

## Endocervical Cytobrush for the Detection of Cervical Dysplasia Before Large Loop Excision of the Transformation Zone (LLETZ)

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**Abstract.** *Background:* We aimed to identify the number of histologically verified cervical intraepithelial neoplasia (CIN)/cervical cancer lesions detected by endocervical cytobrush (EC) which have been missed by repeat PAP smear and colposcopy before large loop excision of the transformation zone (LLETZ). *Patients and Methods:* A retrospective cohort study of 1,676 consecutive patients in a colposcopy clinic undergoing repeat PAP smear, colposcopy, biopsy, and subsequent LLETZ. *Results:* Data were available for 1,421 patients. EC identified 31/1,367 (2.2%) cases of CIN and/or cervical cancer missed by repeat PAP smear and colposcopy. Compared to repeat PAP smear and colposcopy, the combination of repeat PAP smear, colposcopy and EC increased the positive predictive value (PPV) (89.6% vs. 98.9%,  $p=0.07$ ), but this difference was not statistically significant. Sensitivity (93.1% vs. 93.9%;  $p=0.8$ ), specificity (27.7% vs. 27.7%;  $p=1.0$ ), and negative predictive value (NPV) (11.9% vs. 13.1%,  $p=0.9$ ) were also not significantly different. The number needed to screen (NNS) for identifying one additional case of CIN and/or invasive cancer by EC was 45. In a multivariate analysis, presence of CIN/cervical cancer in the LLETZ specimen and human papilloma virus infection, but not age, visibility of the transformation zone or presence of an endocervical lesion were independently associated with the likelihood of CIN and/or invasive cancer detected by EC. *Conclusion:* Adding EC to repeat PAP smear and colposcopy identifies 2.2% more cases of CIN and/or cervical cancer, but does not significantly increase the sensitivity, specificity, PPV or NPV of repeat PAP smear and colposcopy.

The diagnostic work-up of patients with an abnormal PAP smear indicating the presence of high-grade cervical intraepithelial neoplasia (CIN) and/or cervical cancer is controversial. While some suggest that one PAP smear is sufficient as indication for large loop excision of the cervical transformation zone (LLETZ) (1-3), others recommend repeat PAP smear (4), repeat PAP smear combined with colposcopy (5), and repeat PAP smear combined with colposcopy and colposcopically directed cervical punch biopsy (6). The use of additional endocervical cytological sampling has also been suggested, especially in older women and those without an ectocervical lesion visible on colposcopy (7). Adding additional diagnostic procedures, however, increases costs and may not identify a reasonable number of additional cases of CIN and/or cervical cancer. Whether or not adding endocervical sampling by cytobrush to a work-up strategy of repeat PAP smear and colposcopy is a feasible and cost-effective method is unknown.

LLETZ carries significant short- and long-term morbidity. Postoperative complication rates of up to 18% have been described (8, 9). Specifically, hemorrhage, infection and hospital re-admission are observed in 3%, 12%, and 4% of cases, respectively (9, 10). Moreover, LLETZ leads to a significantly increased risk of premature rupture of membranes, preterm delivery and low birth weight (11). Thus, an accurate diagnostic work-up before LLETZ is important. Any additional diagnostic procedure may lead to a loss of specificity and subsequently result in unnecessary additional surgical interventions.

In a retrospective cohort study, we compared the sensitivity and specificity of repeat PAP smear and colposcopy with repeat PAP smear, colposcopy and endocervical cytobrush (EC) for predicting the presence of CIN and/or invasive cancer in the histological specimen after LLETZ. We hypothesized that the combination of repeat PAP smear and colposcopy with EC increases the sensitivity of the diagnostic work-up before LLETZ.

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*Key Words:* Cervical dysplasia, CIN, PAP smear, endocervical cytology, cytobrush.

## Patients and Methods

In a retrospective cohort study, 1,676 consecutive patients undergoing LLETZ for cervical dysplasia from January 1997 to December 2005 were included. Patients were referred to our outpatient clinic by their treating gynecologists. All patients underwent repeat PAP smear, colposcopy with colposcopically directed cervical punch biopsy if a lesion was visible, and EC. All procedures were performed by senior physicians as follows.

After examination of the vulva, perineum and perianal region, a speculum was inserted into the vagina and the cervix was visualized. The examination was started at a low magnification after rinsing the cervix with normal saline and removing any excess cervical mucus. A repeat PAP smear (Cervex-Brush®; Rovers Medical Devices, Oss, The Netherlands) and a separate endocervical cytobrush (Cytobrush® Plus; MedScand Medical Inc., Malmoe, Sweden) on a different slide were obtained. Repeat PAP smear and EC were conventional smears in all cases, liquid-based technology was not used. A complete colposcopic examination was performed including observation of the original squamous epithelium, the transformation zone, the squamocolumnar junction and the columnar epithelium of the cervix. If the squamocolumnar junction was completely visible, that is if the internal – endocervical – limit of the normal or atypical squamous epithelium was entirely apparent, the colposcopy was regarded as satisfactory. A 3% acetic acid solution and Lugol's iodine solution were applied to the cervix. A biopsy was performed under colposcopic guidance if a lesion was visible. Random biopsies were not performed. After completion of the colposcopic examination, all observations were entered in a structured electronic chart.

PAP smears were scored using the Bethesda classification. Histology was reported as negative, condylomata acuminata, mild cervical intraepithelial neoplasia (CIN I), moderate cervical intraepithelial neoplasia (CIN II), severe cervical intraepithelial neoplasia (CIN III), and invasive cervical cancer. Human papilloma virus (HPV) infection was assessed by Hybrid Capture® 2 HPV DNA-Test (Digene Corp., Gaithersburg, MD, USA) according to the manufacturer's instructions.

The decision to perform LLETZ was based on the results of repeat PAP smear, colposcopy and EC. In case of discrepancy between a pathological outside PAP smear result and negative in-house results, the outside pathology lab was contacted and asked to review the slide. If the initial pathological PAP test result was confirmed, LLETZ was performed. Only patients undergoing LLETZ were included in this analysis. LLETZ procedures were carried out as inpatient surgery under general anesthesia. The size and shape of the cone was designed according to the colposcopic picture. The technique of the LLETZ procedure has been described elsewhere (8).

The primary outcome parameter of this study was the rate of histologically verified CIN and/or cervical cancer predicted by repeat PAP smear with colposcopy and repeat PAP smear, colposcopy and EC. For the calculation of sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV), a diagnosis of low-grade squamous intraepithelial lesion (LSIL), high-grade squamous intraepithelial lesion (HSIL) and cervical cancer by repeat PAP smear, EC, or colposcopically directed biopsy were considered correct in the presence of a histological diagnosis of any CIN or cervical cancer in the LLETZ specimen. In cases of a diagnosis of CIN or cervical cancer in the colposcopically directed biopsy specimen and lack thereof in the LLETZ specimen, it was assumed

Table I. Characteristics of the study population.

Characteristic	No. cases
Number of women analyzed	1421
Women's age at study inclusion (years)	35.1 (9.7)*
Transformation zone completely visible	581/1 421 (40.9%)
HPV high-risk positive	656/714 (91.9%)
HPV low-risk positive	95/714 (13.3%)

\*Value is given as mean (standard deviation); HPV, human papillomavirus.

that the complete lesion was removed by biopsy and the biopsy diagnosis was considered correct. A diagnosis of atypical squamous cells of undetermined significance (ASCUS) was rated as a negative test. Values are given as means [standard deviation (SD)] or absolute numbers. Comparisons between groups were made by chi-square test where appropriate. A logistic regression model was performed with CIN and/or cervical cancer predicted by repeat PAP smear, colposcopy and EC as the dependent variable and patient's age, visibility of the transformation zone on colposcopy, presence of an endocervical lesion on colposcopy, presence of high-risk HPV infection, presence of low-risk HPV infection, and histological grade of dysplasia as independent variables. *P*-values of <0.05 were considered statistically significant. The statistical software package SPSS 11.0 for Windows (SPSS Inc., Chicago, IL, USA) was used for statistical analysis.

## Results

In this retrospective cohort study, 1,676 consecutive patients undergoing LLETZ were analyzed. In 90 and 145 patients, PAP smear and EC, respectively, were not performed. In 20 cases, PAP smear and/or EC specimens were considered inadequate by the pathologist. Thus, 255 patients were excluded and 1,421 patients with an adequately sampled PAP smear as well as an adequately sampled EC specimen were available for analysis.

The mean age of the patients was 35.1 (SD 9.7) years. Patient characteristics are shown in Table I. Cytological diagnoses of repeat PAP smear were no dysplasia, ASCUS, LSIL and HSIL in 187, 44, 472 and 718 cases, respectively. Cytological diagnoses of EC were no dysplasia, ASCUS, LSIL and HSIL in 188, 61, 475 and 697 cases, respectively.

On colposcopy, complete visibility of the transformation zone was noted in 581/1,421 (40.9%) cases. The presence of an endocervical lesion on colposcopy was noted in 99/1,421 (7.0%) cases. HPV sampling was performed in 714 patients. Among these, the presence of HPV high-risk infection and HPV low-risk infection was established in 656/714 (91.9%) and 95/714 (13.3%) cases, respectively. Prior to LLETZ, 993/1,421 (69.8%) patients underwent colposcopically directed cervical punch biopsy. Histological evaluation of biopsy specimens found no dysplasia, CIN I, CIN II, CIN III and invasive disease in 82, 155, 283, 466 and 7 cases, respectively.

Table II. Univariate and multivariate regression models for patient and tumor characteristics as predictors for detection of cervical intraepithelial neoplasia (CIN) and cervical cancer by endocervical cytobrush.

Characteristic	Detection of CIN and cervical cancer by endocervical cytobrush	
	Univariate regression <i>p</i> -Value (OR; 95% CI)	Multivariate regression <i>p</i> -Value ( $\beta$ -regression coefficient)
Patient age (<25 years vs. >25 years)	0.3 (1.41; 0.69-2.89)	0.7 (-0.033)
Visibility of the TZ on colposcopy	0.3 (1.21; 0.84-1.77)	0.8 (-0.024)
Endocervical lesion on colposcopy	0.5 (1.26; 0.66-2.45)	0.2 (-0.149)
HPV high-risk infection	0.004 (5.21; 1.48-18.18)	0.02 (-0.294)
HPV low-risk infection	0.06 (1.63; 0.97-2.76)	0.2 (-0.134)
Histologic diagnosis of CIN/cervical cancer*	<0.001 (2.91; 1.78-4.76)	<0.001 (-0.478)

TZ, Transformation zone; OR, odds ratio; CI, confidence interval; HPV, human papilloma virus. \*LLETZ specimen.

Histological evaluation of LLETZ cone specimens found no dysplasia, CIN I, CIN II, CIN III and invasive disease in 54, 189, 341, 752 and 85 patients, respectively. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for predicting CIN and/or invasive cancer were 93.1%, 27.7%, 89.6% and 11.9%, respectively for repeat PAP smear and colposcopy and 93.9%, 27.7%, 98.9% and 13.1%, respectively for repeat PAP smear, colposcopy and EC. Combining repeat PAP smear and colposcopy with EC thus increased the PPV (89.6% vs. 98.9%;  $p=0.07$ ). This difference was of borderline significance. Sensitivity (93.1% vs. 93.9%;  $p=0.8$ ), specificity (27.7% vs. 27.7%;  $p=1.0$ ), and NPV (11.9% vs. 13.1%;  $p=0.9$ ) were not significantly different between groups.

In a univariate analysis, high-risk HPV infection and presence of CIN/cervical cancer in the LLETZ specimen, but not age of the patient, visibility of the transformation zone on colposcopy, presence of an endocervical lesion on colposcopy, or low-risk HPV infection were significantly associated with the likelihood of CIN and/or cervical cancer detected by EC (Table II). In a multivariate analysis, high-risk HPV infection and presence of CIN/cervical cancer in the LLETZ specimen were independent predictors of CIN and/or cervical cancer detected by EC (Table II).

EC identified 59 cases of CIN and/or cervical cancer in addition to repeat PAP smear. The histological diagnoses of these lesions were CIN I, CIN II, CIN III and cervical cancer in 15, 18, 22 and 4 cases, respectively. Of these 59 cases, 28 were diagnosed prior to LLETZ by colposcopically directed biopsy. Thus, 31/1 367 (2.2%) cases of CIN and/or cervical cancer were exclusively detected by EC. The histological diagnoses of these 31 cases were CIN I, CIN II and CIN III in 9, 8 and 11 cases, respectively. Of note, three women were diagnosed with invasive cervical cancer. The number needed to screen (NNS) for identifying one additional case of CIN and/or cervical cancer by adding EC to a repeat PAP smear with colposcopy was 45. On the other hand, 2 out of 1,421

(0.1%) LLETZ procedures were performed solely on the basis of a false-positive EC result. In these two cases, EC indicated the presence of HSIL and the final histology of the LLETZ specimen was negative.

We calculated the additional costs of EC sampling by using the institutional price at our Department of 0.18 USD per EC device and 27 USD for technical handling and interpretation. Thus, the total costs of EC sampling in our patient cohort was 38,622 USD. With an NNS of 45, adding EC to the diagnostic work-up resulted in increased costs of 1,223 USD per additionally identified case of CIN and/or cervical cancer.

## Discussion

In the present study, we found that EC identified 31/1,367 (2.2%) cases of CIN and/or cervical cancer in addition to repeat PAP smear and colposcopy in a patient collective referred to a colposcopy clinic. The NNS for identifying one additional case of CIN and/or cervical cancer by EC was 45 and the associated costs per additionally identified case of CIN and/or cervical cancer were 1,223 USD. The addition of EC, however, did not increase the overall sensitivity, specificity, PPV, or NPV of repeat PAP smear and colposcopy. We conclude that in patients with a pathological PAP smear referred to a colposcopy clinic, adding EC to repeat PAP smear and colposcopy is not necessary prior to LLETZ.

The results of our study are in accordance with previous publications testing the validity of EC in addition to and in comparison with other smear collection techniques within a primary screening setting (12-14). Our data confirm that EC is a valuable diagnostic tool and may provide additional information when combined with other diagnostic modalities such as PAP smear and colposcopy. This difference, however, is too small to justify the routine clinical use of EC in women referred to a colposcopy clinic for a pathological PAP smear.

Our study has limitations. First, the data have been collected in a colposcopy clinic with a specialized gynecopathological department. Therefore, these data may not be representative of other clinical settings. Moreover, it needs to be stressed that these data do not allow any conclusions to be drawn regarding the value of EC within a primary screening programme. All women included in this study were referred to our institution because of an abnormal PAP smear and therefore represent a selected sample. Our study also has strengths, among them the number of patients and the single center study design, ensuring standardized interventions and clinical end-points.

Endocervical cell sampling may be of specific clinical value in older women, who have a higher likelihood of endocervical lesions (6), as well as in those without a colposcopically visible lesion. In our study, however, a multivariate analysis demonstrated that only the histological diagnosis of the LLETZ cone specimen, but not age of the patient, visibility of the transformation zone on colposcopy, or presence of an endocervical lesion on colposcopy was an independent predictor of CIN and/or invasive cancer detected by EC. This indicates that the clinical value of EC is not restricted to a specific subgroup of patients and should therefore not be used in individualized approaches.

In our hands, EC increased the PPV of detecting CIN and/or cervical cancer, but this difference was not statistically significant. Moreover, sensitivity, PPV and NPV of the diagnostic work-up prior to LLETZ were not increased by adding EC to the diagnostic algorithm. In addition, we found that 2/1,421 LLETZ procedures were performed based on a false-positive result of EC. This has to be kept in mind when using the combined work-up strategy of repeat PAP smear, colposcopy and EC. On the other hand, 31 cases of CIN and/or cervical cancer were solely detected by EC. Thus, 31 out of 33 additional LLETZ procedures based on EC results were confirmed in the final histological LLETZ result.

In summary, we found that EC in addition to repeat PAP smear and colposcopy increased the number of women with a pathological test result by 2.2%, but did not increase sensitivity, specificity, PPV, or NPV of repeat PAP smear and colposcopy. We conclude that in patients with a pathological PAP smear referred to a colposcopy clinic, repeat PAP smear and colposcopy are sufficient prior to LLETZ.

### Acknowledgements

Financial Support was kindly provided by Ludwig Boltzmann Institute of Gynecology and Gynecologic Oncology, Vienna, Austria.

### References

- 1 Szurkus DC and Harrison TA: Loop excision for high-grade squamous intraepithelial lesion on cytology: correlation with colposcopic and histologic findings. *Am J Obstet Gynecol* 188(5): 1180-1182, 2003.
- 2 Sadan O, Yarden H, Schejter E, Bilevsky E, Bachar R and Lurie S: Treatment of high-grade squamous intraepithelial lesions: a "see and treat" versus a three-step approach. *Eur J Obstet Gynecol Reprod Biol* 131(1): 73-75, 2007.
- 3 Ng KY, Chang CK, Chen J, Wang PH and Teng SW: Is direct large loop electric excision for the transformation zone reasonable in the investigation of high-grade squamous intraepithelial lesions in cervical smears? *Eur J Gynaecol Oncol* 25(1): 61-65, 2004.
- 4 Dolman G, Tan J and Quinn M: Should the Pap smear be repeated at the first colposcopy visit? *Aust N Z J Obstet Gynaecol* 45(6): 514-517, 2005.
- 5 Byrom J, Douce G, Jones PW, Tucker H, Millinship J, Dhar K and Redman CW: Should punch biopsies be used when high-grade disease is suspected at initial colposcopic assessment? A prospective study. *Int J Gynecol Cancer* 16(1): 253-256, 2006.
- 6 Lindeque BG: Management of cervical premalignant lesions. *Best Pract Res Clin Obstet Gynaecol* 19(4): 545-561, 2005.
- 7 Luzzatto R and Boon ME: Contribution of the endocervical Cytobrush sample to the diagnosis of cervical lesions. *Acta Cytol* 40(6): 1143-1147, 1996.
- 8 Kainz C, Tempfer C, Sliutz G, Breitenacker G and Reinthaller A: Radiosurgery in the management of cervical intraepithelial neoplasia. *J Reprod Med* 41(6): 409-414, 1996.
- 9 Panoskaltzis T, Ind TE, Peryman K, Dina R, Abrahams Y and Soutter WP: Needle versus loop diathermy excision of the transformation zone for the treatment of cervical intraepithelial neoplasia: a randomised controlled trial. *BJOG* 111(7): 748-753, 2004.
- 10 Wright TC, Gagnon S, Richart RM and Ferenczy A: Treatment of cervical intraepithelial neoplasia using the loop electrosurgical excision procedure. *Obstet Gynecol* 79: 17317-17318, 1992.
- 11 Kyrgiou M, Koliopoulos G, Martin-Hirsch P, Arbyn M, Prendiville W and Paraskeva E: Obstetric outcomes after conservative treatment for intraepithelial or early invasive cervical lesions: systematic review and meta-analysis. *Lancet* 367(9509): 489-498, 2006.
- 12 Germain M, Heaton R, Erickson D, Henry M, Nash J and O'Connor D: A comparison of the three most common Papanicolaou smear collection techniques. *Obstet Gynecol* 84(2): 168-173, 1994.
- 13 McCord ML, Stovall TG, Meric JL, Summitt RL and Coleman SA: Cervical cytology: a randomized comparison of four sampling methods. *Am J Obstet Gynecol* 166: 1772-1777, 1992.
- 14 Hoffman MS, Hill DA, Gordy LW, Lane J and Cavanagh D: Comparing the yield of the standard Papanicolaou and endocervical brush smears. *J Reprod Med* 36(4): 267-269, 1991.

Received May 15, 2008

Revised June 24, 2008

Accepted July 1, 2008