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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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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 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 ones using hematoxylin-eosin and immunohistochemical stains. **Results.** The most frequently sampled materials were: transbronchial needle aspiration (TBNA) in 72.2% of the patients and bronchial brushing in 18.54% of the patients, in the following order: bronchial aspirate in 4.88%, tru-cut needle biopsy in 5.37%, and sputum in 2.44% of the patients. In 91.5% (183/200) of the patients cytological diagnosis of SCLC was histopathologically confirmed. Among 17 patients whose cytological diagnosis of SCLC was not confirmed histopathologically, another type of tumor was histopathologically proved for 12 (6%) of them: in 6 cases non SCLC not otherwise specified, and in each *per* one 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:
bronchoscopy; carcinoma, non-small-cell lung; cytological techniques; diagnosis; diagnosis, differential; histological techniques; small cell lung carcinoma.

Apstrakt

Uvod/Cilj. Mikrocelularni karcinom pluća (MCKP) je najagresivnija forma karcinoma pluća. Bolesnici sa MCKP se uglavnom javljaju u lokalno uznapredovalom ili diseminovanom stadijumu, 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. godine postavljena citološka dijagnoza MCKP, na temelju pregleda eksfolijativnog materijala (sputum), kao i abrazivnog i aspiracionog materijala dobijenog prilikom bronhoskopije. Bronhoskopski materijal je u istom aktu uziman za citološku i za histološku dijagnostiku. Citološki materijal bojen je May-Grünwald Giemsa, metodom, a histološki hematoksilin-eozinom i imunohistohemijskim bojenjima. **Rezultati**. Najčešće uzorkovani materijali bili su transbronhijalna iglena aspiracija (TBNA) kod 72,2% bolesnika i bris bronha kod 18,54% bolesnika, a zatim: aspirat bronha kod 4,88%, *true cut* iglena biopsija kod 5,37% i sputum kod 2,44% bolesnika. Kod 183/200 (91,5%) bolesnika citološka dijagnoza MCKP potvrđena je patohistološki. Od 17 bolesnika kojima citološka dijagnoza MCKP nije potvrđena patohistološki, kod 12 (6%) je patohistološkim pregledom dokazan drugi tip tumora: kod 6 nemikrocelularni karcinom pluća (NMCKP) bez druge specifikacije, kod po jednog bolesnika skvamocelularni karcinom, adenokarcinom, karcinom velikih ćelija, mešoviti tumor (NMCKP sa neuroendokrinom komponentom), limfom i sarkom, a kod 5 bolesnika se radilo o lažno negativnom histološkom materijalu. **Zaključak.** Citološka

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dijagnostika MCKP je pouzdana metoda zadovoljavajuće tačnosti. Najbolje je da se interpretira sa histologijom malih biopsija. U spornim slučajevima, kada je na raspolaganju samo citološki materijal, diferencijalno dijagnostički se moraju uzeti u obzir drugi tumori malih okruglih ćelija, ali i slabo diferentovani NMCKP.

Ključne reči:
bronhoskopija; pluća, nesitnoćelijski karcinom; citološke tehnike; dijagnoza; dijagnoza, diferencijalna; histološke tehnike; pluća, sitnoćelijski karcinom.

Introduction

Lung cancer, as the most common type of cancers in the world and the leading cause of mortality among all types of carcinomas, is a global health problem [1]. It is the second most common cancer in both men (after prostate cancer) and women (after breast cancer) [2]. 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 [3, 4].

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 effects of more risk factors in combination with the individual characteristics of the human organism [5, 6]. The main risk factor (in 85% of patients) is tobacco smoking (active and passive) [6, 7].

Lung cancer is a clinical, biological and molecular heterogeneous disease [8]. In the diagnosis of lung cancer, it is essential to separate small cell lung carcinoma (SCLC) from non SCLC (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 cancers [8]. 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 cancers, it opened possibility of personalized therapy, depending on histological diagnosis and molecular tumor status [9, 10]. Unlike the NSCLC group, treatment of SCLC patients has not changed significantly for more than 30 years [11].

Most patients with SCLC have clinically disseminated or extensive disease at the time of diagnosis, when chemotherapy without radiation is recommended method of therapy [12]. In recent years many efforts have been made to discover specific therapeutic goals for SCLC. Immunotherapy tries to find its place in the treatment of SCLC. Increased PD-L1 expression was found in SCLC, underlying potential efficacy of anti PD-1/PD-L1 agents [12].

SCLC is the most aggressive type of lung cancers with a five-year and a 10-year survival rate of about 10% and 5%, respectively [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 [6, 14].

It is believed that SCLC cells are most likely derived from stem cells of the bronchial epithelium, which undergoes partial differentiation to neuroendocrine cells in the process of neoplastic transformation [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 [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 lung tumors 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 cancers are established on samples of small biopsies and cytological samples, due to the disseminated or extensive disease at presentation [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 based on cytological materials compared to resectional or autopsy material ranges from 94%–100%, and concordance of bronchoscopic cytological material and small biopsies up to 97.4% [13, 18].

From small biopsies, it is possible to obtain multiple cuts which allow additional cytochemical and immunocytochemical staining in unclear cases, when the diagnosis can not 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 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 study 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 SCLC established during a two-year period (January 2016 to

December 2017) was correlated with a histopathological 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 needle aspiration (TBNA) of mediastinal or hilar lymph nodes, imprint of bioptic material], as well as for the histopathological 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, Serbia.

The bronchoscopy was performed in the Department of Interventive Pulmology at the Clinic for Pulmonary Disease of the MMA, Belgrade, Serbia. 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 bronchoscopy (Karl StorzGmbH&Co.KG, Tuttlingen, Germany). Percutaneous needle biopsy was done with tru-cut needle under the control of computed tomography.

Material processing

The cytological material was air-dried and stained with 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 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), and neuron-specific enolase (NSE).

Cytological criteria for diagnosing SCLC/suspected SCLC

The cytological diagnosis of SCLC was established if individual cells and/or group of cells were 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, scant cytoplasm with high nucleo-cytoplasmatic ratio, well developed nuclear molding. The main criteria were the absence of the nucleolus and the presence of nucleus molding (Figure 1). The suspicion of SCLC was set if the diagnostic material was scant: if it contained a very small number of cells that 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 nonmicrocellular component.

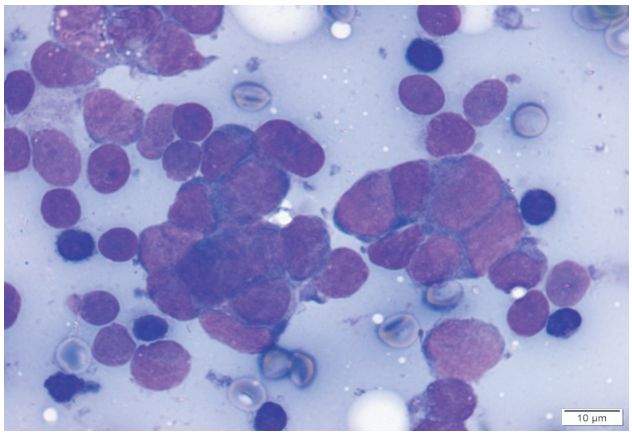

Fig. 1 – Transbronchial needle aspiration finding points out cytology of small cell lung carcinoma: cluster of cells with finely granular and uniformly distributed chromatin, absence of nucleoli, nuclear molding and scant cytoplasm (May-Grünwald-Giemsa, ×1000).

Statistical analysis

The data were statistically processed using descriptive statistics for the age of patients [mean value ± standard deviation (SD)], and the 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 3,773 patients, 5,277 samples of materials for cytological diagnosis of lung lesions and/or hilar and mediastinal lymphadenopathies were taken. There were 3,600 (68.22%) benign and 1,237 (23.44%) malignant samples; 164 (3.1%) of the samples were suspicious to malignancy. Atypical cells were found in 59 (1.12%) of the samples, whereas 217 (4.12%) of the samples were not representative for the analysis.

Out of a total of 1,237 malignant cytological samples taken from 926 patients, in 222 samples taken from 200 (21.59%) of the patients, diagnosis of SCLC/suspected for SCLC was established. They were the subject of this retrospective study. There were 140 (68.3%) men and 65 (31.7%) 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 the mediastinum, as well as the clinical condition of the patient, one or more types of material were sampled. In 18 (8.78%) of the patients, the diagnosis of SCLC was made in several different types of materials, and

in 187 (91.22%) only in one type of material. The most frequently sampled materials were TBNA in 148 (72.2%) of the patients, followed by bronchial brushing in 38 (18.54%) of the patients, and then bronchial aspirate in 10 (4.88%), tru-cut needle biopsy in 11 (5.37%), and sputum in 5 (2.44%) of the patients (Figure 2).

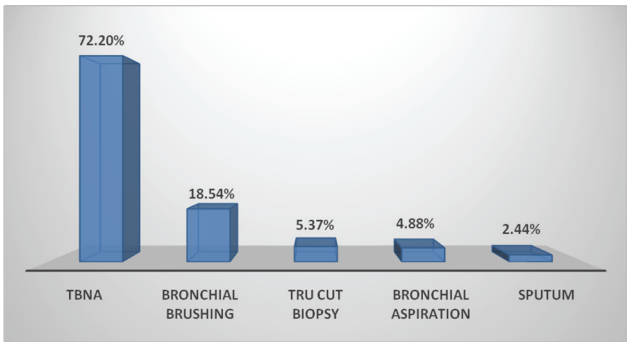

Fig. 2 – Types of the most frequently sampled cytological material for diagnosis of small cell lung carcinoma.

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

Cytological diagnosis of SCLC was not confirmed histopathologically in 17 (8.5%) of the patients. In 12 of them, the other type of tumor was diagnosed: in 6 patients NSCLC not otherwise specified (NOS), and in another 6 patients squamous cell carcinoma, adenocarcinoma, large cell carcinoma (LCC), mixed tumor (NSCLC with neuroendocrine component), lymphoma and sarcoma, each *per* one. In histopathological material of 5 patients, no malignant but benign changes (inflammation, fibrosis) were revealed. Those were the cases of falsely negative histopathological findings.

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

In Figure 3, a-c, cells had round nucleus without visible nucleolus, scant cytoplasm with high nucleo-cytoplasmatic ratio and prominent nuclear molding, but histopathological diagnosis was lymphoma, sarcoma and NSCLC-NOS.

Figure 3d shows the group of cells with increased cytoplasm which lacks definite borders, absence of clear nucleus molding, cell overlap and three-dimensionality; histopathological diagnosis was NSCLC, most probably adenocarcinoma.

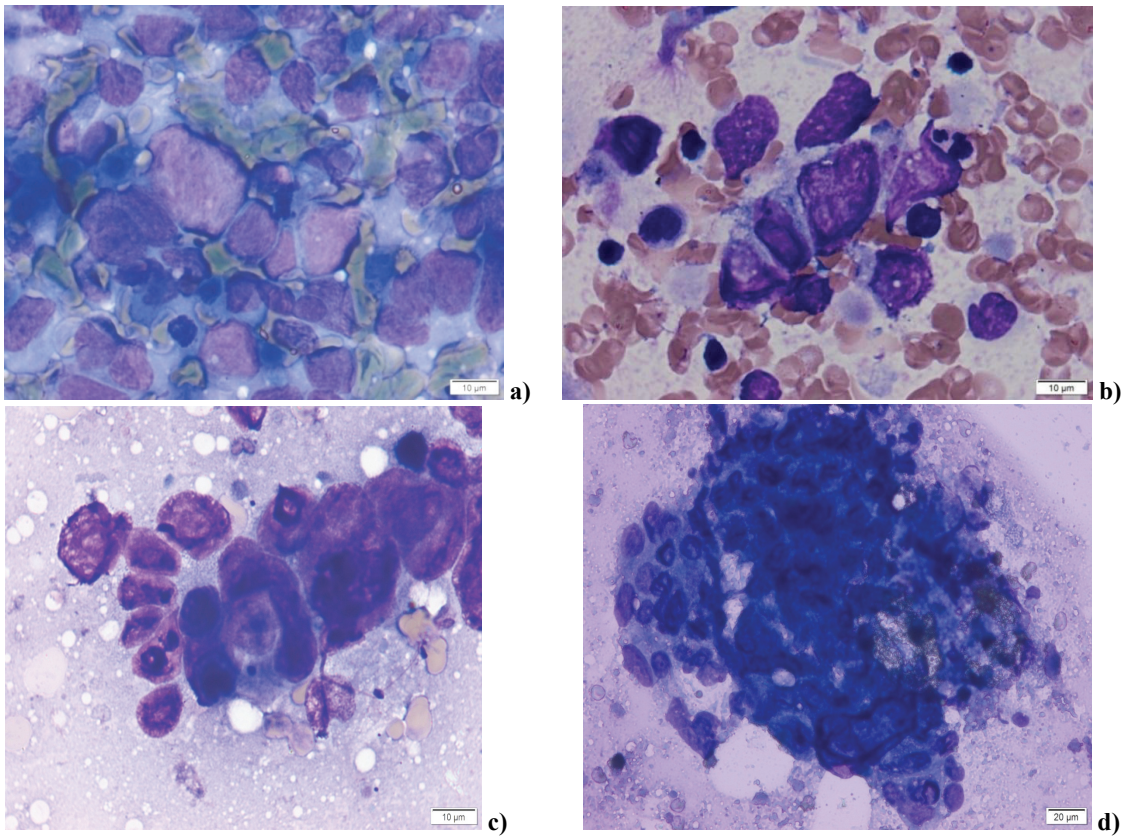

Fig. 3 – Cases with misdiagnosed small cell carcinoma: a) parafollicular T cell lymphoma [transbronchial needle apiration (TBNA), May-Grünwald-Giemsa (MGG), ×1000]; b) nondifferentiated sarcoma (TBNA, MGG, ×1000); c) non small cell lung carcinoma (not otherwise specified – high grade) (tru-cut, MGG ×1000); d) non small cell lung carcinoma, most probably adenocarcinoma (TBNA, MGG, ×400).

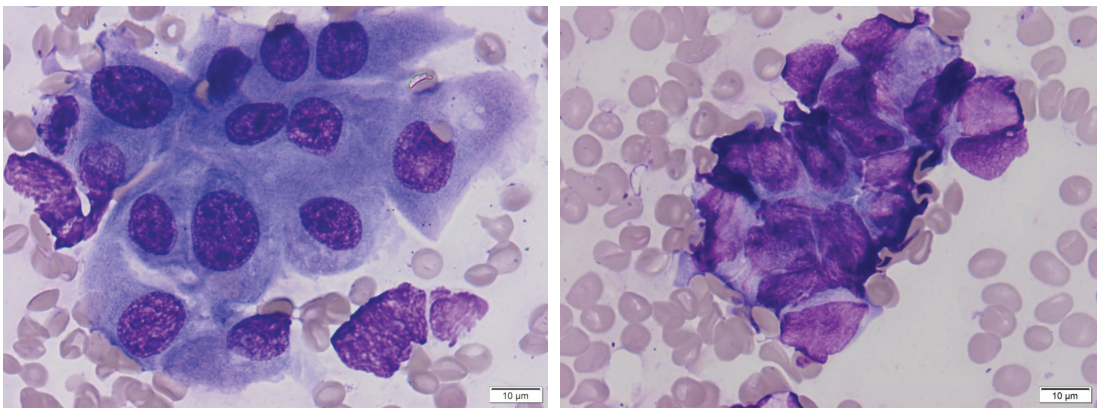

Fig. 4 – Case with misdiagnosed small cell carcinoma: two groups of cells in the same sample of bronchial brushing; nonkeratinizing squamous cell carcinoma intraepithelial basaloid type, with microinvasion (May-Grünwald-Giemsa, ×1000).

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

Figure 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 histopathological analysis, there was no tumor tissue.

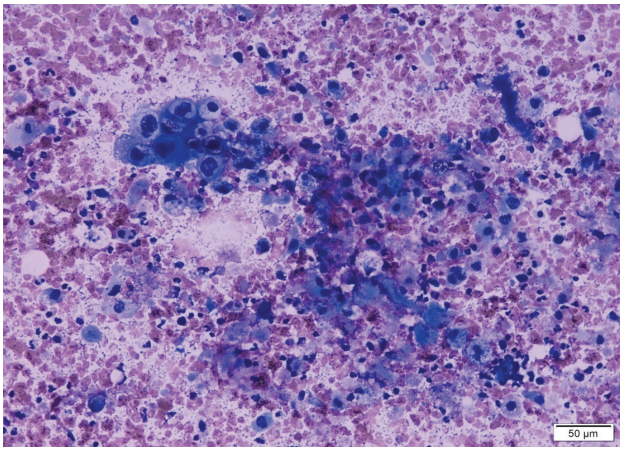

Fig. 5 – Squamous cell lung carcinoma with keratinization (transcarinal puncture, May-Grünwald-Giemsa, ×200).

Discussion

In patients with lung carcinoma, the only significant tumor parameters affecting the therapeutic procedure are the type of malignancy and stage of illness [19].

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 [10, 20]. 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 [21].

The accuracy of SCLC diagnosis on cytologic samples is similar to that achieved with small biopsies, that is, sufficiently high to start with treatment [19]. The most recent study by Li et al. [23], 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) was superior to histology (small biopsy stainded with HE and immunohistochemically) [4].

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 [6, 23]. However, 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 patients. 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 [24, 25].

The most common sampled cytological material in our patients was TBNA lymph node number 7, which is understandable, since SCLC was mainly positioned centrally and submucosally, and in almost all patients the 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 tose of Sakr et al. [26] (83%) and Miličić et al. [24] (76%), but slightly less than Delgado et al. [19] (96%).

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

Unlike our results, and those of Miličić et al. [24] who found disagreement between cytology and histology in 12/50 (23%) of the patients in similar investigation of the value of cytology in SCLC diagnosis, and Sakr et al. [26], who found incorrect cytological diagnoses of SCLC in 1/11 (9%) of the cases, Delgado et al. [19] in their study, comparing the accuracy of fine-needle aspiration cytology in the diagnosis of SCLC with the diagnosis of other lung malignancies, did not have any interpretative error**.** However, in their study, 221 patients had 242 fine-needle aspiration cytology, and all 18 (7%) of the smears interpreted as SCLC were correctly diagnosed, which is a far smaller number of patients with SCLC than in our study.

Rewiew of five misdiagnosed SCLC from 2017, found two cases with a clear interpretive error, that is, the wrong classification of the tumor type. In one case (Figure 3d), it is obvious that morphology and cell architecture did not satisfy cytological criteria for SCLC, in other words, it indicated NSCLC. But in another case, in the same sample (bronchial brushing), besides the groups of poorly differentiated cells (Figure 4, left) there are also other groups of cells with the clear squamocellular differentiation (Figure 4, right). On a bioptic sample, the histopathological diagnosis of nonkeratinized squamous cell carcinoma, basaloid type – intraepithelial with microinvasion, was established. After a month, the cytological finding of a transcarineal puncture performed in the same patient, revealed a clear morphology of keratinized squamous cell carcinoma, but the histopathological 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 Figure 3, a-c, the morphology of cells and the manner of clustering were such, that SCLC could not be excluded only on the basis of morphological criteria, which was also a cytological diagnosis, but pathology revealed lymphoma, sarcoma and NSCLC-NOS.

In cytological 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 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 (Figure 3a).

The main diagnostic problem in our study was to distinguish SCLC form NSCLC-NOS, and in a study of Miličić et al. [24], from squamous cell carcinoma and adenocarcinoma. These authors also had an incorrect diagnosis of SCLC in sarcoma. Domagała-Kulawik et al.[9] had similar difficulties in differentiating SCLC from undifferentiated, anaplastic NSCLC, and Delgado et al. [19] from poorly differentiated squamous cell carcinoma and large cell carcinoma.

In the material obtained by fine needle aspiration, Renshaw et al. [27] studied 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. [28], 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 scant delicate 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 cytological features of NSCLC, as well as some other neoplasms, e.g. lymphoma or sarcoma, may have occasionally some of morphological characteristics of SCLC, such as nucleus molding.

The above-mentioned examples show the complexity of morphological, cytological and also histopathological diagnostics on small biopsies. This complexity comes from the possibility that some of 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 [17].

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. [16] found combined SCLC in 28% cases (16% combined with LCC, 9% with adenocarcinoma and 3% with squamous cell carcinoma). While combined small cell/LCC require at least 10% of the tumor show LCC, no percentage requirement is needed if there is a clear adeno- or squamocellular component [16, 29, 30].

In the most combined tumors, a 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 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 Figures 4). 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 [10]. 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 surguical resection.

In five of our patiens with benign histopathological findings (inflammation, fibrosis), abundant well preserved cytological material with a clear morphological characteristic of SCLC as well as a clinical finding and a further course of the disease, pointed out a false negative histopathological result; there was no tumor tissue in the material for histopathological 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 bioptic material of five patients (nonrepresentative falsely negative bioptic material), and interpretative errors on cytological samples in 12 (6%) of patients. Due to the design of the study, in which the patients with the cytological diagnosis of SCLC were the starting point, we were not able to assess sampling errors on cytological specimens, as well as to evaluate if there were cases with cytological diagnosis of NSLC in histopathologically proved SCLC.

We could say that the part of committed cytological interpretive errors were objective, because they fell into overlapping zone of morphological, cytological characteristics of SCLC and NSCLC, or other types of small cell tumors, which was 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 the cases, even among experienced pathologists involved in the diagnosis of lung cancer [29]. 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 SCLC cells that are approaching the size of LCC cells, or the basaloid variant of LCC and squamous cell carcinoma [30].

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 in 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 [30]. In addition, SCLC should also be separated from small round cell neoplasms, such as neuroblastoma, embryonic rhabdomyosarcoma, desmoplastic small round cell tumor and primitive peripheral neuroectodermal tumor [31].

Conclusion

The cytological diagnosis of 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 invasive procedures are not indispensable in the diagnosis of lung cancer. 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.

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