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LLETZ (Large Loop Excision Transformation Zone) in the management of cervical intraepithelial neoplasia with positive margins: a retrospective comparative analysis

Matteo Terrinoni^{1,2,*,#}, Luisa Alfonsi^{3,#}, Angelo Baldoni¹, Gian Carlo Di Renzo^{4,5}

¹Department of Medicine and Surgery, University of Perugia, Perugia, Italy.

²Department of Obstetrics and Gynecology, "Alto Tevere" Hospital of Città di Castello, USL Umbria 1, Perugia, Italy.

³Department of Obstetrics and Gynecology, "Branca" Hospital of Gubbio-Gualdo Tadino, USL Umbria 1, Perugia, Italy.

⁴PREIS School, International and European School of Perinatal, Neonatal and Reproductive Medicine, Florence, Italy.

⁵Department of Obstetrics, Gynecology and Perinatology, I.M. Sechenov First State University of Moscow, Moscow, Russia.

The authors contributed equally to the work both as first authors

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*Corresponding author: Matteo Terrinoni,

M.D. Department of Medicine and Surgery, University of Perugia, piazzale Settimio Gambulli 1, 06129 Perugia, Italy.

Email: matteo.terrinoni@unipg.it.

ORCID: 0009-0009-5087-0374.

ABSTRACT

Objective. Evaluate the outcomes of LLETZ in patients with cervical intraepithelial neoplasia to determine if conservative postconization followup is reasonable.

Materials and Methods. Retrospective observational study where 370 patients who underwent LLETZ between January 2021 and December 2023 at a single tertiary centre were analysed. Inclusion criteria: colposcopic biopsy confirmed diagnosis of cervical intraepithelial neoplasia, no prior cervical interventions. Exclusion criteria: history of vaginal intraepithelial neoplasia, pregnancy and immunocompromised status. Patients were stratified by margin status in positive margins (n = 48) (endocervical and exocervical) *versus* complete excision (n = 322). Followup included colposcopy, Pap test, and highrisk HPV testing at 3 months (positive margins only), 6 months, and 12 months. Statistical analyses were performed with SPSS 26 with significance set at $p \leq 0.05$.

Results. Among 370 patients, the definitive histology was CIN 1(150 cases), CIN 2(134), CIN 3(82), with a few cases of carcinoma. The 18 patients positive endocervical margins, achieved complete regression at 6 months, the 30 with positive exocervical margins, all had normal colposcopic and PAP findings at 6 months (20% transient positive HPV test). In the complete excision group, follow-up revealed a comparable rate of abnormal findings. Comparative analysis did not reveal a statistically significant difference in recurrence rates between the two groups ($p \leq 0.05$), suggesting that conservative management could be reasonable. No patient in the positive margin group required immediate reexcision.

Conclusions. LLETZ is a safe, effective treatment for CIN. Positive surgical margins do not necessarily predict a higher risk of residual disease. Long-term, prospective studies with HPV genotyping are needed to optimize surveillance guidelines.

INTRODUCTION

Cervical conization has long been established as gold standard treatment for cervical intraepithelial neoplasia [1, 2]. In the evolution of conization techniques, from cold knife to electrosurgical methods, LLETZ (Large Loop Excision of the Transformation Zone) has gained favour for its low blood loss, shorter operative times, and preservation of cervical anatomy and function [3, 4]. Despite its widespread use, positive margins remain a clinical challenge, potentially signifying residual disease [5]. Previous literature has identified risk factors for residual disease, including lesion grade, margin status, and endocervical involvement. Positive margins remain debated because endocervical involvement may reflect deeper glandular spread, whereas exocervical involvement suggests more superficial persistence. Some studies propose that thermal artifacts from electrosurgery may ablate residual microscopic disease at the margin, reducing true recurrence risk [6-8]. However, a clear comparative analysis of outcomes in patients with positive *versus* negative margins is lacking.

Study hypothesis and endpoints: we hypothesize that conservative management with structured followup (including an early 3month colposcopic evaluation for positive margins) leads to similar rates of residual/recurrent highgrade disease and HPV persistence as cases with negative margins. Primary endpoints were residual/recurrent CIN 2+ at 12 months and persistent highrisk HPV at 12 months. Secondary endpoints included transient cytological abnormalities and procedure-related complications.

MATERIALS AND METHODS

Study design and population

We conducted a retrospective analysis at the Lower Genital Tract Pathology Service of the Department of Obstetrics and Gynecology of the University Hospital of Perugia. All patients diagnosed with CIN via colposcopic biopsy and treated with LLETZ between January 2021 and December 2023 were included.

Inclusion criteria were age 18–65 years; colposcopy-guided biopsy-confirmed CIN 1, CIN 2, or CIN 3; no prior cervical interventions.

Exclusion criteria were history of vaginal intraepithelial neoplasia (VaIN); pregnancy during the diag-

nostic–therapeutic process; immunocompromised status (e.g., HIV-positive, organ transplant recipients on immunosuppressive therapy); incomplete medical records; loss to followup before 6 months.

Surgical technique

LLETZ was performed in a day surgery setting using radiofrequency energy (3.8 MHz) with tungsten loop electrodes for cutting and sphere electrodes for coagulation (28–50W). The choice of loop size (10, 15, 20, or 25 mm) was tailored to the lesion's extent and cervical morphology. For visible exocervical lesions, complete removal of the transformation zone was attempted, followed by an endocervical cone excision if indicated. In cases with a non-visible squamocolumnar junction, endocervical cytology was also obtained.

Histopathological examination and margin definition

Resected specimens were oriented with landmarks, fixed in 4% formalin, and evaluated for lesion grade and margin status. We classified margins as “positive” if either the endocervical or exocervical margin were positive for residual disease. Specimens were inked (black for endocervical, red for exocervical) to facilitate precise margin assessment.

Follow-up protocol

Patients with complete excision were scheduled for a 6-month follow-up including colposcopy, PAP test, and HPV test.

Patients with positive margins

Underwent an earlier colposcopic check at 3 months (with Pap test; HPV test only if colposcopy or cytology was abnormal), followed by standard evaluations at 6 months (colposcopy, Pap, HPV) and 12 months (colposcopy and Pap if any prior abnormality; otherwise HPV alone).

Any patient with persistent HSIL cytology, colposcopic evidence of residual disease, or two consecutive positive highrisk HPV tests was offered reexcision (repeat LLETZ or, if indicated, coldknife conization).

Statistical analysis

Continuous variables were analysed using the Kruskal–Wallis and Mann–Whitney tests, while categorical data were assessed using chi-square or Fisher's exact test. A P-value of ≤ 0.05 was considered

statistically significant. Analyses were performed with SPSS26 (IBM Corp., Armonk, NY, USA).

RESULTS

Patient demographics and histological findings

Among 370 patients, the histological breakdown was as follows: 150 cases of CIN 1, 134 CIN 2, 82 CIN 3, with isolated cases of adenocarcinoma in situ (FIGO stage 1A1; n = 2), microinvasive squamous carcinoma (FIGO stage 1A1; n = 1), and infiltrating non-keratinizing squamous carcinoma (FIGO stage 1B1; n = 1) (Figure 1).

The average patient age was 39 years, with CIN 1 patients being significantly older (mean 44 years) than those with higher-grade lesions (mean ≈38-40 years; p < 0.0001) (Table 1).

146 (146/370 equal to 39.5%) were smokers, while 89 (89/370 equal to 24%) were estrogen-progestin pill users. 138 (138/370 equal to 37.3%) were multiparous, defining as multiparity all women who had had ≥1 vaginal birth.

Three cases, equal to 0.8%, presented complications after the operation (cervical stenosis and late cervicorrhagia), while none presented intraoperative complications.

Margin status and group comparison

Positive margin group (n = 48)

- Endocervical (Positive Endocervical Margin): 18 patients
 - At 3month followup, 14 had normal colposcopic findings and Pap; 4 exhibited minor atypia (ASCUS/LSIL) and tested HPVpositive (genotypes 16 or 18 in two).
 - At 6 months, 16 (88.9%) showed complete regression, while 2 (11.1%) had persistent LSIL on cytology but cleared HPV; colposcopy showed only minor acetowhite changes.
- Exocervical (Positive Exocervical Margin): 30 patients
 - At the 6month checkup, all patients demonstrated negative colposcopy and Pap tests. Six (20%) had a positive HPV test (non16/18,

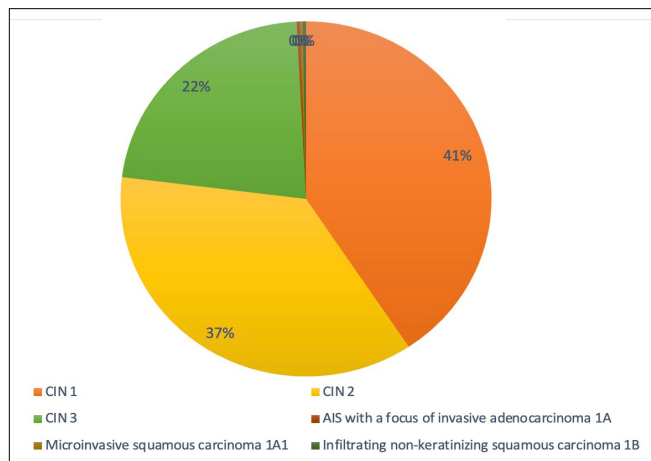


Figure 1. Diagnosis.

low viral load); however, at 12 months, only 1 (3.3%) exhibited persistent HPV positivity (genotype 31) in the absence of cytological abnormalities.

- No patient underwent immediate reexcision; interventions were reserved for persistent HSIL, suspicious colposcopic findings, or two consecutive positive HPV tests.
- Negative margin Group (n = 322)
 - During followup, 289 patients (≈97% of those reevaluated) had normal colposcopic findings at 6 months. Minor cytological abnormalities (LSIL or ASCUS) occurred in 9/298 (≈3%), all with transient HPV positivity (predominantly non16/18 genotypes). Only 1/298 (0.3%) had persistent HPV (genotype 16) at 12 months without cytological or colposcopic abnormalities. No reexcisions were performed (Figure 2).

Comparative analysis

When comparing overall recurrence/residual disease rates between positive-margin (combined endocervical/exocervical) and negative margin groups, no statistically significant difference was observed (0% vs 0.3%; p = 0.63). Persistent HPV at 12 months was 4.2% vs 0.3% (p = 0.08). Transient cytological abnormalities at 6 months were higher in positive margins (27.1% vs 2.8%; p < 0.001) (Table 2).

Table 1. Descriptives and histological findings.

LLETZ	N°	Lower age	Older age	Mean	Standard deviation	Test Statistics ^{a,b}	Age
CIN 1	150	23	67	44.07	10.5	Chi-Square	22.531
CIN 2	134	23	66	38.53	9.2	df	2
CIN 3	81	25	65	39.67	10.6	Asymp. Sig.	0.00

Table 2. Comparison of residual disease and follow-up outcomes.

Parameter	Positive Margin (n = 48)	Negative Margin (n = 322)
Residual disease (histologically confirmed)*	3-? cases (≈6-9 cases overall; see Discussion)	1 case (0.3%)
Abnormal colposcopy/PAP at 6 months	Endocervical: 2/18 (11.1%); Exocervical: 0/30 abnormal, 6/30 HPV+ (transient)	Minor abnormalities in <5% of cases
Persistent HPV positivity at one year	1/30 (exocervical group; none in endocervical group)	1/322 (0.3%)

*Residual disease" was defined as persistence of CIN or invasive disease on follow-up evaluation.

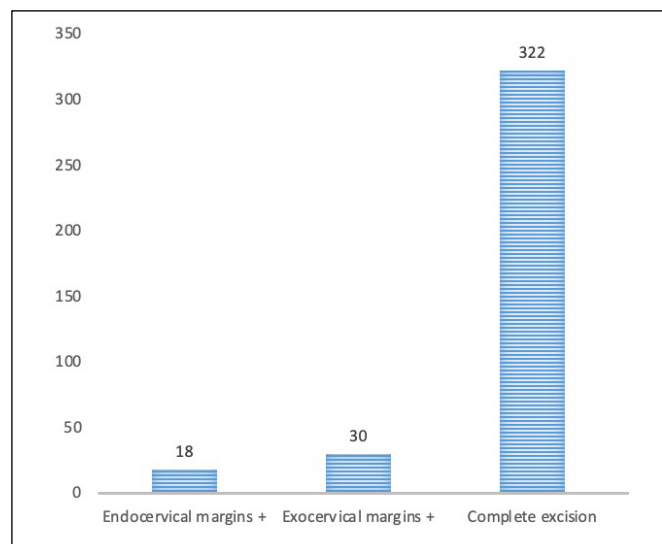


Figure 2. Results of radiofrequency surgical therapy: 322 (87%) complete excision, 18 (4.9%) endocervical margins +, 30 (8.1%) exocervical margins +.

DISCUSSION

Our findings confirm that LLETZ is an effective treatment for CIN, even when positive margins are present. Although 13% of cases had incomplete excision (positive margins), the overall rate of clinically significant residual disease at 12 months was extremely low [9, 10].

Endocervical vs exocervical positive margins

Endocervical involvement suggests deeper glandular spread, historically linked to higher residual risk, whereas exocervical involvement reflects superficial persistence. In our cohort, endocervical positive margins yielded an 11.1% rate of persistent LSIL at 6 months, but all cleared by 12 months without progression. Exocervical margins had a 20% transient HPV positivity at 6 months; only 3.3% remained HPV positive at 12 months, with no cytological or colposcopic abnormalities. These results suggest that the thermal effect of LLETZ may ablate microscopic residual disease at both margin sites, aligning with studies showing low progression rates under conservative surveillance [10-20] (Table 1).

Comparison with existing literature

Existing large prospective cohorts and meta-analysis reported an 8% residual risk with positive margins but ≤ 1% progression to CIN 2+ over two years under conservative followup and ≤ 2% progression with surveillance of positive margins most of all when follow up is conducted with structured methods [21-23]. Our data corroborate these findings, showing no significant difference in 12month outcomes between margin groups.

Followup protocol and reexcision policy

We implemented a 3month colposcopy for positive margins, unlike many guidelines recommending only 6month intervals. This early evaluation enabled detection of minor abnormalities and HPV persistence that regressed by 6 months. No patient required immediate reexcision, thus minimizing overtreatment and preserving cervical integrity. These evidences gain more and more importance when compared with all the cervical cancer fertility-sparing approaches [24-27].

Limitations

1. Retrospective, single center design may limit generalizability; all procedures were performed by a small cohort of experienced colposcopists, which might not reflect broader practice.
2. Positive margin subgroups (n = 48 overall; endocervical n = 18) are relatively small, reducing statistical power for subgroup analyses.
3. HPV genotyping data were incomplete; while we recorded high risk HPV positivity, genotype stratification (16/18 vs others) was unavailable for some.
4. Follow up duration was limited to 12 months; longer term surveillance (24-36 months) is needed, as some recurrences manifest later.
5. Six month follow up data were missing for 24/322 (7.5%) of negative margin patients, introducing potential follow up bias.
6. We did not systematically record HPV vaccination status, which could influence clearance and recurrence.

Despite these limitations, our study supports a conservative followup strategy for positive margins, consistent with recent FIGO and ASCCP guidelines advocating individualized management over routine reexcision [22].

CONCLUSIONS

LLETZ is an efficacious, lowmorbidity treatment for CIN, achieving high complete excision rates. Positive margins (endocervical or exocervical) do not necessarily lead to higher rates of residual or recurrent highgrade disease when managed with a structured surveillance protocol, including an early 3month evaluation.

Conservative followup can minimize overtreatment; reexcision should be reserved for persistent HSIL.

Prospective, multicentre studies with larger positive margin cohorts, comprehensive HPV genotyping, and extended followup (≥ 24 months) are needed to refine postLLETZ surveillance guidelines.

COMPLIANCE WITH ETHICAL STANDARDS

Authors' contributions

A.B., M.T., L.A.: Conceptualization, investigation, methodology, resources, software, visualization, data curation. M.T., L.A.: Formal analysis, writing – original draft, writing – review & editing. A.B., M.T., L.A., G.C.D.R.: Project administration, supervision, validation.

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Study registration

N/A.

Disclosure of interests

The authors declare that they have no conflict of interests.

Ethical approval

N/A.

Informed consent

The enrolled patients gave their informed consent to the study.

Data sharing

Data are available under reasonable request to the corresponding author.

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