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TREATMENT OF CERVICAL PRECANCEROUS LESIONS BY LARGE LOOP EXCISION (LLETZ) IN AN OUTPATIENT SETTING

THESIS SUMMARY

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The public defence of the dissertation will take place on ..... from ..... in…….

The defence materials are available to the interested parties in the Library of MU-Pleven.

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INTRODUCTION

Cervical precancers occur most often after persistent infection with human papillomaviruses. These viruses are widespread among humans and are mainly sexually transmitted. The human body immune system usually neutralises the virus's effect and does not lead to a viral carriage, much less to a persistent infection. In cases when such an infection develops, one of the most commonly affected organs in women is the cervix. Human papillomaviruses are divided into low-, intermediate-, and high-risk, depending on their potential to cause different degrees of precancerous changes in the epithelium of the cervix. Low- and medium-risk strains can lead to so-called low-grade precancerous changes or precancers. They are also called cervical intraepithelial neoplasia (CIN 1) or low-grade squamous intraepithelial lesion (LSIL). Chronic infection with high-risk strains leads to CIN 2-3 or high-grade squamous intraepithelial lesion (HSIL), respectively. If not diagnosed and treated promptly, HSILs progress to Ca colli uteri in situ, Ca colli uteri microinvasiva, and Ca colli uteri invasiva.

Thus, cervical cancer (CC) gets to be a preventable oncological disease, the frequency of which can be minimised with prophylactic measures. These measures include primary and secondary prevention of cervical cancer. Primary prevention consists of vaccinating girls and, in some countries, boys at a certain age (9–13 years) against high-risk and highly oncogenic strains of human papillomavirus (HPV16 and 18). Secondary prevention is aimed at the diagnosis, treatment and follow-up of cervical precancers. National screening programs have been created in different countries for this purpose. Specific age groups of the female population are subject to these screening programs and, at a particular time interval, are subject to tests determining the risk of precancerous disease. Cervical screening and screening programs aim to screen women subject to further diagnostic interpretation. This screening is called organised and can be cytological (so-called pap smear or cervical cytology) or HPV screening, depending on the national screening program. In case of deviations in the screening results, additional diagnostic measures are required (colposcopy, targeted biopsy, COBAS test). Ultimately, the diagnosis of precancers is histological, most often after taking a pinch biopsy from the most suspicious area identified by colposcopy. The role of colposcopy is to determine if there are cellular changes on the cervix and

to locate the biopsy site that will most likely lead to a cervical precancer diagnosis. Treatment of cervical precancers prevents their progression to invasive carcinoma and is the basis of secondary prevention. This treatment includes destructive and excisional techniques, for which precise criteria have been developed. The most commonly used excision method is loop excision or large loop excision of the transformation zone (LLETZ). This is a sparing procedure in terms of cervix recovery and preserving the female's reproductive potential, although these diseases mainly affect young women of childbearing age. The thesis investigates the applicability of this widely used technique in an outpatient setting, without general anaesthesia, and the various prognostic factors for its successful application.

I. AIM AND TASKS

AIM:

To investigate the feasibility, safety, cost-effectiveness, therapeutic outcomes and prognostic factors in the treatment of cervical precancers by the LLETZ procedure in an outpatient setting.

TASKS

1. To study the applicability and economic efficiency of the LLETZ procedure in outpatient settings – analgesia, postoperative pain, postoperative stay; complications: intraoperative (bleeding); early postoperative (bleeding, infection) and late (stenosis of the cervical canal, spotting and incomplete epithelisation).
2. To analyse and present the frequency distribution of histological results after LLETZ (LGSIL; HGSIL; Ca colli uteri in situ; Ca colli uteri microinvasiva; endocervical gland involvement by LGSIL; endocervical gland involvement by HGSIL; resection margin status of the ectocervix, endocervix and apex of the cone: free of dysplasia, artificially thermally damaged, affected by LGSIL, affected by HGSIL, affected by Ca colli uteri in situ).
3. To study the influence of the following prognostic factors: age, parity, hormonal status (premenopausal, postmenopausal), the histological results of targeted biopsy (LGSIL, HGSIL), adequacy of colposcopic examination (satisfactory, unsatisfactory colposcopy), ZT type (type 1, 2, 3), the type of cervical lesion (type 1, 2, 3), the colposcopic impression (diagnosis) of the cervical lesion (LGSIL, HGSIL/ Ca colli uteri in situ), lesion size (up to 1/3; up to 2/3; over 2/3 of the cervical circumference) for the occurrence of LGSIL and HGSIL/Ca colli uteri in situ in the final histological result after the LLETZ procedure.
4. To establish the influence of the forenamed prognostic factors on the resection margin involvement by the pathological process (HGSIL, Ca colli uteri in situ, Ca colli uteri microinvasive).

5. To study cervical precancers' recurrence rate and persistence after the LLETZ procedure.
6. To study the correlation between histological findings after LLETZ and after targeted biopsy under video colposcopy guidance regarding cervical precancer (LGSIL, HGSIL) and to calculate the sensitivity (negative predictive value – NPV) and specificity (positive predictive value – PPV) of the histological result of biopsy with respect to HGSIL in the final histological result after LLETZ.
7. To study the correlation between the colposcopic diagnosis (colposcopic impression) and histological diagnosis after the LLETZ procedure regarding cervical precancer (LGSIL, HGSIL) and to calculate the sensitivity (negative predictive value – NPV) and specificity (positive predictive value – PPV) of the colposcopic diagnosis in relation to HGSIL in the final histological result after LLETZ.

II. MATERIALS AND METHODS

1. Clinical contingent

From Jan. 1st 2017 to July 31st 2021, 189 patients with cervical precancers were treated at Prof. Yavor Kornoski Medical Centre by the LLETZ (loop electrical excision) procedure in outpatient settings.

The indications for performing LLETZ are: targeted biopsy-proven under video colposcopy control histological data for high-grade cervical precancer (intraepithelial lesion) – HGSIL (high-grade squamous intraepithelial lesion), and low-grade cervical precancer – LGSIL (low-grade squamous intraepithelial lesion), which enters the cervical canal and its distal border is not visualised.

2. Methods

2.1. Video colposcopy

Video colposcopy was performed on all patients by one specialist with an additional qualification in colposcopy. Alyn Welch device was used until 10/02/2020, then a Leisegang video colposcope with original software and monitor, model 2020, was used. The examination was performed after treating the cervix with a 5% acetic acid solution prepared every two days and Lugol's solution, which was replaced every month. Each patient's colposcopy examination is saved, documented and archived in the medical centre's patient database.

2.2. Histological examination

2.2.1. Histological examination of the biopsy specimen

- the histological result is reported as LGSIL (in CIN1) and HGSIL (in CIN 2 and CIN 3); the same highly qualified pathologist performed the histological examination.

2.2.2. Histological examination of the specimen preparation after the LLETZ procedure. It is performed by the same highly qualified pathologist who examined the biopsy material.

Information value:

- LGSIL, HGSIL; Ca colli uteri in situ; Adenocarcinoma in situ (AIS); Ca colli uteri microinvasive/invasive;
- Endocervical glands involvement;
- Resection margin status of the ectocervix, endocervix and apex of the cone (free from dysplasia, LGSIL affected, HGSIL/Ca colli uteri in situ affected, thermally damaged).

2.3. Biopsy under video colposcopy control

It is taken with a pinch biopsy from the most suspicious area without anaesthesia; the bleeding is controlled by pressing with gauze, by using Monsel solution-soaked swab, by placing surgical and, in the last case, by sterile gauze tamponade for several hours or 1 day; 10% formalin solution dressing is used.

2.4. LLETZ procedure methodology

It includes the indications for performing LLETZ, patient preparation, description of the procedure, instrumentation and technical parameters, surgical technique and postoperative period.

2.5. Patients' follow-up

2.5.1. Follow-up period in months

It is counted from the date LLETZ was performed until Jan. 31st 2022. The minimum follow-up period is 6 months, and the maximum is 61 months.

The first follow-up examination is within 2–3 months after the LLETZ procedure, and each subsequent examination is at an interval of 6 months. Each examination includes a colposcopy and Pap smear. In addition, the degree of epithelisation of the cervix defect, the presence of cervical stenosis, and the presence of easy bleeding cervical vessels are also considered. The establishment of recurrence is proven by histological confirmation after a targeted biopsy from an atypical colposcopic site. Persistence is defined as the presence of a relapse occurring up to 6 months after LLETZ.

2.6. Cytological examination

Results are reported according to the Bethesda system. The examination is performed by the same highly qualified pathologist who performed the histological examination from the targeted biopsy and the LLETZ procedure.

2.7. Statistical methods

Data were entered and processed with IBM SPSS Statistics 25.0 statistical package and MedCalc Version 19.6.3. The significance level at which the null hypothesis was rejected was $p < 0.05$.

The following methods were applied:

1. Descriptive analysis – the frequency distribution of the considered indicators is presented in a tabular form.
2. Graphical analysis – for visualisation of the obtained results.
3. Fisher-Freeman-Halton exact test, Fisher's exact test and $\chi 2$ test – for testing hypotheses about the presence of dependence between categorical variables.
4. Non-parametric tests of Kolmogorov-Smirnov and Shapiro-Wilk – to check distributions for normality.
5. One-way analysis of variance (ANOVA) – to test hypotheses for differences between the arithmetic means of several independent samples.
6. Kruskal-Wallis non-parametric test – to test hypotheses for differences between several independent samples.
7. Criteria for validation of screening tests.

The following criteria are used to assess the validity of the screening (diagnostic) test:

- Sensitivity;
- Specificity;
- Positive predictive value;
- Negative predictive value;
- Accuracy (% of correct answers).

III. OWN RESULTS AND DISCUSSION

1. To study the applicability and economic efficiency of the LLETZ procedure in outpatient settings – analgesia, postoperative pain, postoperative stay, complications: intraoperative (bleeding); early postoperative (bleeding, infection) and late (cervical canal stenosis, spotting and incomplete epithelisation).

The following issues have been investigated:

1. Duration of the procedure;
2. Analgesia;
3. Intraoperative bleeding;
4. Postoperative pain;
5. Postoperative stay;
6. Early postoperative complications (bleeding, infection);
7. Late postoperative complications (cervical canal stenosis, incomplete epithelisation, spotting before menstrual cycle, inadequate colposcopy).

1.1. Duration of the procedure

The duration of the LLETZ procedure is in the range of 25 – 40 min (average 30 min). This duration includes the time required for disinfection, colposcopy, anaesthesia with local infiltration anaesthesia, the operation itself and haemostasis if necessary. It takes 7 to 10 minutes to aspirate lidocaine into insulin syringes (1 mL) and place infiltration anaesthesia at 10 points on the cervix. Bleeding as an intraoperative complication, although rare in the current contingent of patients, is a factor significantly prolonging the procedure's duration due to the application of specific measures for its control according to the described algorithm. Cai et al. (2020) reported a LLETZ duration of 15.7 ± 9.8 minutes.

1.2. Analgesia (anaesthesia)

Figure 1 presents the frequency distribution of the studied contingent by type of anaesthesia during LLETZ.

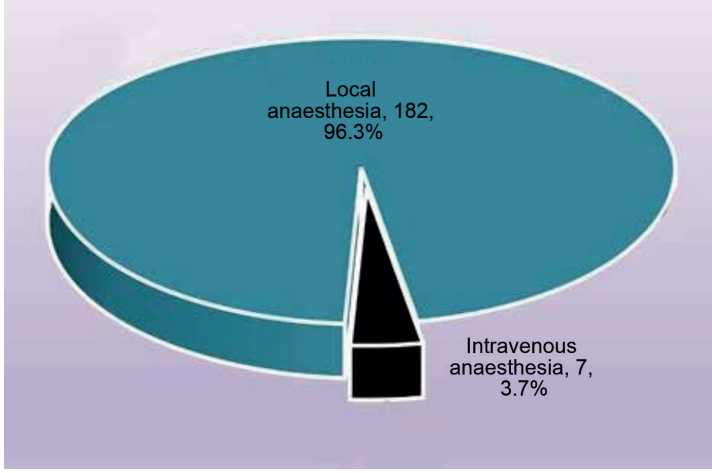

Figure 1. Frequency distribution of the studied contingent by type of anaesthesia during LLETZ

Figure 1 results show that local anaesthesia as a type of analgesia related to LLETZ was applied in 96.3% of the patients, while intravenous anaesthesia was applied only in 7, or 3.7%.

Local anaesthesia, according to the described methodology, ensures a smooth course of the procedure. It was performed in 182/189 patients (96.2%). The only subjective sensation of the patients is warmth during the procedure. The absence of vasomotor symptoms (tinnitus, dizziness) is achieved by avoiding anaesthetic infiltration in the area of the cervicovaginal branches of a. uterinae (3 and 9 o'clock positions). Intravenous anaesthesia was performed in 7/189 patients (3.8%). The indications for intravenous anaesthesia were: allergy to lidocaine on the scarification sample – in 3 patients, and the patients' desire for general anaesthesia – in 4 patients. The UK NHS Cervical Screening Program (NHSCSP) recommendations are that the LLETZ proce-

dure should be performed under local anaesthesia in >80% of cases (Borbolla Foster A, 2012). However, there are few publications on this topic. Yap et al. (2020) administered local anaesthesia to 105 patients undergoing LLETZ procedures in an outpatient setting. The authors reported no postoperative pain and a smooth operation. Borbolla et al. (2012) published perioperative and histological results, including the type of anaesthesia, in 465 LLETZ cases over 4 years (2005 – 2009). 33% of these patients received general anaesthesia, and the rest received local anaesthesia. According to the authors, the type of anaesthesia did not influence the progress and safety of the procedure, postoperative complications and histological results (the dysplastic process affecting the resection lines).

1.3. Intraoperative bleeding (abnormal intraoperative bleeding)

Figure 2 presents the frequency distribution of the studied contingent according to the intraoperative bleeding occurrence

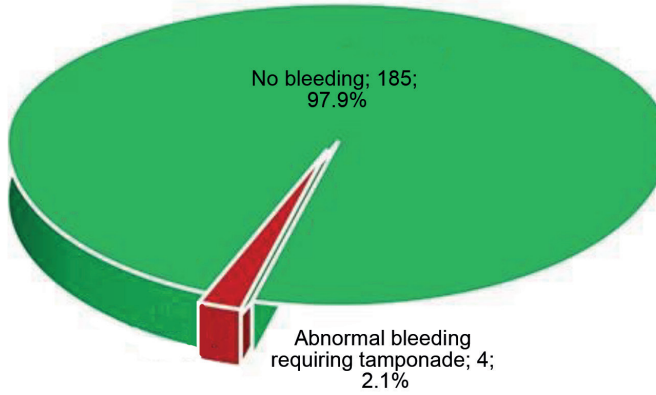

Figure 2. Frequency distribution of the studied contingent according to the occurrence of intraoperative bleeding

We define abnormal intraoperative bleeding as bleeding after the end of the operation, which is not controlled with the measures according to the de-

scribed algorithm and requires the placement of a vaginal tamponade for 24 hours. In the described clinical cohort, such bleeding was reported in 4 of 189 patients or 2.1% (Fig. 2). Causes: HSIL and suspicion of invasive carcinoma, respectively – size and depth of the excised part of the cervix. One of the advantages of the LLETZ procedure over conisation (scalpel and electro-conisation) is less morbidity, including bleeding during the operation, and, accordingly, the possibility of performing it in an outpatient setting. In confirmation, Brun et al. (2002) compared complications after scalpel conisation, electro-conisation, and LLETZ. The lowest incidence of bleeding after LLETZ was 2% (corresponding to that reported in the present study), while after scalpel and electro-conisation, it was 8% and 5%, respectively. The complex of measures described above, including bipolar radiofrequency coagulation, is of great importance for this low frequency. The use of Monsel solution for haemostasis (pressing the wound surface with a Monsel solution-soaked swab) has been the subject of several studies. According to one of them, the Monsel solution reduces the risk of postoperative bleeding after LLETZ but does not prevent the occurrence of severe intra- or postoperative bleeding. The authors of this study concluded that its routine application after cauterisation of the resection surface is unnecessary (Kietpeerakool C, 2007). Another randomised trial compared the use of Monsel solution versus ball electrode coagulation in terms of bleeding control during and after completion of LLETZ, finding no difference between the two methods (Lipscomb GH, 2006). Martin-Hirsch PP et al. (2010) analysed different approaches to limit bleeding in cervical surgeries for CIN. According to them, the combination of adrenaline and local anaesthetic has a good haemostatic effect, in contrast to the use of haemostatic sutures, leading to cervical stenosis and unsatisfactory colposcopy. According to the same study, tranexamic acid has a good effect in scalpel and laser conisation for the prevention of secondary bleeding but does not reduce the risk of primary bleeding and blood loss.

1.4. Postoperative pain

The need for analgesia after LLETZ occurred in only 3.2% of the studied contingent (Fig. 3).

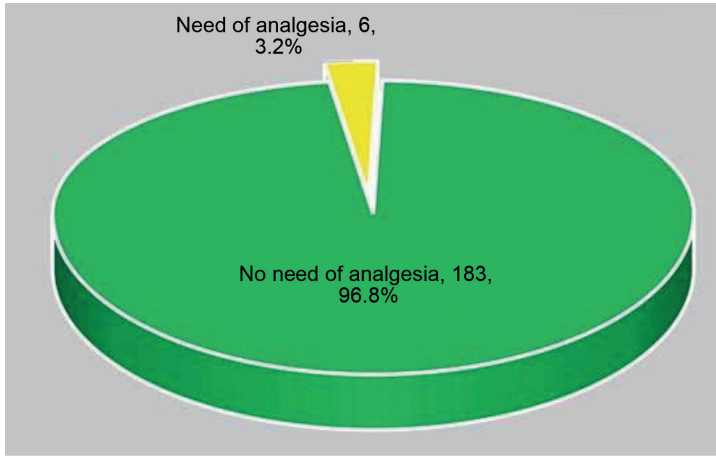

Figure 3. Frequency distribution of the studied contingent according to the need for postoperative analgesia

Reporting pain during the observation period after LLETZ requires NSAIDs analgesia (Dexofen sachet p.o). Such indication occurred in 6 of 189 patients (3.2%). Therefore, local anaesthesia ensures not only a smooth and painless operation but also adequate analgesia during the postoperative period. This is also confirmed by other authors (Yap SJ, 2020).

1.5. Postoperative stay

The postoperative stay of patients after LLETZ was 1 hour for the cases with local anaesthesia (182 of 189 patients) and 4 hours for the cases with intravenous anaesthesia (7 of 189 patients). The main purpose of the observation in the postoperative period is analgesia administration if needed and monitoring for postoperative bleeding. This is one of the factors determining the LLETZ procedure as suitable to be performed in an outpatient setting.

1.6. Early postoperative complications

Figure 4 demonstrates that early postoperative complications (within the 30-day period) were only in 3 cases – two cases of bleeding and one of infection (fever, purulent urethral fluorine).

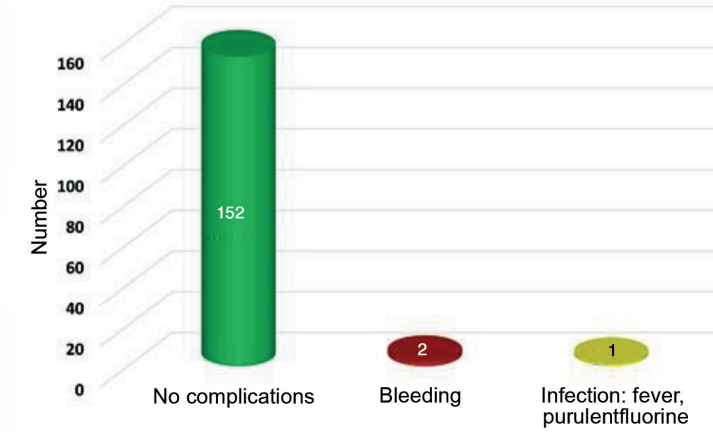

Figure 4. Frequency distribution of the studied contingent by early postoperative complications (within the 30-day period)

Early postoperative complications are defined as complications occurring within 1 month after the procedure. These complications include bleeding from the surgical wound and infection of the wound surface of the cervix. We found bleeding from the operative wound in 2 out of 189 patients (1%) of the studied contingent. The reasons for this bleeding were non-compliance with the postoperative recommendations (heavy lifting or physical work). Its treatment is according to the algorithm described above: bipolar radiofrequency coagulation (in the case of a bleeding vessel), Surgicel (in the case of diffuse bleeding) and tamponade as a last resort. In the literature, the reported incidence of postoperative bleeding ranges from 2% to 23.9% (Cai L, 2020, Brun JL, 2002). In the current study, the low incidence of intra- and postoperative bleeding was due to the characteristics of the loops used (thickness and strength) and the generator's simultaneous cutting and coagulation mode. This approach ensures a bloodless operation. However, it

takes longer, leads to frequent thermocoagulation changes of the cone, and a large amount of smoke is emitted during the procedure. The smoke must be aspirated with a powerful smoke evacuator so as not to interrupt the operator's visual contact with the operative field.

Wound surface infection, clinically manifested by foul-smelling purulent fluorine, was found in 1 of 189 patients (0.5%). The low incidence is due to preoperative vaginal preparation of vaginal discharge and postoperative antibiotic administration. Some authors report an incidence of this complication after LLETZ from 0.8% to 14% (Kietpeerakool C, 2017). However, patient series with a 28% incidence of wound infection have been described in the literature (Cai L, 2020).

1.7. Late postoperative complications

They include: cervical canal stenosis, inadequate colposcopy, incomplete epithelialisation, and premenstrual spotting (patient-reported or objectively determined at the earliest follow-up examination after LLETZ). Figure 5 illustrates the frequency distribution of the study cohort concerning late postoperative complications (after the 30-day period).

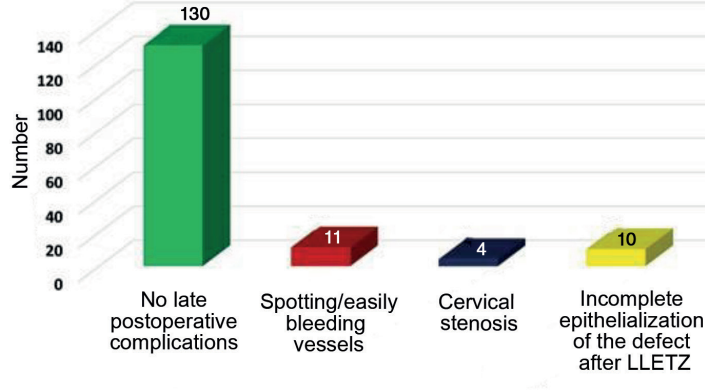

Figure 5. Frequency distribution of the studied contingent regarding late postoperative complications (after the 30-day period).

Cervical canal stenosis was found in 4 out of 189 patients (2.1%). It is defined as difficult or impossible cytology collection from the cervical canal. It occurs in menopausal patients, as well as in cases of deep excision of the cervical canal (more than 2 cm). This is one of the complications and adverse effects of surgical interventions used to treat cervical precancers. It makes taking cytology samples impossible or very painful; thus, the follow-up of patients with high-grade precancers and ca colli uteri in situ becomes risky and unreliable. Therefore, hysterectomy is recommended in menopausal patients diagnosed with HSIL and ca colli uteri in situ after LLETZ. In young women, it can lead to dysmenorrhea, oligomenorrhea, and primary or secondary sterility. Therefore, in young women with incomplete reproductive functions and high-grade endocervical lesions, radicality (concerning the cone's apex) may be bypassed to avoid this complication. Different surgical techniques lead to different rates of cervical stenosis. According to a study, electro-conisation leads to stenosis in 27% of cases, scalpel conisation – in 8%, and LLETZ – in 3% (Brun JL, 2006). El-Nashar et al. (2017) also found that the LLETZ procedure resulted in less cervical stenosis than scalpel conisation. Baldauf JJ et al. (1996, 1997) compared the LLETZ procedure with another surgical technique for the treatment of cervical precancers – laser conisation, in terms of postoperative stenosis occurrence. The study was large-scale and included 277 patients undergoing LLETZ and 255 undergoing laser conisation. The authors determined the factors leading to stenosis – age > 50 years, endocervical lesion, and height of the removed cone > 2 cm. Laser conisation leads to stenosis in 10.2%, while LLETZ – in 4.3%. In addition, according to the authors, the surgical treatment of stenosis (plasty) in most cases leads to restenosis. Lin J et al. (2020) suggest dilatation of the cervical canal at 3, 5 and 8 weeks after the LLETZ procedure in order to prevent cervical stenosis.

Inadequate colposcopy was recorded in 15 of 189 patients (7.9%). In these cases, during colposcopy, the squamocolumnar junction (SCJ) enters the cervical canal and remains unevaluated. Follow-up for these patients is based on endocervical cytology, especially the COBAS test.

Incomplete epithelialisation was manifested in 10 of 189 patients (5.3%). During colposcopy, the wound surface is not covered by a multi-layered squamous epithelium and looks like a “red spot” (erythroplakia). According to some studies, 90% of the wound surface regenerates in 6 months (Song T, 2016, Papoutsis D, 2012).

Premenstrual spotting was a reported complication in 11 out of 189 patients (5.8%). Usually, it is due to easy bleeding vessels at the cervix site where the electrical loop went through (Fig. 6). This is not considered a complication but one of the reportable side effects of LLETZ and tends to diminish and disappear over time. If this condition greatly bothers the patient, cryodestruction or CO_2 laser vaporisation is recommended.

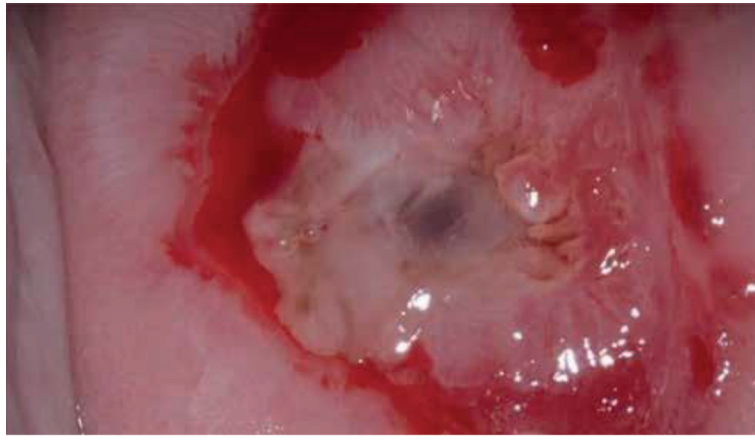

Figure 6. Easy bleeding vessels due to the loop with which LLETZ was performed

2.2. To analyse and present the frequency distribution of histological results after LLETZ (LGSIL; HGSIL; Ca colli uteri in situ; Ca colli uteri microinvasiva; endocervical gland involvement by LGSIL; endocervical gland involvement by HGSIL; resection margin status of the ectocervix, endocervix and the cone’s apex: free of dysplasia, artificial thermal damage, affected by LGSIL, affected by HGSIL, affected by Ca colli uteri in situ).

The following issues have been investigated:

1. Results of the histological analysis of the preparation samples after LLETZ;
2. Frequency of gland involvement by the pathological process;
3. Frequency of thermal damage of the resection lines after LLETZ;
4. Frequency of resection margin involvement by carcinoma and HSIL after LLETZ.

2.1. Results of the histological analysis of the preparation samples after LLETZ

Figure 7 graphically illustrates the frequency distribution of histological findings after LLETZ. The histological results of adenocarcinoma in situ (AIS) and Ca colli uteri invasiva are included in the frequency of Ca colli uteri in situ and Ca colli uteri microinvasiva, respectively, due to their low frequency.

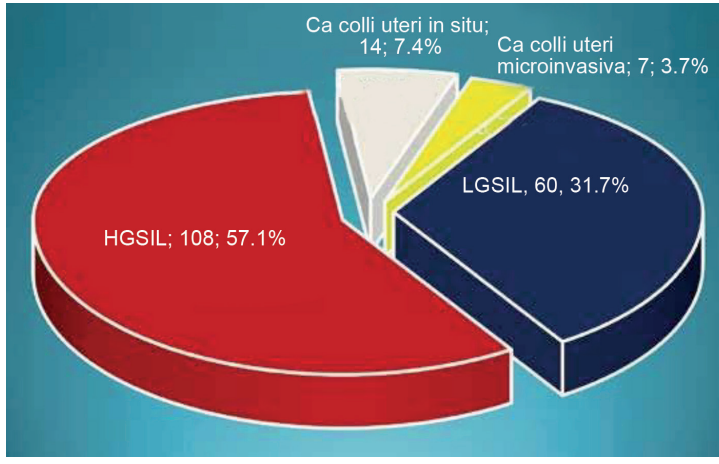

Figure 7. Frequency distribution of the studied contingent according to the histological results after LLETZ

Table 1 presents the histological analysis results of the preparation samples after LLETZ for all histological findings.

Table 1. Distribution of patients according to histological outcome after LLETZ

LSIL	60 (31.7 %)
HSIL	108 (57.1 %)
Ca colli uteri in situ	11 (5.8 %)
AIS(adenocarcinoma in situ)	3 (1.6 %)
Ca colli uteri microinvasiva	4 (2.1 %)
Ca colli uteri invasiva	3 (1.6 %)

LSIL diagnosis (low-grade lesion) is found in nearly 1/3 of LLETZ cases. This is due to compliance with the indications for applying excision techniques. Penetration of the atypical site into the cervical canal, with no options to trace the borders of the atypical site colposcopically, is an indication for LLETZ (Prendiville W, 1995). In all cases of LSIL entering the cervical canal, the LLETZ procedure was performed. The diagnosis before loop excision was made by colposcopy, biopsy and cytology. Ca colli uteri in situ, AIS, and Ca colli uteri microinvasiva were found in 17 patients, showing the loop excision role for diagnosis and often for the treatment of these diseases. In cases of invasive carcinoma, LLETZ is assigned for diagnostic purposes in postmenopausal women with atrophic changes on the cervix to rule out/confirm invasive carcinoma in cases of clinical suspicion and inconclusive pinch biopsy data.

2.2. Frequency of gland involvement by the pathological process

Figure 8 and Table 2 show the frequency of gland involvement by the pathological process.

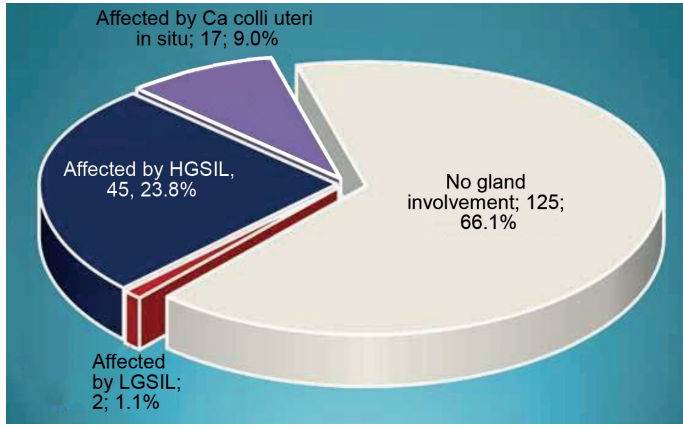

Figure 8. Frequency distribution of the studied contingent by endocervical gland involvement

Table 2. Frequency of involvement of the cervical glands by the pathological process

Involvement of the cervical glands (in total: from LSIL, HSIL, Ca colli uteri in situ, Adenocarcinoma in situ, Ca colli uteri microinvasiva, Ca colli uteri invasiva)	64 (33.9 %)
LSIL	2/64 (3.1%)
HSIL	45/64 (70.3%)
Ca colli uteri in situ	7/64 (10.9%)
Adenocarcinoma in situ	3/64 (4.7%)
Ca colli uteri microinvasiva	4/64 (6.3%)
Ca colli uteri invasiva	3/64 (4.7%)

The highest share of cases with intraglandular involvement is for HSIL (70.3%), as the patients with this diagnosis were 108 out of 189). Additionally, gland involvement in the pathological process can be considered a function of the disease severity. The more severe the disease was, the higher the incidence of cervical gland involvement: in patients with LSIL, the incidence was 2/60 (3.3%); in those with HSIL – 45/108 (41.7%); in Ca colli uteri in situ – 7/11 (63.6%); for AIS – 3/3 (100%); in Ca colli uteri microinvasive –

4/4 (100%); in Ca colli uteri invasive – 3/3 (100%). Thus, this histological indicator can be interpreted as a predictive factor – the involvement of the cervical glands is associated with the severity of the process.

One of the most important histological indicators after LLETZ is the resection margin status. Because of the small number of cases involving ectocervical and endocervical resection lines, as well as those at the cone's apex, Figure 9 presents the overall frequency distribution of resection margin involvement after the LLETZ procedure.

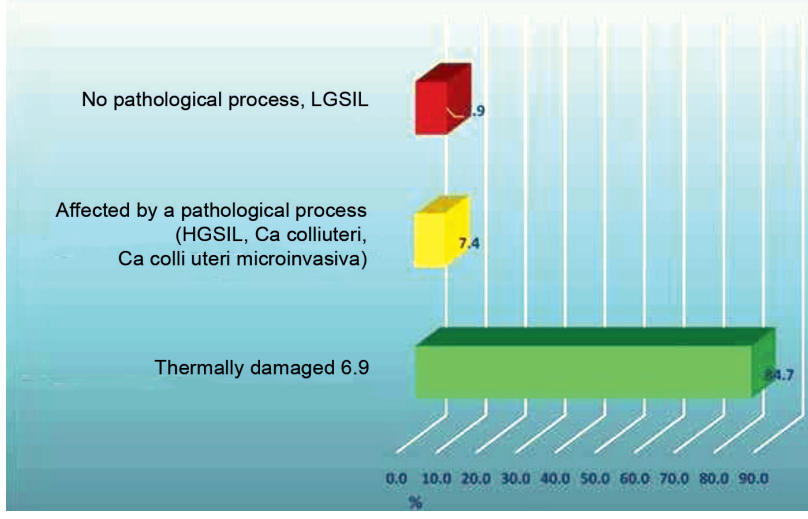

Figure 9. Frequency distribution of the studied contingent by resection margin status

Thermal damage of the resection lines and their involvement by the pathological process have different meanings and, therefore, will be considered separately. In our study, low-grade dysplasia (LGSIL) involved resection lines in only two cases. Because of this, for subsequent analysis, resection line involvement by pathological processes will signify their involvement by carcinoma (Ca colli uteri in situ, Adenocarcinoma in situ, Ca colli uteri microinvasiva/invasive) and high-grade dysplasia (HGSIL)

2.3. Frequency of thermal damage of the resection lines after LLETZ

Table 3 shows the frequency of thermal damage of resection lines after LLETZ.

Table 3. Incidence of thermal damage of the resection lines after LLETZ

Thermal damage of the resection lines (ectocervix, endocervix, tip of cone), free of dysplasia	15 (7.9 %)
Ectocervix	15 (7.9 %)
Endocervix	15 (7.9 %)
Top of the cone	15 (7.9 %)

Changes in the resection lines due to thermocoagulation during electro-loop present a challenge for the pathologist in the histological evaluation; this is considered one of the disadvantages of this excision method. The degree of thermal damage depends on the electricity generator, the amperage, the cutting mode, the time the loop goes through the tissues, and the thickness of the loop. The pathologist's experience in these cases is of utmost importance to assess and evaluate the resection margin status and the presence of dysplasia, despite thermocoagulation damages to the tissue.

In the present study, we report thermal damage without dysplasia in 7.9%. Therefore, thermal changes at the resection lines obstruct adequate histological examination. The damage is due to the characteristics of the loops (thickness and strength) and the mode of simultaneous cutting and coagulation of the generator. This approach ensures a bloodless operation, although it takes longer and leads to these thermocoagulation changes of the cone.

2.4. Frequency of resection margin involvement by carcinoma and HSIL after LLETZ

Table 4 presents the frequency of resection margin involvement by carcinoma and HSIL after LLETZ.

Table 4. Frequency of resection margin involvement by carcinoma and HSIL after LLETZ

Resection lines involvement by carcinoma (number of patients compared to the total number of patients with carcinoma – 21	4 (19%)	HSIL resection lines involvement (number of patients with cone margin involvement)	7 (3.7%)
Involvement of the ecto-, endocervix and the apex of the cone	3	Involvement of the endo-, ectocervix and the apex of the cone	2
Involvement of the endocervix	1	Involvement of the apex of the cone	2
		Involvement of the endocervix	3

The patients with carcinoma in the histological result after LLETZ were 21 (Ca colli uteri in situ – 11; Adenocarcinoma in situ (AIS) – 3; Ca colli uteri microinvasive – 4; Ca colli uteri invasive – 3). Resection margin involvement was found in 4 cases – 3 with invasive carcinoma, and 1– with microinvasive carcinoma. In cases with invasive carcinoma, the LLETZ procedure was performed for diagnostic purposes. This is required in postmenopausal women in whom colposcopy, histological examination and cytology cannot establish a definitive diagnosis due to atrophy, despite the suspicion of invasion. On the other hand, in the patients diagnosed with ca colli uteri in situ, including adenocarcinoma, and the remaining 3 with microinvasive carcinoma, the resected margins were tumour free.

It is interesting to note the histological type and degree of dysplasia in the seven cases of HSIL resection lines involvement: microinvasive carcinoma – 1; adenocarcinoma in situ (AIS) – 1; Ca colli uteri in situ – 2; HSIL – 3. Hence, the resection lines with AIS are affected in 33% (1/3); with ca colli uteri in situ – in 18.2% (2/11); with HSIL – in 2.8% (3/108); ca microinvasive – in 25% (1/4). These results indicate that the LLETZ procedure can be

curative in high-grade squamous cervical lesions and, in most cases, squamous cell carcinoma in situ. The LLETZ technique described above contributes to these results. Also relevant are the colposcopy before the start of the operation, the delineation of the atypical area with Lugol's solution, respectively the selection of an adequately sized loop. For atypical areas on the ectocervix that are larger than the loop, additional excision with a smaller loop is required for cutting the residual dysplastic tissue up to intact, healthy tissue.

The resection margin involvement by the dysplastic process, especially by high-grade dysplasia (HSIL), is the most critical histological parameter after LLETZ. This indicator certifies the radicality and healing effect of the procedure. On the one hand, it is the most important prognostic factor for the onset of recurrence or progression of the precancerous process. In the study cohort, we found an incidence of HSIL resection line involvement in 3.7% of all LLETZ patients (7 of 189).

Different authors report different frequencies, factors and locations of positive resection margins. A meta-analysis of 66 studies of 35 109 women with CIN found 23% resection line involvement. The postoperative recurrence of HSIL was demonstrated in 18% of patients with positive resection margins and only 3% with dysplasia-free resection lines (Ghaem-Maghami S, 2007). These data support the hypothesis of the strong prognostic significance of dysplasia-affected resection lines for the onset of disease recurrence. On the other hand, not all patients (only 18%) with resection line involvement develop recurrence. A study by Chen et al. (2009) found a 13% incidence of resection line involvement (R+) in 1113 patients (141/1113) diagnosed with CIN 3 and treated with LEEP and scalpel conisation. The localisation of the affected resection lines is as follows: ectocervix – 45%, endocervix – 32%, and ecto/endocervix – 23%. Menopausal women had R (+) in 35.4%, and premenopausal women – in 11.6%. The authors identified the factors for the occurrence of R (+): grade of the squamous intraepithelial lesion (SIL), lesion size, excision technique (LLETZ – 24.1%, R+; scalpel conisation – 4.8%, R+) and loop size in LEEP. Panna et al. (2009) reported 26.8% R (+) in 463 women undergoing LLETZ and scalpel conisation. The affected lines are on the ectocervix – 9%, endocervix – 10%, and ecto-endocervix – 7%. According to this study, the factors responsible for R (+) are: the skills of the surgeon; the type of excision technique (R+ were found more often after

LEEP); the histological diagnosis and the purpose of the conisation (diagnostic or therapeutic). In menopausal women, there is often no clear colposcopic and biopsy evidence of invasion due to atrophy, although such may be suspected. In these cases, LLETZ is recommended for diagnostic purposes to confirm or exclude invasive carcinoma. When carcinoma is established, resection lines are usually involved in the process. In another study, R (+) was found in 33%, the ectocervix was affected in 8%, the endocervix – in 22%, and the ecto-endocervix – in 3% (Shaco-Levy R, 2014). According to the study authors, the prognostic factors for R (+) are: age > 35 years, size of the atypical site and intraglandular involvement. Papoutsis et al. (2016) reported a similar frequency – 30.7% of R (+) with the involvement of ectocervix in 10.4%, endocervix – in 18%, and ecto-endocervix – in 2.3%.

The LLETZ procedure is associated with more frequent resection margin involvement compared to scalpel conisation of glandular lesions (adenocarcinoma in situ-AIS) (Costales AB, 2013, Van Hanegem N, 2012). According to one study (Costales AB, 2013), the frequency of R (+) in such lesions after scalpel conisation was 35%, while after LLETZ, it was 56%. Van Hanegem et al. (2012) published similar results for young patients with AIS: R (+) after LLETZ – 27%, and after scalpel conisation – 21%. However, the authors did not find a significant difference between the two excision techniques, although scalpel conisation was the method of the first choice in glandular lesions.

Our study contingent showed an incidence of R (+) for 33% with AIS (3 patients), confirming the hypothesis that loop excision is unsuitable for adenocarcinoma lesions.

3. To study the influence of the following prognostic factors: age, parity, hormonal status (premenopausal, postmenopausal), the histological results of targeted biopsy (LGSIL, HGSIL), adequacy of colposcopic examination (satisfactory, unsatisfactory colposcopy), ZT type (type 1, 2, 3), the type of cervical lesion (type 1, 2, 3), the colposcopic impression (diagnosis) of the cervical lesion (LGSIL, HGSIL/ Ca colli uteri in situ), lesion size (up to 1/3; up to 2/3; over 2/3 of the cervical circumference) for the occurrence of LGSIL and HGSIL/Ca colli uteri in situ in the final histological result after the LLETZ procedure.

Table 5 shows the correlation between patients' age and the histological results after LLETZ.

Table 5. Analysis of the correlation between patients' age and histological outcome after LLETZ (ANOVA, df = 2, F = 0.618, p = 0.540)

Histological result after LLETZ procedure	Age (years)		
	n	$\bar{X}$	SD
LGSIL	60	37.65	8.09
HGSIL	108	36.25	8.07
Ca colli uteri in situ	14	37.21	6.34
Ca colli uteri microinvasiva*	7	44.29	9.98

** The category was not included in the analysis due to a lack of statistical representativeness*

The results in this table show that there is no significant correlation between age and histological outcome after LLETZ. The Ca colli uteri microinvasiva group was not included in the analysis due to the lack of statistical representativeness.

Age is an important factor in the occurrence of high-grade precancerous cervical lesions. The risk of their occurrence after 30 and 50 years of age increases by 4.5 and 11 times, resp ectively (Costa S, 2003). Chen et al. (1995) found an increased risk in patients older than 45 years of age. However, some publications point out that the age below 35 years is associated with a higher risk for high-grade lesions (Kobelin MH, 1998).

Table 6 examines the influence of the factors: parity, hormonal status, biopsy histology results, adequacy of colposcopic examination and diagnostic colposcopic impression on the final histological result after LLETZ.

Table 6. Analysis of the correlation between the histological results after LLETZ and the indicators parity, hormonal status, histological result from targeted biopsy, adequacy of the colposcopic examination and colposcopic diagnosis

Indicators	Frequency	Histological result after LLETZ procedure				P					
		1. LGSIL	2. HGSIL	3. Cacolli uteri in situ	4. Ca colli uteri microinvasiva	1-2	1-3	1-4	2-3	2-4	3-4
Parity											
Parous	n	49	76	13	6	0.155					
	%	81.7	70.4	92.9	85.7						
Non-parous	n	11	32	1	1						
	%	18.3	29.6	7.1	14.3						
Hormonal status											
Perimenopause	n	57	100	14	6	0.471					
	%	95.0	92.6	100.0	85.7						
Postmenopause	n	3	8	0	1						
	%	5.0	7.4	0.0	14.3						
Histological result from targeted biopsy											
LGSIL	n	46	11	0	0	<0.001	<0.001	<0.001	0.357	1.000	-
	%	82.1 a	11.1 b	0.0 c	0.0 c						
HGSIL	n	10	88	13	6						
	%	17.9 a	88.9 b	100.0 c	100.0 c						
Adequacy of colposcopic examination											
Satisfactory	n	42	65	9	4	0.611					
	%	70.0	60.2	64.3	57.1						
Unsatisfactory	n	18	43	5	3						
	%	30.0	39.8	35.7	42.9						
Colposcopic diagnosis											
LGSIL/ grade 1	n	43	4	0	0						
	%	71.7 a	3.7 b	0.0 c	0.0 c	<0.001	<0.001	<0.001	1.000	1.000	-
HGSIL/ grade 2	n	17	104	14	7						
	%	28.3 a	96.3 b	100.0 c	100.0 c						

** The same letters in the horizontal lines indicate the absence of a significant difference, and the different ones – the presence of a significant difference ($p < 0.05$)*

We found a statistically significant correlation between the histological result from targeted biopsy and the colposcopic diagnosis with respect to the final histological result of LLETZ.

In the remaining three indicators: parity, hormonal status and adequacy of the colposcopic examination, no statistically reliable correlation with the histological result after LLETZ was established.

Menopause as a factor influences the occurrence of colposcopic and cytological changes in the cervix. In menopause, as a result of estrogen deficiency, the squamocolumnar junction (SCJ) moves into the cervical canal, making the colposcopic examination often unsatisfactory (Wetrich DW., 1986). In addition, chronic inflammation, reactive atypia, and atrophy occur, which may mask severe precancerous changes and even carcinoma microinvasion. In menopausal patients, cytology can also be misleading. In atrophy of the vaginal mucosa, basal and parabasal cells predominate; they have an altered nuclear-cytoplasmic index, which can lead to false positive Pap test results. In these cases, it is appropriate to test for HPV high-risk strains (COBAS test) before deciding on an excisional biopsy (LLETZ). Moore et al. (2008) found that 30% of the patients they studied over 50 years of age had an unsatisfactory colposcopy, and 50% showed a discrepancy between cytology and colposcopic diagnosis. The authors recommend LLETZ in the absence of a correlation between cytology and biopsy under colposcopic control.

Childbirth as a factor in the occurrence of cervical precancers is also of interest. The reasons are SCJ changes after vaginal delivery. In nulliparous women and women who gave birth by Caesarean section, the cervix and cervical canal were not subjected to trauma, respectively, of a change in the border between the squamous and columnar epithelium (SCJ). In a study among HIV-positive women, it was pointed out that nulliparous women have a higher risk of developing CIN (Lehtovirta P, 2008).

Table 7 shows the correlation between the histological result after LLETZ and the parameters of ZT transformation zone type, cervical lesion type and lesion size.

Table 7. Analysis of the relationship between the histological result after LLETZ and the parameters of the ZT (zone of transformation) types, cervical lesion type and lesion size

Indicators	Frequency	Histological result after LLETZ procedure				P					
		1. LGSIL	2. HGSIL	3. Ca colli uteri in situ	4. Cacolli uteri microin-vasiva	1 - 2	1 - 3	1 - 4	2 - 3	2 - 4	3 - 4
Zone of transformation (ZT)											
Type 1	n	42	65	9	4	0.486					
	%	70.0	60.2	64.3	57.1						
Type 2	n	4	20	2	1						
	%	6.7	18.5	14.3	14.3						
Type 3	n	14	23	3	2						
	%	23.3	21.3	21.4	28.6						
Type of cervical lesion											
Type 1	n	0	6	1	0	0.063	0.039	-	0.822	0.522	0.481
	%	0.0 a	5.6 ac	7.1 bc	0.0 ac						
Type 2	n	0	35	1	1	<0.001	0.039	0.003	0.052	0.319	0.605
	%	0.0 a	32.4 b	7.1 b	14.3 b						
Type 3	n	60	67	12	6	<0.001	0.003	0.003	0.082	0.209	1.000
	%	100.0 a	62.0 b	85.7 b	85.7 b						
Lesion size											
Up to 1/3	n	54	82	9	2	0.445	0.016	<0.001	0.350	0.006	0.132
	%	90.0 a	75.9 ac	64.3 bcd	28.6 bd						
2/3	n	5	24	5	4	0.023	0.007	<0.001	0.266	0.038	0.362
	%	8.3 a	22.2 b	35.7 bd	57.1 cd						
Over 2/3	n	1	2	0	1	0.926	0.626	0.067	0.604	0.049	0.157
	%	1.7 ac	1.9 a	0.0 ac	14.3 bc						

** The same letters in the horizontal lines indicate the absence of a significant difference, and the different ones - the presence of a significant difference ($p < 0.05$)*

Data in Table 7 show that factors such as cervical lesion size and lesion type have a prognostic significance for the histological outcome after LLETZ. In small cervical lesions (up to 1/3 of the size of the cervix), low-grade lesions are more common – in 54/60 (90%) of LGSIL, they are up to 1/3 of the cervix. On the other hand, in cervical lesions occupying 2/3 of the cervix,

HGSIL, Ca colli uteri in situ, and Ca colli uteri microinasiva/invasive were found in 33/38 (87%).

We tentatively separate the cervical lesions into three types: type 1 – located on the ectocervix, fully visible; type 2 – entering the cervical canal (endocervix) but with visible borders; type 3 – entering the cervical canal with invisible borders. The above table results show that in cervical type 2 lesions, HGSIL was found in 35/37 cases (95%). Therefore, endocervix/endocervical gland involvement is a prognostic marker for a high-grade lesion.

In type 3 cervical lesions, 18/21 (86%) carcinomas were diagnosed (Ca colli uteri in situ, Ca colli uteri microinvasive/invasive). In these cases, the LLETZ procedure was performed mainly for a diagnostic purpose – to establish invasion or microinvasion. This is often required in menopausal women with atrophy or because of a process developing in the endocervical canal. In these cases, there is a discrepancy between the colposcopic findings and cytology, which is one of the indications for an excisional procedure. In recent months, the importance of the COBAS test in deciding to perform LLETZ has also increased – especially for women with alerting cytology and inadequate or negative colposcopy.

4. To establish the influence of the forenamed prognostic factors on the resection margin involvement by the pathological process (HGSIL, Ca colli uteri in situ, Ca colli uteri microinvasive).

Table 8 presents the influence of the factors parity, hormonal status, histological result of the targeted biopsy, adequacy of the colposcopic examination and colposcopic diagnosis on the resection margin status.

Table 8. Analysis of the correlation between resection margin status and the indicators parity, hormonal status, histological result of targeted biopsy, adequacy of colposcopic examination and colposcopic diagnosis

Indicators	Frequency	**Resection** margins (RM) **status**			P		
		1. Unaffected by the pathological process	**2. Affected by the pathological process**	**3. Thermally damaged**	**1 - 2**	**1 - 3**	**2 - 3**
Parity							
Parous	n	124	12	8	0,100		
	%	77.5	85.7	53.3			
Non-parous	n	36	2	7			
	%	22.5	14.3	46.7			
Hormonal status							
Perimenopause	n	151	13	13	0,341		
	%	94.4	92.9	86.7			
Postmenopause	n	9	1	2			
	%	5.6	7.1	13.3			
Histological result of targeted biopsy							
LGSIL	n	53	0	4	0.016	0.579	0.113
	%	35.8 a	0.0 bc	26.7ac			
HGSIL	n	95	11	11	0.016	0.579	0.113
	%	64.2 a	100.0bc	73.3ac			
Adequacy of colposcopic examination							
Satisfactory	n	106	7	7	0,176		
	%	66.3	50.0	46.7			
Unsatisfactory	n	54	7	8			
	%	33.8	50.0	53.3			
Colposcopic diagnosis							
LGSIL/grade 1	n	43	0	4	0,060		
	%	26.9	0.0	26.7			
HGSIL/grade 2	n	117	14	11			
	%	73.1	100.0	73.3			

** The same letters in the horizontal lines indicate the absence of a significant difference, and the different ones – the presence of a significant difference ($p < 0.05$)*

We established a significant difference only concerning the factor histological result of targeted biopsy. In HGSIL, detected by targeted biopsy in 11 cases, resection margin involvement by the pathological process was determined, including carcinoma (9.4%). We did not have a single case of resec-

tion margin involvement in a histological LGSIL biopsy result. Therefore, HGSIL from the targeted biopsy is a prognostic factor for the resection margin involvement by a pathologic process after LLETZ.

Table 9 examines the prognostic significance of the factors zone of transformation (ZT) type, cervical lesion type, and lesion size for the resection margin involvement by a pathologic process.

Table 9. Analysis of the correlation between the resection margin status and the parameters ZT type, cervical lesion type and lesion size

Indicators	**Frequency**	**Resection** margins (RM) **status**			P		
		1. Unaffected by the pathological process	**2. Affected by the pathological process**	**3. Thermally damaged**	**1 - 2**	**1 - 3**	**2 - 3**
Transformation zone (TZ)							
Type 1	n	7	7	7	0.791		
	%	50.0	46.7	50.0			
Type 2	n	2	4	2			
	%	14.3	26.7	14.3			
Type 3	n	5	4	5			
	%	35.7	26.7	35.7			
Type of cervical lesion							
Type 1	n	1	1	1	0.818		
	%	7.1	6.7	7.1			
Type 2	n	3	2	3			
	%	21.4	13.3	21.4			
Type 3	n	10	12	10			
	%	71.4	80.0	71.4			
Lesion size							
Up to 1/3	n	9	10	9	1.000		
	%	64.3	66.7	64.3			
2/3	n	4	5	4			
	%	28.6	33.3	28.6			
Over 2/3	n	1	0	1			
	%	7.1	0.0	7.1			

The analysis did not show a statistically significant difference in the influence of the abovementioned factors on the resection margin involvement. This is explained by the LLETZ surgical technique used by us, whose advantages and details are described in item 1 and item 2 of the Results and Discussion section.

5. To study the recurrence rate and persistence of cervical precancers after the LLETZ procedure

Figure 10 shows that only two patients (1.7% of the available data for 118 women) had histologically proven recurrence. There were also 2 patients with histologically proven persistence. However, as a percentage, they were 5.4 since the relative share was calculated based on data from 37 investigated cases (Fig. 11).

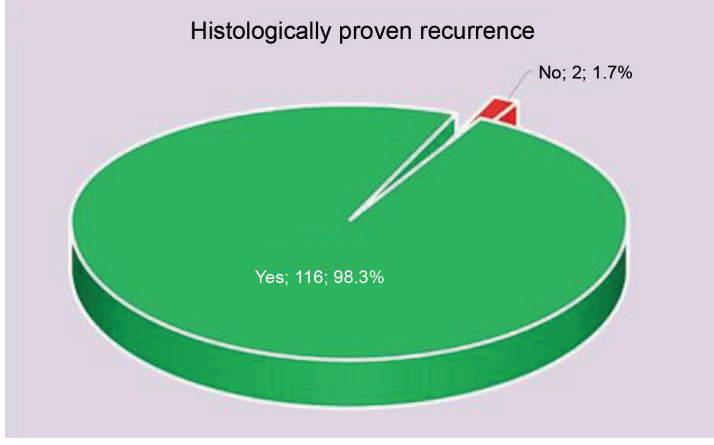

Figure 10. Frequency distribution of the studied contingent by histologically proven recurrence

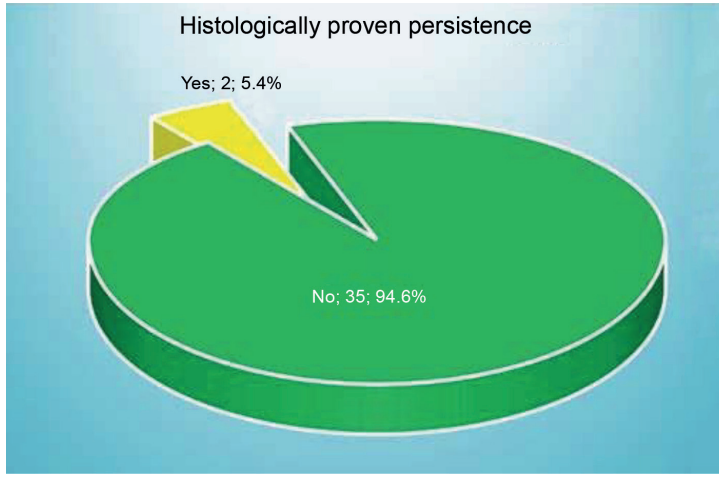

Figure 11. Frequency distribution of the studied contingent according to histologically proven persistence

The way of follow-up after LLETZ is of great importance for detecting and diagnosing recurrences of precancerous changes in the cervix.

Most follow-up protocols after treatment for HSIL by excisional therapy include only cytological examination (conventional or liquid-based cytology) at intervals of 1 to 5 years, depending on the national and regional algorithms and guidelines (Soutter WP, 1997, Kalliala I, 2005). Follow-up protocols by colposcopy, cytology, and gynaecologic examination at intervals of 3 to 12 months until the 2nd year of treatment, then annually for 5 to 20 years, have been reported by a number of authors to detect and promptly treat recurrences after LLETZ (Reich O, 2001, Sangkarat S, 2014). Stasinou et al. applied a follow-up protocol based on colposcopy and cytology every two years for a period of 22 years and found a 1.25% incidence of microinvasive carcinoma. However, at primary treatment, 4.12% of the patients were diagnosed with early invasive, including microinvasive carcinoma.

It is noteworthy that most follow-up protocols requiring colposcopy and cytology after treatment for preinvasive cervical disease do not detect invasive carcinoma for more than 20 years.

The low recurrence rate of cervical precancers reported by our study, albeit for a shorter period of time, establishes the role of colposcopy in combination with cytological examination for these results.

In addition to cytology, the role of colposcopy in the follow-up of patients after treatment for CIN has been studied by a number of researchers. In the United Kingdom, post-treatment patients for cervical precancer are followed up by cytology only, according to the National Guidelines, although some colposcopy clinics perform colposcopy at least once during post-treatment follow-up. Soutter et al. (2006) published a paper emphasising the critical importance of colposcopy in detecting and diagnosing recurrences after CIN treatment. According to the authors, for unclear or dysplasia-affected resection lines after LLETZ, the sensitivity and specificity of colposcopy for detecting recurrence in the follow-up of these patients were 97% and 93.4%, respectively.

Therefore, at least one colposcopy in the follow-up protocols and more than one for patients at higher risk of recurrence is recommended (Soutter WP 2006). Melnikow et al. (2009) also recommend colposcopy 6 months after treatment, as this model is cost-effective, with proven benefits for patient survival and quality of life, and has been a mainstay of clinical practice in the US. Studies by Flannely et al. (1997) and Paraskevaidis et al. (1991) lay emphasis on the need for colposcopy in the follow-up of patients after LLETZ due to the low sensitivity of cytology for detecting recurrences of cervical precancers. In Flannely's study, the recurrence rate after LLETZ was 10.1% over a 4-year period, with 47% of patients with colposcopic atypia showing no abnormal cytology. In conclusion, the authors recommend the combination of colposcopy and cytology over 4 years in order to detect recurrences after treatment of cervical precancers. Paraskevaidis et al. found that 71% of recurrences after CIN treatment occurred in the 1st and 24% in the 2nd year after treatment. In 18% of recurrences, the diagnosis was made by colposcopy, as the cytology of these cases did not show any abnormality. The conclusion and recommendation in this publication are that colposcopy should be applied during the first 2 years of follow-up of patients after treatment, as it significantly increases the detectability of recurrence compared to cytology alone. Bentley et al. (2012) recommended follow-up colposcopy for patients with positive resection lines after LLETZ. These recommendations are based on evidence-based medicine with the highest degree of endorsement – data from systematic reviews, randomised trials and controlled clinical trials.

6. To investigate the correlation between histological findings after LLETZ and after targeted biopsy under video colposcopy guidance regarding cervical precancer (LGSIL, HGSIL) and to calculate the sensitivity (negative predictive value – NPV) and specificity (positive predictive value – PPV) of the histological result of biopsy with respect to HGSIL in the final histological result after LLETZ.

The analysis of the correlation between histological results after LLETZ and the histological results of the targeted biopsy showed that (Table 10):

- There is a significant correlation between the two investigated results;
- There is a strong correlation – Kramer's contingency coefficient is 0.728.

The sensitivity and negative predictive value of targeted biopsy for HGSIL in the final histological outcome after LLETZ were very good (89 and 81%, respectively), whereas the specificity and positive predictive value were lower (61 and 75%, respectively). The precision value is also relatively good – 77% (Table 11).

Table 10. Analysis of the correlation between histological results after LLETZ and the histological results of targeted biopsy (Fisher-Freeman-Halton exact test $p < 0.001$, Cramer's $V = 0.728$)

Indicators	Frequency	Histological results after LLETZ procedure			
		LGSIL	HGSIL	Ca colliuteri insitu	Ca colli uteri microinvasiva
Histological results of targeted biopsy					
LGSIL	n	46	11	0	0
	%	82.1	11.1	0.0	0.0
HGSIL	n	10	88	13	6
	%	17.9	88.9	100.0	100.0

Table 11. Screening test validation criteria values of biopsy histology result in relation to HGSIL in the final histology result after LLETZ

Sensitivity	Specificity	Positive predictive value	Negative predictive value	% correct answers
89	61	75	81	77

Table 10 shows that the correlation rate between the biopsy histologic result and that after LLETZ regarding LGSIL is 80.8% (of 57 cases with an LGSIL diagnosis by biopsy, 46 have the same diagnosis after LLETZ). At the same time, in 19.2% of the LGSIL cases, the biopsy revealed a higher degree of dysplasia – HGSIL in the final histological specimen after loop excision, i.e. hypodiagnostics indicated by targeted biopsy in these cases. This result confirms the appropriateness of loop excision in LGSIL biopsy results and not entirely visible cervical lesion. Otherwise, nearly 20 % with HGSIL would not have been diagnosed accurately.

The same table shows that the correlation rate between the biopsy histologic result and that after LLETZ regarding HGSIL was 75.3%; in 24.7%, the final histological result was different from HGSIL. In only 8.5% of the cases with a discrepancy, the final histological result was LGSIL (10/117). Notably, in 16.2% (19/117) of the HGSIL cases, the biopsy revealed carcinoma in the final histological preparation specimen after LLETZ (Ca colli uteri in situ –13 cases, and Ca colli uteri microinvasive/invasive – in 6 cases). This evidences the diagnostic role of the LLETZ procedure in determining invasion and the extent of invasion in high-grade cervical lesions.

The correlation or comparability between the histological results of the targeted (under colposcopic control) biopsy and the LLETZ procedure as a method of first choice for treating high-grade precancerous cervical lesions has been investigated by several researchers (Chappatte OA 1991, Kjellberg L 2007, Kabaca C 2014, Stoler MH 2011, Jung Y 2018). In the study by Kabaca (2014), the histological results of the targeted biopsy showed a lower degree of change in the cervix compared to the results after LLETZ in 22.9% for CIN 1, 37.03% for CIN 2 and 12.72% for CIN 3/Ca colli uteri in situ. In the same study, the biopsy results were higher in 29.16% for CIN 1, 40.74% for CIN 2 and 15.45% for CIN 3/Ca colli uteri in situ. In a publication by

Stoler et al., the correlation between the histological results of biopsy under colposcopic control and the final histology from LLETZ was 42%. In 21%, the biopsy showed a lower grade, and in 36% – a higher grade of cervical changes. Factors driving the correlation were: age, cervical lesion size, number of biopsies, and HPV-16/18 infection. The factors underlying the lower grades of change shown in biopsy versus the histological results from LLETZ are: the number of biopsies and HPV16/18 infection.

The importance of the number of biopsies for the detection and diagnosis of high-grade lesions was also established in another study (Nicolas W 2015). According to the authors, performing 3 biopsies from 3 sites increased biopsy sensitivity to 96%, regardless of cytology result, HPV status, and colposcopic impression. Costa et al. (2003) pointed out the factors of age, invisible SCJ, number of affected quadrants (lesion size) and cone width for diagnosing severe precancerous changes. Much of the discrepancies are due to the lower detection rates of premalignancies during targeted biopsy compared to that after the excisional procedure (biopsy hypodiagnosis). In a prospective study comparing the histological results from biopsy and LLETZ in 170 cases, the authors found a frequent discrepancy, mainly due to biopsy hypodiagnosis (Byrom J 2006). The sensitivity and specificity of targeted biopsy for HSIL detection were estimated to be 74% and 91%, respectively, and the negative and positive predictive values to be 48% and 97%, respectively. Biopsy failed to diagnose one case of microinvasive carcinoma and one case of the high-grade glandular lesion. The authors conclude that with colposcopic evidence of HSIL, a targeted biopsy is redundant and recommend an excisional procedure in these cases. In another study, the sensitivity of a colposcopic-guided biopsy versus LLETZ histological outcome in LSIL (CIN1) and HSIL (CIN 2–3) was 50 – 70% and 55 – 90%, respectively (Duesing N, 2012).

Three main factors are referenced as reasons for the hypodiagnosis of the biopsy under colposcopic control: the skills of the colposcopist, the qualification (competence) of the histopathologist and the invisible SCJ. The colposcopist's skill has been found to influence the colposcopic assessment of Grade 1 or Grade 2 cervical lesions (Massad LS, 2003). A tendency towards hyperdiagnosis of the cervical lesions was observed with insufficient qualified and experienced colposcopy operator (Baum ME, 2006). More important remains the skill of recognising smaller HSIL areas among larger LSIL

lesions from where to take a targeted biopsy. In the study by Massad et al., colposcopy was performed on 2 825 women, with 2 112 targeted biopsies taken by trainee colposcopists. After comparing the colposcopic diagnosis (impression) and the histological results of the biopsy, it was established: positive predictive value of the colposcopy (regardless of the degree and type of change) – 80%; negative predictive value (i.e., normal colposcopic finding) – 68%; sensitivity of the colposcopic examination compared to the histological result of the biopsy – 89%; specificity of the colposcopic examination compared to the histological result of the biopsy – 52%. The authors reported a low sensitivity for CIN 2–3 of 56%, indicating either a lack of sufficient skill on the part of the training colposcopist or deficiencies on the part of the colposcope apparatus and consumables.

In another study, misjudgement by the colposcopist in choosing the site from which to take the biopsy in larger lesions resulted in hypodiagnosis of the histological outcome after LLETZ (Buxton EJ, 1991). It has been found that targeted biopsy can miss 30 to 50% of existing high-grade lesions and become a misleading factor in deciding on adequate treatment (Denny LA, 1995). In such cases, to escalate the correlation between biopsy results and LLETZ, the role of HPV status is increasing. In HPV 16/18 positive patients, the probability of developing a precancerous disease is 35 times higher, even with normal cytology (Wright TC, 2012). In women negative for high-risk HPV strains before conisation, there is a high probability that the histological result after LLETZ will be negative for precancerous disease (Rodriguez-Manfredi A., 2013). Therefore, immediate conisation (excision biopsy) is recommended in HPV 16 carriers rather than targeted biopsy, which may lead to underdiagnosis and delayed treatment. Another critical factor in the hypodiagnostic evaluation of the histological results of target biopsy compared to the final histology after LLETZ is the ability of the histopathologist to distinguish CIN 1 from CIN 2. The latter is not always an easy task considering the scarce tissue material in the histological preparation specimen of the pinch biopsy (Dalla Palma P, 2009, Martin CM, 2011). The third factor for underdiagnosis of targeted biopsy compared to that after LLETZ is the invisible squamocolumnar junction (SCJ). An ectocervical lesion may be LGSIL but to progress to HGSIL as it enters the cervical canal (Killackey MA, 1986).

Alternatively, the cases in which the colposcopy-guided biopsy gives a higher grade of precancerous disease compared to the histological result after LLETZ can be explained by several hypotheses (Livasy CA, 2004, Ryu A, 2010, Dalla Palma P, 2009, Carrigg A, 2013). A reason for this type of discrepancy is the presence of a small site of high-grade lesion, which is removed by the pinch biopsy itself. So, there would be no other such lesion in the post-LLETZ preparation specimen. A second reason is the presence of multifocal foci of high-grade dysplasia, especially in the cervical canal, that remain outside the resection margins of the cone. This is particularly typical of glandular lesions. The third reason is the subjective evaluation of the preparations by the histopathologist. The role of a highly qualified and competent pathologist in these cases is crucial.

In our results, 19.2% of the LGSIL cases from targeted biopsy were found to have HGSIL in the final histological result. This discrepancy is lower than most of the cited literature data and is due to compliance with the indications for performing LLETZ. One of them is the invisible all-cervical lesion entering the endocervical canal, even though the biopsy proved LGSIL. The discrepancy between HGSIL from targeted biopsy and LGSIL in the final histology is found in a tiny percentage – 8.5%. The explanation for this discrepancy is the fine line in the pathologist's assessment between CIN1 (LGSIL) and CIN2 (HGSIL), the scarce biopsy material, and the multifocality of some high-grade lesions, which cannot be excluded.

7. To study the correlation between colposcopic diagnosis (colposcopic impression) and histological diagnosis after the LLETZ procedure regarding cervical precancer (LGSIL, HGSIL) and to calculate the sensitivity (negative predictive value – NPV) and specificity (positive predictive value – PPV) of the colposcopic diagnosis in relation to HGSIL in the final histological result after LLETZ.

The analysis of the correlation between the histological result after LLETZ and the colposcopic diagnosis showed that (Table 12):

- There is a statistically significant correlation between the two investigated results;
- There is a strong correlation – Kramer's contingency coefficient is 0.739.

The sensitivity and negative predictive value of the HGSIL colposcopic diagnosis in the final histological outcome after LLETZ were excellent (96 and 91%, respectively), while the specificity and positive predictive value were lower (53 and 73%, respectively). The precision value is also very good – 78% (table 13).

Table 12. Analysis of the correlation between histological results after the LLETZ procedure and the colposcopic diagnosis (Fisher-Freeman-Halton exact test $p < 0.001$, Cramer's $V = 0.739$)

Indicators	Frequency	Histological results after LLETZ procedure			
		LGSIL	HGSIL	Ca colli uteri in situ	Ca colli uteri microinvasiva
Colposcopic diagnosis					
LGSIL/grade 1	n	43	4	0	0
	%	71.7	3.7	0.0	0.0
HGSIL/grade 2	n	17	104	14	7
	%	28.3	96.3	100.0	100.0

Table 13. Validation criteria values of the screening tests of colposcopic diagnosis in relation to HGSIL in the final histological result after LLETZ

Sensitivity	Specificity	Positive predictive value	Negative predictive value	% correct answers
96	53	73	91	78

In our study, the correlation rate between colposcopy and histological outcome after LLETZ regarding LGSIL was 91.5%. Only 8.5% of colposcopic findings with signs of low-grade dysplasia were diagnosed as high-grade lesions by the final histology. This is likely due to the invisible all-cervical lesion entering the cervical canal. Regarding the colposcopic findings showing high-grade dysplasia in 88.1%, a correlation was established with

the final histology, i.e. HGSIL, and in 11.9% (17/142) – LGSIL. This discrepancy may be due to the use of lower-magnification colposcopic equipment until 2019 (included). Thus, some lesions could have been perceived as high-grade by colposcopic criteria when they were low-grade. Another explanation is the accumulation of sufficient colposcopic experience during the years of the study.

Nevertheless, these results support the recommendation for histologic verification by targeted biopsy for colposcopic atypia, including cases suspicious of HGSIL. Often, there are conditions, such as atrophy and inflammation, which reflect on the colposcopic diagnosis and cause hyperdiagnosis. In these cases, performing LLETZ without a prior biopsy would be overtreatment. This is confirmed by the results for specificity and positive predictive value of the colposcopic examination in relation to HGSIL compared to the final histological result.

IV. CONCLUSION

The LLETZ procedure is applicable in the outpatient practice with a low incidence of intra- and postoperative complications and minimal downtime. Its economic efficiency in an outpatient setting is determined by two main factors: the application of local anaesthesia instead of general anaesthesia, which requires an anaesthesiologist, anaesthesiology nurse, short-acting intravenous anaesthetics and the cost per bed day – a financial factor in hospital care versus the absence of necessity of a bed-day in ambulatory care. Following the indications for performing LLETZ, LSIL histological results in the postoperative preparation specimen are possible. The LLETZ procedure can be diagnostic to confirm invasive carcinoma in postmenopausal women, clinically and colposcopically suspicious for invasion but with unfavourable histologic findings of targeted biopsy. Intraglandular involvement is a hallmark of high-grade dysplasia and ca colli uteri in situ. Thermal damage to resection lines is a consequence of LLETZ and resulted in 7.9% of the cases in the present study. These damages do not compromise the assessment of the resection lines. The frequency of resection margin involvement after LLETZ of HSIL patients was 2.8%. This is due to the surgical technique. Cone margin involvement in patients diagnosed with Ca colli uteri in situ occurs in 18.2%. This shows the possibilities of the LLETZ procedure for diagnosing and treating this pathology. The histological results of the targeted biopsy and colposcopic diagnosis are significant factors for the final histological outcome after LLETZ. The penetration of the cervical lesion into the endocervical canal is a prognostic factor for HSIL and its invisible borders – for carcinoma (in situ or microinvasive/invasive). A lesion size up to 1/3 of the cervix is a prognostic factor for LSIL, and large lesions (2/3 of the cervix) – for HSIL and Ca colli uteri (in situ, microinvasive/invasive).

The histological result of targeted biopsy (HSIL) is the only significant factor for resection margin involvement after LLETZ. The loop excision technique for larger cervical lesions also contributes to this. Histologically proven recurrences after the LLETZ procedure were 1.7%, and persistence was 5.4%. The sensitivity of the biopsy histological result for HSIL in the final histological result after LLETZ was 89%. The specificity of the biopsy histological result with respect to HSIL in the final histological result after LLETZ was 61%. The positive predictive value of biopsy was 75%, and the negative

predictive value was 81%. The correlation rate between the biopsy histological result and that after LLETZ regarding LSIL was 80.8%. In 19.2% of the cases with LGSIL, HGSIL was found in the preparation specimen after LLETZ – proving the feasibility of loop excision in LSIL from the biopsy and the invisible cervical lesion. The correlation rate between the biopsy histological result and that after LLETZ regarding HGSIL was 75.3%. In 16.2% (19/117) of the HGSIL cases, the biopsy revealed carcinoma in the final histological sample after LLETZ (Ca colli uteri in situ – 13 cases, and Ca colli uteri microinvasive/invasive – 6 cases). This manifests the diagnostic role of the LLETZ procedure in determining invasion and the extent of invasion in high-grade cervical lesions. The sensitivity of the colposcopic diagnosis regarding HSIL in the final histological result was 96%. The specificity of the colposcopic diagnosis with respect to HSIL in the final histological result was 53%. The positive predictive value of colposcopic diagnosis was 73%, and the negative predictive value was 91%. The correlation rate between colposcopy and the histological outcome after LLETZ regarding LGSIL in our study was 91.5%. The correlation rate between colposcopy and the histological outcome after LLETZ for HSIL was 88.1%. For HSIL colposcopic findings, a biopsy is recommended for histologic verification.

V. CONCLUSIONS

1. The LLETZ procedure is applicable in the outpatient practice with a low frequency of intra- and postoperative complications and minimal downtime. Two main factors determine its economic efficiency in an outpatient setting: the application of local anaesthesia instead of general anaesthesia, which requires an anaesthesiologist, anaesthesiology nurse, short-acting intravenous anaesthetics and the cost per bed day – a financial factor in hospital care versus the absence of necessity of a bed-day in outpatient care.
2. Following the indications for performing LLETZ, LSIL histological results in the postoperative preparation specimen are possible. The LLETZ procedure can be diagnostic to confirm invasive carcinoma in postmenopausal women, clinically and colposcopically suspicious for invasion but with unfavourable histologic findings of targeted biopsy. Intraglandular involvement is a hallmark of high-grade dysplasia and ca colli uteri in situ. Thermal damage to resection lines is a consequence of LLETZ and resulted in 7.9% of the cases in the present study. These damages do not compromise the assessment of the resection lines.
3. The frequency of resection margin involvement after LLETZ of HSIL patients was 2.8%. This is due to the surgical technique. Cone margin involvement in patients diagnosed with Ca colli uteri in situ occurs in 18.2%. This shows the possibilities of the LLETZ procedure for the diagnosis and treatment of this pathology.
4. The histological results of the targeted biopsy and colposcopic diagnosis are significant factors for the final histological outcome after LLETZ. The penetration of the cervical lesion into the endocervical canal is a prognostic factor for HSIL and its invisible borders – for carcinoma (in situ or microinvasive/invasive). A lesion size up to 1/3 of the cervix is a prognostic factor for LSIL, and large lesions (2/3 of the cervix) – for HSIL and Ca colli uteri (in situ, microinvasive/invasive).
5. The histological result of targeted biopsy (HSIL) is the only significant factor for resection margin involvement after LLETZ.
6. Histologically proven recurrences after the LLETZ procedure are 1.7%, and persistence is 5.4%.

7. The sensitivity and specificity of the biopsy histological result for HSIL in the final histological result after LLETZ were 89% and 61%, respectively. The correlation rate between the biopsy histological result and that after LLETZ regarding LSIL was 80.8%. In 19.2% of the cases with LGSIL, HGSIL was found in the preparation specimen after LLETZ – proving the feasibility of loop excision in LSIL from the biopsy and the invisible cervical lesion.
8. The correlation rate between the biopsy histological result and that after LLETZ regarding HGSIL was 75.3%. In 16.2% (19/117) of the HGSIL cases, the biopsy revealed carcinoma in the final histological sample after LLETZ (Ca colli uteri in situ – 13 cases, and Ca colli uteri microinvasive/invasive – 6 cases). This manifests the diagnostic role of the LLETZ procedure in determining invasion and the extent of invasion in high-grade cervical lesions.
9. The sensitivity and specificity of colposcopic diagnosis regarding HSIL in the final histological results were 96% and 53%, respectively. The correlation rate between colposcopy and the histological outcome after LLETZ regarding LGSIL in our study was 91.5%. The correlation rate between colposcopy and the histological outcome after LLETZ for HSIL was 88.1%. For HSIL colposcopic findings, a biopsy is recommended for histologic verification.

VI. CONTRIBUTIONS

Original contributions:

1. A study on 189 patients with cervical precancers who underwent loop excision (LLETZ) in an outpatient setting was performed.
2. The applicability and cost-effectiveness of LLETZ in an outpatient setting have been established.

Scientific and practical contributions:

3. Intraoperative, early (within the 30-day period), and late postoperative complications after LLETZ were investigated.
4. The histological findings after loop excision in 189 patients with cervical precancers were evaluated.
5. The prognostic factors for the incidence of different histological findings in the final histological results after LLETZ have been established.
6. The prognostic factors for resection margin involvement by the pathological process (HGSIL, Ca colli uteri in situ, Ca colli uteri microinvasiva/invasiva) after loop excision were established.
7. The frequency of recurrence and persistence of cervical precancers after loop excision were investigated.

Scientific and theoretical contributions:

8. The sensitivity, specificity, and positive and negative predictive values of the histological results of targeted biopsy with respect to HGSIL in the histology after LLETZ were studied and established.
9. The correlation rates of the histological results of a targeted biopsy and those after LLETZ in both LGSIL and HGSIL were studied and established.

10. The sensitivity, specificity, and positive and negative predictive values of colposcopic diagnosis (impression) of HGSIL in histological outcome after LLETZ were studied and established.
11. The correlation rates of colposcopic diagnosis and histological outcome after LLETZ were studied and established in both LGSIL and HGSIL.

VII. DISSERTATION-RELATED PUBLICATIONS

1. Yonka. I. Kornovska; Slavcho T. Tomov; Angel D. Yordanov. LLETZ Procedure in an Out-Patient Setting: Applicability and Cost-Effectiveness. Journal of Biomedical and Clinical Research, vol.15, no.1, 2022
2. Kornovski Y, Ivanova Y, Kostov S, Slavchev S, Yordanov A. Treatment of cervical intraepithelial neoplasia in outpatient practice. Family Medicine & Primary Care Review. 2021;23(3):313-317. doi:10.5114/fmpcr.2021.108196. Web of Science, Scopus (SJR – 0.21); ISSN: 1734-3402
3. Y. Ivanova, D. Metodiev, S. Tomov, Y. Kornovski, S. Kostov, S. Slavchev, A. Yordanov. Histological Results after LLETZ Procedure: An Analysis and Frequency Distribution. Medical Studies/Studia Medyczne. 2022;38(2):109–114. doi:10.5114/ms.2022.117624

DECLARATION OF AUTHENTICITY

I hereby declare that this thesis and all data presented are original and obtained as a result of my research at Prof. Yavor Kornovski Medical Centre.

The results, discussions and conclusions are not written in any medium or sources except where appropriate references have been made.

Declarant:

Yonka Ivanova Kornovska, MD