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Please cite this article VALIDITY OF CYTOLOGY IN THE DIAGNOSIS OF SMALL CELL LUNG CARCINOMA

VREDNOST CITOLOGIJE U DIJAGNOSTICI MIKROCELULARNOG KARCINOMA PLUĆA

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VALIDITY OF CYTOLOGY IN THE DIAGNOSIS OF SMALL CELL LUNG CARCINOMA

VREDNOST CITOLOGIJE U DIJAGNOSTICI MIKROCELULARNOG KARCINOMA PLUĆA

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Cytology in the diagnosis of small-cell lung carcinoma
Citologija u dijagnostici mikrocelularnog karcinoma pluća
Abstract

**Background/Aim.** Small cell lung carcinoma (SCLC) is the most aggressive form of lung cancer. Patients with SCLC generally appear in a locally advanced or disseminated stage, when small biopsies and/or cytological materials are the only possibility for diagnosis. The aim of this study was to evaluate the validity of cytology in the initial diagnosis of SCLC, comparing cytological with histological findings of small biopsies. **Methods.** The retrospective study included 200 patients with cytological diagnosis of SCLC, established in the period from 2016 to 2018 and based on examination of the exfoliative material (sputum), as well as abrasive and aspiration materials obtained during bronchoscopy. In the same act, bronchoscopic materials were taken for cytological and histological diagnosis. Cytological materials were stained by May Grünwald Giemsa and histological using hematoxylin-eosin and immunohistochemical stains. **Results.** The most frequently sampled materials were: transbronchial needle aspiration (TBNA) in 72.2% of patients and bronchial brushing in 18.54% of patients, in the following order: bronchial aspirate in 4.88%, tru-cut needle biopsy in 5.37%, and sputum in 2.44% of patients. In 91.5% (183/200) patients cytological diagnosis of SCLC was pathohistologically confirmed. Among 17 patients whose cytological diagnosis of SCLC was not confirmed pathohistologically, for 12 (6%) of them another type of tumor was pathohistologically proved: in 6 cases non small cell lung carcinoma not otherwise specified (NSCLC - NOS), and in another 6 cases squamocellular carcinoma, adenocarcinoma, large cell carcinoma, mixed tumor (NSCLC with a neuroendocrine component), lymphoma and sarcoma. Finally, in five patients histological material was false-negative. **Conclusion.** Cytological diagnosis of SCLC is a reliable method which yields satisfactory accuracy. The best way is to be interpreted in conjunction with histology of small biopsies. When only cytological materials are available, in doubtful cases, other small round cell tumors, and poorly differentiated NSCLC, must be considered in the differential diagnosis.

**Key words:** small-cell-lung carcinoma; non-small-cell lung carcinoma; diagnosis, differential; cytodiagnosis; sensitivity and specificity.

Apstrakt

**Uvod/Cilj.** Mikrocelularni karcinom pluća (MCKP) je najagresivnija forma karcinoma pluća. Bolesnici sa MCKP se uglavnom javljaju u lokalno nespremetaljenom ili disemionanom stadium, kada su male biopsije i/ili citološki materijali, jedina mogućnost za dijagnostiku. Cilj rada je bio procena validnosti citologije u inicijalnoj dijagnostici MCKP, upoređivanjem citoloških sa histološkim nalazima malih biopsija. **Metode.** Retrospektivnom studijom obuhvaćeno je 200 bolesnika, kojima je u periodu od 2016 do 2018. god. postavljena citološka dijagnoza MCKP, na temelju pregleda eksfolijativnog materijala (sputum), kao i abrazivnih i aspiracionih materijala dobijenih prilikom bronhoskopije. Bronhoskopski materijali su u istom aktu uzimani za citološku i histološku dijagnostiku. Citološki materijali su bojeni May Grünwald Giemsom a histološki hematoksilin-eozinom i immunohistohemijskim bojenjima. **Rezultati.** Najčešće uzorkovani materijali su bili transbronhijalna iglena aspiracija (TBNA) kod 72,2% bolesnika i bris bronha kod 18,54% bolesnika. Sledili su: aspirat bronha kod 4,88% , true cut iglena
biopsija kod 5,37% i sputum kod 2,44% bolesnika. Kod 91,5% (183/200) bolesnika citološka dijagnoza MCKP je potvrđena patohistološki. Od 17 bolesnika kojima citološka dijagnoza MCKP nije potvrđena patohistološki, kod 12 (6%) je patohistološki dokazan drugi tip tumora: kod 6 nemikrocelularni karcinom pluća (NMCKP) bez druge specifikacije, kod po jednog bolesnika skvamocelularni karcinom, adenokarcinom, karcinom velikih čelija, mešoviti tumor (NMCKP sa neuroendokrinom komponentom), limfom i sarkom, a kod 5 bolesnika se radi o lažno negativnom histološkom materijalu. 

**Zaključak.** Citološka dijagnostika MCKP je pouzdana metoda zadovoljavajuće tačnosti. Najbolje ju je interpretirati sa histologijom malih biopsija. Kada je na raspolaganju samo citološki materijal, u spornim slučajevima, diferencijalno dijagnostički se moraju uzeti u obzir drugi tumori malih okruglih čelija, ali i slabo diferentovani NMCKP.

**Ključne reči:** mikrocelularni karcinom pluća; nemikrocelularni karcinom pluća; diferencijalna dijagnoza; citološka dijagnoza; sensitivity and specificity.

**Introduction**

Lung cancer, as the most common type of cancer in the world and the leading cause of mortality among all types of carcinomas, is a global health problem. It is the second most common cancer in both men (after prostate cancer) and women (after breast cancer). A high percentage of deaths from lung cancer is mainly the consequence of the fact that the disease is most frequently diagnosed in the advanced stage.

Serbia belongs to the group of the Central and Eastern European countries with high rates of morbidity and mortality, and also with the trend of increasing incidence of lung cancer.

Besides advanced age, which is the most important risk factor for most cancers, there are a lot of other risk factors for lung cancer. Nowadays it is known that lung carcinoma is a multifactorial disease originated from associate effect of more risk factors in combination with the individual characteristics of the human organism. The main risk factor (in 85% of patients) is tobacco smoking (active and passive).

Lung cancer is a clinical, biological and molecular heterogeneous disease. In the diagnosis of lung cancer, it is essential to separate small cell lung carcinoma (SCLC) from non small cell lung carcinoma (NSCLC) because biological differences between these two types of lung carcinoma cause different clinical course and require different therapeutic modalities.

NSCLC accounts for 80-85% of lung cancers, among which the most common are adenocarcinoma (40%), squamous cell carcinoma (25-30%) and large cell carcinoma (5-10%). SCLC comprises for 15-20% of lung cancer. The development of new treatments based on molecular tumor characteristics (molecular targeted therapy and antiangiogenic agents) led to the necessity of precise diagnosis of the histopathological type in the NSCLC group, and thus, for this group of lung cancer, it opened the possibility of personalized therapy, depending on histological diagnosis and molecular tumor status. Unlike the NSCLC group, treatment of the SCLC has not changed significantly for more than 30 years.
Most patients with SCLC have clinically disseminated or extensive disease at the time of diagnosis, when chemotherapy without radiation is recommended as a method of therapy\textsuperscript{12}. In recent years many efforts have been made to discover specific therapeutic goals for the SCLC. Immunotherapy tries to find its place in the treatment of SCLC. Increased PD-L1 expression was found in SCLC, underlying potential efficacy of the anti PD-1 / PD-L1 agents\textsuperscript{12}.

SCLC is the most aggressive type of lung cancer with a five-year survival rate of about 10\%, and a 10-year of 5\% \textsuperscript{13}. Due to clinical behaviour, systemic nature and good response to chemotherapy and radiotherapy, it is important to distinguish SCLC from other types of lung carcinomas \textsuperscript{6,14}.

It is believed that the SCLC cells are most likely derived from the stem cells of the bronchial epithelium, which undergoes partial differentiation to neuroendocrine cells in the process of neoplastic transformation\textsuperscript{14}.

At about 5 to 30\% of SCLC, a non small cell component can be found, and those are combined SCLC. Most often it is a component of squamous cell carcinoma, adenocarcinoma, and large cell carcinoma (LCC)\textsuperscript{15,16}.

The diversity and complexity of the lung tumor histogenesis led to the need for their classification as precisely as possible. Over time, with new knowledge, the classification of lung tumors has also changed. The latest classification of the lung tumor according to the World Health Organization (WHO) is based not only on the great progress in genetics, immunohistochemistry and lung cancer therapy, but also on the fact that about two thirds (70\%) of lung cancer is established on samples of small biopsies and cytological samples, due to the disseminated or extensive disease at presentation\textsuperscript{17}.

Patients with SCLC are mainly presented in a locally advanced or disseminated stage, when small biopsies and / or cytology materials are the only possibility for diagnosis. Concordance of lung cancer diagnosis on cytological materials compared to resectional or autopsy material ranges from 94-100\%, and the concordance of bronchoscopic cytological material and small biopsies up to 97.4\% \textsuperscript{13,18}.

From small biopsies, it is possible to obtain multiple cuts which allows additional cytochemical and immunocytochemical staining in unclear cases, when the diagnosis can’t be established based on the review of Hematoxylin-Eosin (HE) stained sections. This type of aid is largely not possible in cytodiagnostics. Cytological preparations are commonly stained only by one method, Papanicolaou or Romanowsky, so that the diagnosis is established exclusively on the basis of cell morphology. The question arises now is how much cytological diagnosis is reliable, that is, how much we can rely on well-known and defined cytological criteria in the diagnosis of SCLC.

The aim of this work was to evaluate the validity of cytology in the initial SCLC diagnosis by comparing cytological with histological findings of small biopsies.

**Methods**
Study Design

In this retrospective study, the cytological diagnosis of the SCLC established during a two-year period (January 2016 to December 2017) is correlated with a pathohistological diagnosis.

The cytological diagnosis was based on the examination of the exfoliative material (sputum) as well as the abrasive and aspiration materials obtained during bronchoscopy. The materials were taken in the same act for the cytological (bronchial aspirate and bronchial brushing, transbronchial aspiration biopsy [TBNA] of mediastinal or hilar lymph nodes, imprint of biopsy material), as well as for the pathohistological diagnosis (TBNA, transbronchial and endobronchial biopsy). For both types of diagnostics, the material was also obtained by percutaneous needle biopsy.

The cytological and histological diagnoses were established separately and independently in the Department of Cytology and the Pathology Department of the Institute of Pathology and Forensics Medicine of the Military Medical Academy (MMA) in Belgrade.

The bronchoscopy was performed in the Department of Interventive Pulmology at the Clinic for Pulmonary Disease of the MMA, Belgrade. The bronchoscopic material was taken after a short analgosedation during video bronchoscopy (Olympus BF260, aspirate and bronchial brushing), while TBNA and transbronchial biopsy (histological needle, 19 G, crocodile forceps- type Machida) for both types of diagnostics, was performed during rigid bronchoskopy (Karl StorzGmbH&Co.KG, Tuttlingen, Germany). Percutaneous needle biopsy was done with tru-cut needle under the control of computerized tomography.

Material processing

The cytological material was air-dried and stained with the May-Grünwald-Giemsa (MGG). For histological analysis, the material was processed in the usual manner (fixation in 4% formaldehyde, routine process of incorporation into paraffin and cutting to cuts of thickness of 4 μm). The histopathological diagnosis of the SCLC was first performed on materials stained with HE, and then, in order to confirm the diagnosis, immunohistochemical staining was carried out with chromogranin, sinaptofizin, thyroid transcription factor-1 (TTF-1), cytokeratin 8 (CK8), neuron-specific enolase (NSE).

Cytological criteria for diagnosing SCLC / suspected SCLC

The cytological diagnosis of the SCLC is established if individual cells and / or group of cells are found with subsequent morphological characteristics: nuclear size about 1.5-3 nuclei of small lymphocytes with fine structure of uniformly distributed chromatin without visible nucleolus, scantcytoplasm with high nucleo-cytoplasmatic ratio, well developed nuclear molding. The main criteria were the absence of the nucleolus and the presence of nucleus molding. (Fig. 1). The suspicion of the SCLC was set if the diagnostic material was scant: if it contained a very small number of cells that have had these morphological characteristics with or without the presence of the crash phenomenon, and if in addition to the microcellular component that prevailed, there was also a suspicion of a non-microcellular component.
Statistical analysis

The data were statistically processed using descriptive statistics for the age of patients (mean value ±SD), and Student's *t*-test and Mann-Whitney test, for the evaluation of statistical significance of certain parameters (at the level of *p* < 0.05). Analyses were performed with the computer program IBM SPSS 20 and Microsoft Office Excel 200.

The unit of analysis was a patient. For statistical analysis a finding suspected of SCLC was considered positive.

Results

Over a two-year period, out of a total of 3773 patients, 5277 samples of materials for cytological diagnosis of lung lesions and/or hilar and mediastinal lymphadenopathies were taken. There were 68.22% (3600/5277), benign and 23.44% (1237/5277) malignant samples; 3.1% of the samples (164/5277) were suspicious to malignancy. Atypical cells were found in 1.12% (59/5277) of the samples, whereas 4.12% (217/5277) of the samples were not representative for the analysis.

Out of a total of 1237 malignant cytological samples taken from 926 patients, in 222 samples taken from 200 (21.59%) patients the diagnosis SCLC / suspected for SCLC was established. They were the subject of this retrospective study. There were 68.3% (140/205) men and 31.7% (65/205) women with a mean age(±SD) of 63.41± 11.3 (34-84) years. There was neither statistically significant difference between the number of men and women (*p* = 0.317), nor between the age of male and female patients (*p* = 0.352).

Depending on the localization of lesions in the lungs, hilum of the lungs or in the mediastinum as well as the clinical condition of the patient, one or more types of material have been sampled. In 8.78% (18/205) patients, the diagnosis of SCLC was made in several different types of materials, and in 91.22% (187/205) only in one type of material. The most frequently sampled materials were TBNA in 72.2% (148/205) of patients, followed by bronchial brushing in 18.54% (38/205) of the patients, and then bronchial aspirate in 4.88% (10/205), tru-cut needle biopsy in 5.37% (11/205), and sputum in 2.44% (5/205) of patients (Fig. 2).

A total of 184/200 (92.0%) of patients had a cytological diagnosis of SCLC and 16/200 (8%) and were susceptible to SCLC (cytologically positive). In 183/200 (91.5%) of patients, the SCLC diagnosis was confirmed pathohistologically. There was no statistically significant difference in the number of patients with established diagnosis of SCLC between cytology and pathohistology (*p* = 0.068).

Cytological diagnosis of SCLC was not confirmed histopathologically in 17/200 (8.5%) of patients. In 12 (6%) of these the other type of tumor was diagnosed: in 6 patients NSCLC not otherwise specified, and in another 6 patients squamous cell carcinoma, adenocarcinoma, large cell carcinoma (LCC), mixed tumor (NSCLC with neuroendocrine component), lymphoma and sarcoma. Pathohistological material of 5 patients has not
revealed malignant but benign changes (inflammation, fibrosis). Those were the cases of falsely negative pathohistological findings (Table 1).

Review of 5 misdiagnosed SCLC from 2017. was made by two cytologists. In 3 cases both cytologists confirmed initial cytologic diagnosis of SCLC or suspected SCLC (Fig.3, a,b,c), and in 2 cases the initial diagnosis was not confirmed and NSCLC was diagnosed. (Fig.3d, Fig.4a,b).

In Fig. 3 a,b,c, cells have round nucleus without visible nucleolus, scant cytoplasm with high nucleo-cytoplasmatic ratio and prominent nuclear molding, but the pathohistological diagnosis was lymphoma, sarcoma and NSCLC-NOS.

Fig. 3d shows the group of cells with increased cytoplasm which lacks definite borders, absence of clear nucleus molding, cell overlap and three-dimensionality; pathohistological diagnosis was non small cell lung carcinoma, most probably adenocarcinoma.

Fig. 4 a,b are cytological samples of one patient. The group of cells in Fig. 4a and Fig. 4b belongs to the same sample (bronchial brushing). On the other hand, while a group of cells in Fig. 4a are poorly differentiated and morphologically meet the criteria for SCLC, another group of cells on the same sample (Fig. 4b) is characteristic for squamocellular differentiation (large tumor cells with central, irregular hyperchromatic nuclei and abundant cytoplasm, gaps between cells and distinct cell borders). On a bioptic sample taken in the same act, the pathohistological diagnosis of nonkeratinized squamous cell carcinoma, basaloïd type - intraepithelial with microinvasion, was established.

Fig. 5 presents a smear of transcarineal puncture performed in the same patient after a month. In the background of necrosis and cellular debris there are tumor cells with a clear morphology of keratinized squamous cell carcinoma (mostly isolated bizarre shapes cells with hyperchromatic or pyknotic nuclei and keratinized cytoplasm). In the material taken in the same act for pathohistological analysis, there was no tumor tissue.

Discussion

In patients with lung carcinoma, the only significant tumor parameters affecting the therapeutic procedure are the type of malignancy and stage of illness.

Since two thirds of patients with lung cancer are present in advanced stages when the cancer is unresectable, the decision on therapy is made on the basis of small biopsies and/or cytological samples obtained with less invasive methods, which are the primary method of diagnosis for the majority of lung cancer patients. Due to different therapeutic approaches and different prognoses, the first step in the diagnosis of lung carcinoma is the separation of SCLC from NSCLC.

Previous research has shown that the accuracy in differentiation between SCLC and NSCLC in cytologic diagnosis ranges from 94-100%, with a mean error rate of 9% (range 0 to 33%) for SCLC, and 2% (1-7%) for NSCLC, in comparison with resectional or autopsy samples.

The accuracy of SCLC diagnosis on cytologic samples is similar to that achieved with small biopsies, that is, sufficiently high to start with treatment. The most recent study, Li
et al., based on a comparative analysis of the diagnostic value of cytology and histology taken during the same bronchoscopic procedure, concluded that the value of cytology (bronchial brushing and TBNA) is superior to histology (small biopsy stained with HE and immunohistochemically-IHH).

Of the former 20-25%, today the percentage of patients with SCLC has dropped to around 14-15%, probably due to a reduced number of smokers.

In the examined two-year period the percentage of patients with SCLC diagnosed in our hospital is still high (22.03%). There was neither statistically significant difference by sex nor by age between male and female. SCLC is an older age disease, but among our patients there were ones younger than 40, the youngest was only 34 years old. The mean age of our patients was 63.41 and it is not different from the mean age of patients with SCLC in similar studies.

The most common sampled cytological material in our patients was TBNA lymph node number 7, which is understandable, since the SCLC was mainly positioned centrally and submucosally, and in almost all patients disease was extended to surrounding lymph nodes at the time of diagnosis. Tru-cut needle biopsy was done only in those cases where diagnostic material could not be obtained by any other methods.

In our study, the concordance between cytology and histology (bioptic samples) was 91.5%, slightly higher than in similar studies like Sakr et al. (83%) and Miličić et al. (76%), respectively, but slightly less than Delgado et al. (96%).

The disagreement between cytological and histological diagnosis was found in 17 (8.5%) of our patients. Of this number, 12 (6%) was a pathohistologically proven NSCLC or another type of tumor, confirmed by IHH (Table 1).

Unlike our results, and those of Milicic et al. who found disagreement between cytology and histology in 23% (12/50) patients in similar investigation of the value of cytology in SCLC diagnosis, and Sakr et al., who found the incorrect cytological diagnosis of SCLC in 9% (1/11) cases, Delgado et al. in their study, comparing the accuracy of fine needle aspiration cytology (FNA) in the diagnosis of SCLC with the diagnosis of other lungmalignancies, did not have any interpretative error. However, in their study, 221 patients had 242 FNA, and all 18 (7%) smears interpreted as SCLC were correctly diagnosed, which is a far smaller number of patients with SCLC than in our study.

Review of 5 misdiagnosed SCLC from 2017, found two cases with a clear interpretive error, that is, the wrong classification of the type of tumor. In one case, (Fig.3 d) it is obvious that that morphology and cell architecture do not satisfy cytological criteria for SCLC, in other words, it indicates NSCLC.

But in another case, in the same sample (bronchial brushing) besides the groups of poorly differentiated cells (Fig.4 a) there are also other groups of cells with the clear squamocellular differentiation (Fig.4b). On a bioptic sample the pathohistological diagnosis of nonkeratinized squamous cell carcinoma, basaloid type - intraepithelial with microinvasion, was established. After a month the cytological finding of a transcarietal puncture performed in the same patient reveals a clear morphology of keratinized
squamous cell carcinoma, but the patohistological material was negative for malignancy. This case represents an interpretative error of a cytologist who overlooked a clear nonmicrocellular component in the bronchial brushning, as well as a limitation of small biopsies that represent only a small part of the tumor tissue. It was clear from the cytological sample obtained by transcarineal puncture that it was a keratinized, most likely invasive squamous cell carcinoma, which could not be confirmed histopathologically, as histological sample was false negative.

However, in Fig. 3 a,b,c, the morphology of the cells and the manner of clustering are such, that the SCLC cannot be excluded only on the basis of morphological criteria, which was also a cytological diagnosis, but pathology revealed lymphoma, sarcoma and NSCLC-NOS.

In cytologic samples malignant lymphomas are presented mainly as uniform individual cells, usually with present lymphoglandular bodies. Lymphatic cells, depending on the type of lymphoma can have clearly visible nucleolus, and phenomena of nucleus molding, typical for the SCLC is lacking. However, in the cytological samples, occasionally, tissue fragments or cellular grouping with nucleus molding phenomena can also be obtained, which can objectively lead to the misinterpretation in terms of SCLC, as it happened in our case. (Fig. 3 a).

The main diagnostic problem in our study was to distinguish SCLC form NSCLC-NOS, but in Miličić et al. from squamous cell carcinoma and adenocarcinoma. These authors also had an incorrect diagnosis of SCLC in sarcoma. Domagala-Kulawiki et al. had similar difficulties in differentiating SCLC from undifferentiated, anaplastic NSCLC, while Delgado et al. from poorly differentiated squamous cell carcinoma and large cell carcinoma.

In the material obtained by fine needle aspiration, Renshaw et al. studied the cytological characteristics of those cases of SCLC which are most often incorrectly classified as NSCLC. They concluded that this was mostly often the case with those SCLC that had some NSCLC characteristics, such as increased amounts of cytoplasm, or the presence of paranuclear blue bodies and / or some architectural features such as pseudoglandular or squamous cell grouping.

Sturgis et al., studying the cytomorphologic features useful for separating SCLC from NSCLC in the bronchial brushing and aspirate, found that the three most sensitive and specific cytomorphologic features traditionally used to separate SCLC from NSCLC are nucleus molding, finely granulated chromatin, and scantdelicate cytoplasm. However, they also found that some features which are classically associated with certain types of neoplasms, e.g. 3-dimensional groups with nuclear overlapping in lung adenocarcinoma, were also noted in SCLC (they noted 3-dimensional tumor fragments in 73% and nuclear overlap in 53% of SCLC cases).

These studies have shown that SCLC may have some cytologic features of NSCLC, such as some other neoplasms, e.g. lymphoma or sarcoma, may have occasionally some of the morphological characteristics of small-cell carcinoma, such as nucleus molding.
The above examples show the complexity of morphological, cytological and also pathohistological diagnostics on small biopsies. This complexity comes from the possibility that some of the morphological characteristics of NSCLC could be found in SCLC, as well as from the histological heterogeneity of lung carcinoma. In addition to SCLC and neuroendocrine LCC, the latest WHO classification of lung tumors in the group of neuroendocrine tumors recognizes combined SCLC and combined large cell neuroendocrine carcinoma.

It was estimated that 70% of resected SCLC were pure and 30% combined. In a series of 100 surgical biopsies or SCLC resections, Nisholson et al. found combined SCLC in 28% cases with 16% combined SCLC with LCC, 9% with adenocarcinoma and 3% with squamous cell carcinoma. While combined small cell/large cell carcinoma require at least 10% of the tumor show large cell carcinoma, no percentage requirement is needed if there is a clear adeno or squamocellular component.

In the most combined tumors, the small cell component is predominant. Since the presence of a small cell component will define patient therapy, the most important decision for a pathologist is to determine whether a small cell component is present.

In the light of these facts, except for the possibility of overlapping the morphological characteristics of SCLC and NSCLC, small diagnostic samples do not need to be representative of the entire tumor that may be morphologically heterogenous, consisting of well- and poorly differentiated parts (like in Fig. 4.a,b). If, in these small diagnostic materials, different parts of the tumor are obtained, this may be the reason of an inadequate diagnosis or disagreement in cytological and/or histological diagnosis of small biopsies, with a definite histological diagnosis on the resection material. The most accurate diagnosis can only be set on the resected material. However, this type of material is available only in patients with early stage disease at the time of diagnosis, who are candidates for surgical resection.

In 5 of our patients with benign pathohistological findings (inflammation, fibrosis), abundant well preserved cytological material with a clear morphological characteristic of the SCLC as well as a clinical finding and a further course of disease, pointed out a false negative pathohistological result; there was no tumor tissue in the material for pathohistological analysis, respectively.

The discrepancy between cytological and histological diagnosis can also be the result of sampling (sample quality, size, representativity) or misinterpretation. In our research, we found sampling errors in biotic material of 5 patients (nonrepresentative falsely negative biotic material), and interpretative errors on cytological samples in 12 patients (12/200, 6%). Due to the design of the study, in which the starting point were the patients with the cytological diagnosis of SCLC, we were not able to assess the sampling error on cytological specimens, as well as to evaluate if there were cases with cytological diagnosis of NSLC in pathohistological proved SCLC.

We could say that the part of committed cytological interpretive errors are objective, because they fall into that zone of overlapping of morphological cytological characteristics of SCLC and NSCLC, or other types of small cell tumors, which is difficult to resolve without the aid of immunohistochemistry and/or detailed clinical data.
However, it is well known that problems in the SCLC diagnosis, that is, in the separation of SCLC from NSCLC, exist in the histological HE material in about 5-7% of cases, even among experienced pathologists involved in the diagnosis of lung cancer. Factors that contribute to variability in separating SCLC from NSCLC among pathologists can be of technical nature, such as: extensive crush phenomenon in small biopsies, ischemic changes, poor fixation, too thin or stained preparations, but also a reflection of the variability in the size of the SCLC cells that are approaching the size of the LCC cells, or the basaloid variant of LCC and squamous cell carcinoma.

Besides combined tumors (SCLC with a non small cell component) that may be the reason for misinterpretation (subjective or objective, if only one component of the tumor is on the sample), differential diagnosis of SCLC encompasses NSCLC, lymphoma, melanoma, chronic inflammation, other neuroendocrine lung tumors, metastatic breast and prostate carcinomas and metastatic neuroendocrine carcinomas from other localizations. In addition, the SCLC should also be separated from the small round cell neoplasms, such as neuroblastoma, embryonic rhabdomyosarcoma, desmoplastic small round cell tumor and primitive peripheral neuroectodermal tumor.

**Conclusion**

The cytological diagnosis of the SCLC is a reliable method with satisfactory degree of accuracy. The best way is to be interpreted in conjunction with histology of small biopsies, so that in the diagnosis of lung cancer invasive procedures are not indispensable. When only cytological material is available, in doubtful cases, other type of small round cell tumors, but also poorly differentiated NSCLC must be considered for differential diagnosis. If in these cases it is not possible to do immunohistochemical and molecular studies, then the finding should be interpreted in conjunction with anamnestic, clinical and radiological parameters.
References


Fig. 1.

Fig. 2
Fig. 1. Cytology of small cell lung carcinoma (SCLC): Cluster of cells with finely granular and uniformly distributed chromatin, absence of nucleoli, nuclear molding and scant cytoplasm. (TBNA, MGGx1000)

Fig. 2. Type of the most frequently sampled cytological material for diagnosis of small cell lung carcinoma

Fig. 3. Cases with misdiagnosed small cell carcinoma. (a) Parafollicular T cell lymphoma (TBNA, MGGx1000). (b) Nondifferentiated sarcoma (TBNA, MGGx1000). (c) Non small cell lung carcinoma (not otherwise specified – high grade ) (Tru-cut, MGGx1000). (d) Non small cell lung carcinoma, most probably adenocarcinoma (TBNA, MGGx400).

Fig. 4. Case with misdiagnosed small cell carcinoma. Two groups of cells in the same sample of bronchial brushing. (a,b) Nonkeratinizing squamous cell carcinoma intraepithelial basaloid type, with microinvasion (MGGx1000).

Fig. 5. Squamous cell lung carcinoma with keratinization (transcarinal puncture, MGGx200).

Table 1.

Histological diagnosis in 200 patients in whom the cytological diagnosis was small cell lung carcinoma

<table>
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</tbody>
</table>

SCLC – small cell lung carcinoma; NSCLC – non small cell lung carcinoma; SQA- squamous carcinoma; AD - adenocarcinoma; LCC- large cell lung carcinoma; SAR - sarcoma; LIM - limphoma; MIX – mixed tumor; BEN - benign,*false negative histological findings; T - total.

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