Original paper

Accuracy of cervical cytology and colposcopy-directed biopsy histology in diagnosing squamous intraepithelial lesions

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Key words: abnormal cytology, colposcopy-directed cervical biopsy, large loop excision of transformation zone, accuracy, squamous intraepithelial lesion.

Abstract

Introduction: Discrepancies between cervical cytology, colposcopy-directed biopsy (CDB) histology, and the results of the excisional procedures have been a topic of ongoing debate in the literature.
Aim of the research: This study aims to assess the scale of these discrepancies and to explore the potential clinical factors contributing to such differences.
Material and methods: In this retrospective study, the consistency between Pap smear or liquid-based cytology (LBC), CDB, and large loop excision of transformation zone (LLETZ) results was evaluated. A total of 157 patients who underwent the LLETZ procedure between January 2022 and July 2024 were included in the analysis. Data collected encompassed patient age at LLETZ, menopausal status, hormonal treatment, pregnancies, cytology, HPV test results, histopathology, HPV vaccination, and substance use.
Results: We found statistically significant associations between cytology and biopsy results ($p < 0.05$), cervical cytology and LLETZ histopathology results ($p < 0.05$), and between cervical biopsy results and histopathological findings from the LLETZ procedure ($p < 0.05$). High-grade lesions showed better concordance between cervical cytology, CDB, and excisional procedure, than low-grade lesions. CDB demonstrated higher sensitivity in detecting squamous intraepithelial lesions (SIL) in comparison with cervical cytology (97.06% and 94.85%, respectively). Still, cervical cytology as well as CDB failed to detect cancer (each in one case/one case each).
Conclusions: Transitioning to more reliable HPV-based screening is necessary to achieve global cervical cancer elimination goals. Understanding the impact of uncertainty on diagnostic decisions and clarifying the role of "experience" in colposcopic practice are also crucial.

Introduction

Substantial evidence supports that human papillomavirus (HPV) testing is more sensitive and reproducible than cytology for primary screening, enabling longer screening intervals and the option for self-sampling [1]. The World Health Organization (WHO) guidelines recommend HPV testing, with or without triage, as the preferred method for cervical cancer screening [2]. Nevertheless, cervical cytology remains widely used as the primary tool in cervical cancer screening programs worldwide.

In Poland, the organized cervical cancer screening program, introduced in 2006, targets women aged 25 to 64 who have not undergone a cytology screening test in the past 3 years [3]. A parallel opportunistic screening program also exists, though the number of women participating in it remains unknown. HPV-based screening and co-testing are approved and recommended by the Polish Society of Colposcopy and Cervical Pathophysiology (PTKiPSM). At the time of manuscript submission, the costs of HPV testing were not reimbursed by the national social security system. Consequently, the majority of Polish women – including those with abnormal cytological findings – did not undergo HPV screening.

The 2022 recommendations from PTKiPSM emphasize the "expert stage", which involves assessing the patient's cervical cancer risk based on screening test results and assigning the patient to the appropriate risk group [4]. This stage also includes the final qualification for the colposcopy procedure, with or without cervical biopsy. The PTKiPSM board emphasizes that the decision to perform a colposcopy-directed biopsy (CDB) should be made exclusively by specialists at the expert stage.

Aim of the research

Given the ongoing debate regarding discrepancies between cervical cytology, biopsy histology, and the final histopathological outcomes following exci-

sional treatment, the aim of this study was to assess the accuracy of cervical cytology performed within both the organized cervical cancer screening program and opportunistic screening, as well as the histology of CDB performed by expert colposcopists, in diagnosing squamous intraepithelial lesions (SIL). Additionally, this study examines the scale of these discrepancies and explores the clinical factors contributing to them.

Material and methods

Study design and data collection

This retrospective study analyzed medical records of patients who underwent large loop excision of the transformation zone (LLETZ) at the University Hospital in Kraków, Poland, between January 2022 and July 2024. The study population included women aged 18 and older who were treated at the colposcopy unit of the Gynecological Clinic or the Clinical Department of Gynecological Endocrinology and Gynecological Oncology for SIL and/or a positive HPV test, and who subsequently underwent a LLETZ procedure.

Eligibility for inclusion required complete electronic medical records in the Asseco Medical Management Solutions (AMMS) system. Patients under 18 years of age or those with incomplete medical records were excluded.

Data collected included patient age at the time of the LLETZ procedure, menopausal status, hormonal treatment, number of pregnancies, cytology, HPV test results, histopathology findings, HPV vaccination status, and substance use. All data were entered into a spreadsheet using Microsoft Excel for analysis.

Statistical analysis

Quantitative variables were analyzed using descriptive statistics, including means, standard deviations, medians, quartiles, and minimum and maximum values. Qualitative variables were analyzed by calculating absolute and percentage frequencies for all possible values. Comparisons of qualitative variables were conducted using Fisher's exact test. A significance level of 0.05 was applied, with p-values below 0.05 considered statistically significant. All statistical analyses were performed using R software, version 4.4.1.

Results

Characteristics of the study group

A total of 186 LLETZ procedures were identified at the University Hospital in Kraków, and after excluding 29 cases that did not meet the inclusion criteria, data from 157 women were analyzed. The mean age was 34.7 years (range: 21–79). Of the cohort, 90.5% were premenopausal, and 9.5% were postmenopausal. Most patients (77.7%) were not receiving hormonal therapy, with 1.3% on hormone replacement therapy and 21.0% using hormonal contraception. Pregnancy history revealed a median of one pregnancy per patient, with 41.4% having no reported pregnancies. Most women were non-smokers (74.5%), while 24.9% reported tobacco use, with some also consuming alcohol. HPV vaccination was reported in 6.4% of participants. Cytological examination before LLETZ revealed high grade squamous intraepithelial lesion (HSIL); low grade squamous intraepithelial lesion (LSIL); atypical squamous cells cannot exclude HSIL (ASC-H); atypical squamous cells of undetermined significance (ASC-US); negative for intraepithelial lesion or malignancy (NILM); atypical glandular cells (AGC); adenocarcinoma in situ (AIS); squamous cell carcinoma (SCC) in 1.3%. Regarding HPV testing, 53.5% had never been tested, 20.4% were positive for HPV types 16/18, 12.7% for other types, and 9.6% for both. A negative HPV result was observed in 3.8% of cases (Table 1).

Comparison of cervical cytology with the results of CDB

A statistically significant association was found between cytology and biopsy results ($p < 0.05$), as shown in Table 2. The highest concordance of results was observed for LSIL cytology (56.6%), which was confirmed in CDB, and for HSIL (CIN2/3), which correctly identified 44.1% of HSIL (CIN2) and 59.6% of HSIL (CIN3). However, LSIL in cytology was found to correspond to histopathological HSIL (CIN2) in 32.4% of cases and HSIL (CIN3) in 14% of cases. Conversely, cytological HSIL (CIN2/3) was histopathologically classified as not suspicious for malignancy in 37.5% of cases. Both cytological SCC and AGC were confirmed as HSIL (CIN3) – 3.5% and 1.8%, respectively, while AGC and AIS were found to be not suspicious for malignancy (each 12.5%).

Comparison of cervical cytology with the histopathological results of LLETZ

To assess the accuracy of cervical cytology in diagnosing SIL, we compared the initial cytology results with the histopathological findings from LLETZ specimens. A statistically significant association was observed between cervical cytology and LLETZ histopathology results ($p < 0.05$), indicating a correlation between the two tests. The results are summarized in Table 3. Cytological HSIL (CIN2/3) did not confirm SIL in 42.9% of cases but correctly diagnosed 36.4% of HSIL (CIN2) and 56.7% of HSIL (CIN3) and was present in the only case of SCC in our group. Cytological LSIL was concordant in 40.5% of cases but was found to be a high-grade lesion in 48.5% (CIN2) and 13.3% (CIN3) of cases. Interestingly, 8.3% of histopathological HSIL (CIN3) cases had cytological NILM and 6.7% had ASC-US.

SCC diagnosed by cytology did not confirm cancer and in both cases was HSIL (CIN3) (3.3%). Similarly, AGC and AIS did not confirm glandular pathology.

Sensitivity and specificity of cervical cytology in relation to the histopathological results of LLETZ

Table 4 presents the sensitivity, specificity, accuracy, positive predictive value (PPV), and negative predictive value (NPV) of cervical cytology in comparison to LLETZ histopathological results. The sensitivity of cervical cytology was 94.85%, indicating that in 94.85% of patients with a positive LLETZ result, the cytology test was also positive. The specificity was 0.00%, meaning that in 0.00% of patients with a negative LLETZ result, the cytology test was also negative, failing to identify any true negative cases. An accuracy of 82.17% indicates that cytology and LLETZ results were consistent in 82.17% of patients. The PPV of 86.00% means that in 86.00% of patients with a positive cytology result, the LLETZ result was also positive. The NPV of 0.00% indicates that in 0.00% of patients with a negative cytology result, the LLETZ result was also negative, suggesting that a negative cytology result does not reliably predict a negative LLETZ outcome.

Comparison of histopathological results of CDB with the histopathological resultsof LLETZ

A statistically significant association was observed between cervical biopsy results and the histopathological findings following the LLETZ procedure ($p < 0.05$), indicating a strong correlation between the initial biopsy results and the final histopathological outcomes. The detailed data are presented in Table 5. LSIL diagnosed through CDB was confirmed in the LLETZ specimen in 56.5% of cases, HSIL (CIN2) in 36.8%, and HSIL (CIN3) in 68.4%. However, HSIL (CIN2) identified in the biopsy resulted in CIN3 in 25.0% of cases, while CDB-diagnosed LSIL was a non-oncological case in 30.4%. Negative biopsy results (not suspicious for malignancy) were concordant with the final histological outcome in 50.0% of cases, but also resulted in LSIL and HSIL (CIN3) in 25.0% of cases each.

Sensitivity and specificity of histopathological results of CDB in relation to the histopathological results of LLETZ

Table 6 presents the sensitivity, specificity, accuracy, PPV, and NPV of CDB in relation to LLETZ histopathology. Biopsy accurately identified 97.06% of true positive cases and correctly identified 20.00% of true negative cases. CDB and LLETZ results were consistent in 87.18% of patients. In 89.19% of patients with a positive biopsy result, the LLETZ result was also positive, while in 50.00% of patients with a negative result, the LLETZ result was also negative.

Table 1. Characteristics of the study group (n = 157)

Characteristics	Value
Age on the day of LLETZ surgery, median [IQR], range	34.7 [29.9–44.0], 21–79
Menopausal status, *n* (%)	
Premenopausal	142 (90.5)
Postmenopausal	15 (9.5)
Hormonal treatment, *n* (%)	
None	122 (77.7)
Hormone replacement therapy	2 (1.3)
Contraception	33 (21.0)
Pregnancies, median [IQR]	1 [0–2]
Substance use, *n* (%)	
No	117 (74.5)
Smoking	36 (23.0)
Alcohol	1 (0.6)
Smoking and alcohol	3 (1.9)
HPV vaccination, *n* (%)	
No	147 (93.6)
Yes	10 (6.4)
Cytology results, *n* (%)	
NILM	7 (4.5)
ASC-US	14 (8.9)
LSIL	45 (28.7)
ASC–H	14 (8.9)
HSIL (CIN 2/3)	70 (44.6)
SCC	2 (1.3)
AGC	4 (2.5)
AIS	1 (0.6)
HPV status, *n* (%)	
Not tested	84 (53.5)
Negative	6 (3.8)
Non–16/18	20 (12.7)
16/18	32 (20.4)
Positive (different groups)	15 (9.6)

IQR – interquartile range, n – number of patients.

Discussion

The performance of cytology in detecting cervical precancer and cancer has been extensively studied among women. However, significant discrepancies and underdiagnoses are often observed when cytology results are compared with biopsy and loop electrosurgical excision procedure (LEEP) outcomes

Table 2. Comparison of cervical cytology with the histopathological results of cervical biopsy

Cervical cytology	Biopsy result										*P*-value[1]
	Non–diagnostic (*n* = 1)		Not suspicious for malignancy (*n* = 8)		LSIL (*n* = 23)		HSIL (CIN2) (*n* = 68)		HSIL (CIN3) (*n* = 57)		
	n	%	*n*	%	*n*	%	*n*	%	*n*	%	
NILM	0	0.0	0	0.0	0	0.0	4	5.9	3	5.3	0.001
ASC–US	0	0.0	1	12.5	3	13.0	5	7.4	5	8.8	
LSIL	1	100.0	1	12.5	13	56.6	22	32.4	8	14.0	
ASC–H	0	0.0	1	12.5	2	8.7	7	10.3	4	7.0	
HSIL (CIN 2/3)	0	0.0	3	37.5	3	13.0	30	44.1	34	59.6	
SCC	0	0.0	0	0.0	0	0.0	0	0.0	2	3.5	
AGC	0	0.0	1	12.5	2	8.7	0	0.0	1	1.8	
AIS	0	0.0	1	12.5	0	0.0	0	0.0	0	0.0	

[1]*Fisher's exact test p-value, n – number of patients.*

Table 3. Comparison of cervical cytology with the histopathological results of LLETZ

Cervical cytology	LETZL														*P*-value[1]
	Non–diagnostic (*n* = 0)		Not suspicious for malignancy (*n* = 21)		LSIL (*n* = 42)		HSIL (CIN2) (*n* = 33)		HSIL (CIN3) (*n* = 60)		Cis (*n* = 0)		Squamous cell carcinoma (*n* = 1)		
		%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	
NILM	0	0.0	0	0.0	2	4.8	0	0.0	5	8.3	0	0.0	0	0.0	0.022
ASC–US	0	0.0	4	19.0	5	11.8	1	3.0	4	6.7	0	0.0	0	0.0	
LSIL	0	0.0	4	19.0	17	40.5	16	48.5	8	13.3	0	0.0	0	0.0	
ASC–H	0	0.0	1	4.8	2	4.8	4	12.1	7	11.7	0	0.0	0	0.0	
HSIL (CIN 2/3)	0	0.0	9	42.9	14	33.3	12	36.4	34	56.7	0	0.0	1	100.0	
SCC	0	0.0	0	0.0	0	0.0	0	0.0	2	3.3	0	0.0	0	0.0	
AGC	0	0.0	2	9.5	2	4.8	0	0.0	0	0.0	0	0.0	0	0.0	
AIS	0	0.0	1	4.8	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	

[1]*Fisher's exact test p-value, n – number of patients.*

Table 4. Sensitivity and specificity of cervical cytology in relation to the histopathological results of LLETZ

Sensitivity	Specificity	Accuracy	Positive predictive value (PPV)	Negative predictive value (NPV)
94.85%	0.00%	82.17%	86.00%	0.00%

[5]. A study involving 6,000 Pap smears reported concordance rates with CDB of 100% for SCC, 82.35% for HSIL, 82% for ASC-US, 65.6% for LSIL, and 50% for ASC-H [6]. Our findings revealed slightly different concordance rates between cytology and CDB, with 91.43% for HSIL and 78.57% for ASC-H. In our study, 71.42% of ASC-US cases were confirmed as HSIL (CIN2+) upon biopsy. Interestingly, cytology positive for SCC was not confirmed by histopathology, while LSIL showed a concordance rate of only 28.89%. Overall, our data were characterized by higher-grade histopathological findings in CDB specimens compared to the initial cytological diagnosis, with the exception of two cases where cytology indicated SCC, but CDB showed HSIL (CIN3), and one case of AIS, which was excluded by biopsy.

In terms of cytology's concordance with histopathological LLETZ results, the highest values were

Table 5. Comparison of histopathological results of cervical biopsy with the histopathological results of LLETZ

LLETZ result	Biopsy result										*P*-value[1]
	Non-diagnostic (*n* = 1)		Not suspicious for malignancy (*n* = 8)		LSIL (*n* = 23)		HSIL (CIN2) (*n* = 68)		HSIL (CIN3) (*n* = 57)		
	n	%	*n*	%	*n*	%	*n*	%	*n*	%	
Not suspicious for malignancy	1	100.0	4	50.0	7	30.4	6	8.8	3	5.3	
LSIL	0	0.0	2	25.0	13	56.5	19	27.9	8	14.0	
HSIL (CIN 2)	0	0.0	0	0.0	1	4.3	25	36.8	7	12.3	< 0.001
HSIL (CIN 3)	0	0.0	2	25.0	2	8.8	17	25.0	39	68.4	
SCC	0	0.0	0	0.0	0	0.0	1	1.5	0	0.0	

[1]*Fisher's exact test p-value, n – number of patients.*

Table 6. Sensitivity and specificity of histopathological results of cervical biopsies in relation to the histopathological results of LLETZ

Sensitivity	Specificity	Accuracy	Positive predictive value (PPV)	Negative predictive value (NPV)
97.06%	20.00%	87.18%	89.19%	50.00%

observed for ASC-H and HSIL, with histopathological HSIL (CIN2+) confirmed in 78.57% and 65.71% of cases, respectively. LSIL showed concordance in only 37.78%, confirming that cytology is more accurate in detecting HSIL than low-grade lesions [5]. A recent multicenter cross-sectional cervical cancer screening study from Latin America reported cytology sensitivity for CIN2+ at 48.5% and specificity at 96.5% [7]. The study found substantial variability in performance estimates across study centers, with sensitivity ranging from 32.1% to 87.5% and specificity from 89.2% to 99.5%. Another study involving nearly 13,000 patients who underwent conization reported cytology sensitivity of 84.6%. In our cohort, the sensitivity of cytology was 94.85%, which is comparable to the 93.7% reported in a similar study from Poland, but still, in one case, cytology failed to detect SCC [8]. The literature suggests that the consistency between cytology and LEEP results improves with age, while discrepancies in HSIL detection tend to decrease with age [5].

International guidelines for cervical cancer prevention consider colposcopy the gold standard for diagnosing early cervical cancer and its precursors. Including colposcopy in the diagnostic process for women with abnormal cytology and/or positive HPV tests facilitates the identification of patients requiring therapeutic intervention. According to the European Consensus Statement on Essential Colposcopy, CDB should provide interpretable material for accurate histopathological assessment [9]. The Consensus further states that sensitivity improves with at least two biopsies, while other sources suggest that 3 to 5 punch biopsies may be needed to determine the necessity for conization [10]. The PTKiPSM does not specify the number of biopsies and leaves the decision to the expert colposcopist [4]. For patients with LSIL cytology and grade 1 or negative colposcopic findings, biopsies are not required in every case but are recommended [11, 12]. Reasons for not performing a biopsy should be clearly documented, and if biopsies are inadequate, repeat biopsies should be considered if a residual colposcopic lesion is present [9].

In our analysis, CDB histopathology revealed HSIL (CIN2+) in 79.62% and LSIL in 14.65% of cases. Among the 157 cases, there were 8 non-oncologic biopsy results, with only one biopsy deemed inadequate. Similar results were observed in a study of 229 Polish women during the SARS-CoV-2 pandemic, in which nearly 70% of patients had HSIL in biopsy specimens [8]. In the same study, LEEP diagnosis showed that half of the women were diagnosed with HSIL and one-third with LSIL [8]. In our cohort, HSIL (CIN2+) was confirmed in just over 59% of cases and LSIL in almost 27%. Despite potential differences in physician behavior during the pandemic – leading to more frequent excisional procedures – our results align closely with these findings.

Numerous studies have compared the histologic results of CDB with LLETZ specimens to assess the sensitivity and specificity of biopsies. A systematic review and meta-analysis reported pooled sensitivity for punch biopsies at 91.3% and specificity at 24.6% [13]. Other studies have found CDB sensitivity as high as 95.1% and specificity at 93% [8, 14]. In our study, even though in one case CDB did not detect SCC, the sensitivity of CDB was 97.06%, with speci-

ficity of 20.00%, which is consistent with previous findings. The observed high sensitivity may be influenced by verification bias [13]. Our accuracy (87.18%), PPV (89.19%), and NPV (50.00%) differed from a 2020 study, which reported values of 73.6%, 95.6%, and 32.7%, respectively [15]. The differences may be attributed to the Korean cohort, which included only women with cytologic HSIL. Interestingly, the same researchers found that CDB diagnostic inaccuracy increased in patients ≥ 50 years old. Conflicting results exist regarding factors influencing CDB results. One Korean study found that patients > 45 years old had a higher risk of unexpected low-grade lesions, while patients ≥ 45 years old with HPV 16/18 infections had a lower risk of unexpected high-grade lesions [16]. Age and HPV genotype appeared to contribute to discrepancies between cytology and histology, while other studies suggest age does not affect the consistency rates between cytology and LEEP results. Furthermore, HPV infection did not influence discrepancies between cytology and LEEP results [5].

In our study, all patients underwent biopsy, which is a specific practice of our colposcopy unit. However, various studies have questioned whether biopsies are always necessary before referral for excisional procedures. LEEP or LLETZ may be performed without prior biopsy, particularly in multiparous women in the perimenopausal period, those with extensive visible abnormalities, discrepancies in test results, or suspected invasive cervical cancer [8]. Patients with ASC-H, HSIL, or HPV type 16 may also undergo conization without prior biopsy [10].

A potential limitation of our study is that all patients in the cohort ultimately underwent excisional procedures, introducing selection bias and possibly affecting the results regarding cervical cytology and CDB. Another limitation is the variation in cytological methods used, as the organized cervical cancer screening program, until July 2025, primarily relied on conventional Pap smears, while some women in opportunistic screening had LBC taken. This may have contributed to discrepancies and lower cytology performance.

Conclusions

The limited and highly variable sensitivity of cytology strongly supports the transition to more robust and reproducible HPV-based cervical screening in Poland to ensure progress towards global cervical cancer elimination targets. Additionally, it is crucial to understand how uncertainty affects diagnostic decisions and to define the concept of "experience" in the context of everyday colposcopic practice.

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Ethical approval

This study was conducted in accordance with the principles of the Declaration of Helsinki and received ethical approval from the Jagiellonian University Medical College Research Ethics Committee (Approval No. 118.0043.1.165.2024, dated May 13, 2024). No ethical concerns were raised regarding the conduct of the study.

Conflict of interest

The authors declare no conflict of interest.

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