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Long-term predictors of residual or recurrent cervical intraepithelial neoplasia 2-3 after treatment with a large loop excision of the transformation zone: a retrospective study

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Running title: Long-term predictors of residual or recurrent CIN 2-3 after LLETZ

ABSTRACT

Objective: To assess the long-term risk factors predicting residual/recurrent cervical intraepithelial neoplasia (CIN 2–3) and time to recurrence after large loop excision of the transformation zone (LLETZ).

Design: Retrospective study.

Setting: Colposcopy clinic.

Population: 242 women with CIN 2–3 treated between 1996 and 2006 and followed up until June 2016.

Methods: Age, margins and high risk-human papillomavirus (HR-HPV) were estimated using Cox proportional hazard and unconditional logistic regression models. The cumulative probability of treatment failure was estimated by Kaplan-Meier analysis.

Main Outcome measure: Histologically confirmed CIN 2-3, HR-HPV, margins, age.

Results: CIN 2-3 was associated with HR-HPV (HR =30.5; 95% CI =3.80-246.20); age >35 years (HR =5.53; 95% CI =1.22-25.13); and margins (HR = 7.31; 95% CI = 1.60–33.44). HR-HPV showed a sensitivity of 88.8% and a specificity of 80%. Ecto(+)/endocervical(+)(16.7%), uncertain (19.4%) and ecto(-)/endocervical(+) margins (9.1%) showed a higher risk of recurrence (OR = 13.20(95% CI =1.02-170.96), 15.84(95% CI =3.02-83.01), and 6.60(95% CI =0.88-49.53)), respectively.

Women with involved margins and/or HR-HPV positive had more treatment failure than those who were HR-HPV negative or had clear margins (P-log rank<0.001).

Conclusions: HR-HPV and margins seem essential for stratifying post- LLETZ risk, and enable personalised management. Given that clear margins present a lower risk, a large excision may be indicated in older women in order to reduce the risk.

Funding statement: This study has received no funding.

Keywords: Recurrence, HPV, Margin status, Cervical intraepithelial neoplasia

Tweetable abstract: After LLETZ for CIN 2–3, recurrences appear more often in women with positive HR-HPV and involved margins and aged over 35.

INTRODUCTION

Women with cervical intraepithelial neoplasia 2–3 are treated conservatively, typically by large loop excision of the transformation zone (LLETZ) in order to prevent the development of invasive cervical cancer (1). After treatment, patients require follow-up because of the risk of CIN recurrence or cervical cancer (2) that remains for many years (3)(4).

Several factors are thought to characterise the risk of treatment failure after excisional treatment. Age, smoking, size and severity of the lesion, high-risk human papillomavirus (HR-HPV) type, and persistence of HR-HPV post-treatment have each been shown to predict residual or recurrent CIN (5)(6). In addition, margin involvement is a well-established risk factor for treatment failure (7)(8)(9)(10)(11), However, there are few reports of the prognostic value of HR-HPV when added to the margin status and site of involvement (10)(12), and long-term follow-up data on residual or recurrent CIN after LLETZ are scarce (13).

The aim of this study was to assess the clinical outcomes among women treated for CIN2–3 and followed for a median of 30 months over a 20-year period, to assess the long-term risk of CIN2–3 based on HR-HPV status, and surgical margins at baseline.

METHODS

Study Design

This was a retrospective cohort study of consecutive adult women affected by CIN and treated by LLETZ at the Department of Gynecology of the Hospital Universitari de Bellvitge (Barcelona, Spain). The study was conducted between January 1996 and September 2006, with patients followed to June 2016. This gave a maximum follow-up period of 20 years. We included patients with a histological diagnosis of CIN 2–3 in the surgical specimen and at least one follow-up visit after LLETZ. We excluded women with other histological diagnoses, those without follow-up data, those who underwent re-excision or hysterectomy immediately after LLETZ, and those who were immunosuppressed.

Patients were not involved in the development of the research. A core outcome set was not used when designing the study. However, the Crown database was checked to determine whether a relevant core outcome set existed or was in development for this topic, and no such set was found.

Surgical Procedure

Excision was performed by LLETZ after applying paracervical local anaesthetic and

Lugol's iodine to the cervical surface. The loop size was chosen based on the tissue to be excised. A second selective endocervical sweep was performed with a smaller loop if the transformation zone was type 3 or if the patient was older than 35 years. Electrical coagulation was used to achieve hemostasis. Specimens were orientated with a stitch for pathological examination.

Follow-up

Follow-up was scheduled at 6 and 12 months after LLETZ, with a Pap smear and colposcopy performed at each visit. A sample was taken for HPV detection at 6 months after treatment. If surgical margins were positive, the first control visit was scheduled at 3 months. Women underwent cervical biopsy if they presented with abnormal cytology results (e.g., atypical squamous cells of undetermined significance or worse), a positive HPV result, or an abnormal transformation zone at colposcopy. Women with two normal consecutive Pap smear and colposcopy results were considered negative for residual or recurrent disease and sent back for regular gynaecological control.

The testing procedures for conventional cytology, colposcopy, and HR-HPV were as follows. Ecto- and endocervical smears were obtained and the cytology slides were stained using the Papanicolaou method for conventional cytology. Cytologic findings were then evaluated according to the terminology of the 1989 or 2001 Bethesda System. Colposcopy was performed using a Carl Zeiss binocular Colposcope (Jena, Germany) after applying 5% acetic acid to the cervix with a cotton ball. An endocervical curettage was performed if the transformation zone was not visible (Type 3) or if no abnormality was observed. Specimens were collected for HPV testing with the Digene sampler kit (Digene, Gaithersburg, MD, USA), and HPV was identified with the HC2 system (Digene). This is a signal-amplified hybridisation antibody capture assay that use chemiluminescent detection for each HR-HPV type (16, 18, 32, 34, 36, 39, 45, 51, 52, 56, 58, 59, 68). The chemiluminescence from the conjugated antibody-hybrid was measured by a luminometer as relative light unit (RLU). When the relative light units was greater than or equal to the mean of a positive control (1.0 pg/mL), a sample was deemed positive (14).

Criteria for residual/recurrent disease and treatment of recurrence

Residual/recurrent disease was defined as CIN 2–3 diagnosed by cervical biopsy or endocervical curettage. CIN 2 and CIN 3 were analysed together due to the anticipated small number of cases. Patients affected by residual/recurrent CIN2–3 were referred for a second surgical treatment. Residual lesions were defined as those diagnosed within the first year after LLETZ. CIN 2–3 lesions detected after 1 year were considered recurrences. However, we did not

perform risk assessments to determine whether new CIN2–3 lesions were recurrences or de novo infection.

Data Analysis

An electronic case report form was designed in Microsoft Access for prospective data input. Follow-up data were retrieved at the end of the study period, and the database was verified to evaluate the quality of the collected data. Two-sided P-values <0.05 were considered to indicate a significant difference. Data were analysed using Stata software (Release 15.1, StataCorp, USA).

The relationship between categorical variables was assessed by chi-squared tests or Fischer's exact tests, as appropriate. The relationship between continuous and categorical variables was assessed by analysis of variance or Kruskal–Wallis tests, as appropriate. Predictors of residual/recurrent disease were assessed by estimating odds ratios (ORs) with 95% confidence intervals (CIs). We included the following as predictor variables: age (continuous variable or dichotomised as ≤35 years and >35 years), parity, smoking status, cervical quadrant involved, glandular involvement, margin status, post-LLETZ- HR-HPV status, and semiquantitative measure of the viral load (relative light units by HC2) using unconditional regression analysis.

We assessed the accuracy of the margin status, first HR-HPV detection, and first cytological result after LLETZ by estimating the sensitivity, specificity, positive predicted value (PPV), negative predicted values (NPV), positive likelihood of residual/recurrent CIN2–3. The treatment failure rate time was calculated from the date of LLETZ to the date of residual/recurrent CIN2–3.

The cumulative probability of treatment failure was estimated by Kaplan–Meier analysis, with curves compared using the log-rank test. Univariate Cox proportional hazards models were used to explore the effect of margin status, first HR-HPV detection and first cytological result after LLETZ as prognostic factors.

Multivariate analysis (logistic analysis or cox proportional model) could not be performed due to the small number of cases with residual/recurrent CIN 2-3.

Funding Statement

This study has received no funding.

RESULTS

Study Cohort

We enrolled 471 consecutive adult women treated by LLETZ for cervical intraepithelial neoplasia or cervical neoplasia. Of these, women were excluded because they had low-grade cervical intraepithelial neoplasia (CIN 1) (n = 140), adenocarcinoma or squamous carcinoma (n = 2), no follow-up data (n = 26), undergone re-conisation or hysterectomy immediately after LLETZ (n = 10), immunosuppression (n = 41), or an unknown immunologic status (n = 10). Finally, 242 of the 471 eligible cases were included (51.4%). During routine long-term follow-up, HR-HPV was determined in 42 cases. The flow chart for study participation is shown in Figure S1 (Supporting Information).

The patient characteristics are summarised in Table 1. The median follow-up time was 30 months (range, 2–257 months), with 75% of patients followed for over 149 months. The median age of the population was 35 years (range, 18–77 years).

The indications for the LLETZ procedure were: cervical biopsy with CIN 2-3 in 155 cases (64%), persistent CIN 1 in 45 cases (18,6%), discordance Pap smear-biopsy in 25 cases (10,3%); carcinoma in one case (0,4%), adenocarcinoma in situ, one case (0,4%). unknown, 15 cases (6,2%).

Treatment Success and Residual or Recurrent CIN2–3

In general, LLETZ was highly successful, with 94.6% of cases having no signs of residual or recurrent CIN 2-3 during follow-up. This reached 99.1% for cases that were completely excised. Residual or recurrent CIN2–3 after LLETZ occurred in 13 cases (5.3%). One invasive squamous cervical carcinoma and one vulvar cancer were detected during follow up. There were no cases of adenocarcinoma in situ, but 35 patients (14.4%) developed CIN1.

The median lag time between LLETZ and residual or recurrent CIN2–3 was 13 months (range, 3–212 months; interquartile range, 11–51 months). Five (38.4%) and two (15.3%) CIN2–3 cases were diagnosed during the first and second year, respectively. Another two were diagnosed between 24 and 29 months (15.38%), and four were diagnosed from 30 months onward (30.7%).

Our cohort included 77 cases of CIN 2 (3 CIN 2-3 recurrences) 122 cases of CIN 3 (5 recurrences), and 43 cases of CIN 2-3 (5 recurrences). No differences were found in the proportion of residual/recurrent CIN 2-3 (P = 0.157).

Residual or recurrent CIN was: CIN 3 or CIN 3 with CIN 2 areas in 8 cases (61.5%), CIN 2 in 3 cases (23.1%) and CIN 2-3 in 2 cases (15.4%). All 13 cases with residual/recurrent CIN 2–3 required new treatment: seven underwent repeat LLETZ procedure (53.8%), five underwent hysterectomy (38.6%), and one was lost to follow-up (7.6%).

Predictors of Treatment Failure in Patients with CIN2–3 Disease

Factors associated with treatment failure are summarised in Table 1. Treatment failure was statistically more frequent among women older than 35 years (P = 0.020) and among those with more than four live births (P = 0.016). HR-HPV post LLETZ was positive in 42 cases (23.5%). Post-LLETZ HR-HPV positivity was associated with more residual or recurrent CIN 2–3 than cases without HR-HPV (19% vs 0.7%) (P = 0.001). Cases with treatment failure had higher post-LLETZ RLU HR-HPV values than cases without lesions (P = 0.001). In addition, cases with atypical squamous cells of undetermined significance or worse at the first post-LLETZ cytology had more residual or recurrent CIN2–3 than cases with normal cytology (50% vs 3%) (P = 0.001).

Surgical margins were involved in 75 cases (31%) cases, and uncertain in 31 (12.8%), making a total of 43.8%. The proportions of involvement of the different surgical margins are found in table 1. Statistically significant differences in the proportions of CIN 2–3 treatment failure were observed by margin status (P = 0.003), being higher in margins that had uncertain (19.4%), ecto(+)/endocervical(+) (16.7%), and ecto(-)/endocervical(+) (9.1%) involvement. By contrast, ecto(+)/endocervical (-) and clear margins accounted for 2.4% and 1.5% of CIN2–3 treatment failures, respectively. No difference in residual/ recurrent CIN2–3 was observed by glandular involvement, number of quadrants involved, or smoking status

Predictors of Treatment Failure by Survival and Univariate Logistic Analyses

Univariate logistic analysis showed the ORs related to CIN2–3 treatment failure. Women older than 35 years were at increased risk (OR = 5.45; 95% CI=1.18-25.15; P =0.011) compared to younger women. Margin involvement was significantly related to CIN2–3 treatment failure (P = 0.004), with ORs of 13.20 (95% CI = 1.02-170.96) for ecto(+)/endocervical(+) margins, 15.84 (95% CI = 3.02-83.01) for uncertain margins, and 6.60 (95% CI =0.88-49.53) for ecto(-)/endocervical(+) margins. Ecto(+)/endocervical(-) margins had a non-significant OR of 1.61(95% CI = 0.14-18.21). Women with positive HR-HPV results after LLETZ had an over 32-

fold increased odds (OR =32; P = <0.001) of developing a recurrent or residual lesion than women with a negative result (Table S1 Supporting Information).The crude hazard ratios (HRs) for age, margin status, and first HR-HPV detection after LLETZ are presented in Table 2. Statistically significant worse treatment failure was observed among patients older than 35 years (HR = 5.53; 95% CI =1.22-25.13; P =0.009), with involved margins (HR = 7.31; 95%; CI = 1.60–33.44; P = 0.003), and with HR-HPV positivity (HR =30.58; 95% CI =3.80-246.20 ; P < 0.001). CIN2–3 relapse appeared earlier in HR-HPV positive cases, which had a 30.5 fold higher risk of developing CIN2–3 in the next period of time than HR-HPV negative cases.

Sensitivity and specificity analysis for the Predictors of Treatment Failure

Table 3 shows the sensitivity, specificity, PPV, NPV, and likelihood ratio for margins or HR-HPV as predictors of treatment failure after LLETZ. First, HR-HPV detection after treatment had a sensitivity of 88.8, a specificity of 80, and an NPV of 99.2. The addition of margins or cytology to the HR-HPV result did not substantially improve the diagnostic accuracy.

A significant difference was also observed in HR-HPV positivity in relation to margin status (P = 0.024). If we consider the margins involved, there was lower HR-HPV detection (3 cases, 7.9%) in ecto(+)/endocervical(-) margins (P = 0.010) and higher HR-HPV positivity in uncertain margins (10 cases, 41.7%; P = 0.036). No differences were found in relation to HPV positivity for ecto(-)/endocervical (+) margins (Table S2 Supporting Information).

Kaplan–Meier Estimates

The Kaplan–Meier estimates for the failure rate by HR-HPV post-LLETZ status, margin status, and HR-HPV post-LLETZ status stratified by margins are shown in Figure 1 (a, b, and c, respectively). Women with HR-HPV positive post-LLETZ status (log-rank P < 0.001), involved margins (log-rank P = 0.002), and HR-HPV positivity and involved margins (log-rank P < 0.001) had a higher and earlier failure rate. Separate analysis of the different margin involvements shows differences in failure rates after LLETZ (P < 0.001), with higher failure rates for uncertain, ecto(-)/endocervical(+), and ecto(+)/endocervical(+) margins (Figure S2 e. Supporting Information).

The results for HR-HPV post-LLETZ positivity stratified by margins show differences between the presence of clear margins (log-rank P = 0.025), ecto(+)/endocervical(-) margins (log-rank P < 0.001), and uncertain margins (log-rank P = 0.011). All uncertain margins with HR-HPV positivity with completed follow-up data had treatment failure. No differences were found for

ecto(-)/endocervical(+) involvement with HR-HPV positivity (log-rank P = 0.76) (Figure S2. Supporting Information).

DISCUSSION

Main Findings

CIN2–3 treatment showed a favourable long-term outcome with treatment failure to CIN2–3 in 13 cases (5.7%). More than 50% of lesions were diagnosed during the first 2 years after LLETZ, with 25% found after 51 months and one case after 212 months.

HR-HPV determined after LLETZ was a strong predictive factor for treatment failure (P < 0.001) independently of the effect of margin involvement. CIN 2–3 relapse appeared earlier in HR-HPV positive cases, with a 30.5-fold higher risk to have CIN 2-–3 in the next period of time than HR-HPV negative cases.

As expected, women with involved margins presented recurrence more often than those with clear margins (HR = 7.31; P = 0.003), and the effect was independent of HPV status and age. Interestingly, the type of margin involvement seemed to predict treatment failure, particularly for those women with ecto(+)/endocervical(+) (16.7%) or uncertain margin involvement (19.4%). Being older than 35 years was another predictive factor of recurrence (P =0.009).

Except for ecto(+)/endocervical(-) margins, involved margins were also associated with more HR- HPV positivity than clear margins (P = 0.024). Uncertain margins presented the highest HR-HPV positivity (41%), and ecto(+)/endocervical(-) the lowest positivity (7.9%).

Involved margins with HR-HPV positivity were associated with more recurrences than those that were negative for HR-HPV. Involved margins with HR-HPV negativity had less risk of recurrence.

Strengths and Limitations

Our study has several strengths, not least of which are the inclusion of a large sample with restrictive inclusion criteria and a long follow-up at the same hospital. This allowed us to detect late relapse and to determine the real risk of cervical cancer and CIN. The analysis of factors associated with the time to CIN2–3 recurrence is another strength of our study. To the best of our knowledge, no previous authors have analysed the relationship of different margin types by HPV positivity and the time to CIN 2–3 recurrence.

By contrast, the main study limitations are the retrospective nature and the fact that some data were missing; that said, these weaknesses reflect the realities of clinical practice. Another

major limitation is that we combined CIN2 and CIN3 for the analysis of recurrence due to the small sample size. Another weakness is the large proportion of uncertain margins, which suggests moderate/poor reproducibility of margin assessment after LLETZ. Finally, the confidence interval of the results is wide due to the small number of positive cases. This suggests that the magnitude of the effect is uncertain.

Interpretation

Post-treatment HR-HPV determination has clearly demonstrated a higher sensitivity and NPV than cytology or margins for detecting the residual or recurrent CIN (4)(10)(13). In a meta-analysis, HR-HPV showed a sensitivity of 91% and specificity of 83.8%. The pre-test/post-test probability assessment demonstrated that a post-treatment positive HR-HPV increases the risk of treatment failure to 28.4%, and a negative HR-HPV reduces the risk to 0.8%(10). In our study, the univariate analysis showed that HR-HPV post LLETZ has an OR=32 of treatment failure.

Post- LLETZ HR-HPV was positive in 23.5% of cases, in line with the results of a previous meta-analysis (15).

Our data show that margin involvement is a predictor of treatment failure (10)(16)(17). Two meta-analyses observed that CIN2–3 disease recurred in 18% of cases with involved margins versus 3% with clear margins(10) (16) . The relative risk of CIN2–3 recurrence after incomplete excision has also been reported to be 4.8 (10), observed in a meta-analysis of 97 studies.

Our study showed that resection margins only had limited value in predicting treatment failure. These findings were consistent with the latter meta-analysis (10), which revealed that margins were 38% less sensitive than HR-HPV when predicting treatment failure.

In our series, specimens with involved margins and HR-HPV positivity recurred more frequently than those with clear or involved margins negative for HR-HPV (Figure 1c, d). This pattern was observed in a study of CIN1 and CIN2–3 cases (11).

We observed a lower risk of residual or recurrent CIN2–3 when the ecto(+)/endocervical(-) margin was involved, with a higher risk when there was involvement of the ecto(-)/endocervical(+) or ecto(+)/endocervical (+) margins. Similar results were reported in a meta-analysis of 44,000 women(10). However, the lack of association of ecto(-)/endocervical(+) margins with residual or recurrent CIN2–3 in the present study was an unexpected finding. The available literature suggests that involvement of the endocervical margin implies an increased risk of CIN 2–3 recurrence (7)(10)(12). In the univariate analysis, ecto(-)/endocervical(+) margins

were associated with treatment failure and had shorter time to recurrence, but this association was not significant, possibly because of the small number of cases.

Other unexpected findings were the high proportion of treatment failures and HPV positivity in uncertain margins. The cases of uncertain margins indicate that there was difficulty in the evaluation of the surgical specimen, due to epithelial detachment. The diathermy effect can cause thermal damage and detachment of the epithelium. This can lead to an increase in the number of uncertain margins, and an overestimation of involved margins. The association of uncertain margins with HPV and treatment failure could be related to the lower adhesive capacity of neoplastic epithelium, as for example, the expressions of E-cadherin and β-catenin are altered in CIN and affect epithelial cell adhesion. This altered expression increases with the severity of CIN (18). Detached epithelium can hide a CIN2–3 lesion and may be related to the low reproducibility of margin assessment (10).

Our series shows that involvement of ecto(+)/endocervical(-) margins has lower rates of failure and HR-HPV positivity. Recent thinking suggests that the site of HPV infection affects the pattern of viral gene expression. Infections are more likely to be productive in the ectocervix, and more likely to be non-productive in the endocervix (19)(20). For this reason, our results, and those of others, can be explained as a transient infection, different from that of endocervical infection.

Increasing age is also known to be a predictor of CIN2–3 treatment failure (4)(6)(21)(7), though it is not always observed (22). In the present study, we showed that patients older than 35 years were at higher risk of CIN 2–3 recurrence and had shorter times to recurrence.

Conclusion

Women treated with LLETZ for CIN2–3 show favourable long-term clinical outcomes. Cases positive for HR-HPV recur earlier, as do those with involved margins and those in women older than 35 years. However, HR-HPV appears to be the strongest predictive factor for treatment failure. When margins are involved, recurrence tends to be more frequent when they are HR-HPV positive. Furthermore, the risk of treatment failure and the time to recurrence differs by the type of margin and HPV positivity, with ecto(+)/endocervical(-) margins showing the lowest treatment failure, and the other margins showing higher failure rates.

We believe that HR-HPV and margin statuses can be used to stratify the post-LLETZ risk of recurrence and enable personalised management. This risk-based management has been used to develop new guidelines based on an individualised assessment of risk(23). Given that clear margins present a lower risk of treatment failure and, given that risk increases with age, larger excisions could be indicated in older women.

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CONFLICT OF INTEREST STATEMENT

There are no conflicts of interest to report. Completed disclosure of interest forms are available to view online as supporting information.

AUTHOR CONTRIBUTION

This work was conceived and planned by M.E.F., who also wrote the draft. S.T., G.M., M.C. and A.G. played a role in the data collection, analysis, interpretation of results and made suggestions for revision. S.S. approved the final version. All the authors have read the text and agreed on its contents.

ETHICS STATEMENT

Written informed consent was obtained from patients prior to treatment. Patient data were suitably anonymised and protected according to national standards. The study design was approved by the Clinical Research Ethics Committee of Bellvitge University Hospital (Reference PR345/18) (October 11th, 2018).

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TABLE 1. Follow up time, patient and surgical specimen characteristics. Patients treated with Large Loop Excision of the Transformation Zone (LLETZ) for Cervical Intraepithelial Neoplasia 2-3 (CIN2-3).

PATIENT CHARACTERISTICS		Number of patients (%[1])	No residual/ Recurrent CIN 2-3 (%[2])	Residual/ Recurrent CIN 2-3 (%[2])	P-value[3]
Follow up time (in months)	Median (Min-Max) (IQ range)	30.3 (2-257) (8-149)	35.2 (1.8-256.5) (8.4-149.4)	13.4 (2.8-212.2) (11.5-50.9)	0.428[&]
Age (years)	Median (Min-Max) Mean (SD)	35.5 (18-77) 37.4 (10.9)	35 (29-44) 37.1 (10.9)	40.5 (28-65) 42.7 (9.6)	0.073[$]
Age (Categorized at 35 years)	<35 y 35+y	116 (47.9) 126 (52.1)	114 (98.3) 115 (91.3)	2 (1.7) 11 (8.7)	**0.020**
Parity	Nulliparous ≤4 full-term births >4 full-term births Unknown	41 (16.9) 170 (70.2) 14 (5.8) 17 (7.0)	41 (100.0) 160 (94.1) 11 (78.6) 17 (100.0)	0 (0.0) 10 (5.9) 3 (21.4) 0 (0.0)	**0.016**
Smokers	No Yes Unknown	140 (57.9) 83 (34.3) 19 (7.9)	131 (93.6) 79 (95.2) 19 (100.0)	9 (6.4) 4 (4.8) 0 (0.0)	0.771
HPV AND CITOLOGY RESULTS					
First HR-HPV post-LLETZ	Negative Positive Unknown	137 (56.6) 42 (17.4) 63 (26.0)	136 (99.3) 34 (81.0) 59 (93.7)	1 (0.7) 8 (19.0) 4 (6.3)	**<0.001**
First RLU HR-HPV post-LLETZ (N=167)	Median (Min-Max) IQ range	0.3 (0.1-3250) (0.2-0-7)	0.3 (0.1-3450) (0.2-0.5)	106 (0.1-552) (3-127.5)	**0.001**[&]
First RLU HR-HPV post-LLETZ cat	Negative <1 1-100 >100 *Unknown*	130 (53.7) 26 (10.7) 11 (4.6) *75 (31.0)*	129 (99.2) 23 (88.5) 6 (54.5) 71 (94.7)	1 (0.8) 3 (11.5) 5 (45.5) 4 (5.3 *)*	**<0.001**
First Cytological result post- cone	Normal ASC-US LSIL HSIL	199 (82.2) 18 (7.4) 13 (5.4) 12 (5.0)	193 (97.0) 15 (83.3) 13 (100.0) 8 (66.7)	6 (3.0) 3 (16.7) 0 (0.0) 4 (33.3)	**0.001**
SURGICAL SPECIMEN CHARACTERISTICS					
Margin status	Clear Involved Ecto(+)/Endocervical(-) Ecto (-)/Endocervical(+) Ecto(+)/Endocervical (+) All margin Deep margin Uncertain *Unknown*	134 (55.4) 75 (31.0) 42 (17.4) 22 (9.1) 6 (2.5) 4 (1.7) 1 (0.4) 31 (12.8) *2 (0.8)*	132 (98.5) 71 (94.7) 41 (97.6) 20 (90.9 5 (83.3) 4 (100.0) 1 (100.0) 25 (80.6) *1 (50.0)*	2 (1.5) 4 (5.3) 1 (2.4) 2 (9.1) 1 (16.7) 0 (0.0) 0 (0.0) 6 (19.4) *1 (50.0)*	**0.001**
Glandular involvement	No Yes *Unknown*	11 (4.5) 80 (33.1) *151 (62.4)*	11 (100.0) 78 (97.5) *140 (92.7)*	0 (0.0) 2 (2.5) *11 (7.3)*	1.000
Quadrants involved	1 2 3 4 *Unknown*	37 (15.3) 50 (20.7) 21 (8.7) 54 (22.3) *80 (33.1)*	35 (94.6) 45 (90.0) 20 (95.2) 52 (96.3) *77 (96.3)*	2 (5.4) 5 (10.0) 1 (4.8) 2 (3.7) *3 (3.7)*	0.606
Circumference of the cervical specimen (cm^2) (n=129)	Mean (SD) Median (Min-Max) (IQ Range)	12.6 (5.6) 11.1 (4.6-32.9) (9.2-13.7)	12.5 (5.4) 11.1 (4.6-32.9) (9.1-1307)	16.0 (11.2) 10.8 (9.4-32.8) (9.7-22.2)	0.689[&]
Length of the specimen (cm) (n=131)	Mean (SD) Median (Min-Max) (IQ Range)	0.9 (0.4) 0.9 (0.2-2.7) (0.6-1.2)	0.9 (0.4) 0.9 (0.2-2.7) (0.6-1.2)	4 (1.1) 1.05 (0.7-1.6) (0.85-1.35)	0.310[&]
Endocervical sweep (two-step procedure)	No Yes Unknown	173 (71.5) 69 (28.5) 0 (0.0)	164 (94.8) 65 (94.2) -	9 (5.2) 4 (5.8) -	1.000
TOTAL		**242 (100.0)**	**229 (94.6)**	**13 (5.4)**	

IQ Range: Interquartile range (25%-75%); 1: column percent; 2: row percent; 3: Fisher's exact test P-value; $: ANOVA test P-value; &: Kruskal-Wallis test P-value.Abbreviations: HSIL, high-grade intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion; ASCUS, atypical squamous cells of undetermined significance; HR-HPV, high-risk human papillomavirus; RLU, relative lights units. CIN, Cervical intraepithelial neoplasia. Circumference and length of the cervical specimen determined according to the 2011 International Federation of Cervical Pathology and Colposcopy Colposcopic Terminology.(24) Length is the distance from the distal to the proximal margin of the cervical specimen. Circumference is the perimeter of the excised specimen.

TABLE 2: Univariate Cox Regression Analysis of the factors associated to residual/ recurrent Cervical Intraepithelial eoplasia 2-3 (CIN2-3) after Loop Electrosurgical Excision Procedure (LLETZ)

	Total	CIN 2-3			Univariate analysis		
Characteristics	**N**	**N**	**%**	**[95%CI]**	**HR**	**[95% CI]**	**P-value[1]**
Age					1.0	[0.99-1.1]	0.113
Age (categorized at 35 years)							**0.009**
<35 yo	116	2	1.7	[0.2-6.1]	Ref.		
35+ yo	126	11	8.7	[4.4-15.1]	**5.5**	**[1.2-25.1]**	
Margin status							**0.001**
Clear	134	2	1.5	[0.2-5.3]	Ref.		
Ecto+/Endo-	42	1	2.4	[0.1-12.6]	1.7	[0.2-19.3]	
Ecto-/Endo+	22	2	9.1	[1.1-29.2]	5.7	[0.8-41.3]	
Deep	1	0	0.0	[0.0-97.5*]	-|	-	
Ecto+/Endo+	6	1	16.7	[0.4-64.1]	10.4	[0.9-118.4]	
All	4	0	0.0	[0.0-60.2*]	-|	-	
Uncertain	31	6	19.4	[7.5-37.5]	**19.4**	**[3.8-99.5]**	
Unknown	*2*	*1*					
Margin status (Endo category)							**0.003**
Clear	134	2	1.5	[0.2-5.3]	Ref.		
Ecto+/Endo-	42	1	2.4	[0.1-12.6]	1.7	[0.2-19.3]	
Endo+	28	3	10.7	[2.3-28.2]	6.7	**[1.1-41.2]**	
Deep	1	0	0.0	[0.0-97.5*]	-|	-	
All	4	0	0.0	[0.0-60.2*]	-|	-	
Uncertain	31	6	19.4	[7.5-37.5]	**19.5**	**[3.8-99.7]**	
Unknown	*2*	*1*					
Margin status (Clear *vs* Involved)							**0.003**
Clear	134	2	1.5	[0.2-5.3]	Ref.		
Involved	106	10	9.4	[4.6-16.7]	**7.31**	**[1.6-33.4]**	
Unknown	*2*	*0*					
Margin status (Clear *vs* Involved vs Uncertain)							**<0.001**
Clear	134	2	1.5	[0.2-5.3]	Ref.		
Involved	75	4	5.3	[1.5-13.1]	3.7	[0.7-20.4]	
Uncertain	31	6	19.4	[7.5-37.5]	**20.3**	**[3.9-105.3]**	
Unknown	*2*	*1*					
First HR-HPV post-LLETZ							**<0.001**
Negative	137	1	0.7	[0.0-4.0]	Ref.		
Positive	42	8	19.0	[8.6-34.1]	**30.6**	**[3.8-246.2]**	
Unknown	*63*	11					
First RLU HPV post-LLETZ	167				1.0	[0.999-1.0]	0.374
First RLU HPV post-LLETZ cat							**<0.001**
Negative	130	1	0.8	[0.0-4.2]	Ref.		
1-100	26	3	11.5	[2.4-30.2]	**14.1**	**[1.5-136.1]**	
>100	11	5	45.5	[16.7-76.6]	**190.2**	**[19.4-1832.8]**	
Unknown	*75*	*4*					
Parity							**0.033**
Nulliparous	41	0	0.0	[0.0-8.6*]	-|		
<4 full-term births	170	10	5.9	[2.9-10.6]	**0.3**	**[0.1-0.97]**	
4+ full-term births	14	3	21.4	[4.7-50.8]	Ref.	-	
Unknown	*17*	*0*					
Total	**242**	**13**	**19.8**	**[15.0-25.4]**			

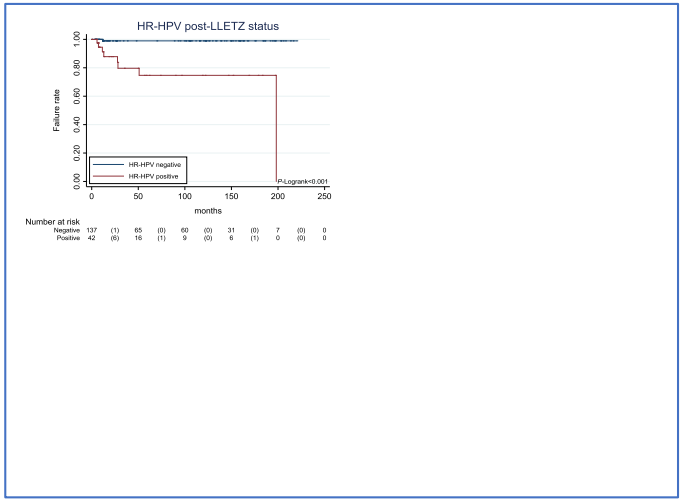
CI: Confidence interval; *: 97.5% CI; P-value1: log-likelihood ratio test P-value; |: No recurrences observed in the category; Endo(+) contains Ecto(-)/Endo(+) and Ecto(+)/Endo(+).

TABLE 3.

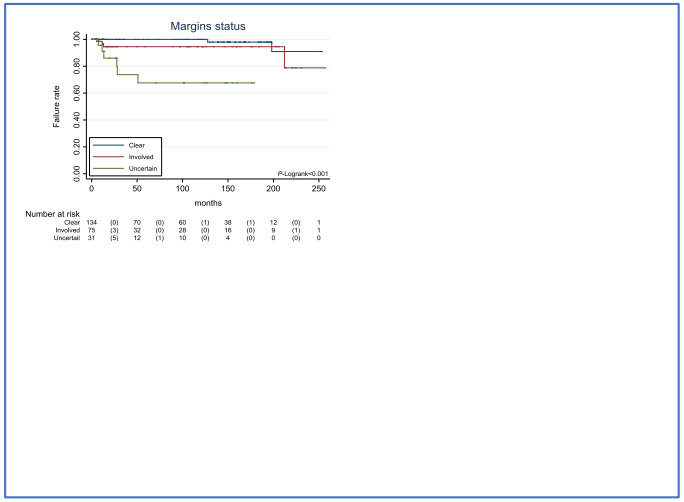
Sensitivity, specificity, positive predicted value, negative predicted value, positive likelihood ratio, of margin status, first HR-HPV detection after Large Loop excision of the Transformation Zone (LLETZ) with regard of residual/recurrent Cervical Intraepithelial Neoplasia 2-3 (CIN 2-3).

Positive post-LLETZ predictors	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	LR+ (%) (Se/(1-Sp)	Fisher's exact test P-value
Margins						**<0.001**
Involved or uncertain	10/12=83.3	132/228=57.8	10/106=9.4	132/134=98.5	1.9	**0.006**
Involved (without uncertain)	4/12=33.3	157/228=68.8	4/75=5.3	157/165=95.1	1.0	1.000
Margins						**<0.001**
Ecto(+)/Endocervical(-)	1/12=8.3	187/228=82.0	1/42=2.3	187/198=94.4	0.4	0.697
Ecto(-)/Endocervical(+)	2/12=16.6	208/228=91.2	2/22=9.0	208/218=95.4	1.9	0.303
Other*	1/12=8.3	218/228=95.6	1/11=9.0	218/229=95.2	1.9	0.438
Uncertain	6/12=50.0	203/228=89.0	6/31=19.3	203/209=97.1	4.5	**0.002**
HR-HPV detection after LLETZ	8/9=88.8	136/170=80.0	8/42=19.0	136/137=99.2	4.4	**<0.001**
Margins AND First HR-HPV detection after LLETZ						
Clear&HPV-	0/9=0.0	99/170=58.2	0/71=0.0	99/108=91.7	0.0	**0.012**
Involved or uncertain&HPV-	1/9=11.1	105/170=61.8	1/66=1.5	105/113=92.9	0.3	0.157
Involved (without uncertain)&HPV-	1/9=11.1	119/170=70.0	1/52=1.9	119/127=93.7	0.4	0.451
Clear&HPV+[1]	1/9=11.1	149/170=87.6	1/22=4.5	149/157=94.9	0.0	1.000
Involved or uncertain&HPV+[1]	7/9=77.7	157/170=92.3	7/20=35.0	157/159=98.7	10.2	**<0.001**
Involved (without uncertain)&HPV+[1]	2/9=22.2	162/170=95.2	2/10=20.0	162/169=95.8	4.7	0.082
Uncertain&HPV+[1]	5/9=55.5	165/170=97.0	5/10=50.0	165/169=97.6	18.8	**<0.001**
Margins OR First HR-HPV detection after LLETZ						
ClearOR HPV+[1]	8/9=88.9	65/170=38.2	8/113=7.1	65/66=98.5	1.4	0.157
Involved or uncertain OR HPV+[1]	9/9=100.0	71/170=41.8	9/108=8.3	71/71=100	1.7	**0.012**
Involved(without incertain)ORHPV+[1]	4/9=44.4	90/170=52.9	4/84=4.8	90/95=94.7	0.9	1.000
UncertainORHPV+[1]	8/9=88.9	122/170=71.8	8/56=14.3	122/123=99.2	3.2	**<0.001**

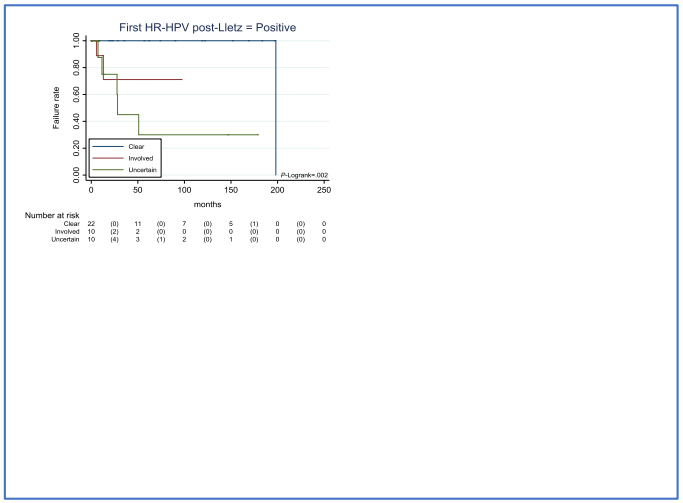
Se: Sensitivity; Sp: Specificity; PPV: Positive predicted value; NPV: Negative predicted value; LR+: Positive likelihood ratio; 1: Considering as negative combinations of -/+, and +/- and -/-, and as positive only combination of +/+; Numbers may not add up to 100 due to missing values; LLETZ: Large Loop Excision of the Transformation Zone. HR-HPV: High-risk human papillomavirus. *Other: Deep, ecto(+)/endo(+) or all margins involvement.



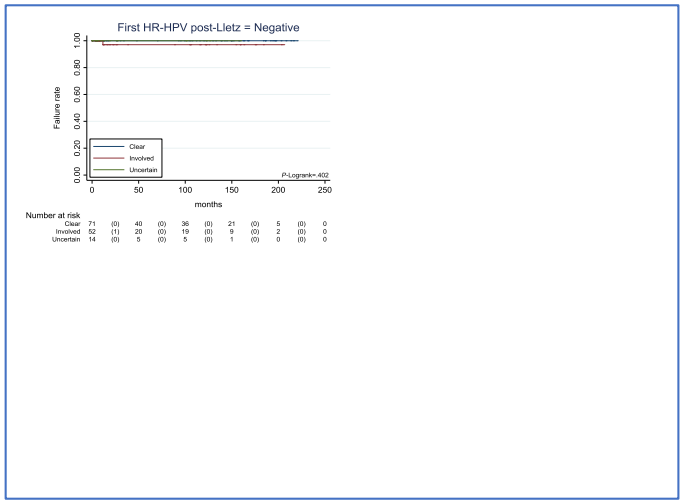
Panel a



Panel b



Panel c



Panel d

FIGURE 1: Cervical Intraepithelial Neoplasia (CIN2-3) Failure Rate after Large Loop Excision of the Transformation Zone (LLETZ) by first high-risk human papillomavirus (HR-HPV) post LLETZ, by margin status, and by margin involvement stratified by HR-HPV status. *Panel a:* Kaplan-Meier curve for CIN 2-3 Failure Rate after LLETZ by HR-HPV post treatment (*P*-Logrank<0.001). *Panel b:* Kaplan-Meier curve for CIN 2-3 Failure Rate after LLETZ by margin status (*P*-Logrank<0.001). *Panel c:* Kaplan-Meier curve for CIN 2-3 Failure rate after LLETZ by margins status for positive first HR-HPV post-LLETZ (*P*-Logrank=0.002). *Panel d*: Kaplan-Meier curve for CIN 2-3 Failure rate after LLETZ by margins status for negative first HR-HPV post-LLETZ (*P*-Logrank=0.40).