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

M-E Fernández-Montoli, S Tous, G Medina, M Castellarnau, A García-Tejedor, S de Sanjosé

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% confidence interval [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 (odds ratio [OR] = 13.20, 95% CI = 1.02–170.96; OR = 15.84, 95% CI = 3.02–83.01; and OR = 6.60, 95% CI = 0.88–49.53, respectively). Women with involved margins and/or who were 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 to reduce the risk.

Keywords Cervical intraepithelial neoplasia, HPV, margin status, recurrence.

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.

Linked article This article is commented on by M Arbyn et al., p. 388 in this issue. To view this mini commentary visit https://doi.org/10.1111/1471-0528.16023.

Introduction Women with cervical intraepithelial neoplasia 2–3 are treated conservatively, typically by large loop excision of the transformation zone (LLETZ) to prevent the development of invasive cervical cancer. After treatment, patients require follow up because of the risk of CIN recurrence or cervical cancer, which remains for many years. 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.\(^7\) 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 CIN 2–3 and followed for a median of 30 months over a 20-year period, to assess the long-term risk of CIN 2–3 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 Gynaecology 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 haemostasis. 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 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 were 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. Cytological 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 uses 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 signal-amplified hybridisation antibody capture assay was measured by a luminometer as relative light unit (RLU). When the relative light units were 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 CIN 2–3 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 CIN 2–3 lesions were recurrences or a \textit{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, College Station, TX, USA).

The relation between categorical variables was assessed by Chi-square tests or Fisher’s exact tests, as appropriate.
The relation 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), and positive likelihood of residual/recurrent CIN 2–3. The treatment failure rate time was calculated from the date of LLETZ to the date of residual/recurrent CIN 2–3.

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.

Results

Study cohort

We enrolled 471 consecutive adult women treated by LLETZ for cervical intraepithelial neoplasia or cervical neoplasia. Of these, women were excluded if 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 immunological status (n = 10). Thus, 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.

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 in one case (0.4%), and unknown in 15 cases (6.2%).

Treatment success and residual or recurrent CIN 2–3

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 CIN 2–3 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 CIN 1.

The median lag time between LLETZ and residual or recurrent CIN 2–3 was 13 months (range 3–212 months; interquartile range 11–31 months). Five (38.4%) and two (15.3%) CIN 2–3 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 (three CIN 2–3 recurrences), 122 cases of CIN 3 (five recurrences), and 43 cases of CIN 2–3 (five 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 eight cases (61.5%), CIN 2 in three cases (23.1%), and CIN 2–3 in two cases (15.4%). All 13 cases with residual/recurrent CIN 2–3 required new treatment: seven underwent a 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 CIN 2–3 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 were cases without HR-HPV (19 versus 0.7%) (P = 0.001). Cases with treatment failure had higher post-LLETZ RLU HR-HPV values than did 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 CIN 2–3 than did cases with normal cytology (50 versus 3%) (P = 0.001).

Surgical margins were involved in 75 cases (31%) cases, and involvement was 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…
| 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 (CIN 2–3) |
|---------------------------------------------------------------|
| **Patient characteristics**                                  |
| Follow-up time (in months)                                    |
| Median (Min–Max)                                              | 30.3 (2–257) |
| IQR                                                          | 8–149 |
| Age (years)                                                   |
| Median (Min–Max)                                              | 35.5 (18–77) |
| Mean (SD)                                                     | 37.4 (10.9) |
| Age (categorized at 35 years)                                 |
| <35 years                                                     | 116 (47.9) |
| 35+ years                                                     | 126 (52.1) |
| Parity                                                        |
| Nulliparous                                                   | 41 (16.9) |
| ≤4 full-term births                                          | 170 (70.2) |
| >4 full-term births                                          | 14 (5.8) |
| Unknown                                                      | 17 (7.0) |
| Smokers                                                       |
| No                                                           | 140 (57.9) |
| Yes                                                          | 83 (34.3) |
| Unknown                                                      | 19 (7.9) |
| **HPV and cytology results**                                  |
| First HR-HPV post-LLETZ                                       |
| Negative                                                      | 137 (56.6) |
| Positive                                                     | 42 (17.4) |
| Unknown                                                      | 63 (26.0) |
| First RLU HR-HPV post-LLETZ (n = 167)                         |
| Median (Min–Max)                                              | 0.3 (0.1–3250) |
| IQ range                                                     | 0.2–0.7 |
| First RLU HR-HPV post-LLETZ category                          |
| Negative < 1                                                 | 130 (53.7) |
| 1–100                                                        | 26 (10.7) |
| >100                                                         | 11 (4.6) |
| Unknown                                                      | 75 (31.0) |
| First cytological result post-cone                            |
| Normal                                                       | 199 (82.2) |
| ASC-US                                                       | 18 (7.4) |
| LSIL                                                         | 13 (5.4) |
| HSIL                                                         | 12 (5.0) |
| **Surgical specimen characteristics**                        |
| Margin status                                                 |
| Clear                                                        | 134 (55.4) |
| Involved                                                     | 75 (31.0) |
| Ecto/endo +                                                   | 42 (17.4) |
| Ecto +/endo +                                                  | 22 (9.1) |
| Ecto+/endo +                                                  | 6 (2.5) |
| All margin                                                   | 4 (1.7) |
| Deep margin                                                  | 1 (0.4) |
| Uncertain                                                    | 31 (12.8) |
| Glandular involvement                                        |
| No                                                           | 11 (4.5) |
| Yes                                                          | 80 (33.1) |
| Unknown                                                      | 151 (62.4) |

*Significance level: *p* < 0.05, **p** < 0.01, ***p** < 0.001

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endocervical\(^+(16.7\%)\), and ecto+/endocervical\(^-\) (9.1\%) involvement. By contrast, ecto+/endocervical\(^-\) and clear margins accounted for 2.4\% and 1.5\% of CIN 2–3 treatment failures, respectively. No difference in residual/recurrent CIN 2–3 was observed in 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 CIN 2–3 treatment failure. Women older than 35 years were at increased risk (OR = 5.45, 95\% CI = 1.18–25.15; \(P = 0.011\)) compared with younger women. Margin involvement was significantly related to CIN 2–3 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 a >32-fold increased odds (OR = 32, \(P < 0.001\)) of developing a recurrent or residual lesion compared with women with a negative result (Table S1). 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; \(P < 0.001\)). CIN 2–3 relapse appeared earlier in HR-HPV positive cases, which had a 30.5-fold higher risk of developing CIN 2–3 in the next period of time than did 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. HR-HPV detection after treatment had a sensitivity of 88.8, a specificity of 80, and

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**Table 1. (Continued)**

| Quadrants involved | Number of patients (%) | Residual/Recurrent CIN 2–3 (%) | P-value*** |
|--------------------|------------------------|-------------------------------|------------|
| 1                  | 37 (15.3)              | 2 (5.4)                       | 0.606      |
| 2                  | 50 (20.7)              | 45 (90.0)                     | 5 (10.0)   |
| 3                  | 21 (8.7)               | 20 (95.2)                     | 1 (4.8)    |
| 4                  | 54 (22.3)              | 52 (96.3)                     | 2 (3.7)    |
| Unknown            | 80 (33.1)              | 77 (96.3)                     | 3 (3.7)    |

Circumference of the cervical specimen (cm\(^2\)) \((n = 129)\)

| Mean (SD) | Median (Min–Max) | IQR | Length of the specimen (cm) \((n = 131)\) | Mean (SD) | Median (Min–Max) | IQR | Endocervical sweep (two-step procedure) |
|-----------|------------------|-----|------------------------------------------|-----------|------------------|-----|----------------------------------------|
| 12.6 (5.6) | 11.1 (4.6–32.9) | 9.2–13.7 | 0.9 (0.4) | 0.9 (0.2–2.7) | 6–1.2 | No 173 (71.5) | 164 (94.8) | 9 (5.2) |
| 12.5 (5.4) | 9.1–1307 | 9.1–13.7 | 0.9 (0.4) | 0.9 (0.2–2.7) | 6–1.2 | Yes 69 (28.5) | 65 (94.2) | 4 (5.8) |
| 16.0 (11.2) | 10.8 (9.4–32.8) | 9.7–22.2 | 4 (1.1) | 1.05 (0.7–1.6) | 0.85–1.35 | Unknown 0 (0.0) | – | – |
| 682 (100.0) | 229 (94.6) | 13 (5.4) | **** | ***** | ANOVA test ** | ** | Fisher’s exact test P-value. |

ASCUS, atypical squamous cells of undetermined significance; CIN, cervical intraepithelial neoplasia; HR-HPV, high-risk human papillomavirus; HSIL, high-grade intraepithelial lesion; IQR, interquartile range (25–75\%); LSIL, low-grade squamous intraepithelial lesion; RLU, relative light units. 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.

Values in bold indicate that there are significant differences between groups studied.

*Column percentage.
**Row percentage.
***Fisher’s exact test \(P\)-value.
****Kruskal–Wallis test \(P\)-value.
*****ANOVA test \(P\)-value.

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### Table 2. Univariate Cox regression analysis of the factors associated to residual/recurrent cervical intraepithelial neoplasia 2–3 (CIN 2–3) after loop electrosurgical excision procedure (LLETZ)

| Characteristics | Total | CIN 2–3 | Univariate analysis |
|-----------------|-------|---------|---------------------|
|                 | n     | n      | %                  | 95% CI | HR   | 95% CI | P-value* |
| Age             |       |        |                    |        |      |        |          |
| Age (categorised at 35 years) |     |        |                    |        |      |        |          |
| <35 years old   | 116   | 2      | 1.7                | 0.2–6.1| Ref. |        |          |
| ≥35 years old   | 126   | 11     | 8.7                | 4.4–15.1| 5.5  | 1.2–25.1| 0.009    |
| Margin status   |       |        |                    |        |      |        |          |
| 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| 0.001    |
| 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) |     |        |                    |        |      |        |          |
| 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| 0.003    |
| Ecto−/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 versus involved) |     |        |                    |        |      |        |          |
| 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| 0.003    |
| Unknown         | 2     | 0      |                    |        |      |        |          |
| Margin status (clear versus involved versus uncertain) |     |        |                    |        |      |        |          |
| 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| <0.001   |
| Uncertain       | 31    | 6      | 19.4               | 7.5–37.5| 20.3 | 3.9–105.3|          |
| Unknown         | 2     | 1      |                    |        |      |        |          |
| First HR-HPV post-LLETZ |     |        |                    |        |      |        |          |
| Negative        | 137   | 1      | 0.7                | 0.0–4.0| Ref. |        | <0.001   |
| Positive        | 42    | 8      | 19.0               | 8.6–34.1| 30.6 | 3.8–246.2|          |
| Unknown         | 63    | 11     |                    |        |      |        |          |
| First RLU HPV post-LLETZ category |     |        |                    |        |      |        |          |
| Negative        | 130   | 1      | 0.8                | 0.0–4.2| Ref. |        | <0.001   |
| 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          |       |        |                    |        |      |        |          |
| Nulliparous     | 41    | 0      | 0.0                | 0.0–8.6**| –***| –     | 0.033    |
| <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.
Values in bold indicate that there are significant differences between groups studied.
*Log-likelihood ratio test P-value.
**97.5% CI.
***No recurrences observed in the category; Endo− contains ecto−/endo− and ecto+/endo−.

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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 (three cases, 7.9%) in ectoendo cervical margins (P = 0.010) and higher HR-HPV positivity in uncertain margins (ten cases, 41.7%; P = 0.036). No differences were found in relation to HPV positivity for ectoendo cervical margins (Table S2).

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, ectoendo cervical, and ectoendo cervical margins (Figure S2e).

The results for HR-HPV post-LLETZ positivity stratified by margins show differences between the presence of clear margins (log-rank P = 0.025), ectoendo cervical 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 ectoendo cervical involvement with HR-HPV positivity (log-rank P = 0.76) (Figure S2).

| Table 3. Sensitivity, specificity, positive predicted value, negative predicted value, and 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) |
|---------------------------------------------------------------|
| **Margins** | **Sensitivity (%)** | **Specificity (%)** | **PPV (%)** | **NPV (%)** | **LR⁺ (%)** | **Fisher’s exact test P-value** |
| Involved or uncertain | 10/12 = 83.3 | 132/228 = 57.8 | 10/106 = 9.4 | 132/134 = 98.5 | 1.9 | <0.001 |
| Involved (without uncertain) | 4/12 = 33.3 | 157/228 = 68.8 | 4/75 = 5.3 | 157/165 = 95.1 | 1.0 | 1.000 |
| Ectoendo cervical | 1/12 = 8.3 | 187/228 = 82.0 | 1/42 = 2.3 | 187/198 = 94.4 | 0.4 | 0.697 |
| Ectoendo cervical | 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 = 88.9 | 136/170 = 80.0 | 8/42 = 19.0 | 136/137 = 99.2 | 4.4 | <0.001 |
| **Margins and first HR-HPV detection after LLETZ** | 0/9 = 0.0 | 99/170 = 58.2 | 0/71 = 0.0 | 99/108 = 91.7 | 0.0 | 0.12 |
| Clear & HPV⁻ | 1/9 = 11.1 | 105/170 = 61.8 | 1/66 = 1.5 | 105/133 = 92.9 | 0.3 | 0.157 |
| Involved or uncertain & HPV⁻ | 1/9 = 11.1 | 119/170 = 70.0 | 1/52 = 1.9 | 119/127 = 93.7 | 0.4 | 0.451 |
| Clear & HPV⁺ | 1/9 = 11.1 | 149/170 = 87.6 | 1/22 = 4.5 | 149/157 = 94.9 | 0.0 | 0.001 |
| Involved or uncertain & HPV⁺ | 7/9 = 77.7 | 157/170 = 92.3 | 7/20 = 35.0 | 157/159 = 98.7 | 10.2 | 0.001 |
| Involved (without uncertain) & HPV⁺ | 2/9 = 22.2 | 162/170 = 95.2 | 2/10 = 20.0 | 162/169 = 95.8 | 4.7 | 0.082 |
| Uncertain & HPV⁺ | 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** | 8/9 = 88.9 | 65/170 = 38.2 | 8/113 = 7.1 | 65/66 = 98.5 | 1.4 | 0.157 |
| Clear or HPV⁺ | 9/9 = 100.0 | 71/170 = 41.8 | 9/108 = 8.3 | 71/71 = 100 | 1.7 | 0.012 |
| Involved (without uncertain) or HPV⁺ | 4/9 = 44.4 | 90/170 = 52.9 | 4/84 = 4.8 | 90/95 = 94.7 | 0.9 | 1.000 |
| Uncertain or HPV⁺ | 8/9 = 88.9 | 122/170 = 71.8 | 8/56 = 14.3 | 122/123 = 99.2 | 3.2 | <0.001 |

HR-HPV, high-risk human papillomavirus; LR⁺, positive likelihood ratio; NPV, negative predicted value; PPV, positive predicted value; Se, sensitivity; Sp, specificity.
Values in bold indicate that there are significant differences between groups studied.
*Other: Deep, ectoendo or involvement of all margins.
**Considering negative combinations of −/−, and +/+ and −/−, and as positive-only combination of +/+ Numbers may not add up to 100 due to missing values.

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Discussion

Main findings

CIN 2–3 treatment showed a favourable long-term outcome with treatment failure of CIN 2–3 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 of having CIN 2–3 in the next period of time compared with 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 endocervical margins (16.7%) or uncertain margin involvement (19.4%). Being older than 35 years was another predictive factor of recurrence (P = 0.009).

Except for 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 endocervical margins had 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.
We observed a lower risk of residual or recurrent CIN 2–3 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. However, the lack of association of ecto-/endocervical margins with residual or recurrent CIN 2–3 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. In the univariate analysis, ecto-/endocervical margins were associated with treatment failure and a 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 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. Detached epithelium can hide a CIN 2–3 lesion and may be related to the low reproducibility of margin assessment.

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. 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 CIN 2–3 treatment failure, though it is not always observed. 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 CIN 2–3 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

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 CIN 2–3 recurrence is another strength of our study. To the best of our knowledge, no previous authors have analysed the relation 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 CIN 2 and CIN 3 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. In a meta-analysis, HR-HPV showed a sensitivity of 91% and a 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%. 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. Our data show that margin involvement is a predictor of treatment failure. Two meta-analyses observed that CIN 2–3 disease recurred in 18% of cases with involved margins versus 3% with clear margins. The relative risk of CIN 2–3 recurrence after incomplete excision has also been reported to be 4.8, 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, 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 recur more frequently than those with clear or involved margins negative for HR-HPV (Figure 1C,D). This pattern was observed in a study of CIN 1 and CIN 2–3 cases.
differs by the type of margin and HPV positivity, with ecto/endocevical+ 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. 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.

**Disclosure of interests**

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

**Contribution to authorship**

This work was conceived and planned by M-EF-M, who also wrote the draft. ST, GM, MC, and AG-T played a role in the data collection, analysis, and interpretation of results, and made suggestions for revision. SS approved the final version. All the authors have read the text and agreed on its contents.

**Details of ethics approval**

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) (11 October 2018).

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**Supporting Information**

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Figure S1.** Algorithm of inclusion/exclusion of cases

**Figure S2.** Cervical intraepithelial neoplasia (CIN 2–3) failure rate after large loop excision of the transformation zone (LLETZ) (a) by first high-risk human papillomavirus (HR-HPV) post-LLETZ status and uncertain margin involvement (P-log-rank = 0.011); (b) by first HR-HPV post-LLETZ and ecto+/endocervical+ margin involvement (P-log-rank = 0.76); (c) by HR-HPV post-LLETZ and clear margins (P-log-rank = 0.025); (d) by HR-HPV post-LLETZ and ecto+/endocervical+ margin involvement (P-log-rank <0.001); (e) by margin status (P-log-rank <0.001); (f) by margin involvement stratified by HR-HPV status (P-log-rank <0.001).

**Table S1.** Univariate logistic analysis of the factors associated to residual/recurrent cervical intraepithelial neoplasia (CIN 2–3) after large loop excision of the transformation zone (LLETZ).

**Table S2.** High-risk human papillomavirus positivity after large loop excision of the transformation zone (LLETZ) in patients with cervical intraepithelial neoplasia (CIN 2–3) according to margin status.

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