Colposcopy in Pregnancy: Directed Brush Cytology Compared With Cervical Biopsy

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Objective: **To evaluate colposcopically directed brush cytology as a substitute for directed biopsy of acetowhite lesions identified during pregnancy.**

Methods: **Pregnant patients eligible for the study were referred for colposcopic evaluation for either newly diagnosed abnormal cervical cytology or follow-up of a previously diagnosed squamous intraepithelial lesion (SIL). All patients with acetowhite lesions underwent colposcopically directed brush cytology followed by directed biopsy.**

Results: **Of 81 pregnant patients referred, 50 paired samples of colposcopically directed brush cytology and directed biopsies were evaluated from 49 patients. One patient was sampled in the first and third trimesters and one patient's brush cytology was unsatisfactory for interpretation because of clumping artifact, leaving 49 brush-biopsy pairs that were satisfactory for examination. One patient in the study group had an intrauterine fetal death of uncertain cause, remote from the time of biopsy. Compared with the corresponding biopsy, the directed brush caused significantly less blood loss ($P < .001$). For all diagnostic categories, directed cytology demonstrated a good degree of correlation with biopsy (kappa = 0.73). The brush technique correctly identified 12 of 14 cases (86%) of biopsy-proved cervical intraepithelial neoplasia II–III as high-grade SIL. If one considers "atypical squamous cells, favor human papillomavirus effect" as a true positive, brush sensitivity was 88 ± 9% and specificity was 74 ± 12%, with an accuracy of 80%.**

Conclusion: **In the absence of lesions suspicious for carcinoma, colposcopically directed brush cytology is a safe substitute for directed biopsy in pregnant patients. (Obstet Gynecol 1999;94:198–203.)**

In women with abnormal Papanicolaou smears, colposcopically directed biopsy is the standard initial method for identifying intraepithelial and occult invasive lesions of the uterine cervix.[1–10] In pregnancy, a clinician may be reluctant to perform a biopsy because of the threat of maternal or fetal complications. Several investigators have concluded that biopsy should be performed only on those patients who show colposcopic evidence of invasive carcinoma.[1–5] Others maintain that biopsy should be performed on all cervical lesions detected colposcopically.[6–10] More recently, the use of a brush sampler has been shown to improve the yield of endocervical cells obtained from the endocervical canal, and it can be used as a substitute for a diagnostic endocervical curettage.[11] Careful use of the endocervical brush has been shown to be safe in pregnancy.[12] In this study, we evaluated brush cytology of colposcopically identified lesions as an adjunct in the evaluation of pregnant women who might otherwise undergo colposcopically directed biopsy.

Materials and Methods

This study protocol was approved by the Clinical Investigation Division at the National Naval Medical Center. Pregnant patients with cytologic diagnoses of atypical cells of undetermined significance and squamous intraepithelial lesions (SIL) are referred for colposcopic evaluation by policy of the department of obstetrics and gynecology. These patients were eligible for entry into this study. Patients with a history of SIL requiring colposcopic follow-up during their gestation were also included. All patients had at least one colposcopic evaluation during their pregnancy. No enrolled patient had cytology consistent with carcinoma or glandular cell abnormalities.

All patients gave informed consent for colposcopic evaluation during pregnancy and were assigned a study number that was logged in the study record, with the key known only to two individuals (RWL, JW). All examinations and biopsies were performed by one

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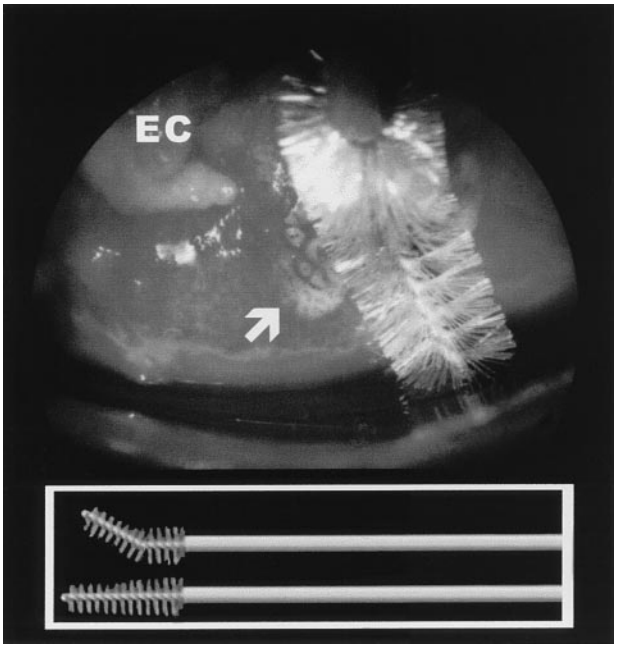

Figure 1. Colposcopic photograph of the directed-brush technique. In allowing for partial rehydration, note that the acetowhite lesion (*arrow*) with punctation and mosaicism has faded slightly. Inset: Cytobrush is bent before obtaining the specimen. EC = endocervix.

individual (RWL). The instrument used in all examinations was the Zeiss OPM Colposcope (Carl Zeiss, Inc., Thornwood, NY) with in-line Polaroid camera attachment (Polaroid Corporation, Cambridge, MA). The sequence of events in the collection of specimens was as follows. The patient was placed in the lithotomy position and a speculum was inserted to allow visualization of the cervix. After a conventional Papanicolaou smear was obtained, 3% acetic acid solution was applied to the cervicovaginal epithelium. Colposcopic photographs were obtained before any brush or biopsy sampling. After identification of the transformation zone and any acetowhite lesions, a determination of the "single worst" appearing area was made, based upon the colposcopist's clinical impression. A directed brush cytology specimen was then collected from this site using a single swab with the Cytobrush (International Cytobrush, Inc., Hollywood, FL) by scraping back and forth across the lesion several times. In most cases, the Cytobrush was bent 30–45° (Figure 1), exposing a larger surface area for sampling. The Cytobrush sample was blotted onto a glass slide and placed in 95% alcohol fixative. After collection of the brush specimens, a cervical biopsy was performed with a Kevorkian biopsy forceps (Cooper Surgical, Shelton, CT) at the same site. All biopsy specimens were placed in 10% formalin solution for fixation. Endocervical curettage was not performed. The colposcopist subjectively assessed the amount of bleeding for the brush and biopsy specimens using the following scoring method: Minimal = spotting or total blood loss less than 5 mL; mild = blood loss estimated at 5–30 mL, but stops with pressure or application of ferric subsulfate solution; moderate = blood loss estimated at 30–50 mL, but stops with pressure and application of ferric subsulfate solution; and marked = bleeding greater than 50 mL, requiring vaginal packing or hospital admission for observation.

The colposcopically directed brush cytology specimens were labeled only with the study number and site of collection. They were processed conventionally, screened by a certified cytotechnologist (SB), and interpreted by a surgical pathologist (WBL) and a cytopathologist (MRH). The corresponding biopsy specimens were processed conventionally, and three 5-μm levels were prepared for examination. These samples were evaluated by a surgical pathologist (WBL) and a gynecologic pathologist (DMO). The terms and criteria used for final diagnoses have been described previously.[13,14]

The patients were divided into three groups based on the following biopsy diagnoses: Group 1 = normal or reactive changes (including atypical cells of undetermined significance, favor a reactive process); group 2 = atypical squamous cells of undetermined significance–human papillomavirus (HPV) and low-grade dysplasias (diagnostic equivalents include low-grade SIL, HPV cytopathic effects, and cervical intraepithelial neoplasia [CIN] I); and group 3 = high-grade dysplasias (including high-grade SIL, CIN II–III). The amount of bleeding and the brush and biopsy diagnoses for each patient were then assigned a weighted numeric value.

Statistical evaluation included analyses of sensitivity and specificity, Wilcoxon signed-rank test of nonparametric pairs, and the kappa statistic using Systat 5.1 for Windows (Systat, Inc., Evanston, IL). Analysis of variance was used to compare differences between groups. $P < .05$ was considered statistically significant.

Results

From September 1, 1992 through April 30, 1994, a total of 81 patients were referred for colposcopic evaluation during pregnancy, including three patients who had previous colposcopy and "normal" Papanicolaou smears who were referred for follow-up colposcopic examination. Ten patients refused to participate in the study for various reasons. Twenty-two patients had no lesions detected on colposcopic examination. Eleven patients had unsatisfactory colposcopy (14%) related to the extent of the lesion or cervical stenosis. Forty-nine patients (60%) had a visible acetowhite lesion detected

Table 1. Summary of Demographic Data

Group*	Age (y)	Gravidity†	Parity	Abortus	Estimated gestational age (wk)
1	25.5 ± 6.6	2.2 ± 1.4	0.3 ± 0.5	0.9 ± 1.2	19.8 ± 5.7
2	23.3 ± 5.7	1.4 ± 0.5	0.2 ± 0.4	0.3 ± 0.5	20.8 ± 7.6
3	24.5 ± 5.2	2.5 ± 1.2	0.6 ± 0.6	0.9 ± 0.7	20.0 ± 9.0

* Group 1 = normal or reactive changes; group 2 = atypical squamous cells of undetermined significance–human papillomavirus and low-grade dysplasias; group 3 = high-grade dysplasias.
† $P = .054$.

colposcopically and underwent directed brush cytology collection followed by biopsy of the worst-appearing area. One patient had brush and biopsy in the first and third trimesters and one patient's brush cytology was unsatisfactory for interpretation because of clumping artifact, leaving 49 brush-biopsy pairs that were satisfactory for examination. These paired brush-biopsy samples constitute the material for this report. The patient demographics are summarized by group in Table 1. There were no significant differences in age, gravidity, parity, abortuses, or mean gestational age among the three groups.

One patient had an intrauterine fetal death at 38 weeks, which was remote from the time of colposcopic directed brush and biopsy. Examination of the infant demonstrated multiple anomalies. Three patients with histories of preterm labor had episodes of premature contractions in the current pregnancies; all episodes were stopped with tocolytic agents and these patients had uncomplicated deliveries at term. All other patients had uneventful pregnancies.

No patient complained of serious discomfort during the brush samplings. Three patients in the third trimester experienced substantial bleeding associated with cervical biopsy. Table 2 presents the subjective assessment of estimated blood loss for brush and biopsy. The decreased blood loss with the directed brush for all gestational ages was highly significant ($P < .001$).

Forty-nine of 50 brush specimens were satisfactory for analysis. Slides from the initial brush samples taken immediately after application of acetic acid demonstrated various degrees of cellular clumping; this was reduced by waiting approximately 30 seconds after colposcopic identification of the abnormal site. The one unsatisfactory smear, obtained early in the study, could not be evaluated because of scant cellularity and distortion from cell clumping. This case was excluded from the paired samples in the statistical calculations.

Table 3 compares the diagnoses from the directed brush and biopsy techniques. The directed brush technique demonstrated good agreement with the corresponding biopsy (kappa = 0.73). The brush identified SIL in 19 of 26 biopsy-proved dysplasias (73%). The brush technique correctly identified 12 of 14 cases (86%) of biopsy-proved CIN II–III as high-grade SIL. For the other two specimens, one brush sample was diagnosed as low-grade SIL, and the other was atypical squamous cells of undetermined significance–HPV. If one considers atypical squamous cells of undetermined significance–HPV as a true positive, the sensitivity of directed brush was 88 ± 9% and specificity was 74 ± 12%, with an accuracy of 82% when compared with biopsy. If atypical squamous cells of undetermined significance–HPV represents a false negative in these calculations, directed-brush sensitivity was 73 ± 12% and specificity was 87 ± 9%, with an accuracy of 80%.

Table 2. Comparison of Blood Loss With Biopsy and Directed Brush Cytology Procedures

Blood loss score	Brush specimens	Biopsy specimens
Minimal	47	9
Mild	3	27
Moderate	0	11
Marked	0	3

$P < .001$, brush versus biopsy, Wilcoxon signed rank test of nonparametric pairs.

Table 3. Comparison of Biopsy Diagnoses and Directed Brush Cytology Diagnoses

	Biopsy diagnosis*		
Brush diagnosis	Group 1	Group 2	Group 3
Normal-ASCUS (reactive)	17	3	0
ASCUS-HPV	3	3	1
LSIL	1	3	1
HSIL (CIN II)	2	2	6
HSIL (CIN III)	0	1	6

ASCUS = atypical squamous cells of undetermined significance; HPV = human papillomavirus; LSIL = low-grade squamous intraepithelial lesion; HSIL = high-grade squamous intraepithelial lesion; CIN = cervical intraepithelial neoplasia.
* For group descriptions, see Table 1.

Papanicolaou smear at the time of colposcopy demonstrated a sensitivity of 58 ± 14%, specificity of 53 ± 14%, and accuracy of 55%. The colposcopic impression was in complete agreement with the biopsy diagnosis in 19 cases (39%), was overcalled in 21 cases (43%), undercalled in nine cases (18%), and within one diagnostic degree in 42 cases (86%).

Discussion

We present a new method for evaluating pregnant women with abnormal cervical cytology using colposcopically directed brushings for cytologic diagnosis. The diagnosis from a brush sample demonstrates good correlation with the corresponding biopsy and appears to predict dysplastic lesions with very good accuracy. This technique is easy to perform and causes significantly less morbidity in the patient than cervical biopsy.

Brush cytology is not a new modality in clinical diagnosis. Numerous investigators have shown that brush cytology is an excellent adjunct to imprint cytology and biopsy for identification of pulmonary lesions.[15,16] In the gastrointestinal tract, the combination of endoscopic biopsy and brush cytology is more sensitive than biopsy alone in making an initial diagnosis.[17,18] When implementing colposcopically directed brush cytology, the gynecologist must communicate clearly with the cytopathologist to ensure proper evaluation of the specimen. The cytopathologist must know that this sample is not a Papanicolaou smear. Although brush slides typically show fewer cells than conventional screening cervical cytology, a diagnostic effort must be made because they are representative of the visualized lesion. Use of standard screening guidelines may otherwise result in a Bethesda-System diagnosis of "less than optimal due to scant cellularity." Cytopathologists routinely render diagnostic interpretations for gastrointestinal and bronchial brush cytology specimens on the basis of a few cells or cell clusters. If the cytopathologist is aware of these characteristics of cervical directed brush cytology, he or she is more likely to render a diagnosis.

In our assessment of the utility of colposcopically directed brush cytology, we used biopsy, the accepted criterion standard, as the diagnostic measure of comparison. Unfortunately, histologic terms do not compare exactly with the Bethesda-System diagnoses of cytology, especially when dealing with atypical squamous cells of undetermined significance–HPV. Consequently, our calculations of sensitivity, specificity, and diagnostic accuracy were performed with atypical squamous cells of undetermined significance–HPV falling both above the cutoff (test positive) and below the cutoff (test negative).

The directed brush confirmed 17 of 23 normal biopsy specimens and 12 of 14 specimens with high-grade dysplasia (86%). It identified SIL in only six of 12 specimens with low-grade dysplasia (50%). The reason for this latter finding is unclear. This may represent subjective differences in identifying cytologic and histologic manifestations of HPV infection,[19] or sampling error. The correlation between brush and biopsy sampling was very high in patients with high-grade dysplasia, suggesting a greater lack of cohesion among these cells and a higher yield of exfoliated cells for review.

Papanicolaou smears obtained at colposcopy correlated poorly with the biopsy diagnoses. This finding supports the conclusion of other investigators that repeated screening Papanicolaou smears alone are inadequate in the follow-up of pregnant patients.[9] Eversion of the endocervix with advancing gestational age may push the transformation zone outside the reach of sampling.

The colposcopic impression predicted the biopsy diagnosis to within one degree of diagnostic severity with reasonable success. Theoretically, many variables can affect the ability of a colposcopist to identify and sample a suspected cervical abnormality.[20] Although physiologic changes during pregnancy can make colposcopic examination difficult, operator experience is the ultimate limitation.

The incidence of squamous cell carcinoma in pregnancy is low (one in 2205 patients[21]) but very real. Progression of CIN to carcinoma during gestation is a theoretical concern and has not been observed. In recent years, there has been a trend toward more conservative management of pregnant patients with cervical neoplasia, even with stage I cervical carcinoma.[22–24] Nevertheless, controversy persists regarding colposcopic biopsy of pregnant women.[10,25]

Adding brush cytology to colposcopy allows one to corroborate the assessment of clinically suspected preinvasive lesions. Because the degree of correlation between directed brush cytology and biopsy was good in this analysis, especially for high-grade lesions, and the trend toward increased morbidity with cervical biopsy was a concern, the biopsy arm of this study was discontinued. Biopsy during pregnancy should be reserved for women who have a suspicion of carcinoma.

After the discontinuation of this protocol, a management algorithm (Figure 2) was proposed and implemented at the National Naval Medical Center for pregnant patients with cytologic intraepithelial abnormalities who are candidates for colposcopy. In this algorithm, colposcopically directed brush cytology is a valuable adjunct in the clinical assessment of the visu-

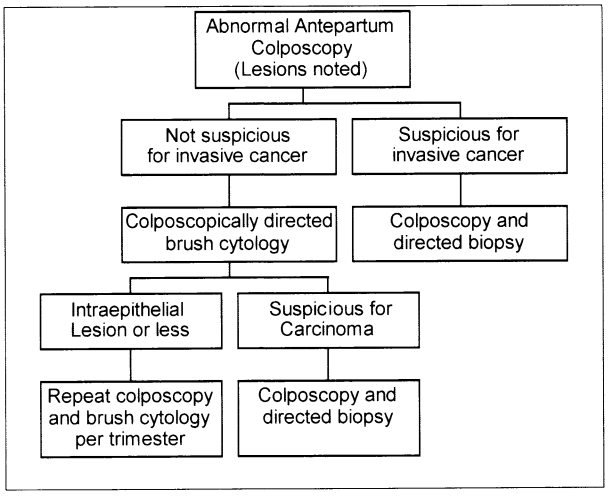

Figure 2. Proposed algorithm for evaluating pregnant patients who present for colposcopic examination.

alized cervix in the absence of lesions clinically suspicious for invasive carcinoma.

The influence of colposcopically directed brush cytology on patient management depends upon the technique of collection and preparation of the slide. Preparation artifacts may make the evaluation of brush cytology difficult. Rapid fixation is essential to prevent air drying. Spray fixative could reasonably substitute for the alcohol fixative used in this study. Allowing time for cervical rehydration (approximately 30 seconds) of the acetowhite areas before brush sampling may help limit artifacts due to acetic acid application. In the future, liquid-based processing techniques may improve the identification of epithelial cell abnormalities.[26] Evaluation of larger patient populations using our proposed algorithm may further establish the value of colposcopically directed brush cytology in pregnancy.

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