

# Predictive factors for persistent and recurrent cervical dysplasia after loop electrosurgical excision procedure in postmenopausal women

## Abstract

The aim of this study was to prospectively evaluate the outcomes of conservative treatment for pathological proven CIN2/3 (cervical intraepithelial neoplasia) in postmenopausal women and the risk factors potentially involved in the persistence/recurrence of cervical dysplasia. A prospective cohort study was performed over an 18-months period on 132 post-climax women with CIN2/3 treated by loop electrosurgical excision procedure (LEEP) attending follow-up visits. We assessed the relationship between age, parity, AFSI (age at first sexual intercourse), smoking status, use of oral contraceptives, high-risk human papillomavirus (HR-HPV) status, surgical margins, glandular involvement and risk of persistent/recurrent disease. Unadjusted and adjusted analyses were performed. Residual/recurrent disease was proved by colposcopically guided biopsy in 25 patients (18.9%). On a multivariate logistic regression analysis, age > 50 years (OR 4.85 95%CI 1.09-21.53,  $p < 0.0001$ ), positive surgical margins (OR 4.26, 95% CI 1.64-11.04,  $p < 0.001$ ) and HR-HPV persistence (OR 5.73, 95% CI 1.93-16.97,  $p < 0.001$ ) were statistically predictive for persistent/recurrent lesions. The most important predictive factors for persistent/recurrent disease in postmenopause women treated conservatively for CIN2/3 are advanced age, positive surgical margins and HR-HPV post treatment persistence; the combined evaluation of these three factors allows placing patients with LEEP into different categories at risk, thus requiring special attention with a rigorous follow-up protocol and preventing a primarily radical therapeutic approach in this particular age - group.

**Keywords:** postmenopause, cervical intraepithelial neoplasia, HR-HPV, LEEP

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## Introduction

Modern society is still confronting with a major health issue: cervical cancer, although a great improvement in prevention and treatment has been achieved for the past few years<sup>(1)</sup>.

In Romania statistics are worrying: cervical neoplasia is the 3rd cause of death in female population (and the 2<sup>nd</sup> in the 15-44 age group), comprising 4.343 cases diagnosed each year, proving that both research and implementing screening programmes are highly needed<sup>(2)</sup>.

The loop electrosurgical excision procedure (LEEP) is the most widely accepted surgical technique for the treatment of high-grade cervical intraepithelial neoplasia (CIN), producing one or more specimens of cervical tissue for histologic interpretation<sup>(3)</sup>; however, it is imperative that the entire transformation zone be excised<sup>(4,5)</sup>.

Postmenopause women represent a challenging group for colposcopic examination and assessment of cervical dysplasia due to changes occurring in the cervix's ap-

pearance resulted from estrogen levels decline. In up to 40% of women >50 years the transformation zone retracts within the endocervix<sup>(6)</sup>. Also, cytologic smears can sometimes be difficult to interpret because the parabasal cells with an increased nuclear: cytoplasmatic ratio predominate<sup>(7)</sup>.

The primary goal of surgery for cervical dysplasia is complete lesion excision; however, women treated for high-grade cervical dysplasia have a long-term risk for cervical cancer higher than the general female population<sup>(8)</sup>. The rates of disease persistence/recurrence reported in literature vary between 5 and 30%<sup>(9)</sup>. Several studies have proposed different risk factors: age, parity, lesion grade and size, surgical margin status, glandular extension and persistence of high-risk human papillomavirus (HR-HPV) for the prediction of persistent/recurrent disease, but variable and sometimes controversial results have been reported and their roles are yet to be established<sup>(10-13)</sup>.

The objective of our prospective study is to evaluate the outcomes of conservative treatment for pathological

proven CIN2/3 in postmenopause women and the risk factors potentially involved in the persistence/recurrence of cervical dysplasia in this particular study group.

## Methods

We conducted a prospective cohort study in the Clinical Hospital of Obstetrics and Gynecology "Prof. Dr. Panait Sîrbu" in Bucharest, Romania from March 2013 to September 2014. A number of 132 postmenopausal (affirmed absence of menstruation for more than a year) women with histological confirmed CIN2/3 were treated by LEEP performed in the Department of Obstetrics and Gynecology of our clinic. Inclusion criteria were: installed menopause, CIN2 or CIN 3 confirmed histologically and attending at least one follow-up visit. Exclusion criteria were: less than one year after cessation of menstruation, overt colposcopic cancer, microinvasive/invasive carcinoma histologic diagnosis, subsequent total hysterectomy, and unavailability of required data. Age, parity (nulliparous/parous=at least one vaginal live birth), age at first sexual intercourse (AFSI), smoking status, use of oral contraceptives were obtained by complete patients history examination. The study was approved by the Committee of Ethics and Research in Humans of our institution.

Patients were scheduled for follow-up visits at 6 months and 12 months after treatment comprising clinical examination, Pap smear, HR-HPV testing, colposcopy and eventually biopsy. If the surgical margins were affected, the first check-up was set at 3 months after the procedure. All of the cervical cytology performed was liquid-based cytology (ThinPrep®) and was interpreted in the Department of Pathology in our clinic. We used for results reporting of HR-HPV infection the Hybrid Capture II system (Digene Diagnostics Inc., Gaithersburg, MD, USA)<sup>(14)</sup>.

All patients signed an informed consent according to the World Medical Association Declaration of Helsinki regarding both colposcopy and LEEP procedure. The colposcopic examination was carried out in our clinics by Colposcopy Department, certified by a gynecologist and diagnosed using Reid Colposcopic Index<sup>(15)</sup>.

All 132 patients underwent a standard LEEP conization performed under intravenous general anesthesia in the operation theater. The cervix was exposed using an adapted speculum which allows smoke evacuation. After delineating the abnormal cervical area with Lugol's iodine, the loop was selected according to the size of the area to be excised and the diathermy power was set at 60 W for "cut" mode and 45 W for "coagulation" mode in "blended" setting. A larger loop (20 mm/0.8 mm) was used for the central pass removing the entire transformation zone and, after identifying the endocervical canal, a second selective endocervical sweep was performed with a smaller loop (10 mm/10 mm). The surgical site was cauterized with a 5 mm cautery ball with a power setting of 50 W to prevent bleeding.

The LEEP specimens were removed, formalin fixed and sent for histologic evaluation in the Department

of Pathology of our hospital where they were paraffin embedded and the slides obtained were hematoxylin-eosin stained. The results were reported at 4 weeks after the intervention and the first post-treatment control was scheduled at 6 months. When surgical margins were affected, the first control was conducted at 3 months. Diagnosis of dysplasia was made according to the World Health Organization Classification of Tumors<sup>(16)</sup> with concurrent surgical margins assessment and glandular involvement.

Criteria for defining persistent/residual disease were based on positive surgical margins at conization and/or abnormal results at the 3-6 months visit and criteria for defining recurrent disease was based on negative surgical margins at conization and first normal check-up. Either persistent or recurrent disease were histological confirmed (CIN of any grade) by positive colposcopy - directed biopsy or endocervical curettage. Women with two consecutive negative PAP smears, negative HR-HPV test and normal colposcopy were considered negative for persistent/recurrent disease. Women with abnormal cytology and/or abnormal transformation zone underwent a colposcopy - directed biopsy. In the case of partially or absent transformation zone visibility or no colposcopic abnormality being identified, an endocervical curettage was performed. For reasons of statistical power patients with persistent/recurrent disease were included in the analysis together.

Statistical analysis was performed using SPSS version 20.0 (IBM, Armonk, NY, USA). Continuous variables were expressed as mean  $\pm$ SD, discrete variables as median (range) and categorical variables as number (percentage). Univariate analyses to identify variables associated with persistence/recurrence were performed using Student's T-test, Mann Whitney test, chi-squared and Fisher's exact test, as appropriate.

Odds ratio (OR) and 95% confidence intervals (CIs) were estimated by logistic regression analysis.

Variables found to be significant by univariate analysis were examined by multivariate analysis to determine independent predictors of disease persistence/recurrence. The level of statistical significance was set at 5% ( $p < 0.05$ ).

## Results

A total of 132 postmenopause women underwent LEEP conization for histologic confirmed CIN2/3 during the study period. Of these women, 98 (74.2%) tested positive for HR-HPV before treatment. The mean age of the patients was  $50.06 \pm 6.55$  years (range 43-62 years), with 58 patients (43.9%) aging over 50 years. 95 (71.9%) patients of the study group were smokers and 32 (24.2%) used oral contraceptives for more than 5 years. About 112 women (84.8%) had at least one prior delivery (i.e. 100 women delivering vaginally and 12 women by cesarean section) with 28 women (21.2%) having parity  $\geq 3$  and 38 women (28.7%) had AFSI  $< 18$  years. The patients characteristics are exposed in Table 1.

**Table 1** Characteristics of patients

Variable	N= 132 (100%)
Mean age (range)	50.06± 6.55 (43-62)
Nulliparous	20 (15.1)
Parity ≥3	28 (21.2)
Smokers	95 (71.9)
Use of oral contraceptives >5 years	32 (24.2)
AFSI <sup>a</sup> <18 years	38 (28.6)
HR-HPV <sup>b</sup> before LEEP <sup>c</sup>	98 (74.2)

<sup>a</sup>AFSI= age at first sexual intercourse; <sup>b</sup>HR- HPV= high-risk human papillomavirus; <sup>c</sup>LEEP= loop electro-surgical excision procedure

**Table 2** Anatomopathological characteristics of cone specimens

Variable	N (%)	N(%) persistence/recurrence	OR <sup>c</sup>	95%CI <sup>d</sup>	P value
Clear surgical margins	88 (66.6)	11 (12.5)	-	-	-
Positive surgical margins	33 (25)	13 (39.3)	4.71	1.64-11.04	.005
H-SIL <sup>a</sup>	26 (78.8)	11 (42.3)			
L-SIL <sup>b</sup>	9 (21.2)	2 (22.2)			
Uncertain surgical margins	11 (8.3)	1 (9)	0.4	0.04-3.31	.02
Glandular involvement	72 (54.5)	19 (26.4)	3.22	1.19-8.7	.02

<sup>a</sup>H-SIL= high-grade squamous intraepithelial neoplasia; <sup>b</sup>L-SIL=low-grade squamous intraepithelial neoplasia; <sup>c</sup>OR= odds ratio; <sup>d</sup>CI=confidence interval

**Table 3** Multivariate analysis of persistent/recurrent disease risk

Variable	Regression coefficient	95% CI <sup>b</sup>	P value
Age >50 years	4.85	1.09-21.53	.0001
Positive surgical margins	4.26	1.64-11.04	.001
HR-HPV <sup>a</sup> persistence	10.26	3.53-28.89	.001

<sup>a</sup>HR-HPV= high-risk human papillomavirus; <sup>b</sup>CI= confidence interval

Residual/recurrent disease was identified in 25 patients (18.9%) during follow-up: 16 (64%) women presented a CIN2/3 and 9 (36%) women developed CIN1, with no case of squamous cell/ endocervical carcinoma.

A total of 66.6% women (88/132) had clear surgical margins and the corresponding percent of patients with positive margins in the operation specimens was 25% (33/132). Thirteen women of the latter category underwent a re-conization procedure.

The other 20 were followed-up without further intervention. In 8.3% of patients (11/132) the resection margins were uncertain. Of the overall group with positive margins, 78.8% of cases (26/33) were positive as high-grade squamous intraepithelial neoplasia and 21.2% of cases (7/33) as low-grade squamous intraepithelial neoplasia. In 54.5% of cases (72/132) there was a positive glandular involvement.

Significant differences in risk of recurrent/persistent disease depending on the involved margins were observed: 39.3% (13/33) patients with positive surgical margins had a recurrent/persistent lesion versus 12.1% (12/99) patients with clear/uncertain margins ( $p < 0.02$ ). Patients with high-grade recurrent/persistent lesions had a greater percentage of affected margins compared to low-grade lesions ( $p < 0.005$ ).

We found a statistically significant correlation between glandular involvement and risk of recurrent/persistent disease (OR=3.22; 95% CI=1.19-8.7,  $p < 0.020$ ). The anatomic-pathological characteristics of cone specimens are exposed in Table 2.

Of the 25 women with recurrence/persistence, 64% of them (16/25) were over 50 years old, with a mean age in this group 57.28 years; age > 50 years was statistically associated with the risk of recurrence/persistence (OR=2.75; 95% CI=1.11-6.79;  $p < 0.028$ ). Smoking status was also correlated with recurrent/persistent disease: 23 of the 95 patients in the smoking group vs. 2 of the 37 patients in the non-smoking group (OR=5.59; 95% CI=1.24-25.06,  $p < 0.001$ ). Parity, AFSI and use of oral contraceptives proved no association with the risk of recurrent/persistent disease.

We found post-treatment HR-HPV positive at 6 months in 51% of patients (50/98); of these patients 40% (20/50) had recurrent/persistent disease. About 87.5% of patients (14/16) with CIN2/3 in follow-up tested positive for HR-HPV in post-treatment in comparison to 33.3% of patients (3/9) with CIN 1 ( $p < 0.003$ ).

In multivariate analysis, only age > 50 years, positive surgical margins and post treatment HR-HPV detection were significant predictor factors of recurrent and persistent disease (OR=4.85 95%CI=1.09-21.53,  $p < 0.0001$ , OR=4.26, 95% CI=1.64-11.04,  $p < 0.001$ , OR=5.73, 95% CI=1.93-16.97,  $p < 0.001$ ). These relationships are depicted in Table 3.

## Discussion

The importance of determining factors that can predict, prior to follow-up, the possible development of recurrent/persistent disease in women treated for CIN

has been underlined by many studies, stating that the risk of cervical cancer remains elevated at least for 20 years after the initial treatment of CIN<sup>(8,17)</sup>. We obtained in our group a rate of 18.9% of disease recurrence/persistence, which falls in the range of the rates reported by other studies (5-30%)<sup>(9,11,18)</sup>.

On the other hand, in the study performed by Lubrano et al. the rate of residual/recurrent disease was 13.9%<sup>(11)</sup>. Persistent disease could be considered as the result of primary treatment incomplete removal of CIN and/or HR-HPV virus persistence.

In our series, a multivariate analysis revealed that age over 50 years, positive surgical margins and HR-HPV persistence were statistically associated with recurrent/persistent lesions after treatment. Lu et al. showed in their study performed on 120 patients with CIN 3 that age > 50 years and parity > 5 two demographic features that predicted post-cone residual disease<sup>(19)</sup>. Also, Brockmeyer et al. proved that advanced age conferred a higher risk of dysplasia recurrence<sup>(20)</sup>.

The fact that a higher proportion of recurrent/persistent disease occurred in older women may be a reflection of the impact of age on the immune system<sup>(13)</sup>, a positive selection over time towards the persistence of a high oncogenic risk virus<sup>(21)</sup> or a tendency of postmenopausal women to have a squamous columnar junction deeper inside the cervical canal, therefore hampering the complete excision of the initial CIN2/3.

In the study performed by Lubrano et al. the rate of residual/recurrent disease was lower than the one obtained in our study (13.9%)<sup>(11)</sup>, but the mean age of the patients was 37.8 ± 8.9 years.

The status of resection margins has been shown in literature to be a predictive factor of persistent disease<sup>(11,12)</sup>. The frequency of positive surgical margins in our study was 25%, which is comparable to the average for cases described in literature.

The relation between affected margins and recurrent/persistent disease was statistically significant ( $p < 0.02$ ). However, although recurrence was more frequent in patients with positive margins, recurrence has also been observed after histological proven complete CIN excision<sup>(13)</sup>. This situation may occur from multifocal disease, endocervical focal lesions or HR-HPV deoxyribonucleotide acid persistence. In our series, recurrence occurred in 39.3% and 12.1% of patients with positive and negative margins, respectively. These percentages are consistent with data reported in the literature<sup>(22)</sup>.

Livasy et al. stated in their paper that a negative LEEP is not a reassuring finding<sup>(23)</sup> and it was associated with a recurrence rate similar to patients with positive LEEPs. Therefore, after an excisional therapy, close follow-up is essential for early detection of persistent/recurrent lesions, whatever the excision status.

Other studies found other predictive indicators of persistence/recurrence such as lesion severity, smoking, and menopausal status<sup>(10,11,24)</sup>.

In our study, neither smoking status, nor parity or AFSI were statistically associated on a multivariate



analysis with risk of having a persistent or recurrent lesion.

In agreement with other recent studies<sup>(12,13,24)</sup>, we observed in our study that there is a significant association between persistent posttreatment infection with HR-HPV and persistent or recurrent disease.

A total of 37.8% of the patients had a positive HR-HPV test at the 6 month follow-up, higher than the one obtained by Lubrano et al. in their study (25%)<sup>(11)</sup>. This can partly be explained by the fact that there are many factors which may increase the risk of persistence and the population that we analyzed was older and therefore the rate of viral clearance may be reduced.

Baser et al. reported a rate of 21.2% HR-HPV post-treatment persistence and multivariate analysis revealed that patient age and cone depth were significant independent predictors<sup>(25)</sup>.

Moreover, they found that HPV persistence was less common in the older age group which contrasts our findings.

Although Serati et al. demonstrated in their series that HPV infection did not represent a risk factor, neither for recurrence nor for margin involvement<sup>(12)</sup>, we are of the same opinion as most of the recent studies that routine post-conization HR-HPV testing together with cervical cytology may provide a better predicti-

on for potential of persistent/recurrent disease<sup>(9,13,26)</sup>. Vintermyr et al. observed in their study that 95.9% of women with primary CIN3 and recurrent CIN2+ had persistent HR-HPV infection, of which 74.5% were HPV 16/18<sup>(13)</sup> and clearly showed that normal cytology between conizations was not indicative of HPV clearance.

Moreover, Park et al. found that pre-conization high-risk HPV load of at least 100 relative light units/positive control was a significant risk factor for persistence or recurrence of disease after conization<sup>(27)</sup>, proving that the utility and cost effectiveness of HPV viral load measurement should be taken into account in future trials.

## Conclusions

In conclusion, we observed that the most important predictive factors for persistent/recurrent disease in post menopause women treated conservatively for CIN2/3 are advanced age, positive surgical margins and HR-HPV posttreatment persistence.

Our results suggest that the combined evaluation of these three factors allows placing patients with LEEP into different categories at risk, thus requiring special attention with a rigorous follow-up protocol and also preventing a primarily radical therapeutic approach in this particular age-group. ■

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