The Significance of Positive Margins in Loop Electrosurgical Cone Biopsies

JUAN C. FELIX, MD, LAILA I. MUDERSPACH, MD, BRIDGETTE D. DUGGAN, MD, AND LYNDA D. ROMAN, MD

Objective: **To determine the interpretability and significance of the endocervical margins of cervical cone biopsy specimens removed by the loop electrosurgical excision procedure (LEEP).**

Methods: **Loop electrosurgical cervical conization was performed on 57 women with biopsy-confirmed, high-grade dysplasias in whom the extent of the lesion could not be determined by colposcopic visualization. Internal endocervical margins of the resected specimens were marked with ink by the operating physician and evaluated microscopically by the pathologist. Endocervical curettage (ECC) was done in all instances, and all subjects were followed for 1 year after the procedure.**

Results: **Histologic evaluation of the inked endocervical margins was possible for all 57 resected specimens and was in no instance hindered by thermal artifact. In 19 patients, dysplasia was present in the inked core margin, the ECC, or both. Each patient had re-excisions of the endocervical area; 12 of the 19 (63%) had dysplasia in the specimen. Of 12 cases in which dysplasia was present in both the endocervical margin and the ECC, nine had residual dysplasia. Two of four patients with positive margins but a negative ECC had residual dysplasia, but only one of three patients with a negative endocervical margin and a positive ECC showed residual dysplasia. In the 38 patients with negative inked margins and a negative ECC, there was only one instance of dysplasia demonstrated during the 1-year follow-up period.**

Conclusion: **Endocervical margins of cone biopsies removed by LEEP can be accurately assessed pathologically and can help predict the presence of persistent dysplasia.** *(Obstet Gynecol 1994;84:996–1000)*

The use of the loop electrosurgical excision procedure (LEEP) is reported to be a safe and efficacious method for treating cervical intraepithelial neoplasia.[1] Its major advantage over destructive or ablative procedures is the ability to examine the tissue histologically, which should increase both the accuracy of grading intraepithelial neoplasia and the ability to detect occult invasive lesions.[2,3] At first, indications for LEEP were limited to cervical lesions in which the entire lesion could be defined by colposcopic examination, with no evidence of disease extension into the endocervical canal. However, its use is currently being expanded to include lesions in which the entire extent cannot be determined by colposcopic examination, as well as other lesions that formerly required cold-knife or laser cervical conization. These indications include patients with an endocervical curettage (ECC) positive for dysplastic squamous epithelium and a discrepancy of more than two grades between cervico-vaginal cytology and tissue histologic findings.

A recent case-control report[4] found that, compared with cold-knife conization, loop excision was significantly quicker and was associated with less intraoperative blood loss and fewer postoperative complications. Despite these results, concern exists regarding the ability to determine histologically if dysplasia is present in the margins of the specimen when using loop electrosurgery for conization. In patients undergoing cold-knife conization, the status of the resection margin is used to guide further management. If loop electrosurgical conization is to be a viable alternative to cold-knife conization, the histologic status of the internal margin must be assessable and predictive of residual disease.

We are currently conducting a randomized study comparing LEEP with cold-knife conization in the therapy for colposcopically unsatisfactory, high-grade cervical intraepithelial neoplasia (CIN). This report summarizes the findings of a pilot project designed to evaluate the accuracy and significance of internal margin determination.

From the Departments of Pathology and Obstetrics and Gynecology, University of Southern California School of Medicine, Los Angeles, California.

Aided in part by grant no. IRG–21–33 from the American Cancer Society.

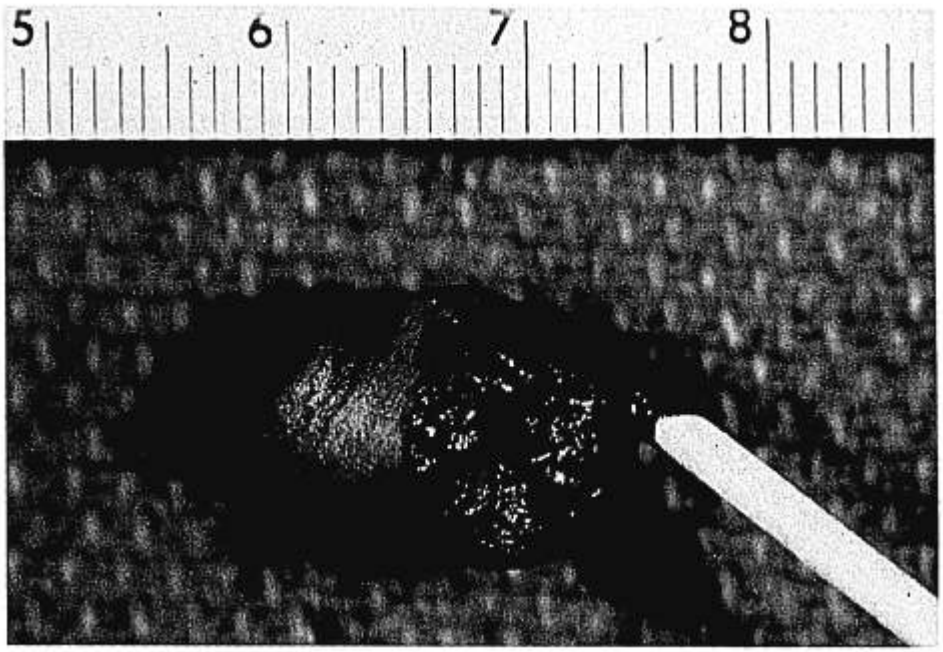

Figure 1. Apical view of a core specimen. Using a wooden applicator, indelible ink is applied along the cut stromal edge of the apex of the specimen. A 2–3-mm rim of ink surrounds the endocervical canal at the apex of resection.

Materials and Methods

Study subjects were selected from the colposcopy clinic of Women's Hospital, Los Angeles County + University of Southern California Medical Center between April 1 and September 30, 1992. Eligible patients presented with cervical dysplasia and met traditional criteria for a diagnostic or therapeutic conization biopsy. In brief, the criteria were biopsy-proven, high-grade squamous intraepithelial lesion (ie, CIN II or III) and either an unsatisfactory colposcopy (inability to visualize the entire squamocolumnar junction or the full extent of the dysplastic lesion) or an endocervical curettage demonstrating high-grade dysplastic epithelium. Exclusion criteria included pregnancy, known human immunodeficiency virus infection or other immunocompromising states, a history of prior cytoablative therapy, or anatomy unsuitable for the safe performance of LEEP (cervix flush with the vaginal fornices or vaginal strictures).

After colposcopic evaluation of the cervical lesion, the cervix was painted with Lugol iodine solution to highlight the resection area. Local anesthetic consisted of circumferential infiltration of the cervical portio with 1% lidocaine hydrochloride with epinephrine at 1:100,000 dilution. A Cooper Surgical Model 6000 unit (Cooper Surgical Instruments, Shelton, CT), equipped with 20 × 8-mm and 10 × 10-mm resecting loops and a 5-mm cautery ball, was used. Excision was performed using the blend setting at a power output of 46 W; cautery was executed using the cautery setting at a power output of 56 W. Several residents in obstetrics and gynecology performed the procedure under faculty staff supervision.

Resection of the transformation zone and all externally visible cervical lesions was accomplished using the 20 × 8-mm loop. In general, two or, occasionally, three sequential passes were required to remove the entire lesion. After the removal of the transformation zone and the external cervical lesion, the canal was inspected and probed with the tip of a moistened cotton swab to determine its full width. The 10 × 10-mm loop was then used to excise a plug of the central cervix; an attempt was made to include the entire breadth of the endocervical canal. The resulting specimen, termed the "core," was removed carefully to preserve proper anatomic orientation and set aside for marking of the internal margin. After excision of the core, ECC was performed using a Kevorkian endocervical curet. Hemostasis was attained using ball cautery and Monsel solution as needed.

After the procedure, the core fragment was wiped gently to remove blood or excess mucus and was oriented with the margin most proximal to the uterine cavity toward the surgeon. Using a wooden applicator, indelible marking ink (The Davidson Marking System, Bradley Products, Bloomington, MN) was applied to the internal cervical surface immediately surrounding the endocervical canal (Figure 1). Care was taken to avoid ink leakage into the endocervical canal of the specimen. The ink was applied until a continuous,

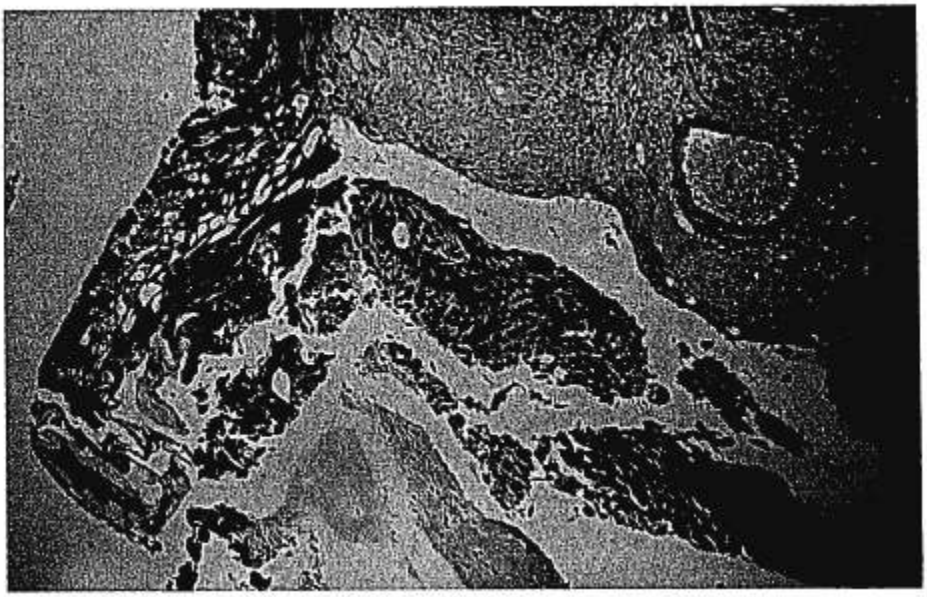

Figure 2. Photomicrograph of a core margin showing cervical intraepithelial neoplasia III extending to the inked margin. Although thermal damage involves approximately 100 μm, dysplastic squamous epithelium is noted within the 500-μm range, within which we consider a margin "positive." Magnification of × 200.

clearly visible ring approximately 2 mm wide was seen around the canal. The specimen was set aside for 3–5 minutes to dry and then placed in formalin.

All loop specimens were sectioned serially and submitted entirely for histopathologic examination. Standard 5-μm sections were placed onto glass slides and stained with hematoxalin and eosin. Dysplasias were graded using the classification of CIN I, II, or III. The core specimen was cut radially in an identical fashion to the cold-knife cone biopsy specimens. We considered a margin to be positive when dysplasia was present at or within one high-power microscopic field (× 400) of the inked margin or approximately 500 μm (Figure 2). Evaluation of the ectocervical margin was done grossly at the time of excision by colposcopy and Lugol staining and was not further analyzed microscopically because colposcopic determination of these margins can adequately predict successful resection. The ECC was considered positive if any amount of dysplastic squamous epithelium was identified. Margin determination was performed by one of three gynecologic pathologists at the Los Angeles County Women's Hospital. One of the authors (JCF) reviewed all margins, but in no instance did the original diagnosis change on review.

Patients with either positive margins, positive ECC, or both were considered excision failures and scheduled for subsequent cold-knife conization 4–6 weeks after loop electrosurgical conization. All subsequent histologic specimens were thoroughly examined for the presence of residual disease. After repeat therapeutic procedures, these patients received follow-up at 3 months, 6 months, and 1 year. Follow-up visits included pelvic examination and Papanicolaou smears.

Patients with negative proximal core margins and negative ECC were followed for 1 year with pelvic examinations, colposcopy, and Papanicolaou smears. Follow-up visits were scheduled at intervals of 2 weeks for cervical inspection only, then at 3 months, 6 months, and 1 year after the procedure for pelvic examination, colposcopy, and Papanicolaou smears.

Complete compliance with the aforementioned schedule did not occur in every instance; however, every patient studied received at least two follow-up visits that included colposcopy and a Papanicolaou smear, the last of which occurred at least 1 year after treatment. At the end of 1 year, all patients with negative follow-up were discharged from our high-risk clinic according to clinic protocol.

Results

Fifty-seven women participated in this study. The mean age was 43 years (range 21–63). The histologic diagnoses ranged from chronic cervicitis to microinvasive squamous cell carcinoma (less than 3 mm depth of invasion and no lymph vascular space invasion), with CIN III accounting for the majority (61%) of findings. Histologic evaluation of the internal endocervical margins revealed that the thermal distortion of tissue was less than 100 μm in depth and did not interfere with margin assessment in any instance.

Table 1 summarizes the evaluations of margins and ECC. Follow-up evaluation of the 19 patients with dysplasia on either the inked margins or ECC revealed that 12 (63%) had residual dysplasia in cervical tissue resected subsequently. Nine of 12 patients with both

Table 1. Follow-up of Patients After Loop Conization by Status of Internal Core Margin and Endocervical Curettage

	No. of cases	Residual dysplasia	No residual dysplasia
+Margin/+ECC	12	9	3
+Margin/−ECC	4	2	2
−Margin/+ECC	3	1	2
−Margin/−ECC	38	1	37

ECC = endocervical curettage.

positive margins and positive ECC had residual dysplasia. Two of four patients with positive endocervical margins but negative ECC and one of three patients with negative endocervical margins but positive ECC had residual dysplasia.

Among the 38 women with negative inked margins and negative ECC, there was only one instance of subsequent dysplasia on follow-up during the first year after loop conization. Cervical intraepithelial neoplasia I with evidence of human papillomavirus (HPV) infection was found in the cervix of this patient 6 months after loop conization for CIN III.

Discussion

Numerous studies[5–8] have established the significance of finding dysplasia at the margins of cone biopsy specimens. Women undergoing conization biopsy for high-grade dysplasia in whom the internal margins are involved, have approximately a 50% risk of residual dysplasia on further evaluation[5,6] and appear to be at increased risk of harboring or developing invasive disease.[7,8] Hysterectomy is usually recommended in such patients if fertility is not an issue.[9,10] Margin status is especially important to ascertain when the conization reveals microinvasive squamous carcinoma or adenocarcinoma in situ. In such cases, the presence of a clear margin would allow conservative follow-up, whereas an involved margin would mandate further therapy.[11–13]

There have been conflicting reports[14–16] regarding the accuracy of margin determination when LEEP is used to perform conization. Furthermore, there is scant information regarding the significance of a positive loop electrosurgical conization margin because follow-up information on such patients has usually not been provided. Baggish et al[14] reported that thermal injury was minimal and did not interfere with the interpretation of margin adequacy in 30 patients undergoing conization with LEEP. Mor-Yosef et al[15] and Oyesanya et al[4] reported similar findings: There were no instances of notable thermal injury in the former study and one instance (2.3%) of margin inassessability in the latter. In contrast, Montz et al[16] found that extensive heat distortion precluded full histologic assessment of endocervical margins in 48% of patients. There was no obvious difference in the power settings or the loop sizes used in these studies. Montz et al theorized that the low rate of thermal injury reported by other authors may reflect their expertise in LEEP technique and that a much higher rate of thermal injury may occur when the procedure is performed by less experienced physicians.

In our study involving thorough evaluation of inked core endocervical margins, in no instance did thermal artifact interfere with histopathologic interpretation and diagnosis. Thermal artifact was less than 100 μm in all instances and usually involved only a few cell layers. This degree of thermal damage correlates well with previously reported measurements for specimens obtained with loop electrosurgical excisions. Baggish et al[14] reported a mean thermal damage of 0.187 μm using loop electrosurgery and stated that such damage made no significant impact on the interpretation of the margins. Similarly, Wright et al[17] reported thermal damage of 150–830 μm in loop electrosurgical specimens and stated that it was possible to evaluate the epithelium and stroma histologically. Our belief that margins with the amount of thermal injury present in Figure 2 can be adequately interpreted is confirmed by the fact that we predicted residual disease with the same accuracy as studies done on cold-knife conization specimens[5,8] when no thermal injury was present. Using the method described, it was easy for the surgeon to ink the internal core endocervical margin. Only the surgeon can optimally orient the core specimen for adequate marking because the initial superficial resection removes part of the lesion and often cuts through dysplasia. Careful marking is essential to allow accurate pathologic interpretation of the internal core margin.

The status of the internal core margin predicted residual disease on subsequent histologic evaluation. Eleven of the 16 patients (69%) with a loop core margin positive for dysplasia had residual dysplasia. Conversely, only two of the 41 in whom the core margin was negative had residual dysplasia. Both cases of recurrence despite negative margins are noteworthy. In one instance, the patient had a positive ECC and was followed aggressively with a cone biopsy. This case probably represents a true false-negative margin and serves to underscore the value of the ECC after conization. In the second instance, a patient treated for CIN III had a Papanicolaou smear showing CIN I with HPV 6 months after her loop cone procedure. We believe that this case most likely represents a reinfection rather than missed residual disease.

The reason for the high rate of thermal damage

observed by Montz et al[16] is unclear. Lack of expert status of the surgeon alone does not appear to be an adequate explanation because most of the procedures in our study were performed by residents in obstetrics and gynecology at various levels of training who were learning the technique from supervising faculty. We believe that adequate maneuverability in removing the core, immediate orientation, and marking of the specimen are key contributors to margin assessability.

We agree with previous authors[14,17] that loop electrosurgical conization can be performed with minimal thermal damage, allowing adequate assessment of the margins. In our study, the significance of having dysplasia at the core margin of a loop electrosurgical conization is comparable to that of the traditional cold-knife conization procedure. Based on the findings of this study, we have begun a randomized controlled trial of loop conization and conventional cold-knife conization as a diagnostic and therapeutic modality for the treatment of CIN.

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Address reprint requests to:
Juan C. Felix, MD
Department of Pathology—1M 19
Women's Hospital
1240 North Mission Road
Los Angeles, CA 90033

Received March 10, 1994.
Received in revised form July 5, 1994.
Accepted July 11, 1994.