Joanna Domagała-Kulawik[1], Barbara Górnicka[2], Rafał Krenke[1], Sylwia Mich[1], Ryszarda Chazan[1]

[1]Katedra i Klinika Chorób Wewnętrznych, Pneumonologii i Alergologii Warszawskiego Uniwersytetu Medycznego
Kierownik: prof. dr hab. n. med. R. Chazan
[2]Zakład Anatomii Patologicznej Warszawskiego Uniwersytetu Medycznego
Kierownik: prof. dr hab. n. med. A. Wasiutyński

The value of cytological diagnosis of small cell lung carcinoma

Znaczenie badania cytologicznego w rozpoznawaniu drobnokomórkowego raka płuca

Abstract

Introduction: Small cell lung carcinoma (SCLC) is a very aggressive neoplasm. Accurate and quick diagnosis is crucial to initiate proper treatment.
The aim of this study was to establish the value of initial cytological diagnosis and to present typical cytological features of SCLC.
Material and methods: We reviewed 116 cases of SCLC confirmed by cytology in: bronchial brushings, pleural fluids, and fine needle aspiration biopsies (FNAB).
Results: In 77% of SCLC cases, the diagnosis was established only by cytology; in 23% of cases, both cytological and histological recognition was possible. Cytology of SCLC was initially uncertain in 12%, and histology was uncertain in 30% of the cases. The morphology of SCLC cells was not uniform, and often a mixture of non-small atypical cells and bronchial epithelial cells with signs of metaplasia was observed. There were four cases of combined cell type with large cell carcinoma and two with adenocarcinoma. The main diagnostic problem was to distinguish small cell lung carcinoma from lymphomas, and from cancer consisting of small cells with the cytological features of non-small cell carcinoma.
Conclusion: Diagnosis of SCLC in cytological smears is accurate, and final diagnosis is based on light microscopy. In the differential diagnosis, other tumours of small cells have to be taken into account.

Key words: small cell lung carcinoma, cytology, diagnosis

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Streszczenie

Wstęp: Drobnokomórkowy rak płuca (SCLC) należy do bardzo agresywnych nowotworów. Szybkie ustalenie właściwego rozpoznania odgrywa kluczową rolę w podjęciu właściwej terapii.
Celem pracy była ocena roli badania cytologicznego we wstępnym rozpoznawaniu SCLC oraz przedstawienie typowych cytologicznych cech tego nowotworu.
Materiał i metody: Analizie poddano 116 przypadków SCLC, które potwierdzono badaniem cytologicznym w następujących materiałach: wymazy szczoteczkowe, płyn opłucnowy, biopsja aspiracyjna cienkoigłowa.
Wyniki: W 77% przypadków rozpoznanie SCLC było możliwe jedynie na podstawie badania cytologicznego; w 23% na podstawie badania cytologicznego i histopatologicznego. Wynik badania cytologicznego był pierwotnie wątpliwy w 12% przypadków, zaś histopatologicznego w 30%. Obraz morfologiczny SCLC nie był jednorodny — obserwowano formy mieszane z obecnością komórek o cechach raka niedrobnokomórkowego oraz komórek nabłonka oskrzelowego z cechami metaplazji płaskonabłonkowej. Rozpoznano formy złożone SCLC z rakiem wielkokomórkowym w 4 przypadkach i rakiem gruczołowym w 2 przypadkach. Głównym problemem diagnostycznym było odróżnienie raka drobnokomórkowego od chłoniaka i raka z drobnych komórek o cechach cytologicznych raka niedrobnokomórkowego.
Wniosek: Klasyczne badanie cytologiczne w mikroskopie świetlnym umożliwia właściwe rozpoznanie SCLC. W diagnostyce różnicowej należy brać pod uwagę inne nowotwory zbudowane z drobnych komórek.

Słowa kluczowe: drobnokomórkowy rak płuca, badanie cytologiczne, diagnoza

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Adres do korespondencji: dr hab. n. med. Joanna Domagala-Kulawik, Katedra i Klinika Chorób Wewnętrznych, Pneumonologii i Alergologii, Warszawski Uniwersytet Medyczny, ul. Banacha 1a, 02–097 Warszawa, tel./faks: (48 22) 599 28 53, e-mail: domagalakulawik@gmail.com

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Introduction

Lung cancer affects about 1.3 million people worldwide and is responsible for more than 1 million deaths per year. Two main histological types are distinguished, namely non-small cell lung carcinoma (NSCLC) and small cell lung carcinoma (SCLC) [1]. They both differ markedly in their biology and clinical course, with SCLC being highly aggressive and highly responsive to chemotherapy. By the end of the twentieth century SCLC composed about 20% of lung carcinomas, although this proportion has recently decreased to about 13% (as reported in the US) [2]. Nevertheless, SCLC is still responsible for the poor prognosis of lung malignancies as a whole. There are two main stages of the disease, limited (LD) and extensive (ED), which differ significantly in their prognosis and response to treatment [3]. The introduction of new chemotherapeutics promises improvement in SCLC therapy [3, 4]. However, as in most malignancies, early diagnosis (*i.e.* in the stage of LD) is the key point for optimal therapeutic effect. Since the onset of the disease is insidious, early diagnosis of SCLC is a rare entity. The primary tumour is often a hilar mass or submucosal infiltration [4], in many cases there are difficulties in obtaining an adequate tissue sample for histological diagnosis, and only cytological diagnosis is possible. The role of cytological diagnosis may be essential in the early and accurate diagnosis of SCLC. Many studies performed in the 1980s and 1990s confirmed the value of diagnostic cytology in lung cancer diagnosis [5–11]. However, apart from the textbook descriptions, there are few papers discussing the morphological pattern of SCLC in cytological samples and the problems in differential diagnosis [1, 12, 13]. The aim of this work is to present the value of cytology in the initial diagnosis of SCLC compared with histological biopsy, and to present the typical features of SCLC in different cytological samples and the main problems of differential diagnosis.

Material and methods

In this retrospective study we analyzed and reviewed the cytological samples in which diagnosis of SCLC was established. We compared cytological diagnosis with histological diagnosis, if performed. During the diagnostic procedure, bronchoscopy was performed in each patient and one or more cytological and/or histological specimens were taken. Informed consent was obtained from each patient before the diagnostic procedures. The examination was approved by the Ethics Committee of Warsaw Medical University. Bronchial brushings (BB), fine needle aspirates (transbronchial and transthoracic), and pleural fluid samples were used for the cytological studies. BB and transbronchial needle aspiration biopsies (TBNA) were collected (using a disposable bronchial cytology brush — Con Med Endoscopic Technologies, Inc., USA and a Wang transbronchial cytology needle 21 G — Con Med Endoscopic Technologies, Inc., USA, respectively) during awaken fibre-optic bronchoscopy (performed with Pentax EB-1830T2, FB-19TV and FB-18V bronchoscopes). A fine needle (21 G) was used to obtain aspirates from palpable peripheral lymph nodes. For other extrapulmonary metastases (*e.g.* thyroid gland, non- palpable lesions of neck, or tumours of visceral organs), ultrasound visualization was used. Transthoracic needle aspiration (TTNA) of peripheral lung tumours was performed under fluoroscopic or ultrasound guidance using 21 G needles (minimal tumour size 2 cm). After brushing or aspiration, cytological smears were immediately fixed in alcohol and then stained with haematoxylin and eosin. Pleural fluid samples (100 ml), collected during diagnostic thoracentesis, were centrifuged (10 min, 300 × g) and then smears were prepared, fixed, and stained as described above.

For SCLC diagnosis, the morphological criteria defined by the WHO 2004 classification were applied. This classification defines SCLC as „a malignant epithelial tumour consisting of small cells with scant cytoplasm, ill-defined cell borders, finely granular nuclear chromatin, and absent or inconspicuous nucleoli. The cells are round, oval, and spindle shaped, and nuclear molding is prominent" (Fig. 1) [4, 12, 13]. All samples were reviewed by two independent experienced pathologists. Uncertain cases were resolved by a third expert review.

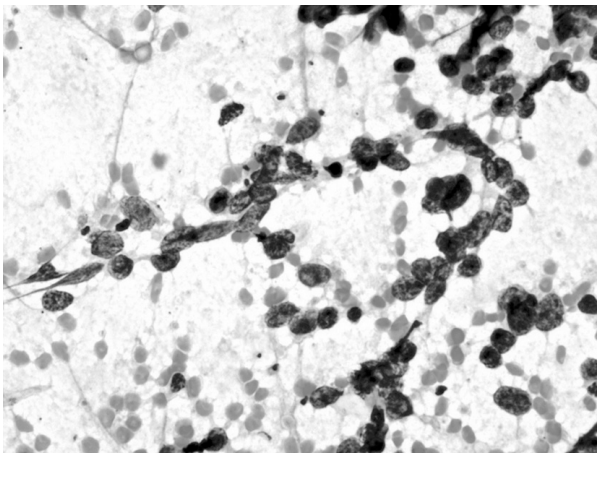

Figure 1. Typical features of small cell lung cancer cells in fine needle aspiration biopsy (haematoxylin- eosin, × 400)

Histological samples were collected with forceps (PRECISOR bronchopulmonary disposable biopsy forceps — ConMed Endoscopic Technologies, Inc., USA, diameter 1.8 or 2.3 mm) from endobronchial lesions during fibre-optic bronchoscopy. The slides were stained routinely with haematoxylin-eosin. As an additional method, immunohistochemistry (IHC) with monoclonal antibodies was used. The following antibodies were used: cytokeratins (CKAE1, E3), leukocyte common antigen (LCA), chromogranin, and synaptophysin (DAKO, Denmark).

Results

Of 4000 cytological examinations from respiratory tracts performed from 2000 to 2006, we selected cases diagnosed as SCLC. This included cytological samples obtained from 116 patients (53 F, 63 M, mean age 65 years). We have not presented any negative cytological examinations, only those in which SCLC was confirmed. Therefore, the number of samples and cases did not differ. Figure 2 presents the source of the samples: the highest number came from bronchial brushings (n = 36), while the lowest was from pleural fluid (n = 12). Needle aspirates included: transbronchial (n = 24), transthoracic (n = 12), and FNAB of extrapulmonary metastases (n = 32). Of these, 25 samples were taken from peripheral lymph nodes, mainly from the supraclavicular group (17, *i.e.* 68% of all lymph nodes). Other sites for FNAB included tumours of the thoracic wall, thyroid gland, head, and neck. SCLC diagnosis was certain in 102 cases; in 14 (12%) cases it was initially doubtful. Further analysis with the help of a third expert led to the final diagnosis of SCLC (6 cases) or SCLC combined with large carcinoma (LC) in 4 cases. In 2 cases adenocarcinoma was found in histological biopsy, which confirmed another 2 combined types of SCLC. The doubtful cases in each sample type and differential diagnosis taken into consideration are characterized in Table 1.

In these 116 cases of SCLC diagnosed by cytology, histological specimens were possible to obtain only in 71 patients (61%), all of them being bronchial biopsies. In 39 of these samples cancer was present, whereas in 32 (45%) there were no malignant cells in the biopsy specimens.

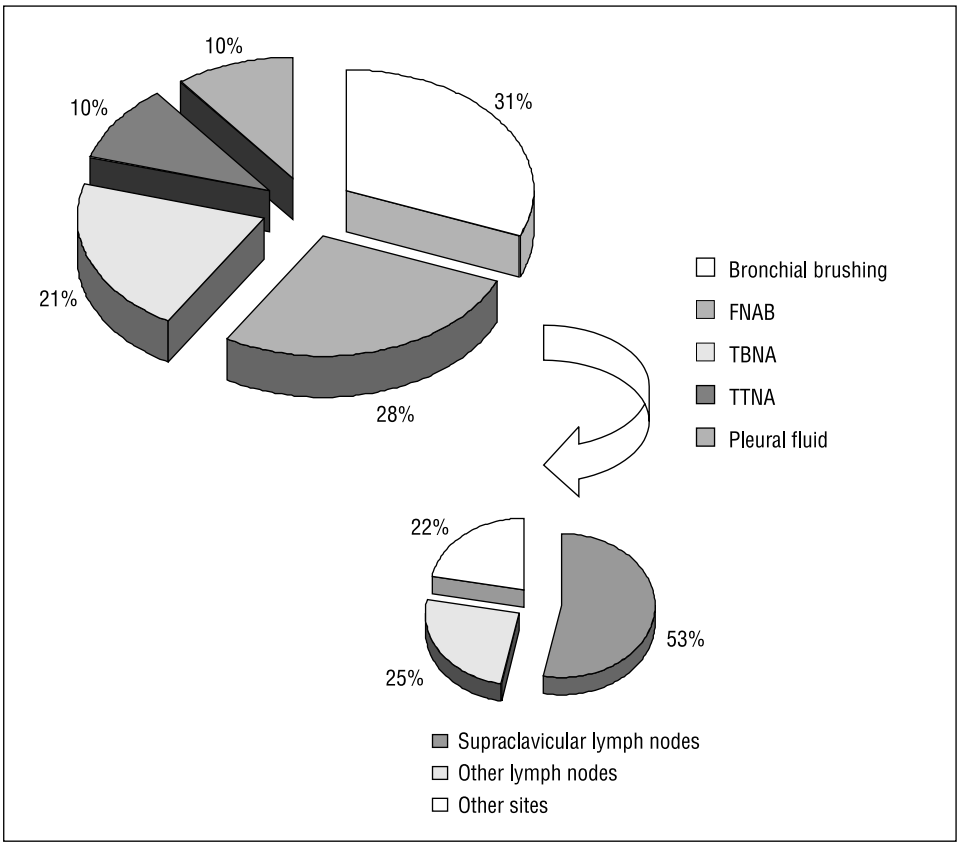

Figure 2. Type of cytological material for light microscopy diagnosis of small cell lung carcinoma. FNAB — fine needle aspiration biopsy of extrapulmonary metastases (supraclavicular lymph nodes being most frequent in our material), TBNA — transbronchial needle aspirates, TTNA — transthoracic needle aspirates

Table 1. Number of initially doubtful cases and the main causes of interpretative errors in cytological and histological diagnosis of small cell lung carcinoma

	Number of doubtful cases	Differential diagnosis and interpretative problems
Bronchial brushings [n = 36]	0	Squamous metaplasia
Transbronchial needle aspiration biopsy [n = 24]	3	Lymphoma
		NSCLC of small cells
Transthoracic needle aspiration biopsy [n = 12]	2	NSCLC of small cells
Fine needle aspiration biopsy of metastases [n = 32]	8	Lymphoma
		NSCLC of small cells
Pleural fluid [n = 12]	1	Metastases of a small cell neoplasm of other than lung cancer
Histological bronchial biopsy [n = 39]	12	Crush artefacts, inflammation

NSCLC — non-small cell lung carcinoma

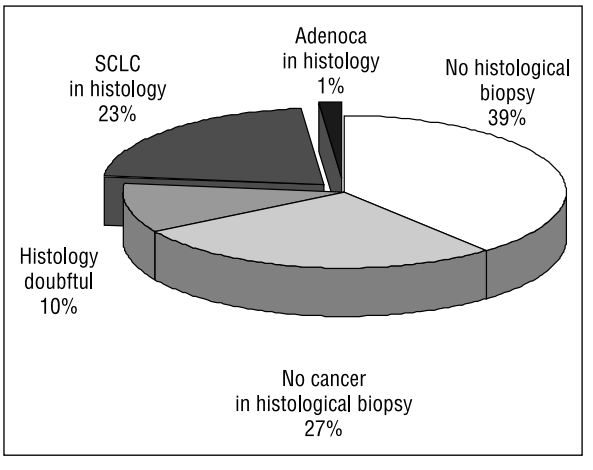

Figure 3. Cytological *v.* histological diagnosis of small cell lung carcinoma (SCLC)

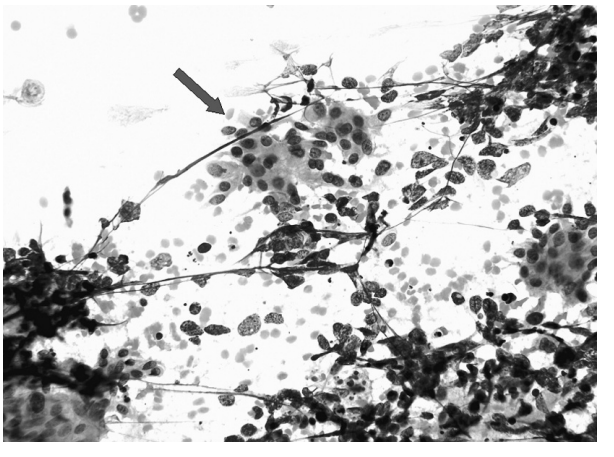

Figure 4. Bronchial cells with features of squamous metaplasia (arrow) and SCLC (haematoxylin-eosin, × 400)

Diagnosis of SCLC was certain in 25 cases (35% of collected bronchial biopsies), in 2 adenocarcinoma was found, whereas in 12 cases the examination was non-conclusive due to crush artefacts. IHC was applied in 74% of the histological biopsies, and in all of them SCLC was confirmed.

To summarize, in our group of 116 patients with SCLC, in 89 cases (77%) the only diagnosis was cytology (Fig. 3).

Bronchial brushing

Bronchial brushing represents an exfoliative type of cytology with the presence of a mixture of cancer cells and normal ciliated cells, a few basal and goblet cells, and finally inflammatory cells (neutrophils and lymphocytes). In our material, smears of good quality revealed well-preserved SCLC cells with typical cytological features easy to recognize. If taken from the tumour surface, the brushing samples contained numerous malignant cells, sufficient for accurate diagnosis. Rarely, necrotic masses or the presence of blood caused interpretative problems. More often, nuclear smearing was visible; this occurred when smears were performed with too much force. However, this feature was useful as a clue suggestive of SCLC. This nuclear smearing was usually accompanied by well-preserved SCLC cells, and the latter were the basis of the final diagnosis. Especially because there was more than one slide from each patient, and we never diagnosed SCLC only based on nuclear smearing. Very often, small cells were accompanied by epithelial cells with features of squamous metaplasia (Fig. 4), dysplasia, or marked atypia similar to the appearance of NSCLC. Thus, the cytological picture might have been erroneously interpreted as squamous cell carcinoma. In our study, in 36 samples from bronchial brushings, no uncertain cases were present.

Transbronchial needle aspirates (TBNA)

SCLC often manifested as a mediastinal mass, and distinguishing the primary tumour from mediastinal lymph node involvement by metastases was sometimes difficult. Metastases to the lymph nodes were characterized by the presence of mature and young forms of lymphocytes which coexisted with cancer cells. This was the cause of the main problem in the differential diagnosis: SCLC versus lymphoma. Cancer cells were larger in size than mature lymphocytes, whereas lymphoma cells were similar to SCLC cells in size. The main difference between SCLC and lymphoma is the presence of necrosis, the absence of cytoplasm, and slightly more pronounced cell polymorphism of cancer cells versus lymphoma cells (which depends of the kind of lymphoma). Nuclear smearing of cancer cells but not of lymphocytes was observed, which was useful in SCLC diagnosis.

Other interpretative challenges included distinguishing SCLC from undifferentiated, anaplastic NSCLC and recognizing a combined type. In our study, of 24 TBNAs, 3 were initially doubtful. In two samples, we found the cells with morphology of NSCLC and we recognized the presence of large cells (LC). LCs were larger than SCLCs and had a visible cytoplasm, marked nuclear boarding, and conspicuous nucleoli [14]. Finally, in these two cases the combined type with LC was recognized while in the third case the SCLC was confirmed by an independent expert.

Fine needle aspiration biopsy (FNAB) of extrapulmonary metastases

FNABs of metastatic lesions usually contained malignant cells and sometimes necrotic mass. In SCLC, aspirates were full of cancer cells, and the admixture of lymphocytes, even in lymph node metastases, was not dominant. The morphology of SCLC in the FNABs was typical, as described above. Nuclear smearing was sometimes present. There were no bloody samples. However, differential diagnosis with lymphomas and NSCLC of small cells or combined types had to be taken into consideration. In this study, of 32 FNABs, 8 were initially doubtful. In two of them, combined types with LC were finally diagnosed and in the others the clinical data and histological diagnosis of SCLC in bronchial biopsy were useful in resolving the doubts.

Transthoracic needle aspiration (TTNA)

As in other FNABs, in TTNA samples cancer cells were well preserved and presented typical cytological features, as described above. Necrosis and inflammatory cells with pulmonary macrophages were sometimes visible, but this did not alter the very good quality of the smears. However, an admixture of blood caused some interpretative problems. Initially there were 2 doubtful cases of 12 TTNA from our patients, which needed additional analysis and discussion with another pathologist.

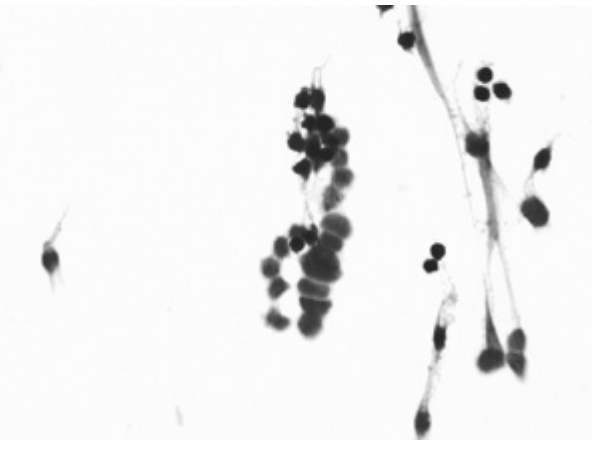

Figure 5. SCLC cells in pleural fluid (haematoxylin-eosin, × 400)

Pleural fluid

In our study, SCLC cells in the pleural fluid were not numerous (Fig. 5). They were found in mixtures of inflammatory and mesothelial cells and formed small aggregates with cell molding. The nuclei of the cancer cells were dark and the nuclear structure was poorly visible. The cell diameter seemed to be smaller than in samples from bronchial brushing or FNABs. In our study, 1 case in 12 was doubtful. In this case, only a few cancer cells were found; however, the clinical data were very useful to minimize the risk of misinterpretation.

Discussion

In this study, we presented the value of cytological diagnosis of SCLC and we confirmed its role in the final diagnosis of this very aggressive neoplasm. We analyzed the results of the examination of different kinds of cytological samples with special attention paid to the morphology of cancer cells. Difficulties in the differential diagnosis and interpretative problems were discussed. SCLC recognition was most accurate in bronchial brushing, while in FNABs we found a relatively high number of uncertain cases.

The role of cytological diagnosis in lung cancer was established in the last thirty years. In 1982 Pilotti et al. presented very good results of diffe-

rent cytological methods in lung cancer diagnosis (samples included various materials, from sputum to FNAB) [5–7, 15]. In recent decades, new techniques of visualization and penetration of the bronchial tree and lung parenchyma, as well as new methods of lung tissue sampling, have been developed. The main drawback of this work is that it not very innovative, but we presented the role of microscopic examination in SCLC, which still exists in the new era of diagnostic methods in imaging and endoscopy. In spite of the development of new techniques, light microscopic examination remains the gold standard in lung cancer diagnosis. Lung cancer classification is based on the histological characteristics of tumours and focuses on light microscopy. A review of the literature revealed the lack of large studies concerning SCLC; however, in studies on lung cancer cytopathology, a high diagnostic accuracy for SCLC was noted. Sharafkhaneh et al. reported a higher yield of TBNA for SCLC than for NSCLC (87% *v.* 64%, respectively) [16]. He explained this observation by a lower cellular adherence of small cells and higher size of primary tumour resulting from the aggressiveness of SCLC. On the other hand, in a review by Schreiber et al., the authors showed that misclassification in cytology was higher for SCLC than for NSCLC: 9% *v.* 2%; however, the accuracy in distinguishing these two types of cancer by cytology was high: from 0.94 to 1.00 (mean 0.98) in a large group of 6305 patients [10]. In this *résumé*, the yield of cytobrushing had a higher sensitivity than TBNA, which is in agreement with our observation. Delgado et al., in a study on the role of FNAB in the diagnosis of SCLC, described 100% specificity and 67% sensitivity and a high ability to distinguish SCLC from other lung malignancies. He found 12% of all 259 samples to be SCLC [13]. In other studies, SCLC was confirmed in 8% to 70% of all TBNA [11, 16, 17] and 8% of TTNA performed [8]. In the study of Steffee et al., a higher accuracy of SCLC than of NSCLC diagnosis was documented [11]. Recently, the role of ultrasound guided biopsy has been widely presented [17–20].

There are two main types of errors in pathological diagnosis: sampling errors, when "the diagnostic material was not present on the slide", and interpretative errors [21]. In cytology, the latter type is more frequent. As we have shown, some features of small carcinoma cells need to be taken into consideration in light microscopy diagnosis of SCLC. In samples from bronchial brushing and FNAB, the cell morphology is well preserved; however, the admixture of other cells may cause important interpretative difficulties — metaplastic bronchial epithelial cells in brushing [22] or lymphocytes in FNAB [20] serve as examples. The morphology of the same small cancer cells varied in different samples. The main difference was between cells in FNAB and body fluids. Presently, the method of liquid cytology is widely used, and this preparation may influence the differences in the cell morphology [23].

At the time of SCLC diagnosis, extensive disease is recognized in 70% of the patients [3]. Very often FNAB of a metastatic tumour is the first diagnostic procedure, and in some exceptional cases may be the final diagnosis before treatment. In our study, accurate diagnosis of SCLC was established by FNAB of extrapulmonary metastases in 27.5% of the cases. Metastases to the supraclavicular lymph nodes were most frequent. In a review by Jackman, the frequency of this site of metastases varied from 17% at presentation to 42% in autopsy [4].

Pleural fluid rarely serves as a good material for the initial diagnosis of SCLC, more often for the confirmation of cancer spread. Involvement of the pleura was reported in 20–30% of patients with SCLC [4]. Small carcinoma cells may be detected accidentally during the diagnosis of pleural effusion. In our series, SCLC cells in pleural fluid are not numerous. The presence of small carcinoma cells may indicate metastases from other small cell tumours (such as breast carcinoma), and therefore the interpretation of pleural fluid cytology should be cautious, and clinical data always needs to be considered in the final diagnosis [24].

As we have shown in our results, it may be difficult to distinguish SCLC from undifferentiated, anaplastic NSCLC. Delgado et al. noted similar difficulties in the differentiation of SCLC and poorly differentiated squamous cell and large cell lung carcinoma [13]. An admixture of cells with features of non-small cell origin may indicate a combined type of SCLC (Fig. 6). SCLC combined with non-small cell carcinoma is quite frequent (estimated at 10–26%) and is currently classified as a subtype of SCLC [1, 14]. The combined type of SCLC is defined as cancer of small cell origin in which at least 10% of the cancer tissue is composed of large carcinoma cells [12]. A mixture with large carcinoma (LC) or large cell neuroendocrine lung carcinoma (LCNEC) is reported to be the most frequent. In a series of 113 neuroendocrine lung carcinomas, Asamura et al. found 26% combined and, of these, 13% with LCNEC; 14 cases were borderline [14]. As mentioned above, combined SCLC is characterized by the presence of at least 10% of large cells in the cancer tissue. Thus, in the

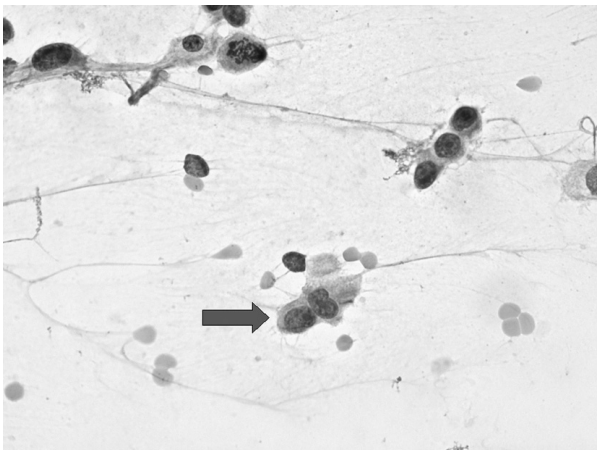

Figure 6. Admixture of NSCLC, arrow (haematoxylin- eosin, × 400)

cytology of small samples this admixture may be missed. In our material there were four combined cell types, all with LC and all in the needle aspirates of metastases. LC cells are larger than SCLC and have a visible cytoplasm, marked nuclear boarding, and conspicuous nucleoli [25]. Cell size alone seems to be insufficient as a criterion for small cell/large cell differentiation, as proposed by some authors [14]. In spite of the some initial problems, we recognized these cells among SCLC cells. Even if combined type SCLC is not diagnosed, clinically this may have little significance: no difference between pure small cell and the combined type in the prognosis was observed [11, 12, 14]. Nitadori et al. showed the usefulness of immunohistochemistry in LCNEC diagnosis: a higher expression of cytokeratines CK7, 18, E-cadherin, and beta-catenin in the LCNEC cells when compared with SCLC [26] was noted.

The diagnostic yield of histological biopsy in SCLC is disappointing: Clee et al. reported 25% [9]; in our study it reached 35%. The main problem in histology is sampling error, usually due to the primary tumour localization and crush artefacts [12]. In our series of 71 bronchial biopsies, cancer tissue was found in 39 samples, and in 12 of them SCLC was not certain. In the study of Nicholson, crush artefacts caused interpretative problems in 14% of the cases [12]. In uncertain histological cases, certain antibodies may be used to increase the specificity. These include cytokeratins to distinguish SCLC from lymphomas and CD56--NCAM, synaptophysin, and chromogranin to confirm the neuroendocrine origin of the small cells [1]. Recently, a positive reaction of thyroid transcription factor 1 (TTF1) with SCLC cells was found in about 80–90%, which may be of value in differential diagnosis with NSCLC and carcinoid. However, in the opinion of many pathologists, the histological diagnosis of SCLC should be based on light microscopy, and immunohistochemistry (IHC) is not generally recommended [1, 4, 12]. Cytological samples are usually too small to apply IHC. However, sometimes it may be useful and necessary in differential diagnosis with lymphomas.

We conclude that the diagnosis of SCLC in cytological smears is accurate, and final diagnosis may be based on light microscopy. In the differential diagnosis, other tumours of small cells have to be taken into account.

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References

1. World Health Organization. Tumors of the lung, pleura, thymus, and heart. In: Travis W.D., Brambilla E., Muller-Hermelink H.K. et al. (eds.). Classification of tumors. Lyon, France, IARC Press, 2004: 9–124.
2. Govindan R., Page N., Morgensztern D. et.al. Changing epidemiology of small-cell lung cancer in the United States over the last 30 years: analysis of the surveillance, epidemiologic, and end results database. J. Clin. Oncol. 2006; 24: 4539–4544.
3. Krzakowski M., Orłowski T., Roszkowski K. et al. Drobnokomórkowy rak płuca — zalecenia diagnostyczno-terapeutyczne Polskiej Grupy Raka Płuca. Pneumonol. Alergol. Pol. 2007; 75: 88–94.
4. Jackman D.M., Johnson B.E. Small-cell lung cancer. Lancet 2005; 366: 1385–1396.
5. Pilotti S., Rilke F., Gribaudi G., Damascelli B. Fine needle aspiration biopsy cytology of primary and metastatic pulmonary tumors. Acta Cytol. 1982; 26: 661–666.
6. Pilotti S., Rilke F., Gribaudi G., Spinelli P. Cytologic diagnosis of pulmonary carcinoma on bronchoscopic brushing material. Acta Cytol. 1982; 26: 655–660.
7. Pilotti S., Rilke F., Gribaudi G., Damascelli B., Ravasi G. Transthoracic fine needle aspiration biopsy in pulmonary lesions. Updated results. Acta Cytol. 1984; 28: 225–232.
8. Larscheid R.C., Thorpe P.E., Scott W.J. Percutaneous transthoracic needle aspiration biopsy: a comprehensive review of its current role in the diagnosis and treatment of lung tumors. Chest 1998; 114: 704–709.
9. Clee M.D., Duguid H.L., Sinclair D.J. Accuracy of morphological diagnosis of lung cancer in a department of respiratory medicine. J. Clin. Pathol. 1982; 35: 414–419.
10. Schreiber G., McCrory D.C. Performance characteristics of different modalities for diagnosis of suspected lung cancer: summary of published evidence. Chest 2003; 123: 115S–128S.
11. Steffee C.H, Segletes L.A., Geisinger K.R. Changing cytologic and histologic utilization patterns in the diagnosis of 515 primary lung malignancies. Cancer 1997; 81: 105–115.
12. Nicholson S.A., Beasley M.B., Brambilla E. et al. Small cell lung carcinoma (SCLC): a clinicopathologic study of 100 cases with surgical specimens. Am. J. Surg. Pathol. 2002; 26: 1184–1197.
13. Delgado P.I., Jorda M., Ganjei-Azar P. Small cell carcinoma versus other lung malignancies: diagnosis by fine-needle aspiration cytology. Cancer 2000; 90: 279–285.
14. Asamura H., Kameya T., Matsuno Y. et al. Neuroendocrine neoplasms of the lung: a prognostic spectrum. J. Clin. Oncol. 2006; 24: 70–76.
15. Pilotti S., Rilke F., Gribaudi G., Ravasi G.L. Sputum cytology for the diagnosis of carcinoma of the lung. Acta Cytol. 1982; 26: 649–654.
16. Sharafkhaneh A., Baaklini W., Gorin A.B., Green L. Yield of transbronchial needle aspiration in diagnosis of mediastinal lesions. Chest 2003; 124: 2131–2135.

17. Annema J.T., Versteegh M.I., Veselic M., Voigt P., Rabe K.F. Endoscopic ultrasound-guided fine-needle aspiration in the diagnosis and staging of lung cancer and its impact on surgical staging. J. Clin. Oncol. 2005; 23: 8357–8361.
18. Szlubowski A., Kużdżał J., Pankowski J. et al. Przezoskrzelowa biopsja węzłów chłonnych śródpiersia i wnęk płuca pod kontrolą ultrasonografii wewnątrzoskrzelowej w czasie rzeczywistym w diagnostyce raka płuca i sarkoidozy. Pneumonol. Alergol. Pol. 2008; 76: 229–236.
19. Herth F.J., Rabe K.F., Gasparini S., Annema J.T. Transbronchial and transoesophageal (ultrasound-guided) needle aspirations for the analysis of mediastinal lesions. Eur. Respir. J. 2006; 28: 1264–1275.
20. Kramer H., Sanders J., Post W.J., Groen H.J., Suurmeijer A.J. Analysis of cytological specimens from mediastinal lesions obtained by endoscopic ultrasound-guided fine-needle aspiration. Cancer 2006; 108: 206–211.
21. Nodit L., Balassanian R., Sudilovsky D., Raab S.S. Improving the quality of cytology diagnosis: root cause analysis for errors in bronchial washing and brushing specimens. Am. J. Clin. Pathol. 2005; 124: 883–892.
22. Domagala-Kulawik J., Droszcz P. Squamous metaplasia of bronchial epithelium — role in the pulmonary tumors diagnosis. Medical Science Monitor 1998; 4: 993–997.
23. Hoda R.S. Non-gynecologic cytology on liquid-based preparations: A morphologic review of facts and artifacts. Diagn. Cytopathol. 2007; 35: 621–634.
24. Koss L.G. Effusions in the presence of cancer. In: Diagnostic Cytology and Its Histopathologic Bases. Philadelphia: JB Lippincott Company, 1992: 1116–1184.
25. Domagała-Kulawik J., Olszewski W.T. Atlas cytopatologii układu oddechowego. Alfa Medica Press, Bielsko-Biała, 2009.
26. Nitadori J., Ishii G., Tsuta K. et.al. Immunohistochemical differential diagnosis between large cell neuroendocrine carcinoma and small cell carcinoma by tissue microarray analysis with a large antibody panel. Am. J. Clin. Pathol. 2006; 125: 682–692.