassification can occur when the worst lesion is not sampled. However, achieving unbiased estimates of HSIL would require loop electrosurgical excision of all women, independent of biopsy result, which is not ethical. We minimized verification bias by taking multiple biopsies and by imputing additional biopsy results for women with fewer than four biopsies. We observed a plateauing yield of HSIL with increasing number of biopsies, suggesting that few additional HSILs were missed in our study.

More sensitive screening and biopsy approaches may preferentially detect lesions with a low risk of progression to cervical cancer. This is a challenge in all studies evaluating cervical precancer end points. We used a biomarker-enhanced end point of HSIL to achieve higher specificity for cervical cancer precursors.<sup>16</sup>

Our study was highly efficient, because each considered intervention was evaluated in each woman. Despite the additional biopsies required, the participation rate of our study was 50.3%, comparable to similar studies, such as the ASCUS-LSIL Triage Study.<sup>21</sup> Our results are generalizable because we have provided risk and sensitivity estimates stratified by and adjusted for important clinical criteria, such as cytology, HPV status, and colposcopic impression, that can be applied to other populations.

For clinical recommendations, the benefits and harms of taking additional biopsies need to be considered. Taking more biopsies and increasing the sensitivity for HSIL will allow earlier management decisions and provide greater reassurance for women with negative results. Currently, women with negative biopsies undergo repeated colposcopies until HSIL is detected or screening tests turn negative,<sup>4</sup> which is burdensome, emotionally stressful for women, and costly. Potential harms of taking multiple biopsies are discomfort and increased cost of pathology processing and evaluation. In our study, taking additional biopsies with sharp high-quality biopsy forceps was well tolerated. No adverse events occurred in our study; we previously demonstrated that taking more biopsies did not increase the risk of subsequent HPV infections.<sup>22</sup>

The efficiency data presented in our study represent the essential first step to move to consideration of population effectiveness of standardized colposcopy-biopsy protocols. The optimal number of biopsies depends on prior risk, as determined by cytology status, colposcopy impression, and HPV type status.<sup>23</sup> In almost all patients, a second biopsy appears particularly useful. A recent study of endocervical curettage in more than 13,000 women found a yield of only 1% for endocervical sampling,<sup>24</sup> much lower compared with the 10% yield of a second biopsy observed in our study.

Analogously, positive prostate cancer screening results are followed up with biopsies. Studies have demonstrated that conventional biopsy protocols miss up to 30% of prostate cancers, which has led to recommendations of more biopsies.<sup>25</sup>

In summary, our findings reaffirm the importance of colposcopy and lesion-directed biopsy and have important implications for clinical practice—taking multiple directed biopsies during colposcopy can increase detection of HSIL. The full benefit of earlier detection of HSIL by screening using HPV testing<sup>3</sup> will depend on improvement and standardization of colposcopy. Rather than sampling only the worst-appearing site, at least two or three biopsies should be taken

when distinct abnormal sites, including acetowhitening as abnormal, are present.

#### AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at [www.jco.org](http://www.jco.org).

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**AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST**

**Multiple Biopsies and Detection of Cervical Cancer Precursors at Colposcopy**

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## Appendix

Table A1. Observed and Adjusted Marginal Yields of HSIL for Targeted and Random Biopsies

Biopsy	No. of Patients	No. of HSILs				
		Observed Counts	Marginal Yield	Adjusted Counts	Adjusted Marginal Yield (predictive margins)	95% CI
All	419					
Targeted		108	25.78	108	25.78	21.8 to 30.2
Random		8	1.91	8	1.91	0.96 to 3.78
Colposcopy impression normal/acetowhitening	101					
Targeted		6	5.94	11.9	11.79	5.56 to 23.26
Random		3	2.97	2.6	2.55	0.8 to 7.88
Colposcopy impression low grade	235					
Targeted		63	26.81	62.7	26.68	21.67 to 32.38
Random		5	2.13	5.8	2.48	1.01 to 5.97
Colposcopy impression high grade	83					
Targeted		39	46.99	26.3	31.63	23.61 to 40.92
Random		0	0	0	0	0 to 0
Cytology ASCUS	110					
Targeted		17	15.45	19.7	17.91	11.92 to 26.04
Random		0	15.45	0	0	0 to 0
Cytology LSIL	190					
Targeted		37	19.47	43.7	23.01	17.57 to 29.53
Random		3	1.58	2.7	1.41	0.45 to 4.31
Cytology HSIL	119					
Targeted		54	45.38	41.8	35.12	27.58 to 43.47
Random		5	4.2	6.8	5.75	2.39 to 1.32
HPV-16 negative	316					
Targeted		58	18.35	65.2	20.64	1.64 to 25.63
Random		5	1.58	5.1	1.61	0.67 to 3.82
HPV-16 positive	103					
Targeted		50	48.54	40.3	39.15	30.82 to 48.15
Random		3	2.91	3.1	2.98	0.99 to 8.67
No targeted biopsy	26					
Targeted		NA	NA	NA	NA	
Random		1	3.85	1.9	7.47	0.94 to 40.81
One targeted biopsy	83					
Targeted		11	13.25	14.8	17.86	10.65 to 28.39
Random		3	3.61	2.8	3.33	1.14 to 9.29
Two targeted biopsies	172					
Targeted		32	18.6	33.4	19.42	14.21 to 25.96
Random		3	1.74	2.9	1.69	0.54 to 5.17
Three targeted biopsies	138					
Targeted		65	47.1	51.9	37.58	30.0 to 45.83
Random		1	0.72	1.1	0.78	0.1 to 6.06

NOTE. Observed count and yield are based on biopsy results without imputation. Adjusted count and yield are based on predictive margins from multinomial logistic regression, mutually adjusted for colposcopy impression, cytology result, HPV-16 status, and number of targeted biopsies. Differences in numbers between Table 2 and Appendix Table A1 are related to missing covariates, which leads to exclusion of patients for this analysis.

Abbreviations: ASCUS, atypical squamous cells of undetermined significance; HPV, human papillomavirus; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion; NA, not applicable.

**Table A2.** Observed and Adjusted Yield of HSIL With Increasing Number of Biopsies

Biopsy	No. of Patients	No. of HSILs				95% CI
		Observed Counts	Marginal Yield	Adjusted Counts	Adjusted Marginal Yield (predictive margins)	
All	624					
Biopsy 1		152	24.36	152	24.36	21.14 to 27.89
Biopsy 2		60	9.62	60	9.62	7.53 to 12.20
Biopsy 3		18	2.88	18	2.88	1.82 to 4.54
Biopsy 4		4	0.64	4	0.64	0.24 to 1.70
Colposcopy impression normal/acetowhitening	86					
Biopsy 1		3	3.49	7.2	8.41	3.14 to 20.63
Biopsy 2		3	3.49	5.1	5.94	1.97 to 16.54
Biopsy 3		1	1.16	1.3	1.51	0.22 to 9.78
Biopsy 4		0	0	0	0	0 to 0
Colposcopy impression low grade	326					
Biopsy 1		64	19.63	76.3	23.42	19.16 to 28.29
Biopsy 2		27	8.28	28.2	8.66	6.02 to 12.32
Biopsy 3		6	1.84	5.9	1.81	0.78 to 4.14
Biopsy 4		3	0.92	3.4	1.04	0.31 to 3.46
Colposcopy impression high grade	212					
Biopsy 1		85	40.09	63.4	29.89	24.74 to 35.61
Biopsy 2		30	14.15	27.6	13.03	9.19 to 18.17
Biopsy 3		11	5.19	11.8	5.55	2.89 to 10.40
Biopsy 4		1	0.47	0.9	0.42	0.05 to 3.5
Cytology ASCUS	146					
Biopsy 1		16	10.96	24.8	17.02	1.22 to 23.24
Biopsy 2		12	8.22	10.2	6.98	4.02 to 11.84
Biopsy 3		4	2.74	4.5	3.10	1.31 to 7.16
Biopsy 4		0	0	0.8	0.53	0.07 to 3.70
Cytology LSIL	253					
Biopsy 1		46	18.18	48.7	19.26	13.79 to 26.23
Biopsy 2		24	9.49	32.8	12.98	8.51 to 19.30
Biopsy 3		8	3.16	10.6	4.19	1.88 to 9.08
Biopsy 4		2	0.79	3.8	1.49	0.37 to 5.79
Cytology HSIL	202					
Biopsy 1		83	41.09	105.6	52.29	43.47 to 60.98
Biopsy 2		24	11.88	33.5	16.58	11.10 to 24.04
Biopsy 3		6	2.97	6.1	3.02	1.16 to 7.65
Biopsy 4		2	0.99	1.6	0.81	0.12 to 5.24
HPV-16 negative	444					
Biopsy 1		68	15.32	84	18.93	15.41 to 23.02
Biopsy 2		39	8.78	43.9	9.88	7.30 to 13.23
Biopsy 3		13	2.93	14.1	3.18	1.88 to 5.33
Biopsy 4		3	0.68	3	0.69	0.23 to 2.00
HPV-16 positive	180					
Biopsy 1		84	46.67	62.8	34.86	28.99 to 41.24
Biopsy 2		21	11.67	18.5	10.26	6.72 to 15.35
Biopsy 3		5	2.78	4.7	2.59	1.11 to 5.92
Biopsy 4		1	0.56	1.1	0.59	0.1 to 3.44
Age 21-24 years	233					
Biopsy 1		52	22.32	54.3	23.29	18.66 to 28.67
Biopsy 2		30	12.88	30.7	13.18	9.46 to 18.05
Biopsy 3		8	3.43	8.5	3.63	1.82 to 7.1
Biopsy 4		0	0	0	0	0 to 0
Age 25-29 years	195					
Biopsy 1		50	25.64	47	24.10	19.36 to 29.58
Biopsy 2		18	9.23	16.4	8.40	5.34 to 12.97
Biopsy 3		6	3.08	5.3	2.73	1.25 to 5.88
Biopsy 4		2	1.03	1.9	0.96	0.24 to 3.85

(continued on following page)

**Multiple Biopsies and Detection of Cervical Precancers**

**Table A2.** Observed and Adjusted Yield of HSIL With Increasing Number of Biopsies (continued)

Biopsy	No. of Patients	No. of HSILs				95% CI
		Observed Counts	Marginal Yield	Adjusted Counts	Adjusted Marginal Yield (predictive margins)	
Age $\geq$ 30 years	176					
Biopsy 1		41	23.3	41.4	23.53	18.26 to 29.78
Biopsy 2		12	6.82	12.7	7.23	4.20 to 12.16
Biopsy 3		4	2.27	4.2	2.39	0.89 to 6.28
Biopsy 4		2	1.14	2.5	1.43	0.34 to 5.73

NOTE. Observed count and yield are based on biopsy results without imputation. Adjusted count and yield are based on predictive margins from multinomial logistic regression, mutually adjusted for colposcopy impression, cytology result, HPV-16 status, and age group. Biopsies 1-4 = marginal yield of HSIL found with biopsies 1, 2, 3, or 4. Differences in numbers between Table 3 and Appendix Table A2 are related to missing covariates, which leads to exclusion of patients for this analysis. Abbreviations: ASCUS, atypical squamous cells of undetermined significance; HPV, human papillomavirus; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion.

**Table A3.** Sensitivity for and Yield of CIN 2/CIN 3 and CIN 3 Alone With Increasing Number of Lesion-Directed Biopsies

Biopsy	Cumulative Sensitivity (%)	95% CI (%)	No. of CIN 2 and CIN 3 Detected	Cumulative Yield
All (N = 653), CIN 2/CIN 3 end point				
Biopsy 1	62.4	55.8 to 68.6	174	0.27
Biopsies 1-2	86.1	80.4 to 90.8	240	0.37
Biopsies 1-3	95.9	91.4 to 99.3	267	0.41
Biopsies 1-4	100	100 to 100	278	0.43
All (N = 653), CIN 3 end point				
Biopsy 1	65.4	54.4 to 76.4	50	0.08
Biopsies 1-2	90.2	83.1 to 96.1	69	0.11
Biopsies 1-3	96.1	91.4 to 100	73	0.11
Biopsies 1-4	100	100 to 100	76	0.12

Abbreviation: CIN, cervical intraepithelial neoplasia.

**Table A4.** Sensitivity for and Yield of HSIL With Increasing Number of Lesion-Directed Biopsies by Colposcopist

Biopsy	No. of Patients	Cumulative Sensitivity (%)	95% CI (%)	No. of HSILs Detected	Cumulative Yield
Colposcopist 1	172				
Biopsy 1		58.1	45.8 to 70.0	36	0.21
Biopsies 1-2		83.9	75 to 92.3	52	0.30
Biopsies 1-3		96.8	91.6 to 100	60	0.35
Biopsies 1-4		100	100 to 100	62	0.36
Colposcopist 2	84				
Biopsy 1		63.3	45.7 to 80	19	0.23
Biopsies 1-2		90.0	78.3 to 100	27	0.32
Biopsies 1-3		93.3	82.4 to 100	28	0.33
Biopsies 1-4		100	100 to 100	30	0.36
Colposcopist 3	68				
Biopsy 1		32.3	14.2 to 62.1	12	0.18
Biopsies 1-2		56.6	29.1 to 93.5	21	0.31
Biopsies 1-3		68.5	36.5 to 100	25	0.37
Biopsies 1-4		100	100 to 100	37	0.54
Colposcopist 4	72				
Biopsy 1		71	53.1 to 87.5	22	0.31
Biopsies 1-2		93.5	83.9 to 100	29	0.40
Biopsies 1-3		100	100 to 100	31	0.43
Biopsies 1-4		100	100 to 100	31	0.43
Colposcopist 5	175				
Biopsy 1		69.2	56.8 to 80.3	50	0.29
Biopsies 1-2		91.3	81.2 to 98.7	66	0.38
Biopsies 1-3		95.4	85.5 to 100	69	0.39
Biopsies 1-4		100	100 to 100	72	0.41
Colposcopist 6	53				
Biopsy 1		53.3	32.4 to 75	12	0.23
Biopsies 1-2		88.9	73.2 to 100	20	0.38
Biopsies 1-3		100	100 to 100	22	0.42
Biopsies 1-4		100	100 to 100	22	0.42

Abbreviation: HSIL, high-grade squamous intraepithelial lesion.