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Original Study • DOI: 10.2478/ahem-2022-0015 • AHEM • Vol. 76 Issue 1/2022 • 149-156

AHEM

Advances in Hygiene and Experimental Medicine
Postępy Higieny i Medycyny Doświadczalnej

Correlation between brush cytology results and histopathological examination in diagnostic evaluation of precancerous conditions and laryngeal cancer: A prospective study

Original Study

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Received 09 September, 2021, Accepted 03 February, 2022

Abstract

Introduction. The aim of the paper was to demonstrate that brush cytology can be useful in diagnostic evaluation of precancerous states and laryngeal cancer.
Materials and Methods. Ninety-two patients were analyzed. The control group included patients with benign laryngeal lesions, while the study group was composed of patients in whom a precancerous condition or laryngeal cancer was suspected. Material for histopathological and cytological examination was collected during a laryngeal microsurgery. The authors analyzed the consistency of the results of cytological and histopathological examination in the diagnostic evaluation of precancerous conditions and laryngeal cancer.
Results. Comparing diagnoses based on cytological examination with the results of histopathological examinations, the authors observed that there was a strong and statistically significant ($p<0.001$) correlation between the results. The accuracy of brush cytology in the conducted study indicates that the sensitivity and specificity of the test reaches 90.09% and 93.5% with 4 false negative and 3 false positive results. Positive prediction reached 93% and negative prediction reached 91.5%.
Conclusions. Brush cytology of the larynx may be useful in screening and as an auxiliary test in diagnostic evaluation of precancerous conditions and laryngeal cancer.

Keywords

brush cytology • histopathological examination • dysplasia • laryngeal squamous carcinoma • screening

1. Introduction

Squamous cell carcinoma (SCC) constitutes approximately 95% of laryngeal malignancies and is the most frequent malignancy in the neck and head region, with a 5-year survival rate of 50% [1, 2]. SCC is diagnosed on the basis of histopathological examination of the compromised tissue. The examination, however, is not used to monitor the condition of patients after cancer treatment. Frequent biopsies conducted under general anesthesia are associated with perioperative risk as well as a risk of bleeding or infection. They also require hospitalization and recovery after the procedure. Material for histopathological examination can also be obtained during videolaryngoscopy conducted under local anesthesia in an outpatient setting; however, it should be taken into account that bleeding or even laryngospasm may occur during the procedure [3]. In patients who underwent cancer treatment, follow-up consists in conducting laryngological examinations and imaging. Follow-up of a patient with a history of laryngeal cancer is essential, both due to the high relapse rate and frequent occurrence of a second primary cancer in this area, which is a result of the negative effect of carcinogenic factors on the entire mucous membrane of the upper digestive and respiratory tract [4, 5, 6]. Considering the 5-year survival period, the considerable percentage of laryngeal cancer relapses as well as the economic aspects associated with hospitalization and recovery in the case of surgical biopsy, the search for other, less invasive, repeatable and comparably sensitive diagnostic methods seems legitimate. Attempts to use brush cytology in the case of laryngeal diseases were described decades ago, and the first reports published by J. W. Ayre are from 1954 [7]. Currently, the method is used in screening and early diagnostic evaluation of intraepithelial uterine cervix neoplasia, lung and bronchial cancer, as well as dysplastic lesions and cancer of the oral cavity [8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18].

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2. Materials and Methods

The study involved 92 patients who were treated at the Clinic of Otolaryngology Head and Neck Surgery, 4th Military Clinical Hospital with Polyclinic in Wroclaw, in the years 2019 – 2020. Approval of the Bioethics Committee of the Wroclaw Medical University was obtained. The study was conducted in accordance with the recommendations of the Helsinki Declaration, Clinical Trial Registration Number KB-519/2018. All patients received detailed information on the assumptions and stages of the study and gave written consent to participate therein. The inclusion criteria were morphological lesions in the larynx observed during an outpatient laryngological examination. A history of radiotherapy treatment of the head and neck region was the exclusion criterion. Data on patients were obtained from their medical records: medical history, surgical protocol, results of histopathological examination. Within the framework of preoperative diagnostic evaluation, patients underwent indirect laryngoscopy as well as endoscopic examination of the larynx with archiving of photographic documentation. Patients underwent a diagnostic and surgical procedure: laryngeal microsurgery under general anesthesia with the use of a microscope and Kleinsasser set. First, material for cytological examination was collected with the use of a sterile, standard cytological brush of 195 mm in length. After reaching the level of the examined lesion, the physician pressed the brush against it and performed several 360-degree rotational movements while moving the brush along the long axis. The material collected during this procedure was applied to a microscope slide by shaking the material off the brush and gently spreading it. It was then preserved with the use of Cytofix, applied to the slide from a distance of 25 cm for 1 second. After being dried for 4 minutes, the material was stained with hematoxylin and eosin. Tissue material for histopathological examination was collected from the same site. It was immediately preserved in a 10% solution of buffered formalin. Cytology evaluation was conducted with the use of the scale shown in Table 1. This scale is the authors' own modification of the Bethesda scale, which is commonly used for the evaluation of cervical cytology.

Table 1. Cytological evaluation of laryngeal smear

Type of cytological preparation	Conventional Made using a different method
Overall quality of the smear	Suitable for cytological evaluation Suitable for cytological evaluation, dried Not suitable for cytological evaluation (technical procedure was not performed, or technical procedure was performed but material is too dry, incorrectly preserved, cell concentration is low, material is not legible due to numerous inflammatory cells, erythrocytes)
Number of cells in the smear	High cell concentration K1 Moderate number of cells K2 Low cell concentration K3
Erythrocyte count in the smear	High erythrocyte count E1 Low/moderate erythrocyte count E2 Erythrocytes absent E3
Severity of inflammation	Minor inflammation INF1 Moderate inflammation INF2 Severe inflammation INF3
Type of inflammatory cells	Lymphocytes L Granulocytes G Lymphocytes and granulocytes L and G
General characteristics of the smear	No intraepithelial neoplasia or cancer, only normal stratified squamous epithelial cells are present Abnormal cytology Cells other than stratified squamous epithelial cells are present in the smear, glandular epithelium cells are present
Abnormal epithelial cells	Atypical squamous cells of undetermined significance ASC-US Low-grade intraepithelial lesion LSIL High-grade squamous intraepithelial lesion HISL High-grade squamous intraepithelial lesion with features that raise suspicion of invasion (keratin pearl, keratin bundles, pathological mitoses)

All smears were performed by conventional methods. Two out of 92 preparations were not suitable for evaluation. The remaining 90 preparations were evaluated. Results of cytological examination were categorized into 5 main groups:

I cytologically benign lesions
II LSIL lesion
III HSIL lesion
IV HSIL lesion with features of invasion
V ASC-US

An analogous division was applied while grouping the results of histopathological examination:

I histopathologically benign lesion
II low-grade intraepithelial neoplastic lesion
III high-grade intraepithelial neoplastic lesion
IV invasive cancer

The collected sociodemographic data were statistically analyzed with the use of the STATISTICA vs. 13.3 PL software. Nominal qualitative values (e.g., *sex*) are presented in cross tabulations as size (*n*) and proportion (%). Pearson's chi-square test was used to assess the significance of the relationship between two qualitative variables. Quantitative variables (e.g., *age*) are presented in tables as mean values (*M*) and standard deviations (*SD*) as well as statistics of location: medians (*Me*), lower quartiles (*Q*51), upper (*Q3*), lowest (*Min*) and highest values (*Max*). The consistency of the empirical distribution of quantitative variables with the theoretical Gaussian distribution was verified with the use of the *W* Shapiro-Wilk normality test. Homogeneity of variance was verified using the Levene's test. The significance of differences of average values of quantitative variables in two groups was verified with the use of the *U*Mann-Whitney test. Cohen's kappa reliability coefficient was used to assess the consistency of the results of cytological and histopathological examinations of surgical biopsy collected from the same site. Coefficients weighted with quadratic weights were used due to the categorical nature of the diagnoses.

3. Results

3.1 Patients' characteristics

The study involved 92 patients, including 34 women (37.0%), aged 26 to 85 (mean *M8* = 62.0; standard deviation *SD* = 11.0 years), treated for laryngeal diseases. Laryngeal cancer or leukoplakic lesions were suspected in 67 (72.8%) patients, who were assigned to the study group (B). In 25 (27.2%) patients diagnosis was benign (polyp, cyst, Reinke's edema), and they were assigned to the control group (K). (See Table 2.)

Clinical characteristics of both groups of patients are presented in Table 3.

Due to the adopted division criterion, statistically significant correlation occurs between the initial diagnosis made on the basis of the results of cytological (Fig. 1) and the histopathological examination (Fig. 2) as well as whether patients were assigned to group B or K ($p < 0.001$) based on final diagnosis.

Comparison of diagnoses made on the basis of cytological examination with histopathological results is presented in Table 4.

A strong, statistically significant ($p < 0.001$) correlation was observed between diagnoses made on the basis of cytological and histopathological examinations (Fig. 3).

Spearman's correlation coefficient and the value of Cohen's kappa reliability coefficient confirm that there is a strong correla-

Table 2. General characteristics of patients

Feature (variable)	Group B		Group K		Total		B vs K p
	N = 67		N = 25		N = 92		
	n	(%)	n	(%)	n	(%)	
Sex:							<0.001
Female	16	23.9%	18	72.0%	34	37,0%	
Male	51	76.1%	7	28.0%	58	63,0%	
Age:							0.011
M ± SD	64.1 ± 8.6		56.3 ± 14.4		62.0 ± 11.0		
Me [Q1; Q3]	66 [60; 70]		59 [42; 67]		64 [57; 69]		
Min – Max	26 - 79		34 - 85		26 - 85		

n - group size; % - proportion; *M* - mean; *SD* - standard deviation; *Me* - median (50. percentile); *Q*1 - lower quartile (25. percentile); *Q*3 - upper quartile (75. percentile); *Min* - lowest value; *Max* - highest value; *p* - test significance level

Table 3. Clinical characteristics of patients

Feature (variable)	Group B N = 67		Group K N = 25		Total N = 92		B vs K p
	n	(%)	n	(%)	n	(%)	
Diagnosis:							<0.001
Laryngeal cancer	57	85.1	0	0.0	57	62.0	
Leukoplakia	10	14.9	0	0.0	10	10.9	
Polyp	0	0.0	10	40.0	10	10.9	
Reinke's edema	0	0.0	10	40.0	10	10.9	
Cyst	0	0.0	5	20.0	5	5.3	
Results of histopathological examination							<0.001
FI - benign lesion	21	31.3%	25	100.0%	46	50.0%	
LSIL	7	10.4%	0	0.0%	7	7.6%	
HSIL	6	9.0%	0	0.0%	6	6.5%	
Malignant lesion	33	49.3%	0	0.0%	33	35.9%	
Results of cytological examination:	N = 67		N = 23		N = 90		<0.001
FI - benign lesion	20	29.9%	23	100.0%	43	47.8%	
LSIL	11	16.4%	0	0.0%	11	12.2%	
HSIL	12	17.9%	0	0.0%	12	13.3%	
HISL* (with features of invasion)	21	31.4%	0	0.0%	21	23.3%	
ASC-US	3	4.5%	0	0.0%	3	3.3%	

Table 4. Number (proportion) of diagnoses in 90 patients with laryngeal diseases made based on results of cytological and histopathological examinations

Result of brush cytology	Result of histopathological examination			
	Benign lesion N = 44	LSIL N = 7	HSIL N = 6	Malignant lesion N = 33
FI - benign lesion	40 (44.4%)	0 (0.0%)	0 (0.0%)	3 (3.3%)
LSIL	4 (4.4%)	7 (7.8%)	0 (0.0%)	0 (0.0%)
HSIL	0 (0.0%)	0 (0.0%)	6 (6.7%)	6 (6.7%)
HISL* - with features of invasion	0 (0.0%)	0 (0.0%)	0 (0.0%)	21 (23.3%)
ASC-US - abnormal result	0 (0.0%)	0 (0.0%)	0 (0.0%)	3 (3.3%)

tion between diagnoses made on the basis of the results of histopathological and cytological examination (Fig. 4).

The following values of diagnostic accuracy indices of the test were estimated in order to estimate the accuracy of diagnosis based on cytological examination in relation to the results of histopathological examination (the gold standard): sensitivity, specificity, accuracy of diagnosis, actual disease rate, predicted frequency of the disease in individuals with a positive test result (+) (positive prediction) and predicted frequency in individuals with a negative test result (-) (negative prediction).

Results of cytological and histopathological examination (Table 3) were transformed into dichotomous variables:

- positive results (+): LSIL, HSIL or HSIL with features of invasion (in cytology),
- negative result (-): benign lesion

Table 5 presents diagnostic accuracy evaluation based on a study of 87 patients.

The following designations are used in Table 5:

- PD - true positive results, (TP)
- FD - false positive results, (FP)
- FU - false negative, (FN)

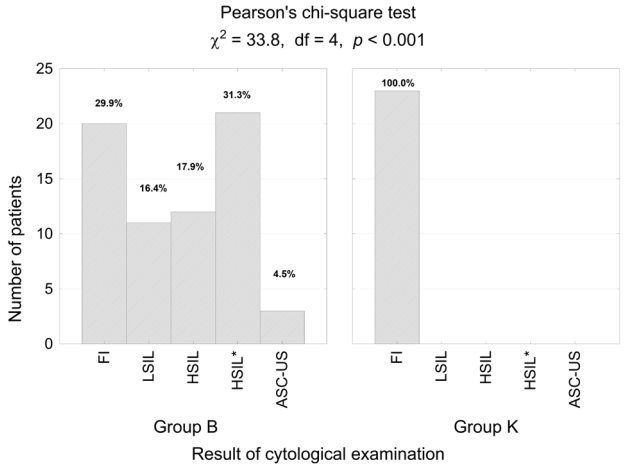


Fig. 1. Number (percentage) of patients in groups differing in terms of final and initial diagnosis on the basis of cytological examination results (FI – normal result, benign lesions; LSIL – low-grade lesion; HSIL – high-grade lesion; HSIL – high-grade lesion with features of invasion; ASC-US – atypical cells of undetermined significance) and results of independence test*

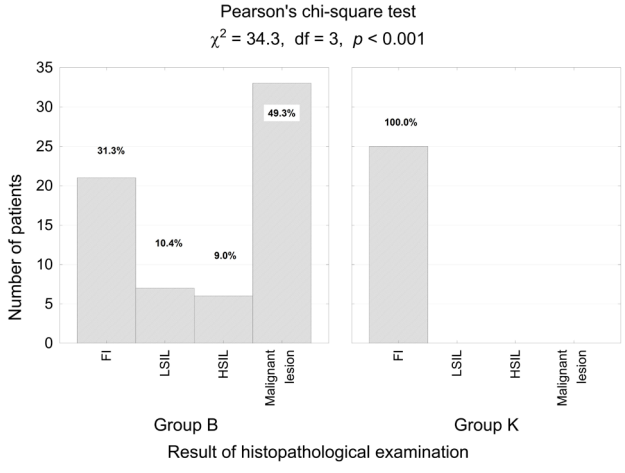


Fig. 2. Number (percentage) of patients in groups differing in terms of clinical diagnosis and result of histopathological examination as well as result of independence test

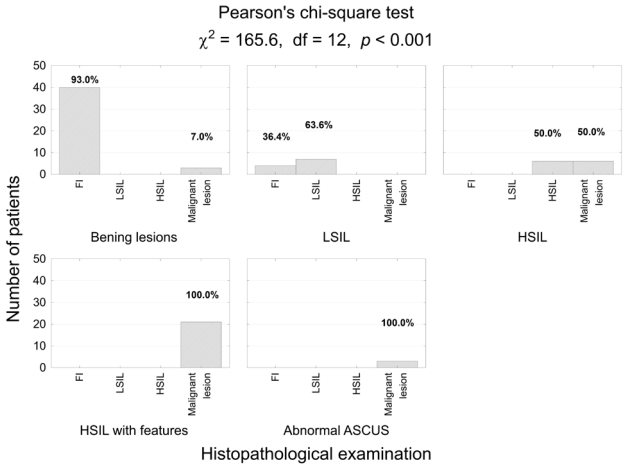


Fig. 3. Number (percentage) of patients in groups differing in terms of initial diagnosis and diagnosis made based on results of histopathological and cytological examination and results of independence test

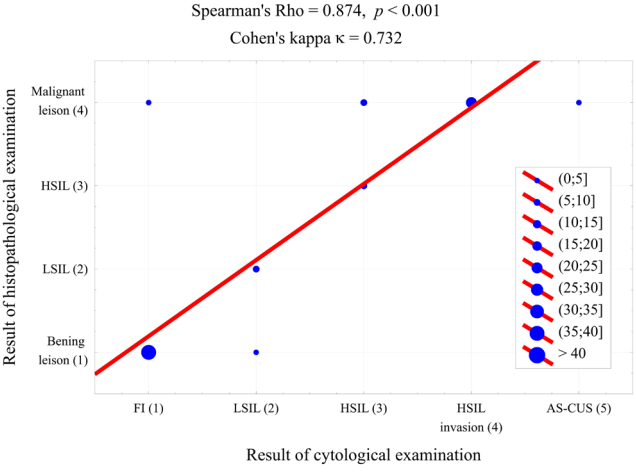


Fig. 4. Scatter plot of cytology and histopathology results

- PU - true negative, (TN)
- Test sensitivity = PD/(PD + FD)
- Test specificity = PU/(FD + PU)
- Accuracy of diagnosis = (PD + PU)/(PD + FD + FU + PU)
- Actual frequency of the disease = (PD + FU)/(PD + FD + FU + PU)
- Predicted frequency of the disease in individuals with a positive test result (+) positive prediction PPV = PD/(PD + FD)
- Predicted frequency of lack of the disease in individuals with a negative test result (-) negative prediction NPV = FU/(FU+ PU)
- *Likelihood ratio for a positive test result*, LR+ = (sensitivity)/(1 - specificity)
- *Likelihood ratio for a negative test result*, LR- = (sensitivity)/(1 - specificity)

It is assumed that tests with LR+ around 10 and above, or LR- from 0.1 and below are often decisive. Cytological examination meets both of these criteria. In addition, weighted Cohen's kappa coefficient was used for the assessment of the reliability of double examinations of collected tissues. Results of the comparisons are presented in Table 6.

Weighted Cohen's kappa for 87 patients diagnosed using two methods is $\kappa = 0.732$ ($p < 0.001$). Cohen's kappa coefficient with quadratic weights is the same as intraclass correlation coefficient. The obtained result (p-value < 0.001) makes it pos-

Table 5. The accuracy of brush cytology as a diagnostic test for laryngeal cancer

Cytological examination	The actual status – "gold standard" Histopathological examination		
	(+)	(-)	Total
(+)	40 (PD)	3 (FD)	43 (PD + FD)
(-)	4 (FU)	43 (PU)	47 (FU + PU)
	44 (PD + FU)	46 (FD + PU)	87 (PD + FD + FFU + PU)

Test sensitivity = (40/44) x100 = 90.9%
Test specificity = (43/46) x 100 = 93.5%
Accuracy of diagnosis = (83/87) x 100 = 92.2%
Positive prediction = (40/40) = 93.0%
Negative prediction = (15/25) = 91.5%
LR+ reliability index = 13.94
LR- reliability index = 0.10

Table 6. Observed numbers of the classification of laryngeal findings conducted by a cytologist and histopathologist, and Cohen's kappa value with quadratic weights and standard errors for κ

Cytologist	Histopathologist			
	Benign lesion	LSIL	HSIL	Malignant lesion
Benign lesion	**40**	0	0	3
LSIL	4	7	0	0
HSIL	0	0	**6**	6
HISL with features of invasion	0	0	0	**21**
N = 87; Cohen's kappa coefficient κ = 0.732; SE = 0.020; p < 0.0001				

sible to reject the null hypothesis on the lack of consistency between these evaluations, which indicates that opinions of both diagnosticians (cytologist and histopathologist) are consistent. Based on the classification proposed by Altman, Landis, and Koch, revealed in Table 7, we can conclude that results of cytology and histopathology are highly consistent.

4. Discussion

Analysis of the general characteristics of the patients involved in the described study revealed that their age and sex were statistically significant (p<or=0.001). In the study group, most patients histopathologically diagnosed laryngeal squamous cell carcinoma were in their sixties with a significant predominance of the male sex. The majority of patients in the control group, on the other hand, were women and the mean age in the group was 56.3. Comparing the initial clinical diagnosis with the result of histopathological examination in the control group, we obtain a consistency of 100%. In the case of cytological examination, 2 out of 25 smears were not fit for analysis, and no signs of cellular atypia were observed in any of the 23 analyzed images.

In the case of the study group, which included 67 individuals in whom the morphological image of the larynx suggested a potentially malignant lesion, histopathological examination revealed benign lesions in 21 cases and in 46 cases showed low-degree dysplasia (7.46), high-degree dysplasia (6/46), or squamous cell cancer (33/46). In the analysis of the results of cytological examination conducted in the study group, benign lesions were observed in 20 smears, while 3 examinations revealed ASC-US, atypical squamous cells of undetermined significance. Potentially malignant or malignant lesions were observed in 43 examinations, 11, 12, and 21 results for LSIL, HSIL, and HSIL with features of invasion (Table 3). Comparing diagnoses based on cytological examination with the results of histopathological examination, we observed a strong, statistically significant (p < 0.001) correlation between diagnoses made on the basis of cytological and histopathological examination (Table 5, Fig. 4) The accuracy of brush cytology as a diagnostic test for laryngeal cancer in the conducted study indicates that the sensitivity and specificity of the test reaches 90.09% and 93.5% with 4 false negative and 3 false positive results. Positive prediction, the predicted frequency of the disease in individuals who tested positive (+) is 93%, and negative prediction, the predicted frequency of the lack of the disease in individuals who tested negative (-), is 91.5%. The likelihood index was: LR+ 13.94 i LR- 0.10. Such values of likelihood indices are frequently decisive, and cytological examination meets both of these criteria. In addition, we obtained kappa coefficient (k = 0.732) with quadratic weights identical to the intraclass correlation coefficient. The obtained result (p-value < 0.001) shows that diagnoses made by both diagnosticians, the cytologist and the histopathologist, are consistent. Referring to Altman, Landis, and Koch classification, we can conclude that

the results of histopathology and cytology are highly consistent (Table 7).

In the case of laryngeal cancer, the most common malignancy of the head and neck, we can achieve satisfactory results of treatment when the disease is diagnosed at an early stage [19]. In the advanced stage of the disease, only 22% to 50% of patients survive 5 years [4]. Histopathological examination constitutes the "gold standard" in diagnostic evaluation of laryngeal cancer [20]. It is a reliable, yet time-consuming examination, in the case of which tissue is collected with the use of laryngeal microsurgery performed under general anesthesia during hospitalization. Histopathological diagnoses of low- or moderate-grade dysplasia require careful observation of the patient, due to the risk of intraepithelial transformation into high-grade dysplasia, carcinoma in situ, or an invasive carcinoma. Sometimes clinicians also deal with the problem of a discrepancy between the results of clinical and histopathological examination. In such circumstances, clinicians repeat surgical biopsies, which carries further risk of perioperative complications, may contribute to impairing the function of the voice organ and constitutes an economic burden on the health care system. A diagnostic tool that is widely available, minimally invasive, cheap and simple to use, and that can be applied both in diagnostic evaluation conducted on an outpatient basis and at the hospital, is needed for early diagnostic evaluation of precancerous conditions and laryngeal malignancies as well as the follow-up after surgical treatment.

For years, brush cytology has been used as a screening examination in diagnostic evaluation of uterine cervical cancer, even though the diagnostic accuracy of cytological smears ranges from 41% for ASC-US diagnoses to 73% for HSIL diagnoses, with many false positive and false negative results [21]. The main drawback of cytological examination is that during the examination it is impossible to obtain the level of the basal membrane and the dispersion of cancer cells in relation thereto, which makes it impossible to differentiate between high-grade dysplasia, carcinoma in situ, or invasive carcinoma. Therefore, in the case of cytological examinations, a different classification should be used for categorizing cytologically benign lesions, LSIL, HSIL, and HSIL with features of invasion or ASC-US.

The role of brush cytology in diagnostic evaluation of laryngeal diseases has been studied for years. In the 1980s, in a large group of 350 patients, Lundgreen et al. obtained sensitivity and specificity of brush cytology of 83% and 84% in diagnostic evaluation of moderate- and high-degree dysplasia and laryngeal cancer. The results obtained in this study are much better. False negative results obtained from patients previously treated with radiotherapy due to head and neck cancer may be different. In the present study, such criterion was used to exclude patients from the study group [22]. Results of analysis similar to those described were obtained in 1994 by Waloryszak and Makowska, whose research revealed examination consistency of 91% in a group of 70 patients with laryngeal cancer; the other cases were false negative results [23]. In the study conducted by Malamou-Mitsi et al., the obtained specificity and sensitivity were equal to 100% and 93%, with one false negative result in the case of laryngeal non-Hodgkin lymphoma [24]. In 2006, researchers conducted a study aimed at diagnosing cancer of the glottis with the use of brush cytology and laryngostroboscopy performed prior to the procedure of cellular material and specimen collection. The sensitivity of this diagnostic method combining both examinations was as high as 97% [25]. Chatziavramidis et al. analyzed the consistency of brush cytology and liquid base cytology (LBC) with histopathological examination. Exclusion criteria in the study group included a history of radiotherapy of the head and neck region and cardiovascular or pulmonary comorbidities. Despite the restrictions in the selection of the study group and adding liquid base cytology to diagnostic evaluation, the authors obtained a specificity of 90% and sensitivity of 85%, which is worse than in the conducted analysis.

5. Conclusions

In the case of laryngeal cancer, brush cytology does not compete with histopathological examination and should not be decisive in the making of a cancer diagnosis. The described analysis, however, shows that the results of the discussed studies are significantly consistent. Such indicators as sensitivity and specificity as well as positive and negative prediction exceeding 90% prove that brush cytology may be a very good auxiliary diagnostic tool in the case of laryngeal cancer. It could also be used in screening diagnostic evaluation for laryngeal cancer when monitoring patients with dysplasia, or evaluating distant effects of surgical treatment performed within the framework of both inpatient and outpatient procedures.

Abbreviations

ASC-US - atypical squamous cell of undetermined significance, **HSIL** - high-grade squamous intraepithelial lesion, **LSIL** - low-grade squamous intraepithelial lesion, **SCC** - squamous cell carcinoma.

Authors' Contribution

P.L.: research concept and design, carrying out the experiment, aquisiting of date, data analysis and interpretation, writing – original draft preparation, visualization, literature review; **T.Z.**: research concept and design, supervising the project, writing – review and editing, final proofreading and approval of version for publication; **S.B.**: supervising the project, visualization, final proofreading and approval of the version for publication; **P.L.**: data analysis and interpretation, visualization, literature review, final proofreading and approval of the version for publication; **A.H.**: research concept and design, supervising the project, ac-

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Conflict of Interest

The authors have no potential conflicts of interest to declare.

Ethics Approval

Approval of the Bioethics Committee of the Wroclaw Medical University was obtained (number of approvement KB-519/2018).

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