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Porównanie wyników cytologii szczoteczkowej z wynikami badania histopatologicznego w stanach przednowotworowych i nowotworach krtani

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Rozprawa doktorska

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Drugi promotor: prof. dr hab. Agnieszka Hałoń

Panu dr hab.n.med. Tomaszowi Zatońskiemu
i Pani prof. dr hab. n. med. Agnieszce Hałoń
składam serdeczne podziękowania za zaangażowanie
i wskazanie właściwej drogi w tworzeniu niniejszej dysertacji.
Panu dr n.med. Szczepanowi Barnasiowi składam szczególne podziękowania
za stworzenie możliwości przeprowadzenia badań będących bazą dla tej pracy.

Mojej wspaniałej Rodzinie

SPIS TREŚCI:

1. WYKAZ SKRÓTÓW

ASCUS - atypical squamous cell of undetermined significance, nieprawidłowe komórki nabłonka wielowarstwowego płaskiego o nieokreślonym znaczeniu

CT - computed tomography, tomografia komputerowa

FI - cytologically benign lesions, prawidłowy wynik badania cytologicznego

HPV - human papillomavirus, wirus brodawczaka ludzkiego

HSIL - high-grade squamous intraepithelial lesion, zmiana śródnabłonkowa dużego stopnia

LSIL - low-grade squamous intraepithelial lesion, zmiana śródnabłonkowa małego stopnia

MRI - magnetic resonance imaging, obrazowanie rezonansu magnetycznego

PET - positron emission tomography, pozytonowa tomografia emisyjna

SCC - squamous cell carcinoma, rak płaskonabłonkowy

SSE - stratified squamous epithelium, nabłonek wielowarstwowy płaski

VEGF - vascular endothelial growth factor, czynnik wzrostu śródbłonka naczyniowego

2. STRESZCZENIE

Według danych epidemiologicznych rak krtani stanowi 1,1% wszystkich chorób nowotworowych i jest najczęstszym nowotworem w zakresie głowy i szyi. O rozpoznaniu raka krtani decyduje wynik badania histopatologicznego tkanki uzyskanej podczas zabiegu diagnostyczno-leczniczego, którym jest bezpośrednia laryngoskopia wykonywana w znieczuleniu ogólnym dotchawiczym w warunkach sali operacyjnej. W celu minimalizowania ryzyka, jakie niesie za sobą zabieg operacyjny w znieczuleniu ogólnym, obniżania kosztów leczenia oraz skrócenia czasu oczekiwania zarówno na sam zabieg, jak i wynik badania, można rozważyć zastosowanie innych, pomocniczych procedur. Zarówno w ginekologii, jak i pulmonologii, narzędziem stosowanym w diagnozowaniu oraz kontrolowaniu raka szyjki macicy oraz raka płuca i oskrzela jest cytologia szczoteczkowa.

Celem pracy doktorskiej było:

1. Stwierdzenie, czy w cytologii szczoteczkowej jest możliwa ocena stanów przednowotworowych oraz nowotworowych krtani.
2. Określenie korelacji wyników cytologii szczoteczkowej z badaniem histopatologicznym materiału z biopsji chirurgicznej pobranej z tej samej okolicy krtani.
3. Wykazanie, czy cytologia szczoteczkowa jako procedura małoinwazyjna, nieczasochłonna i tania, może być wykorzystywana jako badanie wstępne w określeniu charakteru zmian w krtani oraz czy możliwe jest utworzenie algorytmu dla wstępnego etapu diagnostyczno-terapeutycznego w chorobach krtani, w którym cytologia szczoteczkowa byłaby elementem towarzyszącym badaniu endoskopowemu krtani.
4. Charakterystyka pacjentów ze zmianami przednowotworowymi i nowotworami krtani.

Cele te realizowano poprzez przeprowadzenie dwóch projektów badawczych, które stanowiły podstawę do artykułów współtworzących cykl.

W projektach tych w latach 2019-2020 wzięło udział 92 pacjentów hospitalizowanych w Klinice Otolaryngologii Chirurgii Głowy i Szyi 4 Wojskowego Szpitala Klinicznego z Poliklinika we Wrocławiu w latach 2019-2020. Kryterium włączenia do badania była choroba krtani, wymagająca zabiegu diagnostyczno-leczniczego, czyli mikrochirurgii krtani w zestawie Kleinsassera. Z badania wyłączono natomiast osoby, które przebyły radioterapię okolicy głowy i szyi. W trakcie procedury diagnostyczno-leczniczej ze zmienionej chorobowo okolicy pobierany był materiał do badania cytologicznego, a następnie tkanka do badania histopatologicznego.

W pierwszym projekcie badawczym 92 badania cytologiczne zostały ocenione przez autorkę pracy przy współudziale doświadczonego patologa i cytologa pani prof. dr hab. Agnieszki Hałoń. Cytologię oceniano na podstawie skali własnej, która polegała na modyfikacji skali Bethesda. Spośród 92 rozmazów 2 badania nie nadawały się do oceny, pozostałe 90 rozmazów poddano ocenie. Oceniano liczne parametry cytologiczne, przyporządkowując uzyskane wyniki do jednej z pięciu grup:

- I zmiany cytologicznie łagodne (FI)
- II zmiana LSIL
- III zmiana HSIL
- IV zmiana HSIL z cechami inwazji
- V ASCUS

Wyniki badań cytologicznych poddano dokumentacji fotograficznej, a otrzymane fotografie stanowią reprezentatywny zbiór obrazów cytologicznych krtani. Badanie potwierdziło możliwość uzyskania materiału komórkowego nabłonka krtani do badania cytologicznego i potencjał rozpoznania na podstawie cytologii stanów przednowotworowych i nowotworów krtani.

W drugim projekcie badawczym wyniki 90 wykonanych badań cytologicznych zostały zestawione z wynikami badań histopatologicznych poszczególnych pacjentów.

Podczas grupowania wyników badania histopatologicznego zastosowano podział:

- I zmiana histopatologicznie łagodna
- II zmiana o charakterze śródnabłonkowej neoplazji niskiego stopnia
- III zmiana o charakterze śródnabłonkowej neoplazji dużego stopnia
- IV rak inwazyjny

Porównując diagnozy oparte na badaniach cytologicznych z wynikami badań histopatologicznych, zaobserwowano silną, istotną statystycznie (p<0,001) współzależność pomiędzy rozpoznaniami na podstawie badań cytologicznych i histopatologicznych. Trafność cytologii szczoteczkowej jako testu diagnostycznego raka krtani w przeprowadzonym badaniu wykazuje czułość i swoistość testu na poziomie 90,9% i 93,5%. Wyniki analizy statystycznej wykazują, że cytologia szczoteczkowa może być bardzo dobrym pomocniczym narzędziem diagnostycznym w diagnozowaniu stanów przednowotworowych i raka krtani. Mogłaby być również stosowana w diagnostyce przesiewowej w kierunku raka krtani, w przypadku monitorowania pacjentów z dysplazją lub do oceny odległych skutków leczenia chirurgicznego zarówno w ramach procedur szpitalnych, jak i ambulatoryjnych.

Publikacją rozpoczynającą cykl jest praca poglądowa przedstawiająca dotychczasowe doniesienia dotyczące badań cytologicznych materiału pobranego z krtani i porównania wyników z rezultatami badania histopatologicznego. Omówiono wady i zalety cytologii szczoteczkowej jako badania diagnostycznego w rozpoznawaniu chorób krtani.

Cytologia szczoteczkowa jako metoda ogólnodostępna, nieinwazyjna i tania może być z powodzeniem stosowana w diagnostyce łatwo dostępnych okolic. Wyniki przeprowadzonych projektów wskazują, że możliwe jest uzyskanie reprezentatywnego materiału do badania cytologicznego z nabłonka krtani, gdy cytologia pobierana jest w znieczuleniu ogólnym pacjenta. Rozwiązaniem, które pozwoliłoby cytologii szczoteczkowej stać się szeroko stosowanym narzędziem diagnostycznym w chorobach krtani byłoby uzyskanie materiału do badania w znieczuleniu miejscowym pod kontrolą nasofiberoskopu. Tak więc otrzymane wyniki mogą stanowić punkt wyjścia do dalszych badań dotyczących roli cytologii szczoteczkowej w diagnostyce stanów przednowotworowych i nowotworów krtani.

Publikacje wchodzące w skład cyklu:

1. Paulina Lepka, Agnieszka Hałoń, Szczepan Barnaś, Tomasz Zatoński: The combination of brush cytology with the results of histopathological examination in laryngeal cancer diagnosis. Advences in Hygiene and Experimental Medicine, DOI: 10.2478/ahem-2021-0030. IF: 0,878, MEiN: 40.
2. Paulina Lepka, Tomasz Zatoński, Szczepan Barnaś, Emilia Jaśkiewicz-Burnejko, Agnieszka Hałoń: Analysis of laryngeal brush biopsy-based cytology results in patients of the 4th Military Teaching Hospital and Polyclinic in Wrocław in years 2019–2020. Otolaryngologia Polska 2022;76(2):1-9, DOI: 10.5604/01.3001.0015.7083. MEiN:100.
3. Paulina Lepka, Tomasz Zatoński, Szczepan Barnaś, Piotr Lepka, Agnieszka Hałoń: Correlation between brush cytology results and histopathological examination in diagnostic evaluation of precancerous conditions and laryngeal cancer-a prospective study. Advences in Hygiene and Experimental Medicine, DOI:10.2478/ahem-2022-0015. IF: 0.878, MEiN: 40.

ABSTRACT

According to epidemiological data, laryngeal cancer accounts for 1.1% of all neoplastic diseases and is the most common cancer in the head and neck area. The diagnosis of laryngeal cancer is determined by the result of histopathological examination of tissue obtained during a diagnostic and therapeutic procedure, namely direct laryngoscopy performed under general endotracheal anaesthesia in operating room conditions. Other auxiliary procedures may be considered to minimise the risk associated with surgery under general anaesthesia, reduce treatment costs and shorten the waiting time for both the procedure itself and the examination result. In both gynaecology and pulmonology, the tool used for the diagnosis and monitoring of cervical, lung and bronchial cancers is brush cytology.

The aim of the doctoral dissertation was:

1. To determine whether precancerous and cancerous conditions of the larynx can be evaluated through brush cytology.
2. To determine the correlation between brush cytology results and histopathological examination of surgical biopsy material sampled from the same laryngeal region.
3. To demonstrate whether brush cytology, as a minimally invasive, time-effective and inexpensive procedure, can be used as a preliminary examination to determine the nature of laryngeal lesions and whether it is possible to create an algorithm for the initial diagnostic and therapeutic stage in laryngeal diseases in which brush cytology would be an adjunct to the endoscopic examination of the larynx.
4. To characterise patients with precancerous lesions and cancers of the larynx.

These objectives were pursued by carrying out two research projects that formed the basis for the articles co-creating this series.

These projects, conducted in 2019–2020, involved 92 patients hospitalised at the Department of Otolaryngology, Head and Neck Surgery of the 4th Military Teaching Hospital and Polyclinic in Wrocław. The inclusion criterion was a laryngeal disease that required a diagnostic and therapeutic procedure, namely laryngeal microsurgery with a Kleinsasser set. However, the study excluded patients who had received radiotherapy to the head and neck area. During the diagnostic and therapeutic procedure, two samples were taken from the affected area: material for cytological examination and then tissue for histopathological examination.

In the first research project, 92 cytological examinations were evaluated by the author of the paper in cooperation with an experienced pathologist and cytologist, prof. dr hab. Agnieszki Hałoń. The cytology was evaluated using an original scale that was a modification of the Bethesda scale. Out of 92 smears, two were not evaluable. The remaining 90 were evaluated. Numerous cytological parameters were evaluated and the obtained results were assigned to one of the five groups:

- I cytologically benign lesions (FI)
- II LSIL lesion

- III HSIL lesion
- IV HSIL lesion with features of invasion
- V ASCUS

The results of cytological examinations have been documented as photographs, which constitute a representative collection of cytological images of the larynx. The study confirmed the possibility of obtaining cellular material from laryngeal epithelium for cytological examination and the potential for cytology-based diagnosis of precancerous conditions and cancers of the larynx.

In the second research project, the results of the 90 cytological examinations performed were compared with the results of the histopathological examinations of individual patients.
The following division was used when grouping the histopathological examination results:

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

When comparing diagnoses based on cytological examinations with the results of histopathological examinations, a strong, statistically significant ($p < 0.001$) correlation was observed between diagnoses made on the basis of cytological and histopathological examinations. 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 reach respectively 90.9% and 93.5%. The results of the statistical analysis show that brush cytology can be a very good auxiliary diagnostic tool for diagnosing precancerous conditions and cancers of the larynx. It could also be used in screening diagnosis for laryngeal cancer, in monitoring patients with dysplasia, or to evaluate the long-term effects of surgical treatment provided as part of both inpatient and outpatient procedures.

The publication opening in the series is a review paper presenting previous reports on cytological examinations of material sampled from the larynx and comparisons of the results with those of histopathological examination. It discusses the advantages and disadvantages of brush cytology as a diagnostic test for the recognition of laryngeal diseases.

As a generally accessible, non-invasive and inexpensive method, brush cytology can be successfully used in the diagnosis of easily accessible areas. The results of the projects carried out indicate that it is possible to obtain representative material for cytological examination from the laryngeal epithelium when the smear is sampled under general anaesthesia. A solution that would allow brush cytology to become a widely used diagnostic tool in laryngeal diseases would be obtaining the examination material under local anaesthesia using a nasofiberoscope. Thus, the results obtained may provide a starting point for further research on the role of brush cytology in the diagnosis of precancerous conditions and cancers of the larynx.

Publications included in the series:

1. Paulina Lepka, Agnieszka Hałoń, Szczepan Barnaś, Tomasz Zatoński: The combination of brush cytology with the results of histopathological examination in laryngeal cancer diagnosis. Advances in Hygiene and Experimental Medicine, DOI: 10.2478/ahem-2021-0030. IF: 0,878, MEiN: 40.
2. Paulina Lepka, Tomasz Zatoński, Szczepan Barnaś, Emilia Jaśkiewicz-Burnejko, Agnieszka Hałoń: Analysis of laryngeal brush biopsy-based cytology results in patients of the 4th Military Teaching Hospital and Polyclinic in Wrocław in years 2019–2020. Otolaryngol Pol 2022; 76(2):1-9, DOI: 10.5604/01.3001.0015.7083.
3. Paulina Lepka, Tomasz Zatoński, Szczepan Barnaś, Piotr Lepka, Agnieszka Hałoń: Correlation between brush cytology results and histopathological examination in diagnostic evaluation of precancerous conditions and laryngeal cancer – a prospective study. Advances in Hygiene and Experimental Medicine, DOI:10.2478/ahem-2022-0015. IF: 0.878, MEiN: 40.

3. WSTĘP

Według Krajowego Rejestru Nowotworów liczba zachorowań na nowotwory złośliwe krtani w 2010 roku wynosiła 2200 i obejmowała 1900 mężczyzn i około 300 kobiet, co stanowi około 2,7% zachorowań na nowotwory złośliwe u mężczyzn i 0,4% u kobiet. Przeżycia 5-letnie wśród pacjentów z nowotworami krtani utrzymują się na poziomie 51% u mężczyzn i 63% u kobiet. Zarówno zachorowalność jak i umieralność z powodu raka krtani w Polsce jest wyższa niż w innych krajach Unii Europejskiej. Rak krtani, według amerykańskich statystyk, jest jedną z niewielu chorób onkologicznych, w których wskaźnik przeżycia zmniejszył się w ciągu ostatnich lat, z 66% do 63%, chociaż ogólna zapadalność spada. Przyjmuje się, że u ponad 60% pacjentów choroba w momencie rozpoznania jest w III-IV stadium zaawansowania, co wiąże się z niekorzystnym rokowaniem. Ujawnia to potrzebę dalszych badań i innowacji w tej dziedzinie. Palenie tytoniu i spożywanie wysokoprocentowych alkoholi to główne czynniki rozwoju raka krtani. Wpływ infekcji onkogennym typem wirusa brodawczaka ludzkiego na proces transformacji nowotworowej w procesie raka krtani jest znacznie mniejszy niż w przypadku patogenezy raka ustnej części gardła. Objawy takie jak: chrypka, osłabienie głosu, zaburzenia połykania, duszność, guz na szyi czy otalgia powinny skłonić do dalszej diagnostyki w kierunku raka krtani. Najczęstszym typem histologicznym wśród nowotworów złośliwych krtani jest rak płaskonabłonkowy. Rozwój raka płaskonabłonkowego jest poprzedzony zmianami w komórkach nabłonka, proces ten determinuje powstanie dysplazji. Leukoplakia, erytroplakia czy owrzodzenie nabłonka to obrazy kliniczne, które budzą podejrzenie transformacji nowotworowej, a w rozpoznaniu histopatologicznym przybierają postać różnego stopnia dysplazji, carcinoma in situ czy raka inwazyjnego. Celem oszacowania zaawansowania choroby wykonuje się badania obrazowe, tomografię komputerową, rezonans magnetyczny, pozytonową tomografię emisyjną. Stadium zaawansowania choroby w momencie rozpoznania jest bardzo ważnym czynnikiem predykcyjnym, dlatego ważne jest usprawnienie diagnostyki raka krtani i wykrywanie zmian we wczesnym stadium rozwoju. W procesie rozpoznania raka krtani „złotym standardem" jest badanie histopatologiczne materiału pobranego podczas mikrochirurgicznego zabiegu diagnostyczno-leczniczego w znieczuleniu ogólnym. Trudności diagnostyczne pojawiają się w procesie monitorowania skutków leczenia u pacjentów z rakiem krtani lub w przypadku - często długoletnich - obserwacji pacjentów, u których rozpoznano stan przednowotworowy. Istotą obserwacji pacjenta z przebytym rakiem krtani jest wgląd na duży odsetek wznów choroby, jak i częste występowanie drugiego, pierwotnego raka tej okolicy, co spowodowane jest negatywnym wpływem czynników kancerogennych na całą błonę śluzową górnego odcinka drogi oddechowej i pokarmowej. Częste biopsje chirurgiczne w znieczuleniu ogólnym przyczyniają się do traumatyzacji delikatnych tkanek krtani i narażają pacjenta na ryzyko związane z zabiegiem w znieczuleniu ogólnym. Jednym z rozwiązań tego problemu jest ocena krtani w obrazowaniu wąską wiązką światła (narrow band imaging). Badanie to umożliwia wykrycie nawet niewielkich zmian nabłonka krtani. Endoskop dający możliwość obrazowania wąską wiązką światła jest urządzeniem drogim i niestety nie stanowi standardu wyposażenia oddziału czy poradni laryngologicznej. Zarówno w ginekologii, jak i pulmonologii, narzędziem pomocniczym w diagnozowaniu i kontrolowaniu chorób nowotworowych szyjki macicy, oskrzela i płuca jest cytologia szczoteczkowa. Biorąc pod uwagę okres 5-letniego przeżycia, niemały odsetek wznów raka krtani oraz aspekty ekonomiczne związane z hospitalizacją i rekonwalescencją pacjenta w przypadku biopsji chirurgicznych krtani, celowe wydaje się poszukiwanie innych, mniej inwazyjnych, powtarzalnych, powszechnie dostępnych i czułych metod diagnostycznych.

4. CELE I ZAŁOŻENIA PRACY

Celem pracy doktorskiej było określenie przydatności cytologii szczoteczkowej do oceny stanów przednowotworowych i nowotworów krtani, w szczególności:

1. Stwierdzenie, czy w cytologii szczoteczkowej jest możliwa ocena stanów przednowotworowych oraz nowotworowych krtani.
2. Określenie korelacji wyników cytologii szczoteczkowej z badaniem histopatologicznym materiału biopsji chirurgicznej pobranej z tej samej okolicy krtani.
3. Wykazanie, czy cytologia szczoteczkowa jako procedura małoinwazyjna, szybka i tania, może być wykorzystywana jako badanie wstępne w określeniu charakteru zmian w krtani oraz czy możliwe jest utworzenie algorytmu dla wstępnego etapu diagnostyczno-terapeutycznego w chorobach krtani, w którym cytologia szczoteczkowa byłaby stałym elementem towarzyszącym badaniu endoskopowemu krtani.
4. Charakterystyka pacjentów ze zmianami przednowotworowymi i nowotworami krtani.

Założone cele badawcze zrealizowano poprzez przeprowadzenie dwóch projektów badawczych, z których każdy stanowił podstawę dla artykułu współtworzącego cykl.

5. MATERIAŁ I METODY

Materiał stanowi 92 pacjentów hospitalizowanych w Klinice Otolaryngologii Chirurgii Głowy i Szyi 4 Wojskowego Szpitala Klinicznego z Polikliniką we Wrocławiu, którzy poddani zostali zabiegowi diagnostyczno-leczniczemu, jakim jest mikrochirurgia krtani w zestawie Kleinsassera w znieczuleniu ogólnym w warunkach Zintegrowanego Bloku Operacyjnego. Do badania zakwalifikowani zostali pacjenci, którzy z powodu choroby krtani wymagali zabiegu mikrochirurgicznego, kryterium wyłączenia natomiast była przebyta radioterapia okolicy głowy i szyi. Uzyskano zgodę Komisji Bioetycznej Uniwersytetu Medycznego we Wrocławiu o numerze KB-519/2018 na przeprowadzenie badań. Badania te przeprowadzone były zgodnie z rekomendacjami Deklaracji Helsińskiej. Pacjenci otrzymali wnikliwe informacje dotyczące badania i wyrazili pisemną zgodę na udział w badaniu. Ochrona wrażliwych danych osobowych odbyła się z zaleceniami Europejskiej Rady Ochrony Danych Osobowych. W większości przypadków mikrochirurgia krtani odbyła się w trybie planowym, u 11 pacjentów połączona była z pilną tracheotomią.

5.1. Materiał i metodyka analizy badania cytologicznego krtani.

Podczas zabiegu diagnostyczno-leczniczego, jakim jest mikrochirurgia krtani w zestawie Kleinsassera w znieczuleniu ogólnym, w pierwszej kolejności ze zmienionej chorobowo błony śluzowej krtani pobierano materiał cytologiczny przy pomocy jednorazowej, jałowej szczoteczki cytologicznej z prostą końcówką wykonaną z włosia, producenta SoftMed o długości 195 mm. Pobrany materiał nanoszony był na szkiełko podstawne Thermo Scientific, Superftost i utrwalany preparatem Cytofix producenta Sanko. Osuszony materiał poddawany był barwieniu hematoksyliną i eozyną. 92 badania zostały ocenione przez autora pracy doktorskiej przy współudziale doświadczonego cytologa i patologa pani prof. dr hab. Agnieszki Hałoń. Do oceny badań służył wielostanowiskowy mikroskop firmy Leica model DM4B z powiększeniem od 2,5x do 60x i z możliwością fotograficznej archiwizacji. Cytologię oceniano na podstawie skali własnej, która polegała na modyfikacji skali Bethesda używanej do oceny szyjki macicy.

Tabela 1. Cytologiczna ocena rozmazu z krtani

Typ preparatu cytologicznego	• Konwencjonalny. • Wykonany inną metodą.
Ogólna jakość rozmazu	• Nadaje się do oceny cytologicznej. • Nadaje się do oceny cytologicznej, podsuszony. • Nie nadaje się do oceny cytologicznej (nie wykonano procedury technicznej albo preparat opracowany technicznie, ale zbyt podsuszony, źle utrwalony, ubogo komórkowy, nieczytelny z powodu licznych komórek zapalnych, erytrocytów).
Liczebność komórek w rozmazie	• Bogatokomórkowy. • O umiarkowanej liczbie komórek. • Ubogo komórkowy.
Ilość erytrocytów w rozmazie	• Liczne erytrocyty. • Mało/umiarkowana ilość erytrocytów. • Brak erytrocytów.
Stopień nasilenia stanu zapalnego	• Niewielkie nasilenie stanu zapalnego. • Umiarkowany stopień nasilenia stanu zapalnego. • Znaczny stopień nasilenia stanu zapalnego.
Typ komórek zapalnych	• Limfocyty. • Granulocyty. • Limfocyty i granulocyty.
Ogólna charakterystyka rozmazu	• Nie stwierdza się śródnabłonkowej neoplazji ani raka, widoczne jedynie prawidłowe komórki nabłonka wielowarstwowego płaskiego. • Obraz cytologiczny nieprawidłowy. • W rozmazie obecne inne komórki niż komórki nabłonka wielowarstwowego płaskiego, obecne komórki nabłonka gruczołowego.
Nieprawidłowe komórki nabłonka	• Nieprawidłowe komórki nabłonka wielowarstwowego płaskiego o nieokreślonym znaczeniu ASCUS. • Zmiana śródnabłonkowa małego stopnia LSIL. • Zmiana śródnabłonkowa dużego stopnia HSIL. • Zmiana śródnabłonkowa dużego stopnia z cechami budzącymi podejrzenie inwazji (perły rogowe, zwijki keratyny, patologiczne mitozy).

Wszystkie rozmazy wykonane były metodą konwencjonalną. Spośród 92 rozmazów 2 badania nie nadawały się do oceny z powody złej jakości rozmazu, a tym samym braku czytelności. Pozostałe 90 rozmazów poddano ocenie. Wyniki badań cytologicznych podzielono na 5 grup:

- I zmiany cytologicznie łagodne (FI)
- II zmiana LSIL
- III zmiana HSIL
- IV zmiana HSIL z cechami inwazji
- V ASCUS

Do każdej z grup dołączono odpowiednią ilustrację fotograficzną.
Zebrane dane poddano analizie statystycznej.

5.2. Materiał i metodyka oceny korelacji pomiędzy wynikiem cytologii szczoteczkowej, a wynikiem badania histopatologicznego w rozpoznaniu stanów przednowotworowych i nowotworów krtani.

U 92 pacjentów podczas zabiegu diagnostyczno-leczniczego mikrochirurgii krtani, po pobraniu z chorobowo zmienionej tkanki cytologii szczoteczkowej, dokonywano biopsji wycinającej lub diagnostycznej materiału tkankowego przeznaczonego do badania histopatologicznego. Materiał utrwalano natychmiastowo w 10% roztworze formaliny buforowanej. Oceny histopatologicznej dokonywali specjaliści z Zakładu Patomorfologii 4 Wojskowego Szpitala Klinicznego z Polikliniką we Wrocławiu pod kierownictwem lek. Edmunda Prudlaka. Podczas grupowania wyników badania histopatologicznego zastosowano podział analogiczny do podziału stosowanego podczas grupowania wyników badania cytologicznego:

- I zmiana histopatologicznie łagodna
- II zmiana o charakterze śródnabłonkowej neoplazji niskiego stopnia
- III zmiana o charakterze śródnabłonkowej neoplazji dużego stopnia
- IV rak inwazyjny

Wyniki uzyskane podczas badania cytologicznego i histopatologicznego porównano i poddano analizie statystycznej.

Dokonano również ogólnej charakterystyki pacjentów ze zmianami przednowotworowymi i rakiem krtani. Dane o pacjentach uzyskano z dokumentacji lekarskiej: wywiad, historia choroby, protokół operacyjny, wynik badania histopatologicznego.

5.3. Analiza statystyczna

Zebrane dane socjodemograficzne i kliniczne poddano analizie statystycznej. Wykorzystano program STATISTICA vs. 13.3 PL.

Zmienne jakościowe nominalne (np. *płeć*) i porządkowe (np. *wykształcenie*) przedstawiono w tabelach wielodzielczych w postaci liczności (*n*) i proporcji (%). Do oceny istotności związku między dwiema zmiennymi jakościowymi wykorzystano test niezależności chi-kwadrat Pearsona.

Zmienne ilościowe (np. *wiek*) przedstawiono w tabelach w postaci wartości średnich (*M*) i odchyleń standardowych (*SD*) oraz statystyk pozycyjnych: median (*Me*), kwartyli dolnych (*Q*1), górnych (*Q*3), wartości najmniejszych (*Min*) i największych (*Max*). Zgodność rozkładów empirycznych zmiennych ilościowych z teoretycznym rozkładem gaussowskim weryfikowano za pomocą testu normalności *W* Shapiro-Wilka. Jednorodność wariancji weryfikowano za pomocą testu Levene'a.

Istotność różnic przeciętnych wartości zmiennych ilościowych w dwóch grupach weryfikowano za pomocą testu *U* Manna-Whitneya.

Do oceny zgodności wyników badań cytologicznych i histopatologicznych z biopsji chirurgicznej pobranej z tej samej okolicy wykorzystano współczynnik rzetelności kappa Cohena k. Z uwagi na kategorialny charakter diagnoz wykorzystano współczynniki ważone wagami kwadratowymi.

6. CYKL PUBLIKACJI STANOWIĄCYCH PODSTAWĘ PRACY DOKTORSKIEJ

Wynikiem pracy jest cykl trzech publikacji:

- 6.1. Paulina Lepka, Agnieszka Hałoń, Szczepan Barnaś, Tomasz Zatoński: The combination of brush cytology with the results of histopathological examination in laryngeal cancer diagnosis. Advences in Hygiene and Experimental Medicine, DOI: 10.2478/ahem-2021-0030. IF: 0.878, MEiN:40.
- 6.2. Paulina Lepka, Tomasz Zatoński, Szczepan Barnaś, Emilia Jaśkiewicz-Burnejko, Agnieszka Hałoń: Analysis of laryngeal brush biopsy-based cytology results in patients of the 4th Military Teaching Hospital and Polyclinic in Wrocław in years 2019–2020. Otolaryngologia Polska 2022;76(2): 1-9; DOI: 10.5604/01.3001.0015.7083. MEiN:100.
- 6.3. Paulina Lepka, Tomasz Zatoński, Szczepan Barnaś, Piotr Lepka, Agnieszka Hałoń: Correlation between brush cytology results and histopathological examination in diagnostic evaluation of precancerous conditions and laryngeal cancer-a prospective study. Advences in Hygiene and Experimental Medicine, DOI:10.2478/ahem-2022-0015. IF: 0.878, MEiN:40.

Łączna ocena publikacji cyklu wynosi:
IF: 1,756
Pkt. MEiN: 180

Ad 6.1. Wymieniony artykuł poglądowy stanowi podsumowanie aktualnego stanu wiedzy na temat zastosowania cytologii szczoteczkowej w diagnostyce chorób krtani, w tym stanów przednowotworowych i raków krtani. W pierwszej części artykułu opisano epidemiologię, histologię, czynniki ryzyka, objawy, diagnostykę, leczenie i rokowanie w przypadku raka krtani. Opisano stany przednowotworowe krtani oraz prognozowanie potencjału ich transformacji. W kolejnej części artykułu przedstawiono przegląd badań, w których oceniano przydatność cytologii szczoteczkowej jako badania służącego do diagnostyki chorób krtani, wyszczególniono różnice w metodologii pobierania materiału do badania cytologicznego, skupiono się na ukazaniu zalet i wad tej metody jako narzędzia diagnostycznego.
Ad 6.2. i 6.3. Opisy projektów badawczych zostały przedstawione w pkt. 5.

7. PUBLIKACJE

PRACA NAUKOWA

NR 1

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Postępy Higieny i Medycyny Doświadczalnej / Advances in Hygiene and Experimental Medicine

The combination of brush cytology with the results of histopathological examination in laryngeal cancer diagnosis

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Abstract

Recently, the incidence rate of head and neck cancer (HNC) has been increasing significantly. It is estimated that there are over 550,000 new cases per year, of which over 130,000 are laryngeal cancers. It is assumed that in more than 60% of patients the disease is diagnosed late, at stages III–IV, which is associated with unfavorable prognoses: the average survival ranges from 15% to 45%. The mainstay of successful tumor therapy is the early detection of neoplastic tissue. The laryngological examination with the use of traditional instruments should be expanded with an endoscopic examination of the larynx using optics in the outpatient clinics. This procedure is sufficient to select patients who need a direct laryngoscopy with a surgical biopsy, usually under general anesthesia in operating room conditions. However, it may bear potential complications. In 1941, Papanicolaou and Traut showed that brush cytology could be useful in detecting precancerous conditions and cervical cancers. For decades, research on the usefulness of brush cytology in diagnosing precancerous conditions and laryngeal cancers has been conducted. This paper aims to enable the reader to understand the issues related to laryngeal cancer and present the results of the previous use of brush cytology in the diagnostic process.

Keywords

Brush cytology • histopathological examination • laryngeal cancer

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Introduction

According to the National Cancer Registry, the laryngeal cancer incidence in 2010 reached 2,200 cases (1,900 men and approx. 300 women), representing about 2.7% of malignancies in men and 0.4% in women. More than 90% of cases occur in patients over 50; the risk increases with age, reaching its peak in men over 70 and women at the turn of the fifth and sixth decade of life. The incidence of laryngeal cancers in men constantly increased until the mid-1990s, after which an incidence decline was observed; however, it remains stable in women. The 5-year survival rate is 51% for men and 63% for women. In Poland, both the morbidity and mortality due to larynx cancer are higher than in other European Union countries [1, 2]. It is estimated that laryngeal cancer incidence in the United States of America in 2016 reached 13,430, with more than 3,620 deaths reported [3]. According to American statistics, it is one of the few oncological diseases for which the 5-year survival rate decreased over the last 40 years (from 66% to 63%). However, the overall incidence rate is decreasing [3]. It is assumed that in more than 60% of patients, the disease is diagnosed at stages III–IV, which is associated with unfavorable prognoses: the average survival ranges from 15% to 45% [4, 5]. This reveals the need for further research and innovation in this area. The risk factors for laryngeal cancer development include alcohol consumption and smoking [6]. Smoking is linearly related to laryngeal cancer; the risk among smokers is 10–15 times higher than among non-smokers. The heaviest smokers have up to 30 times higher risk of developing this illness [7, 8]. HPV plays a significant role in the pathogenesis of cancer of the oral pharynx. In contrast, the impact of

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oncogenic HPV infections (16,18,31,33,35) on the cancer transformation process in laryngeal malignancies appears to be much smaller and not fully understood. It is estimated that viral DNA incidence ranges from 20% to 30%, but this percentage varies among researchers and depends on the detection method applied [9, 10]. The most prevalent is squamous cell carcinoma (SCC). SCCs are divided into well differentiated (G1), moderately differentiated (G2), poorly differentiated (G3), and anaplastic (G4) carcinomas, depending on keratosis and several miotic figures [11]. Lesions in epithelial cells precede the development of SCC, which determines the formation of dysplasia. Roegan introduced the term *dysplasia* in 1958; it refers to atypical proliferation and lesions in both cell and epithelial structures [12, 13]. Leucoplakia, erythroplakia, and epithelial ulceration are clinical diagnoses that raise suspicions of neoplastic transformation; in histopathological diagnosis, they indicate dysplasia at various stages, carcinoma in situ, or invasive cancer [14]. In dysplasia, the neoplastic transformation grade is about 36.4% and represents a relatively late multistage carcinogenesis phase [15]. Forecasting of the transformation potential depends on the histological evaluation, which involves the quantitative determination of the degree of epithelial architecture abnormalities and cellular ones above the basal membrane [14]. Such symptoms as hoarseness, weakening of the voice, dysphagia, dyspnea, neck tumors, or ear pain should lead to further diagnostic processes for laryngeal cancer. The diagnosis should be based on a physical examination, taking risk factors into account, and on a laryngological analysis using a nasofiberscope. An optical technique, narrow band imaging (NBI), based on the modification of white light using special filters, is instrumental in detecting the microvascular abnormalities associated with the preneoplastic and neoplastic changes in the mucosal lining. This procedure can be performed as a diagnostic and during endoscopic laser cordectomy, as it allows a more precise assessment of the status of surgical margins of early-stage and locally advanced laryngeal cancers [16, 17]. Narrow band imaging is also a practical tool in laryngeal cancer follow-up after surgical procedures such as transoral laser microsurgery or radiotherapy [18]. This diagnostic process is not always sufficient; however, it can be supplemented with direct laryngoscopy using the collection of samples for histopathological examination, usually performed under general endotracheal anesthesia in operating room conditions. In cases of general anesthesia contraindications or cervical spine alterations, an alternative procedure should be applied. Videolaryngoscopy during conscious sedation might be considered as a surgical approach in patients who are not suitable for phonosurgery by microlaryngoscopy. It is essential to mention that during surgical biopsy of the larynx under local anesthesia, unexpected bleeding or laryngospasm may occur [19].

After receiving the histopathological diagnosis, it is necessary to evaluate the extent of the disease—that is, the disease stage at the time of diagnosis. Standard imaging techniques include computed tomography (CT), magnetic resonance imaging (MRI), and positron emission tomography (PET). The advantage of CT over MRI for laryngeal cancer is debatable; CT usually shows a higher specificity in the identification of thyroid cartilage invasion compared to MRI [20]. The disease stage at the time of diagnosis is a critical predictive factor. It is estimated that the T3–T4 stage patients are twice as likely to die compared to those at an earlier stage; the 5-year survival rate for T1 is about 95.6%, while for T4 it is only 52.1% [21]. Therefore, the effective diagnostic process leading to early-stage laryngeal cancer diagnosis through histopathological examination is of great importance. The histopathological examination is a gold standard in laryngeal SCC diagnosis. However, it is difficult to monitor the effects of treatment or conduct the follow-up of a patient with low- or high-grade dysplasia by performing regular laryngeal microsurgeries, which, in addition to tissue traumatization, carry the risk of infection or perioperative bleeding. Moreover, the follow-up of a patient with a history of laryngeal cancer is essential, due to the high rate of relapse and the frequent occurrence of a second primary cancer of 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 [22, 23]. To minimalize the risks associated with surgery under general anesthesia, other auxiliary procedures may be considered. In both gynecology and pulmonology, brush cytology is an additional tool for diagnosing and monitoring cervical, lung, and bronchial cancers [24].

Material and methods

This review aims to introduce the contribution of exfoliative cytology to the diagnosis of precancerous conditions and laryngeal cancers. It considers the following question: ”Is brush cytology a highly reliable and valuable diagnostic procedure for clinically suspected laryngeal lesions?”

A comprehensive and detailed literature search was conducted to answer the above question. The abstracts of the studies identified in the search were screened, and subsequently, full texts were selected. The primary source of literature included search engines and online databases with articles regarding medicine and life science, such as PubMed and Google Scholar. The other source of data was the Wroclaw Medical University library service.

Keywords or phrases used in the research included *brush cytology, exfoliative cytology of larynx*, and *otolaryngology*, combined with *cytology* and *histopathology*, *squamous cell carcinoma of the larynx*. The included criteria combined the use of histopathology with touch smear cytology in suspected malignant lesions of the larynx. The studies concerning only benign diseases were excluded. As a result, 11 relevant articles were identified across 66 years, from 1951 to 2017.

Discussion

In 1941, Papanicolaou and Traut showed that brush cytology could be useful in detecting precancerous conditions and cervical cancers [12]. The role of brush cytology in the diagnosis of laryngeal diseases has been explored for years. It appears that Morrison et al. were the first to present the results of the study (1964), which compared cytology and histopathology results in terms of laryngeal cancer – 8 out of 9 compared results were consistent [25]. In 1951, Friedmann compared brush cytology and histopathology results in 212 patients with suspected malignant lesions within the head and neck, 106 of whom were patients with suspected laryngeal cancer. The clinical diagnosis was confirmed histopathologically in 110 cases and cytologically in 105 cases. Friedmann also raised the importance of brush cytology for laryngeal diseases, concluding that laryngeal swabs have fewer non-specific cells than nasal, nasopharyngeal, or sinus ones [26]. In 1968, W. F. Frable and M. A. Frable obtained a positive result from brush cytology in 14 out of 19 laryngeal cancer cases. The authors also emphasize that the presence of hyperkeratosis in a brush smear should raise suspicion of SCC cells [27]. Glennie et al. (1976) obtained full compliance in cytological and histopathological diagnoses in benign lesions of the larynx. In malignancies, the result was consistent in 20 out of 22 cases [28]. In the early 1980s, in their prospective study involving 350 patients, Lundgren et al. achieved brush cytology sensitivity and specificity of 83% and 84% to detect moderate- and high-grade dysplasia, carcinoma in situ, or invasive cancer. The authors noted that false-negative swab results were found in patients who previously underwent radiotherapy of the head and neck region or were diagnosed with moderate-grade dysplasia. In this study, a positive result was obtained in the smear, whereas the surgical biopsy was negative. The result was confirmed by the next surgical biopsy [29]. In 1994, Waloryszak and Makowska, willing to evaluate the usefulness of brushing smears in detecting laryngeal cancer, examined 70 patients, finding consistent results in 91% of cases; the remaining 9% were false-negative results [30]. In 2000, Malamou-Mitsi et al. obtained 100% specificity of brush cytology in a similar study involving a group of 30 patients, without obtaining false-positive results and with a sensitivity of more than 93%, with one false-negative result of smear for non-Hodgkin’s lymphoma of the larynx [31]. In 2006, Ustundag et al., analyzing the results of 135 patients, obtained sensitivity and specificity of brush cytology of the order of 82.5% and 94.5% in the diagnosis of benign and malignant laryngeal lesions [32]. In 2006, Gugatschka et al. recruited 130 patients who underwent laryngostroboscopic examination before microsurgery of the larynx with the brushing smear collection. The results of the study showed the sensitivity of cytology as an isolated test in the range of 74%, while the combination of this diagnostic method with laryngostroboscopic examination made it possible to obtain over 97% sensitivity in the detection of glottic cancer [33]. In 2015, in their study involving 30 patients, Chatziavramidis et al. achieved 85% sensitivity and over 90% specificity using restrictive exclusion criteria. The study group did not include patients with radio- or chemotherapy for HNC nor patients with chronic cardiovascular and lung diseases. Brush cytology, liquid-based cytology, and surgical biopsy were performed. Additionally, VEGF expression was determined in the material from liquid-based cytology. The high VEGF expression is associated with a higher relapse rate and faster disease progression [34]. In the framework of the 2017 research conducted by Ajayan et al. involving a group of 50 patients, slightly over 70% sensitivity of brush cytology and 100% specificity were obtained. The sensitivity of brush cytology was significantly higher in the diagnosis of poorly differentiated squamous cell carcinomas (SCCs) [35]. In many cases, directly following the administration of anesthesia and applying a Kleinsasser laryngoscope, a brush smear was first performed, and then the histopathological examination sample was collected from the same region [25, 29, 31, 35]. Ustundag applied the reverse order in his study by first collecting the histopathological sample and then performing a brushing smear from the collected sample [32]. Despite differences in smear sample collection, the results reported by Ustundag do not differ significantly from others. In most cases, the sample collected during brush cytology was applied to a glass slide, fixed in 95% ethyl alcohol, and stained using Papanicolaou [25, 29, 31, 35] or May–Grünwald–Giemsa staining techniques [32]. Despite different staining techniques, Ustundag received comparable results.

The authors agree that brush cytology can be a useful auxiliary examination in the diagnostic process of precancerous conditions and laryngeal cancers. Lundgren et al. emphasize that a positive result of the brush cytology together with a negative biopsy result prompts the performance of a repeat biopsy. In contrast, a negative cytology result does not exclude cancer. Lundgren also noted that brush cytology results are less reliable in patients after

radiotherapy or with moderate-grade dysplasia [29]. On the other hand, Malamou-Mitsi et al. claim that brush cytology can be used as a stand-alone diagnostic method where a surgical biopsy is either contraindicated or impossible or frequently repeated biopsies can adversely affect the larynx structure and function [31]. Ustundag et al. state that the best diagnostic method combines histopathological and cytological examination [32]. At the same time, Gugatschka et al. highlight the role of stroboscopic examination, which in combination with brush cytology can be both a satisfactory screening test for detecting early glottic cancer and useful for long-term patient follow-up [33]. Ajayan et al. stress that brush cytology is a cheap and minimally invasive examination that provides a quick, simple, and reliable result and should be widely used in the diagnostic process of precancerous conditions and laryngeal cancers. Nevertheless histopathological examination remains the gold standard in making the diagnosis [35].

Conclusion

The results of brush cytology in detecting precancerous conditions and laryngeal cancers are ambiguous and differ in studies. Its sensitivity ranges from 70% to 93%; however, it usually reaches approximately 80% and can be increased to almost 97% when combined with another diagnostic tool such as laryngostroboscopy. Moreover, false-negative swab results are found in patients who previously underwent radiotherapy of the head and neck region. Therefore, brush cytology may not be a suitable diagnostic tool for all patients. It is necessary to underline that exfoliative cytology and histology are not competitive diagnostic procedures. Histopathology examination of laryngeal precancerous conditions and malignancy is a gold standard. Furthermore, brush cytology appears to be a very cheap, simple, cost-effective, and relatively accurate test that gives a speedy diagnosis. It may be performed under local anesthesia, and even consecutive cytology procedures do not lead to vocal folds, scars, or a voice impairment, which may occur after subsequent biopsies. To summarize, exfoliative cytology is not suitable for a final diagnosis, but it may be included as an additional tool in the early diagnostic process at the prehospitalization stage.

Abbreviations

CT - computed tomography, **HPV** - human papillomavirus, **MRI** - magnetic resonance imaging, **PET** - positron emission tomography, **SCC** - squamous cell carcinoma, **VEGF** - vascular endothelial growth factor.

Authors' Contribution

P.L.: reserch concept and design, acquisition of data, data analysis and interpretation, writing: original draft preparation, visualization, literature review; **A.H.**: supervising the project, data analysis and interpretation, writing: review and editing, final proofreading, and approval of the version for publication; **S.B.**: supervising the project, visualization, literature review, final proofreading and approval of the version for publication; **T.Z.**: supervising the project, data analysis and interpretation, writing: review and editing, final proofreading, and approval of the version for publication

Conflict of Interest

The authors have no potential conflicts of interest to declare.

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INDEX COPERNICUS
INTERNATIONAL

ORIGINAL ARTICLE

Analysis of laryngeal brush biopsy-based cytology results in patients of the 4th Military Teaching Hospital and Polyclinic in Wrocław in years 2019–2020

Authors' Contribution:
A – Study Design
B – Data Collection
C – Statistical Analysis
D – Data Interpretation
E – Manuscript Preparation
F – Literature Search
G – Funds Collection

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ABSTRACT:

Introduction: Cytological examination of exfoliated epithelial cells of the uterine cervix, oral cavity, or rectum has been successfully used in the diagnostics of pathological conditions of these organs for many years. In these cases, the test material is collected from the available regions.

Aim: The aim of the study consisted in the analysis of cytological smears of laryngeal epithelial cells from patients hospitalized at the Department of Otolaryngology, Head and Neck Surgery of the 4th Military Teaching Hospital and Polyclinic in Wrocław in years 2019–2020. The analysis was aimed at demonstrating whether representative laryngeal epithelial material could be obtained from brush biopsies.

Material and methods: The study was carried out in 92 subjects aged between 26 and 85 years, including 34 women (37.0%), from whom material for cytological examination had been collected from the larynx in the course of microsurgical procedures carried out using the Kleinsasser laryngeal instrument set in 2019–2020.

Results: Analysis was performed on 90 out of 92 cell smears (97.8%). Two smears were not qualified for analysis due to illegibility. The smears were assessed using a proprietary scale consisting in a modification of the Bethesda system. Abnormal results of cytological examinations were obtained in a majority of cases. HSILs with invasive features were the most common abnormal results of cytological examinations.

Conclusions: Laryngeal epithelial cells can be successfully evaluated by means of cytological examination. Abnormal presentation of cytological smear is frequently hypercellular, with inflammatory cells being observed less frequently. No statistically significant relationship was observed between the results of the cytological examination and the overall quality of the smear, number of cells, number of erythrocytes, or the severity of inflammation.

KEYWORDS: brush biopsy-based cytology, cytological smear, intraepithelial neoplasia, laryngeal brush biopsy-based cytology

ABBREVIATIONS

ASC-US – atypical squamous cells of undetermined significance
DNA – deoxyribonucleic acid
FI – unremarkable cytology
HE – hematoxylin/eosin
HSIL – high-grade squamous intraepithelial lesions
LSIL – low-grade squamous intraepithelial lesion
SD – standard deviation
SSE – stratified squamous epithelium

INTRODUCTION

Exfoliative cytology is a diagnostic technique based on microscopic evaluation of epithelial cells following their fixation and appropriate staining. The indirect method consists of examination of epithelial cells following spontaneous exfoliation while the direct method consists in the biopsy material being collected using a dedicated brush. In the conventional method, exfoliated cells are placed on a microscopic slide, fixed with the preservative, and evaluated under microscope following appropriate staining [usually the HE (hematoxylin/eosin) staining] [1, 2].

Brush biopsy-based cytology has been successfully used for many years in the diagnostics of cervical cancer [3–5]. It is also used as an aid in the diagnostics of rectal, bronchial, oral or nasal diseases [6–14].

Microscopic analysis of the biopsy material allows for evaluation of cytological parameters (according to the Papanicolau classification), the shape and appearance of epithelial cells, as well as other

morphological characteristics [15]. An abnormal result of cytological examination requires enhanced diagnostics for preneoplastic and neoplastic disorders and facilitates appropriate treatment if the diagnosis is confirmed [6, 7]. Laryngeal mucosa is characterized by a specific structure resulting from its location at the junction of the respiratory and digestive tracts. Stratified squamous epithelium (SSE), partially keratinized, covers the laryngeal surface of the glottis, vestibular folds, laryngeal vestibule, and vocal folds. The remaining parts of the larynx are covered by pseudostratified ciliated epithelium [16].

Reliable results of cytological examinations can be obtained by obtaining cell material from readily accessible areas, including the epithelium of the cervix, rectum, mouth, and nose.

AIM

The aim of this study was to determine whether representative laryngeal epithelial material could be obtained from brush biopsies for subsequent cytological examination.

MATERIAL AND METHODS

The study group consisted of 92 patients hospitalized at the Department of Head and Neck Surgery of the 4th Military Teaching Hospital and Polyclinic in Wrocław in years 2019–2020, in whom microsurgical procedures were performed within the larynx using the Kleinsasser laryngeal instrument set in general anesthesia. Qualified for the study were patients requiring a microsurgical procedure due to laryngeal disease. Excluded from the study were patients with a history of radiation therapy within the head and neck region. The study was approved by the Bioethics Committee of the Wrocław Medical University, decision no. KB-519/2018. The study was carried out in accordance with the recommendations of the Helsinki Declaration. Patients had received extensive information about the study and provided their written, informed consent to participate. Sensitive personal data were subject to protection pursuant to the recommendations of the European Data Protection Board. In most cases, laryngeal microsurgery was performed in an elective setting; in 11 cases, it was performed along with emergency tracheotomy. Microsurgery was carried out as a diagnostic procedure in cases when histopathological diagnosis was of key importance or as a diagnostic/therapeutic procedure when involving the resection of benign lesions. Indirect laryngoscopy and laryngeal endoscopy were performed during hospitalization and before the microsurgical procedure in all patients. The material for cytological examination was collected using a cytological biopsy brush applied to the affected area and rotated around its axis while being moved along the longitudinal axis. The collected material was transferred to microscopic slides (Thermo Scientific, Superfrost). The material was shaken off the brush and gently smeared on the surface of the slide. Next, the material was fixed using the Samko Cytofix preservative applied onto the slide over 1 second from a distance of 25 cm. The drying time was 4 minutes. The next step consisted in HE staining of the specimens. A total of 92

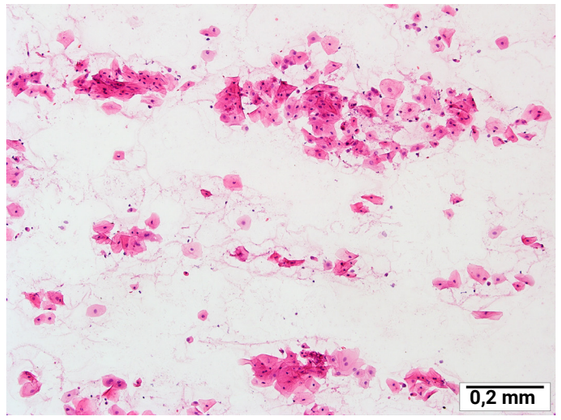

Fig. 1. Normal squamous epithelium.

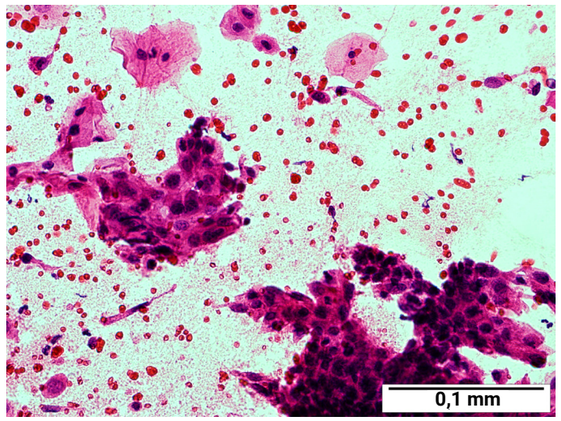

Fig. 2. Low-grade squamous intraepithelial lesions (LSILs).

cytological specimens were evaluated by the article's author with the help of an experienced pathologist and cytologist, prof. Agnieszka Haloń MD, PhD. Specimens were evaluated using a Leica multistation microscope with image archiving. The smears were assessed using a proprietary scale consisting in a modification of the Bethesda system as used in evaluation of cervical smears. All smears were performed using the conventional method. Out of the total 92 smears, two smears were unevaluable due to being excessively dried, poorly fixed, hypocellular, or illegible due to the presence of numerous a large number of inflammatory cells or erythrocytes. The remaining 90 smears were subjected to assessment. The results of cytological examinations were divided into 5 groups:

1. Cytologically benign lesions (FI),
2. LSILs,
3. HSILs,
4. HSILs with invasive features,
5. ASC-US.

Example of photographs material specimens are presented in the article (Fig. 1.–5.).

Tab. I. Number (percentage) of patients in groups presenting with different cytological examination results and clinical features.

VARIABLE	TOTAL N = 90	CYTOLOGY (BETHESDA SYSTEM)				
		FI N = 43	LSIL N = 11	HSIL N = 12	HSIL* N = 21	ASCUS N = 3
Tracheotomy						
Yes	11 (12.2%)	1 (2.3%)	0 (0.0%)	2 (16.7%)	7 (33.3%)	1 (33.3%)
No	79 (87.8%)	42 (97.7%)	11 (100.0%)	10 (83.3%)	14 (66.7%)	2 (66.7%)

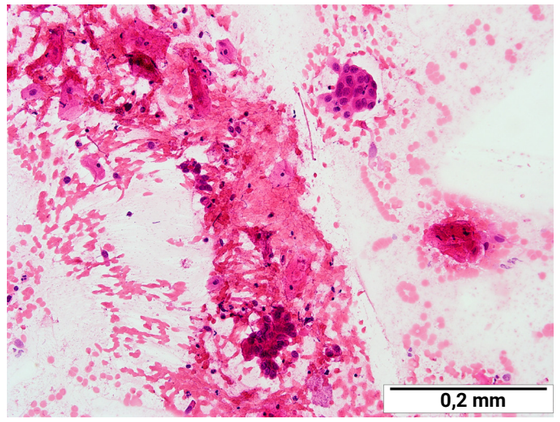

Fig. 3. High-grade squamous intraepithelial lesions (HSILs).

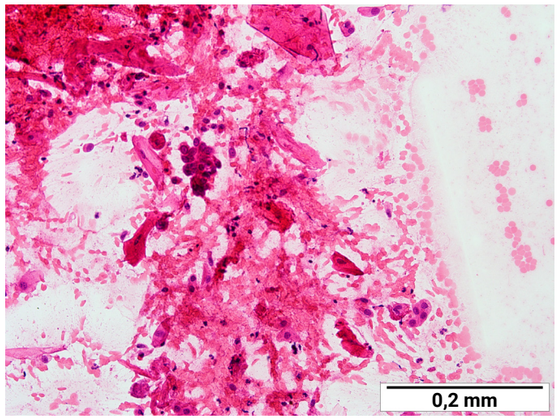

Fig. 5. Atypical squamous cells of undetermined significance.

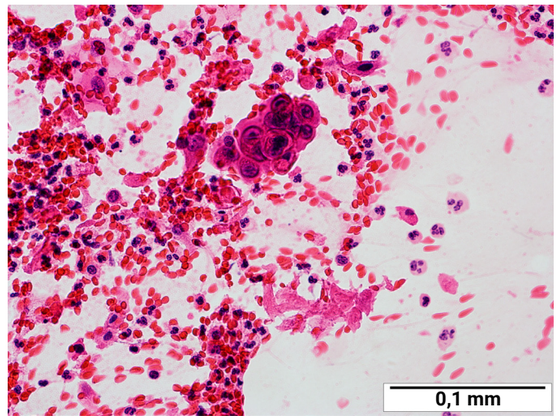

Fig. 4. High-grade squamous intraepithelial lesions with invasive features – cancer bulb visible.

Numbers of cells, numbers of erythrocytes, severity of inflammation and inflammatory cell types (lymphocytes, granulocytes, lymphocytes and granulocytes) were assessed in the smears. Smears were also classified using the overall smear types as follows: A – only normal SSE cells visible, no intraepithelial neoplasia or cancer; B – abnormal cytological presentation (karyopyknosis, perinuclear halo, cytoplasmic vacuolization, cell shape changes – ballooning degeneration, rounded shape, cellular cannibalism, cancer spheres); C – non-SSE cells present, glandular epithelial cells present.

The collected data was subjected to statistical analysis using the STATISTICA vs.13.3 PL package.

Pearson's chi-squared independence test was used to assess the significance of correlation between pairs of qualitative variables. Quantitative variables are presented in tables as mean values (M) and standard deviations (SD) along with positional statistics: medians (Me), lower quartiles (Q1), upper quartiles (Q3), minimum (min) and maximum values (max).

RESULTS

The test material consisted of 92 patients including 34 women (37.0%), all patients aged between 26 and 85 years (mean M = 62.0; standard deviation SD = 11.0) and undergoing treatment for laryngeal disorders. A total of 90 laryngeal epithelial smears were evaluated, the remaining 2 being discarded due to lack of legibility.

Specimen collection was performed in the course of emergency tracheotomy in 11 patients (12%). Tab. I. presents the clinical characteristics of patients in groups presenting with different cytological examination results.

A statistically significant relationship was observed between the necessity for tracheotomy and the results of cytological examination. Acute dyspnea was significantly more common in patients with HSILs presenting with invasive features than in

Tab. II. Number (percentage) of patients in groups presenting with different cytological examination results and smear features.

VARIABLE	TOTAL N = 90	CYTOLOGY (BETHESDA SYSTEM)				
		FI N = 43	LSIL N = 11	HSIL N = 12	HSIL* N = 21	ASCUS N = 3
Overall smear quality:	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Evaluable	61 (67.8%)	30 (69.8%)	8 (72.7%)	5 (41.7%)	16 (76.2%)	2 (66.7%)
Evaluable when air-dried	29 (32.2%)	13 (30.2%)	3 (27.3%)	7 (58.3%)	5 (23.8%)	1 (33.3%)
Smear cells:						
Abundant	66 (73.3%)	31 (72.1%)	10 (90.9%)	8 (66.7%)	15 (71.4%)	2 (66.7%)
Moderate	16 (17.8%)	5 (11.6%)	1 (9.1%)	4 (33.3%)	5 (23.8%)	1 (33.3%)
Scarce	8 (8.9%)	7 (16.3%)	0 (0.0%)	0 (0.0%)	1 (4.8%)	0 (0.0%)
Smear erythrocytes:						
Numerous	21 (23.3%)	6 (14.0%)	2 (18.2%)	5 (41.7%)	8 (38.1%)	0 (0.0%)
Sparse/Moderate	20 (22.2%)	8 (18.6%)	2 (18.2%)	4 (33.3%)	4 (19.0%)	2 (66.7%)
None	49 (54.4%)	29 (67.4%)	7 (63.6%)	3 (25.0%)	9 (42.9%)	1 (33.3%)
Severity of inflammation:						
Significant	45 (65.2%)	25 (80.7%)	5 (71.4%)	4 (40.0%)	11 (61.1%)	0 (0.0%)
Moderate	16 (23.2%)	5 (16.1%)	1 (14.3%)	4 (40.0%)	4 (22.2%)	2 (66.7%)
Mild	8 (11.6%)	1 (3.2%)	1 (14.3%)	2 (20.0%)	3 (16.7%)	1 (33.3%)
Cell type:						
Lymphocytes	51 (73.9%)	25 (80.6%)	4 (57.1%)	8 (80.0%)	12 (66.7%)	2 (66.7%)
Granulocytes	3 (4.4%)	0 (0.0%)	0 (0.0%)	2 (20.0%)	1 (5.5%)	0 (0.0%)
Lymphocytes and granulocytes	15 (21.7%)	6 (19.4%)	3 (42.9%)	0 (0.0%)	5 (27.8%)	1 (33.3%)
Smear type:						
Type A	14 (15.6%)	14 (32.6%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Type B	47 (52.2%)	0 (0.0%)	11 (100%)	12 (100%)	21 (100%)	3 (100%)
Type C	29 (32.2%)	29 (67.4%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)

FI – normal result, benign lesions; LSIL – low-grade squamous intraepithelial lesion; HSIL – high-grade squamous intraepithelial lesion; HSIL* – high-grade squamous intraepithelial lesion with invasive features; ASC-US – atypical squamous cells of undetermined significance; Type A – only normal SSE cells visible in the field; Type B – abnormal cytological presentation; Type C – non-SSE cells present, glandular epithelial cells present

patients with benign lesions (33.3% vs 2.3%; p = 0.004, Fig. 6.). Tab. II. presents the structural characteristics of all groups of smears.

The Pearson's chi-squared independence tests revealed no statistically significant relationship between the results of cytological examinations and the overall quality of the smear (p = 0.796), the number of cells in the smear (p = 0.281), the number of erythrocytes in the smear (p = 0.080), the severity of inflammation (p = 0.116), or the type of inflammatory cells (p = 0.138).

However, a strong correlation was observed between the results of cytological examination and smear type (p < 0.001). In the group of patients with unremarkable cytological examination results, normal SSE cells (type A) as well as non-SSE cells (type C) were detected with no signs of neoplasia (type B). Abnormal cytological presentation was observed in the remaining patients (Fig. 7.).

A statistical relationship was observed between the type of smear and the number of cells in the smear (p < 0.001). The cell counts in type A smears were significantly lower than in types B and C smears (Tab. III., Fig. 8.).

In type B smears, the severity of inflammation was milder and granulocytes were more common than in type A and type C smears (Tab. IV.), albeit the differences in structural indicators were not statistically significant (p > 0.05).

DISCUSSION

In 1941, Papanicolaou and Traut demonstrated that brush cytology could be useful for the detection of preneoplastic conditions and cancer of the uterine cervix [17]. The importance of exfoliative cytology in laryngeal diseases has been studied for decades.

Tab. III. Number (percentage) of patients in groups presenting with different smear types and features and different and independence test results.

VARIABLE	SMEAR TYPE			*P TEST RESULT*
	A *N = 14*	B *N = 47*	C *N = 29*	
Overall smear quality:				0.633
Evaluable	12 (85.7%)	31 (66.0%)	18 (62.1%)	
Evaluable when air-dried	2 (14.3%)	16 (34.0%)	11 (37.9%)	
Smear cells:				**<0.001**
Abundant	6 (42.9%)	35 (74.5%)	25 (86.2%)	
Moderate	3 (21.4%)	11 (23.4%)	2 (6.9%)	
Scarce	5 (35.7%)	1 (2.1%)	2 (6.9%)	
Smear erythrocytes:				0.172
Numerous	2 (14.3%)	15 (31.9%)	4 (13.8%)	
Sparse/Moderate	2 (14.3%)	12 (25.5%)	6 (20.7%)	
None	10 (71.4%)	20 (42.6%)	19 (65.5%)	

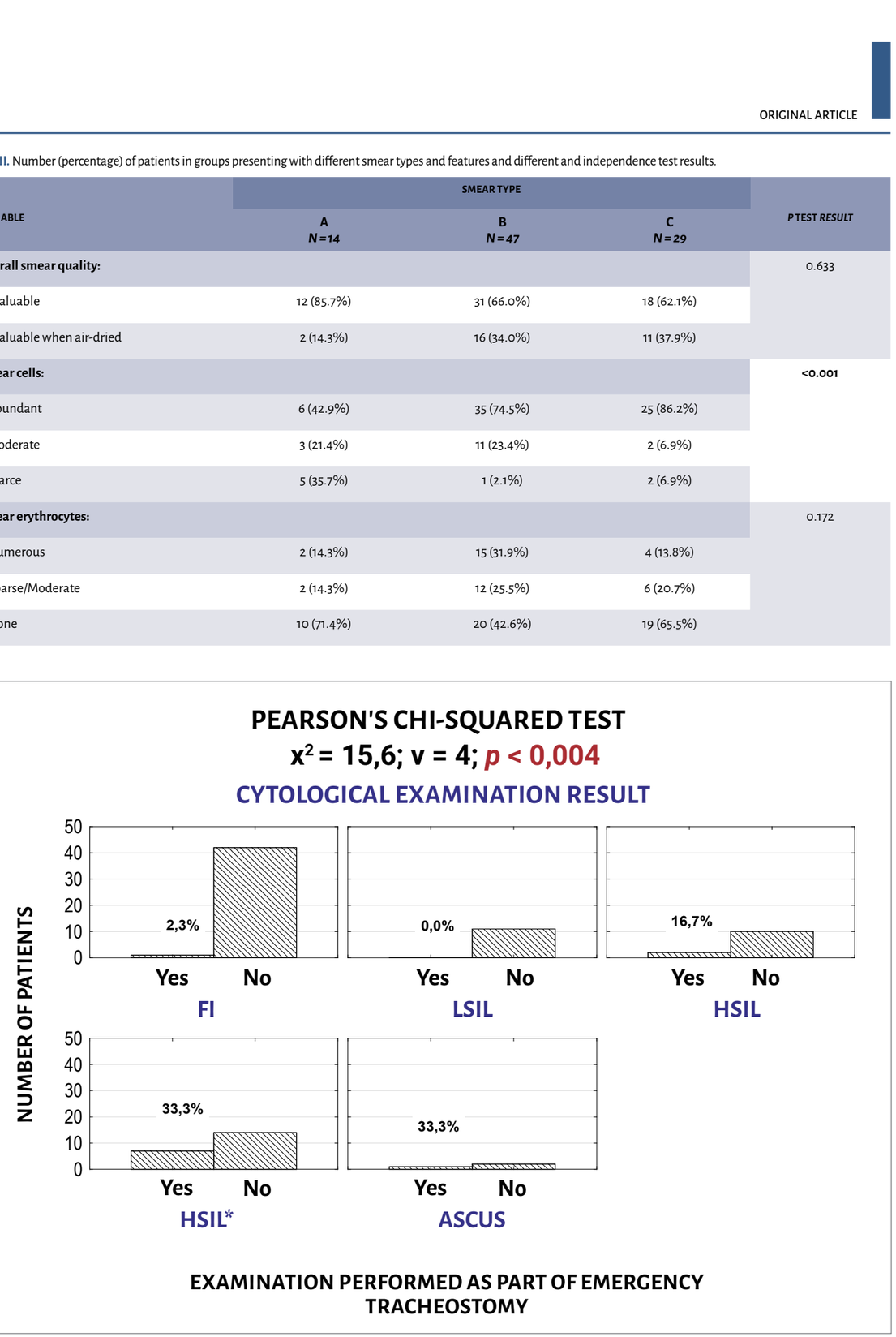

Fig. 6. Number (percentage) of patients in groups presenting with different preliminary diagnosis as determined from cytological examination results, tracheotomy status, and independence test results.

Tab. IV. Liczba (odsetek) pacjentów w grupach różniących się typem rozmazu i jego cechami oraz wyniki testu niezależności.

VARIABLE	SMEAR TYPE			*P* TEST *RESULT*
	A *N = 10*	B *N = 38*	C *N = 21*	
Severity of inflammation:				0.124
Significant	9 (90.0%)	20 (52.6%)	16 (76.2%)	
Moderate	1 (10.0%)	11 (29.0%)	4 (19.0%)	
Mild	0 (0.0%)	7 (18.4%)	1 (4.8%)	
Cell type:				0.567
Lymphocytes	8 (80.0%)	26 (68.4%)	17 (80.9%)	
Granulocytes	0 (0.0%)	3 (7.9%)	0 (0.0%)	
Lymphocytes and granulocytes	2 (20.0%)	9 (23.7%)	4 (19.0%)	

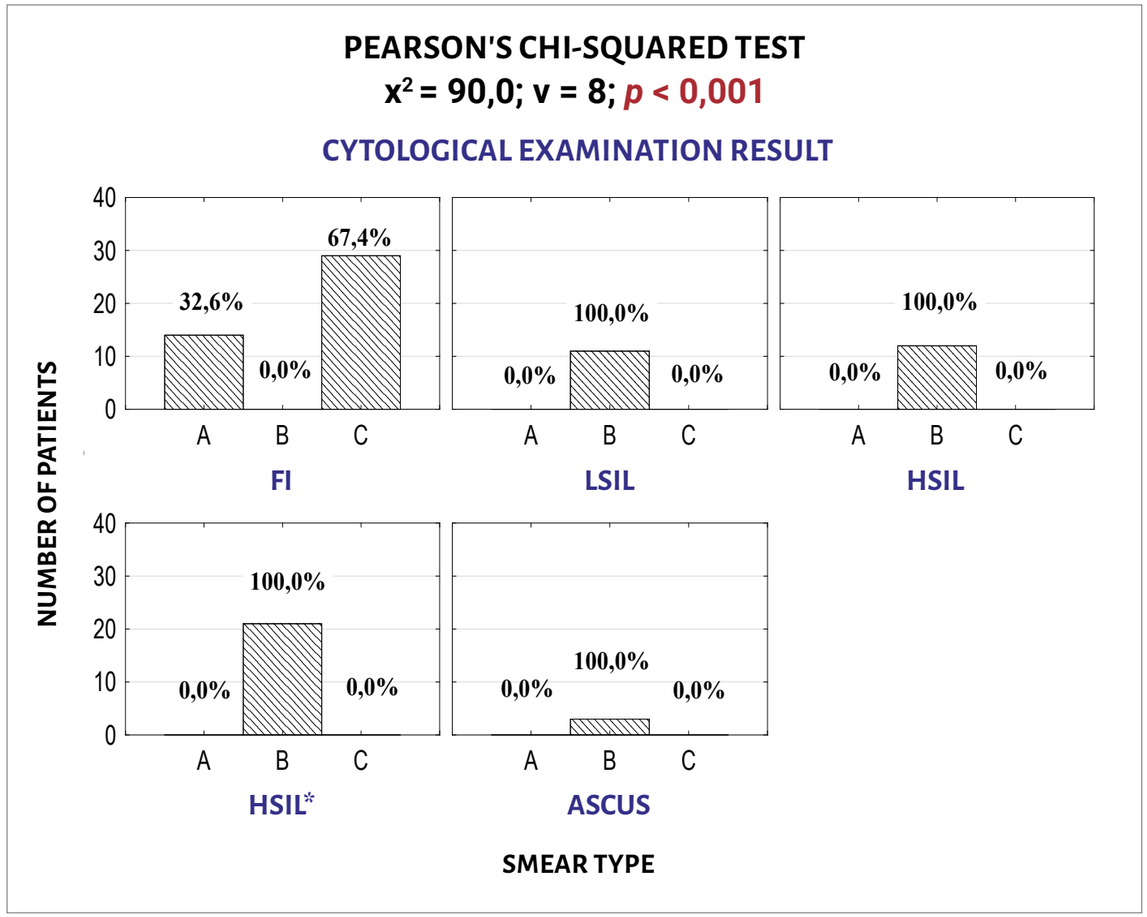

Fig. 7. Number (percentage) of patients in groups presenting with different cytological examination results, smear types (A – no intraepithelial neoplasia, only normal SSE cells visible; B – abnormal cytological presentation the cytological image is abnormal; C – non-SSE cells present, glandular epithelial cells present), and independence test results.

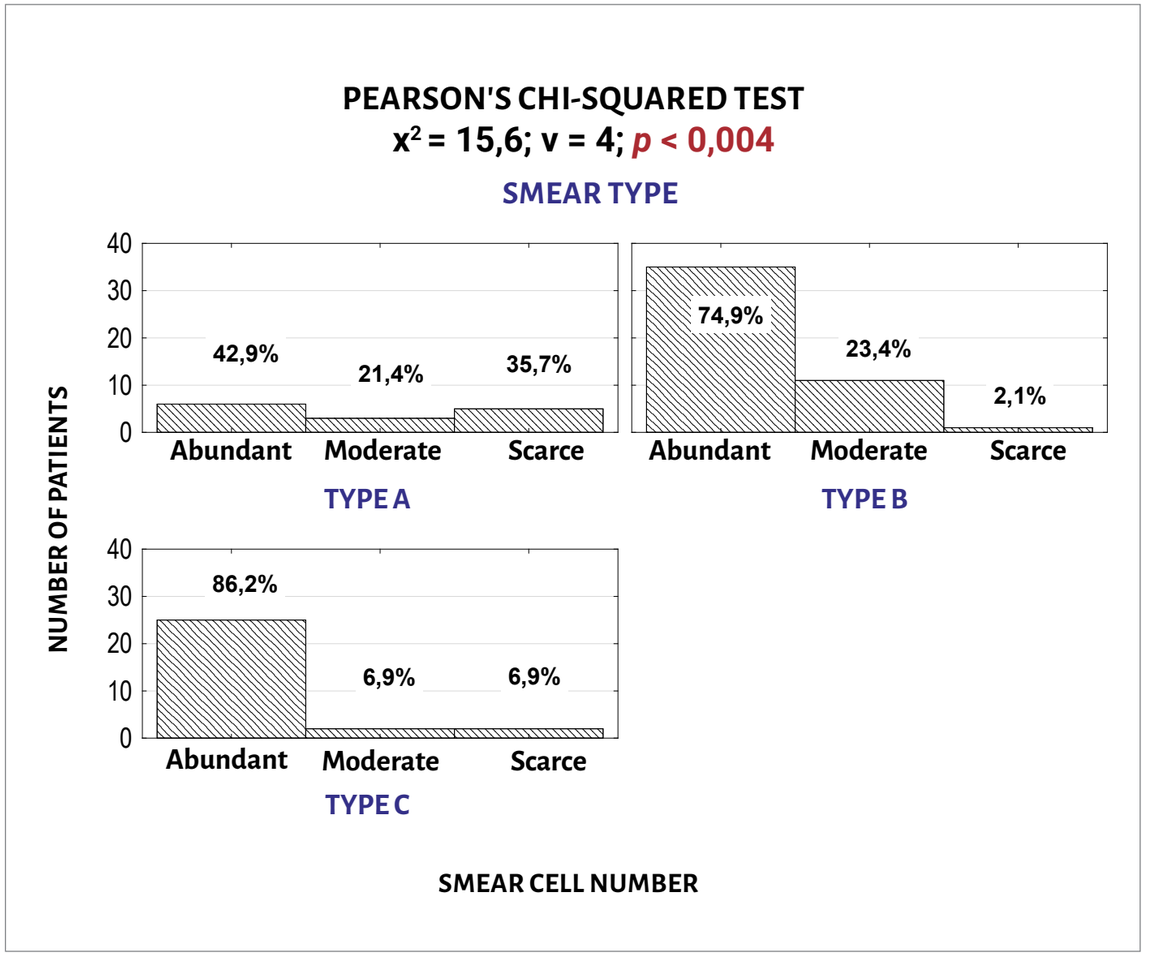

Fig. 8. Number (percentage) of patients in groups presenting with different smear types, smear cell counts, and independence test results.

As early as in 1964, Morison et al. presented the results of their study comparing the results of the cytological and histopathological examinations of larynx [18]. Friedmann et al. compared the results of histopathological examinations with those of cytomorphological examinations in a group of 212 patients with suspected head and neck cancer. Friedmann also stressed the importance of brush cytology in laryngeal diseases stating that laryngeal swabs contained fewer non-specific cells than swabs collected from the nose, nasopharynx or sinuses [19]. Studies are continued to determine the sensitivity and specificity of brush cytology as a diagnostic method. Ajahan et al. presented a review of the current knowledge on the subject [20]. As shown by the review authors in their analysis of available material, it is possible to obtain representative results of exfoliative cytological examinations of laryngeal specimens. When evaluating cytological images, one is able to determine the size of cells, the severity of inflammation, the degeneration process occurring within the cells, or the features suggesting the invasive process (cancers spheres/beads, or cellular cannibalism).

A disadvantage of exfoliative cytology consists in its inability to evaluate cellular pathology against basal membrane and to differentiate between high-grade intraepithelial neoplasia and invasive cancer [21]. The future of brush cytology may be related to examinations based on the analysis of cellular DNA isolated from cytological specimens of cancerous lesions. Mutation typing may be helpful in determining the sensitivity of lesions to traditional as well as novel treatments [12]. An unquestionable advantage of brush biopsy-based cytology consists in its general accessibility, low cost, and non-invasive character; the method is therefore successfully used in the diagnostics of conditions affecting easily accessible areas. The results of our study suggest that highly representative cytological material can be obtained from laryngeal epithelium when cytological specimens are collected from a patient in general anesthesia. Ability to obtain examination specimen using fiberoscopic guidance in a patient in local anesthesia would extend the applicability of exfoliative cytology so that it could become a widely used diagnostic tool in laryngeal diseases.

CONCLUSIONS

1. A total of 90 of 92 smears (97.8%) were subjected to cytological examination confirming the possibility of obtaining representative laryngeal epithelial cells for cytological purposes;

2. Acute laryngeal dyspnea was significantly more frequent in patients with HSILs presenting with invasive lesions as compared to patients with cytologically benign lesions;

3. No statistically significant relationship was observed between the results of the cytological examination (FI, LSIL, HSIL, HSIL with invasive features) and the overall quality of the smear, number of cells, number of erythrocytes, or the severity of inflammation;

4. Abnormal results of cytological examinations were obtained in a majority of cases. HSILs with invasive features were the most common abnormal results of cytological examinations;

5. An abnormal presentation of a cytological smear is usually hypercellular; in cases of intraepithelial neoplasia, features of intense inflammation are less commonly observed in cytological smears.

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Correlation Between Brush Cytology Results and Histopathological Examination in Diagnostic Evaluation of Precancerous Conditions and Laryngeal Cancer: A Prospective Study

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ABSTRACT

Aim. 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%.

Conclusion. 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

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].

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.

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.

RESULTS

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 correlation 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)
- 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 possible 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.

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]aged between 25 and 65, with abnormal pap smears were included into the study. All patients had a control pap smear and in each case punch biopsy with endocervical curettage were performed under the control of a colposcope. The presence of p16 and Ki67 proteins was detected using the CINtecPlus test. The results of the research were statistically assessed. Results: Abnormal pap smears were found in 82.5% (520/630. 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.

CONCLUSION

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, acquisition of data, writing – review and editing, final proofreading and approval of version for publication.

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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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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

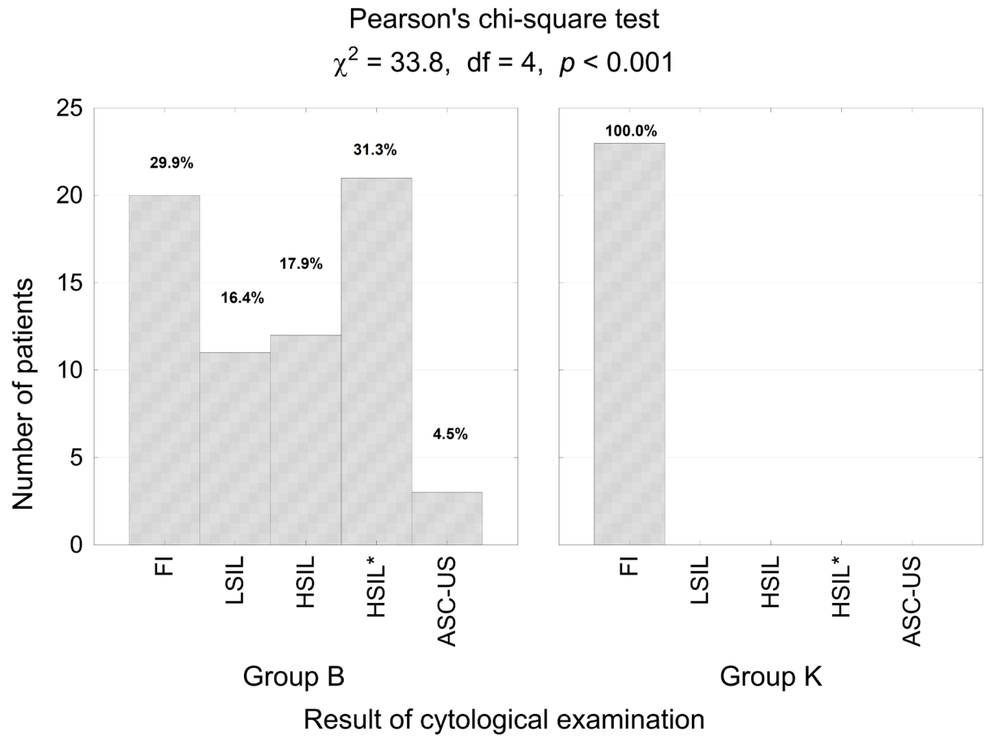

Fig. 2. Number (percentage) of patients in groups differing in terms of clinical diagnosis and result of histopathological examination as well as result of independence test

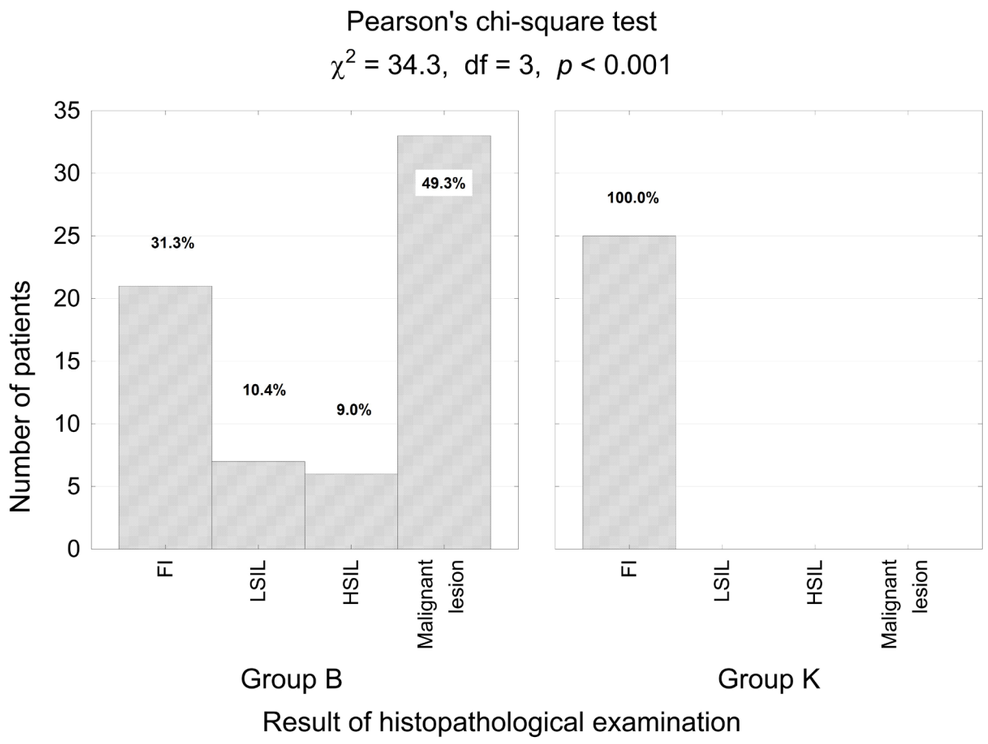

Fig. 3. Number (percentage) of patients in groups differing in terms of initial diagnosis and diagnosis made based on results of histopathological and cytological examination and results of independence test

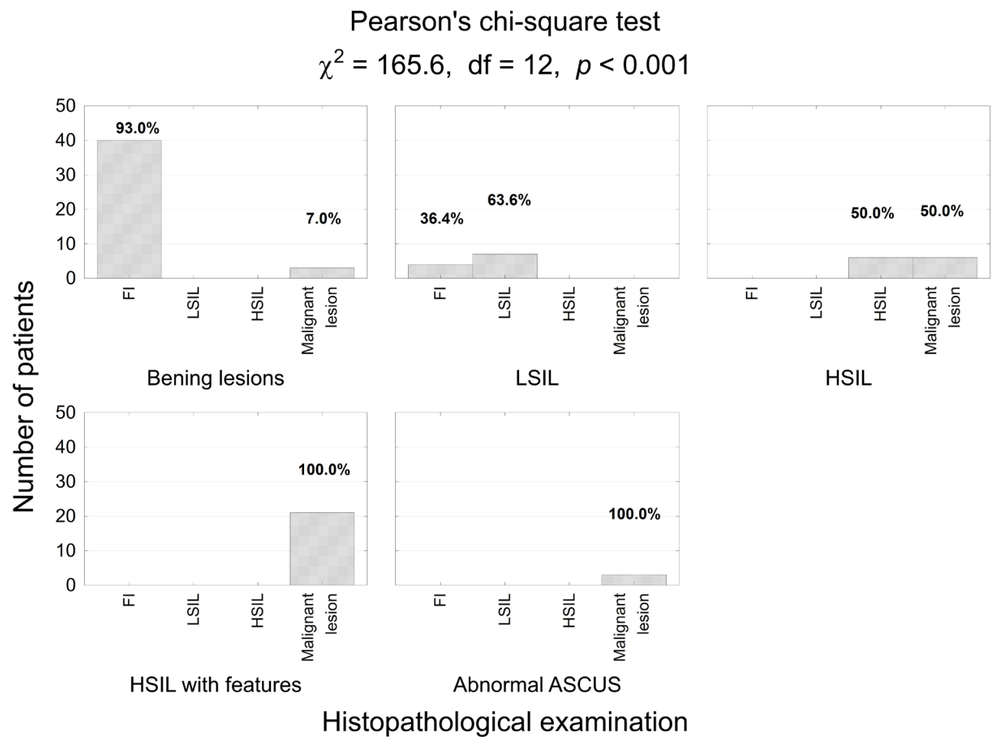

Fig. 4. Scatter plot of cytology and histopathology results

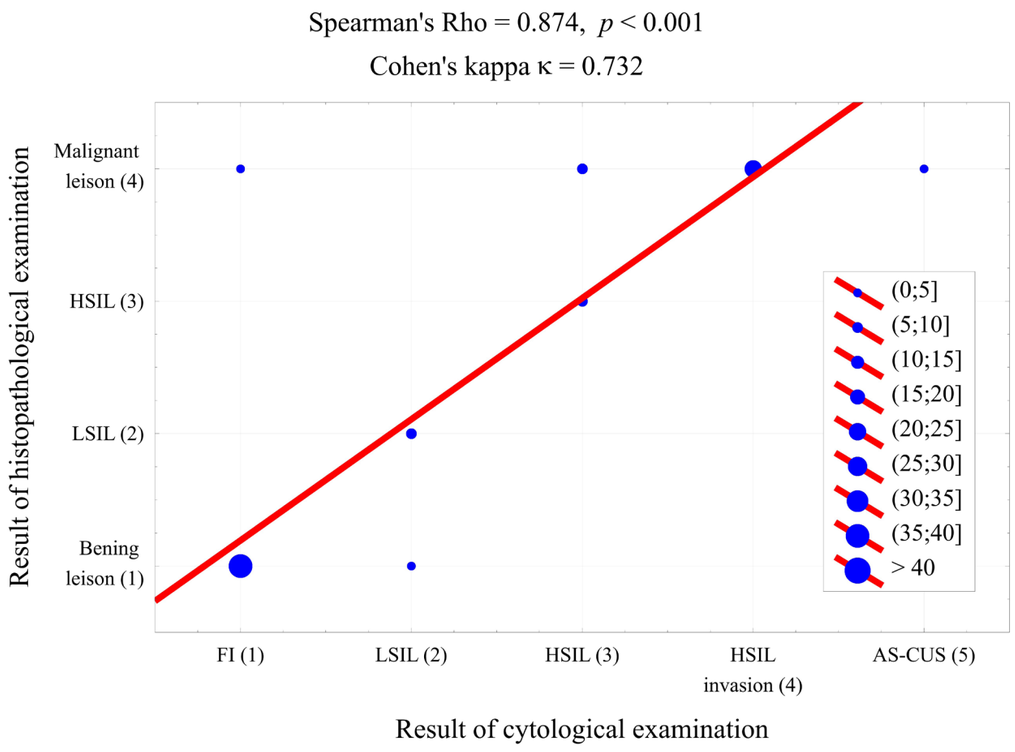

Table 1. Cytological evaluation of laryngeal smear

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)

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 [*Q*1; *Q*3]	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		Group K		Total		B vs K *p*
	N = 67		*N* = 25		*N* = 92		
	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 oedema	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:							**<0.001**
	N = 67		*N* = 23		*N* = 90		
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%)

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) ´ 100 = 90.9%
Test specificity = (43/46) ´ 100 = 93.5%
Accuracy of diagnosis = (83/87) ´ 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 k

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 k = 0.732; SE = 0.020; $p < 0.0001$

Table 7. Interpretation of Cohen's kappa coefficient values

Value k	Assessment
< 0,20	Poor consistency
0,21 – 0,40	Acceptable consistency
0,41 – 0,60	Moderate consistency
0,61 – 0,80	Substantial consistency
0,81 – 1,00	Very good consistency

8. PODSUMOWANIE WYNIKÓW I WNIOSKI

Materiał badawczy stanowiło 92 pacjentów, w tym 34 kobiety (37,0%), w wieku od 26 do 85 lat, leczonych z powodu chorób krtani. Analizie poddano 90 rozmazów cytologicznych (97,82 %), 2 rozmazy (2,18%) nie zostały ocenione z powodu nieczytelności. Wyniki badania cytologicznego zakwalifikowano do odpowiednich grup na podstawie obrazu komórkowego.

W analizowanej grupie u 11 pacjentów (12%) procedura pobierania wycinków połączona była z pilną tracheotomią. Zaobserwowano istotny statystycznie związek między potrzebą wykonania tracheotomii a wynikiem badania cytologicznego. Ostra duszność występowała istotnie częściej u pacjentów z wynikiem HSIL z cechami inwazji niż u pacjentów ze zmianami łagodnymi (33,3% vs 2,3%; p=0,004). Wyniki testów niezależności chi-kwadrat Pearson wykazały brak istotnego statystycznie związku wyników badań cytologicznych z ogólną jakością rozmazu (p=0,796), z liczebnością komórek w rozmazie (p=0,281), ilością erytrocytów w rozmazie (p=0,080), ze stopniem nasilenia stanu zapalnego (p=0,116) oraz z typem komórek zapalnych (p=0,138). Natomiast silną współzależność zaobserwowano pomiędzy wynikiem badania cytologicznego i typem rozmazu (p<0,001). W grupie pacjentów z wynikiem prawidłowym (FI) stwierdzono obecność prawidłowych komórek nabłonka WWP (typ A) oraz innych komórek oprócz WWP (typ C), nie stwierdzono natomiast śródnabłonkowej neoplazji (typ B). Obraz cytologiczny nieprawidłowy stwierdzono u wszystkich pozostałych pacjentów. Zaobserwowano statystyczny związek między typem rozmazu a liczebnością komórek w rozmazie (p<0,001). Liczebność komórek w rozmazie typu A była istotnie mniejsza niż w rozmazach typu B i C. W rozmazie typu B stopień nasilenia stanu zapalnego był niższy, a granulocyty występowały częściej niż w rozmazach typu A i C, ale różnice wskaźników struktury nie są statystycznie istotne (p>0,05). Wyniki badania cytologicznego porównano z wynikami badania histopatologicznego. Zaobserwowano silną, istotną statystycznie (p<0,001) współzależność między rozpoznaniami na podstawie badań cytologicznych i histopatologicznych. Silny związek między diagnozami opartymi na wynikach badań cytologicznych i histopatologicznych potwierdza współczynnik korelacji rang Spearmana *rho* oraz wartość współczynnika rzetelności kappa Cohena k. W celu oceny trafności diagnozy w badaniu cytologicznym w odniesieniu do wyników badań histopatologicznych (złoty standard), oszacowano wartości wskaźników trafności diagnostycznej testu: czułość, swoistość, dokładność rozpoznania, rzeczywistą częstość choroby, przewidywaną częstość choroby u osób z testem (+) (predykcja dodatnia) i przewidywaną częstość choroby u osób z testem (-) (predykcja ujemna). Trafność cytologii szczoteczkowej jako testu diagnostycznego raka krtani w przeprowadzonym badaniu wykazuje czułość i swoistość testu na poziomie 90,9% i 93,5% przy 4 wynikach fałszywie ujemnych i 3 fałszywie dodatnich. Predykcja dodatnia to 93% a predykcja ujemna to 91,5%. Wskaźnik wiarygodności wyniósł: LR+ 13,94 i LR- 0,10. Takie wartości wskaźników wiarygodności są często decydujące, a badanie cytologiczne spełnia oba te kryteria. Dodatkowo uzyskano współczynnik Kappa (k=0,732) z wagami kwadratowymi tożsamy ze współczynnikiem korelacji wewnątrzklasowej. Uzyskany wynik (wartość p<0,001) świadczy o zgodności opinii dwóch diagnostów, cytologa i histopatologa. Powołując się na klasyfikację Altmana, Landisa i Kocha można stwierdzić, że zgodność wyników cytologii i histopatologii jest bardzo dobra. Zgodnie z założonymi celami pracy przeprowadzono również ogólną charakterystykę pacjentów, u których rozpoznano stan przednowotworowy lub raka krtani. W grupie tej większość (76,1%) stanowili mężczyźni, wiek pacjentów wahał się pomiędzy 26 a 79 rokiem życia. Były to głównie osoby z wykształceniem zasadniczym, palacze tytoniu, najczęściej palący między 20-40 papierosów dziennie i spożywający alkohol raczej sporadycznie.

Na podstawie przeprowadzonych badań wysunięto następujące wyniki:

1. Ocenie cytologicznej poddano 90 z 92 pobranych rozmazów (97,82 %), co potwierdza możliwość uzyskania reprezentatywnych komórek nabłonka krtani do badania cytologicznego.
2. Nie wykazano statystycznie istotnego związku pomiędzy wynikiem badania cytologicznego (FI, LSIL, HSIL, HSIL z cechami inwazji, ASCUS) a ogólną jakością rozmazu, liczebnością komórek, ilością erytrocytów czy stopniem nasilenia stanu zapalnego.
3. Wynik badania cytologicznego częściej był nieprawidłowy. Spośród nieprawidłowych wyników badania cytologicznego najczęściej stwierdzono HSIL z cechami inwazji.
4. Nieprawidłowy obraz rozmazu cytologicznego najczęściej jest bogato komórkowy, a w przypadku występowania śródnabłonkowej neoplazji, rzadziej stwierdza się cechy nasilonego stanu zapalnego w rozmazie cytologicznym.
5. Cytologia szczoteczkowa nie jest badaniem konkurencyjnym dla badania histopatologicznego w przypadku rozpoznania raka krtani i nie powinna stanowić o rozpoznaniu onkologicznym. Jednak wyniki przeprowadzonych badań wykazują znaczącą zgodność pomiędzy omawianymi badaniami.
6. Cytologia szczoteczkowa może być bardzo dobrym pomocniczym narzędziem diagnostycznym w przypadku rozpoznawania stanów przednowotoworowych i raka krtani, o czym świadczą wskaźniki takie jak: czułość, swoistość, predykcja dodatnia czy predykcja ujemna powyżej 90%.
7. Cytologia szczoteczkowa mogłaby być również stosowana w diagnostyce przesiewowej w kierunku raka krtani, w przypadku monitorowania pacjentów z dysplazją lub do oceny odległych skutków leczenia chirurgicznego zarówno w ramach procedur szpitalnych, jak i ambulatoryjnych.
8. Istnieje potrzeba dalszych badań nad zastosowaniem cytologii szczoteczkowej w diagnostyce stanów przednowotworowych i raka krtani z uwzględnieniem prób pobierania materiału do badania w znieczuleniu miejscowym pod kontrolą endoskopii.
9. Wśród badanych pacjentów stan przednowotworowy czy raka krtani rozpoznano częściej u mężczyzn, u osób z wykształceniem zasadniczym, palących ponad 20 papierosów w ciągu dnia i pijących alkohol raczej sporadycznie.

Na podstawie przeprowadzonych badań wysunięto następujące wnioski:

1. W badaniu cytologicznym możliwa jest ocena stanów przednowotworowych i nowotworowych krtani.
2. W badanym materiale stwierdzono istotną statystycznie korelację pomiędzy wynikami cytologii szczoteczkowej a wynikami badania histopatologicznego materiału z biopsji chirurgicznej pobranej z tej samej okolicy.
3. Cytologia szczoteczkowa jako procedura małoinwazyjna, nieczasochłonna i tania mogłaby być wykorzystywana jako badanie wstępne w określeniu charakteru zmian w krtani.
4. Możliwe jest utworzenie algorytmu dla wstępnego etapu diagnostyczno-terapeutycznego w chorobach krtani, w którym cytologia szczoteczkowa byłaby elementem towarzyszącym badaniu endoskopowemu krtani jednak istnieje potrzeba dalszych badań z uwzględnieniem prób pobrania materiału do badania w znieczuleniu miejscowym pod kontrolą endoskopii.

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10. SPIS RYCIN

11. SPIS TABEL

12.ANEKSY:

a) opinia Komisji Bioetycznej

OPINIA KOMISJI BIOETYCZNEJ Nr KB – 519/2018

Komisja Bioetyczna przy Uniwersytecie Medycznym we Wrocławiu, powołana zarządzeniem Rektora Uniwersytetu Medycznego we Wrocławiu nr 133/XV R/2017 z dnia 21 grudnia 2017 r. oraz działająca w trybie przewidzianym rozporządzeniem Ministra Zdrowia i Opieki Społecznej z dnia 11 maja 1999 r. (Dz.U. nr 47, poz. 480) na podstawie ustawy o zawodzie lekarza z dnia 5 grudnia 1996 r. (Dz.U. nr 28 z 1997 r. poz. 152 z późniejszymi zmianami) w składzie:

dr hab. Jacek Daroszewski (endokrynologia, diabetologia)
prof. dr hab. Krzysztof Grabowski (chirurgia)
dr Henryk Kaczkowski (chirurgia szczękowa, chirurgia stomatologiczna)
mgr Irena Knabel-Krzyszowska (farmacja)
prof. dr hab. Jerzy Liebhart (choroby wewnętrzne, alergologia)
ks. dr hab. Piotr Mrzygłód (duchowny)
mgr prawa Luiza Műller (prawo)
dr hab. Sławomir Sidorowicz (psychiatria)
dr hab. Leszek Szenborn (pediatria, choroby zakaźne)
Danuta Tarkowska (pielęgniarstwo)
prof. dr hab. Anna Wiela-Hojeńska (farmakologia kliniczna)
dr hab. Andrzej Wojnar (histopatologia, dermatologia) przedstawiciel Dolnośląskiej Izby Lekarskiej)
dr hab. Jacek Zieliński (filozofia)

pod przewodnictwem
prof. dr hab. Jana Kornafela (ginekologia i położnictwo, onkologia)

Przestrzegając w działalności zasad Good Clinical Practice oraz zasad Deklaracji Helsińskiej, po zapoznaniu się z projektem badawczym pt.

„Porównanie wyników cytologii szczoteczkowej z wynikiem badania histopatologicznego w stanach przednowotworowych i nowotworach krtani"

zgłoszonym przez **lek. Paulinę Lepkę** zatrudnioną w Oddziale Otolaryngologicznym 4 Wojskowego Szpitala Klinicznego z Poliklinika we Wrocławiu oraz złożonymi wraz z wnioskiem dokumentami, w tajnym głosowaniu postanowiła **wyrazić zgodę** na przeprowadzenie badania w Oddziale Otolaryngologicznym 4 Wojskowego Szpitala Klinicznego z Poliklinika oraz Klinice Otolaryngologii, Chirurgii Glowy i Szyi USK we Wrocławiu pod nadzorem dr hab. Tomasza Zatońskiego **pod warunkiem zachowania anonimowości uzyskanych danych.**

Pouczenie: W ciągu 14 dni od otrzymania decyzji wnioskodawcy przysługuje prawo odwołania do Komisji Odwoławczej za pośrednictwem Komisji Bioetycznej UM we Wrocławiu.

Opinia powyższa dotyczy projektu badawczego będącego podstawą rozprawy doktorskiej.

Wrocław, dnia 21 września 2018 r.

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Wrocław, 10.03.2022 r.

OŚWIADCZENIE

1. Oświadczam, że w pracy: *The combination of brush cytology with the results of histopathological examination in laryngeal cancer diagnosis. Advences in Hygiene and Experimental Medicine, DOI: 10.2478/ahem-2021-0030.* mój udział polegał na analizie i interpretacji zgromadzonych danych, nadzorze nad projektem, współpracy przy tworzeniu manuskryptu oraz akceptacji jego ostatecznej wersji.

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mój udział polegał na współtworzeniu projektu badań oraz przygotowaniu tekstu manuskryptu.

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