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The yield of bronchoscopy in the diagnosis of lung diseases

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Abstract

Introduction

Bronchoscopy is the main method in the diagnosis of various lung diseases. EBUS-TBNA is the most modern bronchoscopic technique useful in diagnosis and staging of lung cancer (LC).

Objectives

The aim of the study was to assess the yield of bronchoscopy in the diagnosis of various respiratory diseases. Especially, we examined the possibility of obtaining diagnostic material using bronchoscopic techniques in patients with various pathomorphological types of LC.

Patients and Methods

The results of pathomorphological examinations from 5279 bronchoscopies performed in 2016-2018 were analyzed. Clinical and demographic factors were analyzed using the Fisher χ^2 test.

Results

5279 patients (1892 women, 3387 men) were diagnosed due to various respiratory symptoms. LC was confirmed in 36.42% of patients, reactive lymph nodes – in 16.06%, sarcoidosis – in 4.12%, IPF – in 0.19%, non-LC metastases – in 2.39%. 40.81% of patients had no definitive diagnosis. SCC was most often diagnosed (32.07%), then AC, (30.61%), SCLC (25.83%) and NSCLC NOS (11.49%). Diagnosis of SCC was obtained significantly more often (χ^2=43.143, p<0.000001) by endobronchial (41.09%) than by transbronchial biopsies (26.62%). Diagnosis of AC or NDRP NOS was significantly more often (χ^2=20.394, p<0.000007 and χ^2=3.902, p<0.05, respectively) observed in transbronchial biopsies (34.31% and 12.6%) than in endobronchial biopsies (24.52% and 9.64%).

Conclusion

The use of bronchoscopy in the diagnosis of various lung diseases has many limitations. SCLC and SCC may be more common in Poland than in other European countries and in US. Effectiveness of EBUS-TBNA and forceps biopsy is strongly affected by tumor localization and type of cancer.

Key words: lung cancer; bronchoscopy; endobronchial biopsy; EBUS-TBNA; EUS-FNA

Article summary

Strengths and limitations of this study

1. This is one of the largest bronchoscopy methods effectiveness research in routine clinical practice.

2. Endobronchial biopsy is more effective in the diagnosis of squamous cell carcinoma (central tumors). Whereas, EBUS-TBNA and EUS-FNA has higher efficiency in the detection of adenocarcinoma (peripheral tumors).

3. Study shows that advanced small cell lung cancer may be underestimated.

4. The study revealed that EBUS-TBNA value in daily clinical practice differed from that in clinical trials.

5. It was not possible to determine the final diagnosis for all patients in this large group (5279 cases). Not distinguish between material collected by EBUS-TBNA and EUS-FNA. The lack of detailed clinical and radiological characteristics of all patients who underwent the bronchoscopy procedures.

Introduction

Epidemiological analyses indicate that lung cancer (LC) is the most common cause of cancer-related death. It is usually diagnosed in an unresectable, advanced stage. [1, 2] LC is the most common cancer in men and the third most common in women. In 2018, there were more than 2 million new cases of lung cancer worldwide. [3]

In the United States from 2004 to 2009, a total of 1,096,276 lung cancer cases were diagnosed and reported. This American study investigated the histologic type of lung cancer and demographic characteristics of the patients. The incidence of individual types of LC was as follows: small cell lung cancer (SCLC) – 14.9% of cases, squamous cell carcinoma (SCC) – 21.9% of cases, adenocarcinoma (AC) – 37.1% of cases and large cell carcinoma (LCC) – 3.2% of patients. [4]

It is difficult to obtain accurate epidemiological data on the occurrence of individual pathomorphological types of LC in advanced stages. Such data is only available on patients undergoing surgery in earlier stages of the disease. 17,783 patients diagnosed and operated in

Polish thoracic surgery centers were registered in the National Lung Cancer Registry in the years 2014-2018. This group includes: 48.7% of patients with AC, 40.8% of patients with SCC, 6.45% of patients with LCC, 2.1% of patients with adenosquamous cell carcinoma, 1.05% of patients with SCLC and 0.9% of patients with non-otherwise specified (NOS) non-small cell lung cancer (NSCLC). This material included a low percentage of patients with SCLC (usually an inoperable type of lung cancer) and patients with NSCLC-NOS (large surgical material is easy for pathomorphological examination). In addition, LCC should be diagnosed only in the surgical material. Therefore, this type of cancer is almost absent in patients with advanced lung cancer (it could not be diagnosed in the small specimens). [2]

Due to different locations and tumor types, varied approaches are required to cancer diagnosis in different compartments of the lung or metastatic lymph nodes. Bronchoscopy is an appropriate method for detecting LC. Endobronchial ultrasound-guided with transbronchial needle aspiration (EBUS-TBNA) and endoscopy ultrasound with fine needle aspiration (EUS-FNA) are preferred for mediastinal lymph nodes assessment for evaluation of N-stage in patients with NSCLC. [5] However, endobronchial forceps biopsy or cryobiopsy may be preferred mainly in diagnosis of the central tumors. These two methods could be used in sampling visible tumors localized in central airways but also peripherally to the main bronchi, i.e. in lobar or even segmental bronchi. The sensitivity of detecting lung cancer by bronchoscopy varies from 34% to 88% depending on the size and location of the tumor and preliminary diagnosis of the patients. [6] Meta-analysis of 18 studies which included a total of 1,201 patients with LC diagnosed by ultrasound-guided fine needle aspiration showed sensitivity of 83% (range 45-100%) and specificity of 97% (range 88-100%) of these methods. [7] However, all false negative results delay cancer diagnosis and force the repetition of diagnostic procedures including surgery. While, earlier detection of the lung cancer gives patients the chance for better treatment. [8]

Aim

The aim of our study was to assess the yield of bronchoscopy in the diagnosis of different lung diseases in daily clinical practice. Especially, we devoted special attention to the possibility of diagnosis of individual pathomorphological types of LC with various techniques use for collecting materials during bronchoscopy.

Material and methods

In our observational cross-sectional study, we analyzed the results of pathomorphological examination carried out on the material obtained by the 5279 bronchoscopies performed in 2016-2018. Those bronchoscopies were performed in three

Polish pulmonology departments. The study included 1892 women and 3387 men with median age of 65 years.

Various diseases of the respiratory system were indication for bronchoscopy: 3127 (59.2%) patients had suspicion of chest tumor in computed tomography, 882 (16.7%) patients demonstrated hilar lymphadenopathy, 205 (3.9%) patients had suspicion of sarcoidosis and 20 (0.4%) patients had suspicion of pulmonary fibrosis. Other indications for bronchoscopy occurred in 1045 (19.8%) patients (e.g. suspicion of tuberculosis, chronic cough, hemoptysis, etc.). After receiving the diagnosis, we selected a population of patients with lung cancer and divided them to groups of patients with different cancer types (squamous cell lung cancer, adenocarcinoma, large cell carcinoma, not otherwise specified NSCLC and small cell lung cancer). Than we assessed the prevalence of different types of LC in the materials obtained with various bronchoscopic procedures.

In 3565 (67.5%) patients, EBUS-TBNA and EUS-FNA was performed and cytological material archived in a cytoblock was obtained. In 1346 (25.5%) patients, EBUS-TBNA (without EUS-FNA) was the only diagnostic procedure. In the remaining patients, EBUS-TBNA was supplemented by EUS-FNA. There were no patients in whom EUS-FNA would be the only diagnostic method. 1714 (32.5%) patients had non-ultrasound-guided bronchoscopy with the forceps biopsy of endobronchial lesions allowing to obtain a small histological specimen. The method for tissue sampling was chosen based on the location of the lesions and mediastinal or hilar lymph node status. Forceps biopsies were performed using Olympus BF-1T180 and Pentax EB-1970K bronchoscopes, EBUS-TBNA – using Olympus BF-UC180F and Pentax EB-1970UK bronchoscopes (22-gauge needles), and EUS-FNA – using Olympus GF-UCT180 endoscope. Premedication for bronchoscopy was under local or general anesthesia, depending on the situation.

Statistical analysis

Clinical and demographic factors were analyzed using the Pearson's Chi-square test. P-values below 0.05 were considered significant. The percentages reflect the relative number of all lung cancer patients diagnosed with a particular procedure. The evaluation of relative diagnostic yield (sensitivity) of different bronchoscopic procedures could not be done because, in our study, it was not possible to verify the final diagnosis of analyzed patients. We only analyzed the results of the first-time bronchoscopy. Further diagnostic procedures were often not performed in our clinical centers. The following diagnostic procedures were carried out in various clinical centers throughout Poland.

Ethics approval

The protocol of the study was approved by the Committee of Ethics and Research at the Medical University of Lublin (KE-0254/5/2018).

Results

Lung cancer was confirmed in group of 1923 (36.42%) patients, including 1280 men and 643 women. Only reactive lymph nodes were found in 16.06% of the patients, sarcoidosis was diagnosed in 4.13% of the patients, idiopathic pulmonary fibrosis – in 0.19% of the patients, metastases to the lungs from other organs – in 2.39% of the patients. 40.81% of the patients had no definitive diagnosis (Figure 1). Lung cancer was confirmed in 51% of patients with suspected tumor in computed tomography. While, in the group of patients with hilar lymphadenopathy, lung cancer was diagnosed in only 10.1% of cases.

Among patients with lung cancer, squamous cell carcinoma (32.07%) was most often diagnosed, then adenocarcinoma (30.61%), small cell lung cancer (25.83%) and NSCLC-NOS (11.49%) (Figure 2). SCLC and AC were significantly more frequent ($\chi^2 = 8.649$, $p = 0.0033$ and $\chi^2 = 21.128$, $p < 0.000005$, respectively) in women (29.97% and 37.42% of women with lung cancer) than in men (23.75% and 27.19% of male patients with lung cancer). Squamous cell carcinoma appeared significantly more often ($\chi^2 = 41.881$, $p < 0.000001$) among male (36.95%) than among female (22.36%) patients with lung cancer (Figure 3). SCC was significantly more often ($\chi^2 = 4.17$, $p = 0.041$) diagnosed in the group of patients older than 65 years than in younger patients. Other pathomorphological types of lung cancer occurred with similar frequency in these two age groups.

Endobronchial biopsies significantly more often ($\chi^2 = 7.566$, $p = 0.0059$) provided material for the diagnosis of lung cancer than the EBUS-TBNA and EUS-FNA procedures. 42.35% of endobronchial biopsies and 33.6% of transbronchial and transesophageal biopsies provided material sufficient to diagnose lung cancer. Fine needle biopsy of lymph nodes enabled the diagnosis of lung cancer in 29.2% of cases, fine needle biopsy of lung tumor – in 66.6% of cases, and forceps biopsy of bronchial mucosa lesions – in 48.2% of cases. These differences were statistically significant.

Among patients with lung cancer, transbronchial or transesophageal biopsies compared to endobronchial biopsies were similarly effective ($\chi^2 = 0.656$, $p = 0.418$) in detecting SCLC (26.5% vs. 24.8%). On the other hand, the diagnosis of AC and NSCLC-NOS was obtained significantly more frequently ($\chi^2 = 20.394$, $p = 0.000006$ and $\chi^2 = 3.902$, $p = 0.0482$) in EBUS-TBNA and EUS-FNA compared to endobronchial biopsies (34.3% vs. 24.52% and 12.6% vs. 9.6%, respectively). SCC was significantly more often ($\chi^2 = 43.143$, $p < 0.000001$) diagnosed in material obtained from forceps biopsy than in material from EBUS-

TBNA and EUS-FNA (41.09% vs 26.62%) (Figure 4). Table 1 shows the effectiveness of bronchoscopy in the diagnosis of individual pathomorphological types of lung cancer depending on place of collecting the material.

Discussion

Our study on the yield of EBUS-TBNA and forceps biopsy in obtaining material for the diagnosis of various lung diseases is among the largest worldwide. The study points to numerous problems arising from the use of these techniques in routine clinical practice. We are aware of the many limitations of our study. First, we cannot determine the sensitivity and specificity of our methods, because it was not possible to determine the final diagnosis in this large group of patients (5279 cases). In addition, we could not distinguish between material collected by EBUS-TBNA and EUS-FNA. Thirdly, diagnosis of large cell carcinoma was not possible in small specimens (LCC was probably qualified to the NSCLC-NOS group). A limitation of our study was also the lack of detailed clinical and radiological characteristics of all patients who underwent the bronchoscopy procedures.

However, we found that advanced small cell lung cancer may be more common in Poland than previously thought. This tumor is characterized by rapid growth and metastases, therefore, more often it could be diagnosed in advanced stages using bronchoscopic techniques. USG-guides transbronchial biopsy of lymph nodes and endobronchial biopsy had similar efficiency in SCLC detection. Furthermore, the difference in the percentage of patients with squamous cell carcinoma and adenocarcinoma diagnosed with endo- and transbronchial biopsies is noteworthy. In our study, most patients with adenocarcinoma were diagnosed with EBUS-TBNA or EUS-FNA of lymph nodes, while patients with squamous cell carcinoma were diagnosed more often based on examination of material from endobronchial biopsy (forceps biopsy). Endobronchial biopsy is more effective in the diagnosis of squamous cell carcinoma, because patients often had a centrally located tumor. Thus, we point to the problem that effective bronchoscopic procedures depends on the location of the primary tumor and the presence of metastases in the lymph nodes.

Schmid-Bindert et al. performed prospective study in group of 106 patients with NSCLC diagnosis. Small biopsies were collected by three different methods: forceps biopsy (44.6%), EBUS-TBNA (32.7%), and CT-guided core biopsy (22.8%). 38% of adenocarcinoma, 51% of squamous cell carcinoma and 11% of NSCLC-NOS were diagnosed using forceps biopsy. EBUS-TBNA results were 45% for adenocarcinoma, 30% for squamous cell carcinoma and 24% for NSCLC-NOS. [9]

Many authors emphasize that the diagnosis of NSCLC-NOS is the most common in the case of material obtained from EBUS-TBNA. Esterbrook et al. found that NSCLC-NOS rate was 20.8% in EBUS-TBNA samples. Similar results were received by Navani et al. In group of 774 patients with known or suspected lung cancer, 23% of patients had a final diagnosis of NSCLC-NOS. [10, 11]

Chin et al. reported that EBUS-TBNA is the most sensitive diagnostic methods for SCLC, because it allows to sample of specimens from mediastinal as well as submucosal lesions. They also mentioned that the quality of specimens obtained by needle aspiration is better than by forceps biopsies, which may contain crush artifacts. [12] In addition, other studies noticed that the sensitivity of EBUS-TBNA for SCLC detection was higher than for NSCLC diagnosis. [13, 14]

Many authors raise the problem that forceps biopsy has low sensitivity in the diagnosis of lung cancer. Forceps biopsy has a diagnostic yield ranging between 65-82% [15, 16]. In our preliminary study conducted in 212 patients with lung cancer suspicion, we compared sensitivity and accuracy of routine bronchoscopy with endobronchial biopsy, EBUS-TBNA, and combination of EBUS-TBNA and EUS-FNA techniques. Sensitivity and accuracy of endobronchial biopsy vs EBUS-TBNA vs combination of transbronchial biopsies were 43% vs 44.3% vs 93.7% and 93.8% vs 94.7% and 94.8%, respectively. This demonstrates high usefulness of the combination of EBUS-TBNA and EUS-FNA in the diagnosis of lung cancer [17]. Verma et al. demonstrated sensitivity of EBUS-TBNA in cancer diagnosis of 91.4% in small group of 37 patients with lesions located adjacent to the trachea or lesions located adjacent to the main bronchi. [18] Tournoy et al. indicated that EBUS-TBNA has a sensitivity of 82% and low negative predictive value (23%). [19] The similar results received Zhao et al. for lesions located near the central airways. [20] Oki et al. showed that the combined endoscopic method with EBUS-TBNA and EUS-FNA with a single bronchoscope gives better results in staging of NSCLC than each technique alone. However, they mentioned that significant number of patients had false-negative EBUS-TBNA and EUS-FNA results. Moreover Oki et al. suggested that very important issue is bronchoscopists experience, which may cause differences in the results. [21] On the other hand, Wallace et al. showed a EBUS-TBNA sensitivity of only 69% in a group of 150 patients with lung cancer suspicion. [22]

Despite relatively low negative predictive value of EBUS-TBNA, there is an indication to perform other procedures (e.g. surgical procedures) for final diagnosis in a significant group of patients. In our study, 56.9% of patients who underwent bronchoscopy did not receive a definitive diagnosis of the diseases and they have been subjected to other

diagnostic procedures or observations. We showed the results of all performed bronchoscopies and three different method of material collection (endobronchial biopsy and combination EBUS-TBNA and EUS-FNA). Moreover, unselected and heterogeneous patient population were recruited in three different hospitals that employ a total of 8 bronchoscopists. In most studies, the evaluation of the usefulness of EBUS-TBNA and EUS-FNA for detecting malignancy was conducted in selected patients with high clinical suspicion of the tumor. Small, preselected groups of patients with high risk of lung cancer could be the reason of the low negative predictive value of bronchoscopic procedures in these studies. Thus, these observation may not reflected the real clinical situation.

In studies where population was heterogenic regarding the disease (lung cancer, sarcoidosis, tuberculosis), EBUS-TBNA had diagnostic value only in 60-75% of patients. [23] Lange et al. showed diagnostic results of EBUS-TBNA in only 61.4% of unselected patients undergoing routine diagnostic procedures. [24] Fournier et al. examined 185 patients with extrathoracic malignancy and mediastinal lymphadenopathy in real life practice. Pathomorfological types of malignancy were successfully identified using EBUS-TBNA in only 93 patients (50.3%). The diagnostic sensitivity, specificity, negative predictive value, and positive predictive value were 68.4%, 100%, 53.3%, and 100%, respectively. [25]

Conclusions

Comparing all these data we could conclude that bronchoscopy is not an ideal technique in the routine diagnosis of respiratory diseases. Our study proved that 41% bronchoscopy material was insufficient to perform reliable pathomorphological examination. This mainly concerned patients with suspected lung tumor or lymphadenopathy. Especially, here is a problem in the diagnosis of advanced lung tumors (mainly adenocarcinomas) using bronchoscopy if it is performed as the only procedure. We found that the EBUS-TBNA value in daily clinical practice differed from that in clinical trials. Therefore, precise estimation of the frequency of individual pathomorphological types of lung cancer based on material obtained bronchoscopically is not possible. It seems that the incidence of lung cancer with a typical peripheral localization (adenocarcinoma) may be underestimated in comparison to the incidence of lung cancer with a typical central localization (squamous cell carcinoma, SCLC).

Authorship contribution statement:

Conception and design of the study: JB, MF, PK, JP. Administrative support: PK, JP, AP, JB, AS, JS, MM, PK, RK, JM. Provision of study material or patients: JP, AP, JB, AS, JS, MM, PK, RK, JM. Collection and assembly of data: JB, MF, PK, JP, AP, JB, AS, JS, MM, PK, RK. Data analysis and interpretation: JB, MF, PK. Manuscript writing: JB, MF, PK. All authors gave final approval of the published manuscript.

Data Availability Statement:

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest:

The authors declare no potential conflicts of interest.

Foundings:

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Table 1. The effectiveness of bronchoscopy in the diagnosis of individual pathomorphological types of lung cancer depending on place of collecting the material and nodal station.

Material	**SCLC**	**Adenocarcinoma**	**Squamous cell carcinoma**	**NOS**	**Total lung cancer**

EBUS-TBNA/EUS-FNA of lymph nodes	250 (27.7%)	331 (36.7%)	209 (23.1%)	114 (12.5%)	904 (100%)
EBUS-TBNA/EUS-FNA of tumor	88 (24.7%)	94 (25.8%)	135 (37.2%)	44 (12.3%)	361 (100%)
EBUS-TBNA/EUS-FNA metastases to adrenal gland	3 (42.9%)	1 (14.3%)	2 (28.6%)	1 (14.3%)	7 (100%)
Forceps biopsy of tumor	156 (22%)	163 (25%)	270 (41.5%)	62 (9.5%)	651 (100%)

Figure 1. Results of pathomorphological examination carried out on material obtained from 5279 bronchoscopies (entire study population)

Figure 2. The incidence of individual pathomorofological types of LC in the entire study group of lung cancer patients

Figure 3. The incidence of individual pathomorofological types of LC according to the gender of patients with lung cancer

Figure 4. Percentage of patients with different types of lung cancer detected in materials collected with different bronchoscopic techniques. Frequency of different types of lung cancer was calculated in the whole group of patients undergoing a given bronchoscopic procedure (100%), which resulted in the diagnosis of lung cancer

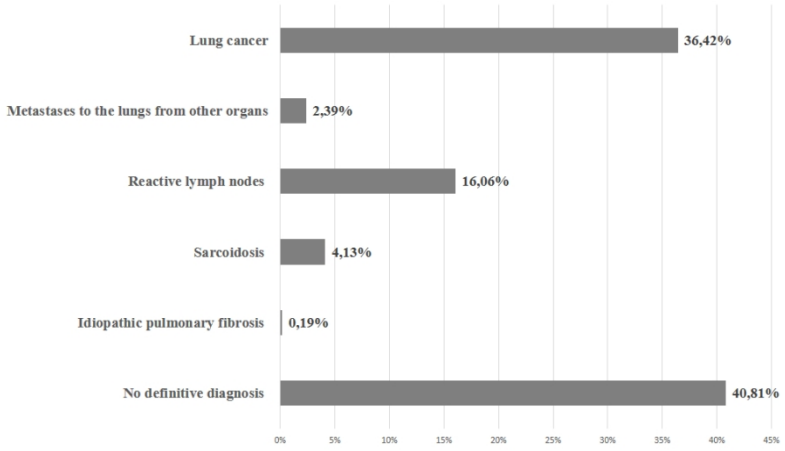

Figure 1. Results of pathomorphological examination carried out on material obtained from 5279 bronchoscopies (entire study population)

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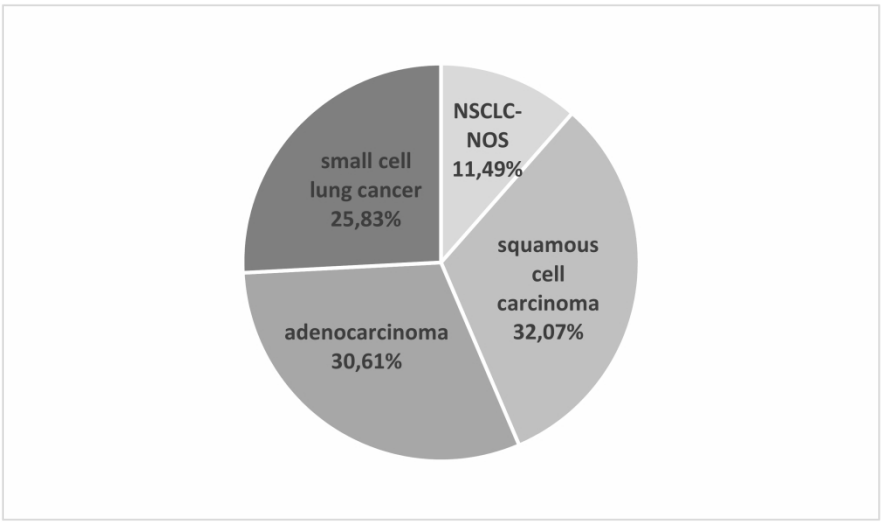

Figure 2. The incidence of individual pathomorofological types of LC in the entire study group of lung cancer patients

296x209mm (300 x 300 DPI)

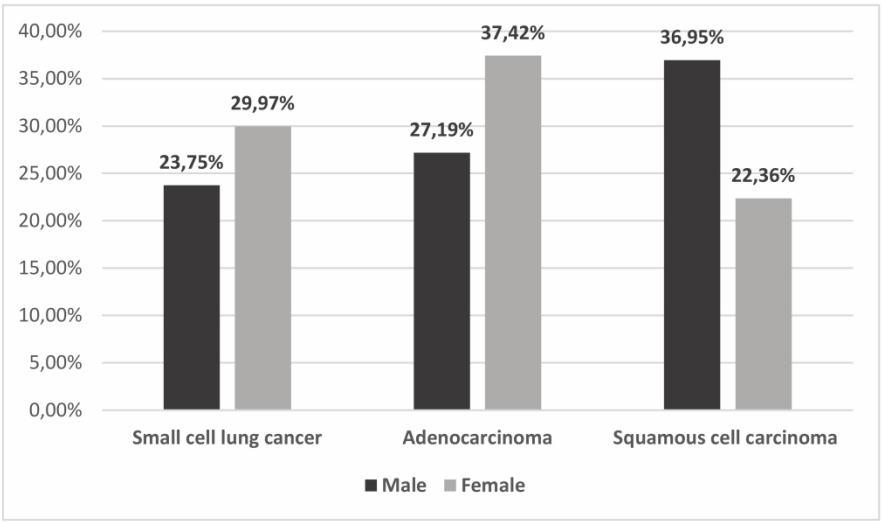

Figure 3. The incidence of individual pathomorofological types of LC according to the gender of patients with lung cancer

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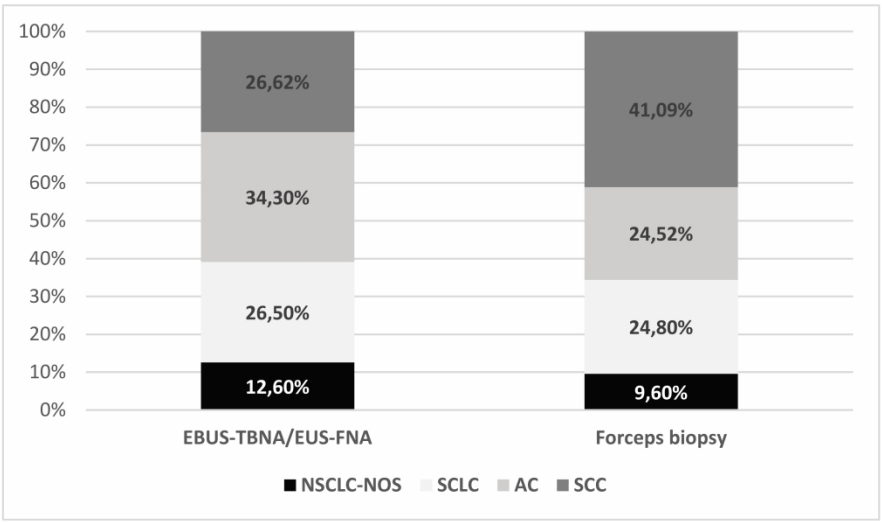

Figure 4. Percentage of patients with different types of lung cancer detected in materials collected with different bronchoscopic techniques. Frequency of different types of lung cancer was calculated in the whole group of patients undergoing a given bronchoscopic procedure (100%), which resulted in the diagnosis of lung cancer

296x209mm (300 x 300 DPI)

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The analysis of 5279 bronchoscopy results for the efficiency of various biopsy techniques in the diagnosis of lung cancer

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The analysis of 5279 bronchoscopy results for the efficiency of various biopsy techniques in the diagnosis of lung cancer

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Short title: **The yield of bronchoscopy in the diagnosis of lung cancer**

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Abstract

Introduction

Bronchoscopy is the main method in the diagnosis of various lung diseases. Endobronchial ultrasound transbronchial needle aspiration (EBUS-TBNA) is the most modern bronchoscopic technique useful in diagnosis and staging of lung cancer.

Objectives

The aim of the study was to assess the yield of bronchoscopy in patients with suspected various respiratory diseases including lung cancer. In particular, we examined the efficiency of different biopsy techniques in the diagnosis of lung cancer in correlation with its localization and pathomorphological type.

Patients and Methods

The results of pathomorphological examinations from 5279 bronchoscopies performed in 2016-2018 were analyzed. The material was collected with endobronchial ultrasound transbronchial needle aspiration (EBUS-TBNA), endoscopic ultrasound guided fine needle aspiration (EUS-FNA) and endobronchial forceps biopsy. Clinical and demographic factors were analyzed using the Fisher χ^2 test.

Results

5279 patients were diagnosed due to various respiratory symptoms. Lung cancer was confirmed in 36.42% of patients. 40.81% of patients had no definitive patomorphological diagnosis. Among patients with lung cancer, the most frequent diagnosis was non-small cell lung cancer: squamos cell lung cancer (SCC) - 32.07% and adenocarcinoma (AC) - 30.61%, then small cell lung cancer (SCLC) - 25.83% and not otherwise specified non-small cell lung cancer (NSCLC NOS) - 11.49%. Diagnosis of SCC was obtained significantly more often (χ^2=43.143, p<0.000001) by endobronchial (41.09%) than by EBUS-TBNA/EUS-FNA (26.62%). On the contrary, diagnosis of AC or NSCLC NOS was significantly more often (χ^2=20.394, p<0.000007 and χ^2=3.902, p<0.05, respectively) observed in EBUS-TBNA/EUS-FNA (34.31% and 12.6%) than in endobronchial biopsies (24.52% and 9.64%).

Conclusions

The use of bronchoscopy in the diagnosis of various lung diseases is vital but also has many limitations. Effectiveness of EBUS-TBNA and endobronchial forceps biopsy in diagnosis of lung cancer is strongly affected by tumor localization and type of cancer.

Key words: lung cancer; bronchoscopy; endobronchial biopsy; EBUS-TBNA; EUS-FNA

Article summary

Strengths and limitations of this study

1. 5 279 patients were enrolled to the study group which makes it one of the largest studies in the world with assement of bronchoscopy effectiveness in routine clinical practice.
2. We analyzed bronchoscopies underwent in 2016-2018 in a few polish medical centers across the country in diversified population which ensure a high level of generalisability.
3. The study is an important contribution to the epidemiological data of advanced lung cancer in Poland.
4. Bronchoscopies were performed by 8 different bronchoscopists. This fact may cause human-dependent variation in results.
5. Results of pathomorphological assessment of the material obtained during single bronchoscopy was analyzed. Patients without diagnosed disease in the bronchoscopic material underwent further diagnostics using other methods or in other centers. Therefore, we were unable to report a definitive diagnosis in patients with inconclusive results of diagnostic prcedures.

Introduction

Epidemiological analyses indicate that lung cancer (LC) is the most common cause of cancer-related death. It is usually diagnosed in an unresectable, advanced stage. [1, 2] Lung cancer is the most common cancer in men and the third most common in women. In 2018, there were more than 2 million new cases of lung cancer worldwide. [3]

In the United States from 2004 to 2009, a total of 1,096,276 lung cancer cases were diagnosed and reported. This American study investigated the histologic type of lung cancer and demographic characteristics of the patients. The incidence of individual types of LC was as follows: small cell lung cancer (SCLC) – 14.9% of cases, squamous cell carcinoma (SCC) – 21.9% of cases, adenocarcinoma (AC) – 37.1% of cases and large cell carcinoma (LCC) – 3.2% of patients. [4]

It is difficult to obtain accurate epidemiological data on the occurrence of individual pathomorphological types of advanced LC in Poland. Up to date, no epidemiological studies have been conducted on a sufficiently large group of patients in advanced lung cancer to obtain reliable results. Statistics on pathomorphological diagnoses of lung cancer in material from bronchoscopy have not been conducted so far. In Poland, the main source of such data is the National Lung Cancer Registry which only contains details about patients undergoing surgery in earlier stages of the disease. Till today, there where 17 783 patients diagnosed and

operated in Polish thoracic surgery centers and registered in the National Lung Cancer Registry in the years 2014-2018. This group includes: 48.7% of patients with adenocarcinoma (AC), 40.8% of patients with squamous cell carcinoma (SCC), 6.45% of patients with large cell carcinoma (LCC), 2.1% of patients with adenosquamous cell carcinoma, 1.05% of patients with small cell lung cancer (SCLC) and 0.9% of patients with non-otherwise specified (NOS) non-small cell lung cancer (NSCLC). This material included a low percentage of patients with SCLC (usually an inoperable type of lung cancer) and patients with NSCLC-NOS (large surgical material is easier for pathomorphological examination which is a factor of reducing misdiagnosis). According to IASLC (International Association for the Study of Lung Cancer) recommendations, large cell carcinoma should be diagnosed only in the surgical materials extracted from the entire resected tumor. [5] Therefore, this type of cancer was recorded in surgical pathology in non-advanced NSCLC patients and almost absent in patients with advanced lung cancer diagnosed with tumor biopsy. [2]

Due to different locations of tumor types in the lungs or metastatic lymph nodes, varied approaches are required to cancer diagnosis. The peripheral location is characteristic for adenocarcinoma, while squamous and small cell carcinomas most often are located centrally. Bronchoscopy is an appropriate method for detecting LC and the endobronchial ultrasound-guided with transbronchial needle aspiration (EBUS-TBNA) procedure has an essential role in the invastigation of lung cancer. If the tumor is centrally located and infiltrated the bronchus, the most optimal procedure seems to be the endobronchial biopsy using a brush or forceps. In contrast, adenocarcinoma frequently metastasizes to the mediastinal lymph nodes, which may be available on EBUS-TBNA or EUS-FNA procedures.

It is advisable to go along with the most easily available material, the collection of which will be associated with the lowest possible risk for the patient. In this connection, in everyday practice, the pathomorphological diagnosis is often achieved by metastatic lymph nodes. It is vital in diagnosing peripheral located tumors as adenocarcinoma.

Metastasis to the mediastinal lymph nodes is typical for each of types of lung cancer. We distinguish compartments of the mediastinal lymph nodes: superior mediastinal nodes (stations 2, 3 and 4), aortic nodes (stations 5 and 6), inferior mediastinal nodes (stations 7, 8, 9), hilar and interlobar lymph nodes (stations 10 and 11) and peripheral lymph nodes (station 12 for lobar nodes, station 13 for segmental nodes and station 14 for subsegmental nodes). EBUS-TBNA is most often used in diagnosis of superior mediastinal nodes, station 7 of inferior mediastinal nodes and stations 10, 11, 12 lymph nodes. EUS-FNA is preffered in diagnosis of superior mediastinal nodes and stations 7, 8 and 9 of inferior mediastinal nodes.

Moreover EUS-FNA is used to sampling subphrenic lymph nodes and metastates in liver and left adrenal gland. Both methods – EBUS-TBNA and EUS-FNA are preferred for mediastinal lymph nodes assessment for evaluation of N-stage in NSCLC patients. [6] These two methods could be used in sampling visible tumors localized in central airways but also peripherally to the main bronchi, i.e., in lobar or even segmental bronchi. These sampling principles were also applied in our study in all the centers that participated in the study.

Lung cancer staging is initially assessed in imaging studies. Normal mediastinum lymph nodes are defined below 10 mm in computed tomography (CT). Such a size of the lymph nodes suggests the advancement of clinical stage N0. Currently, sampling for N0 nodes is not recommended, while surgery is the primary treatment method for stage N0/N1 lung cancer. However, everyday practice shows that it is worth collecting non-enlarged nodes for pathomorphological examination, because cancer cells are often found in such nodes. [7,8]

The sensitivity of detecting lung cancer by different bronchoscopy methods varies from 34% to 88% depending on the size and location of the tumor and preliminary diagnosis of the patients. [9] Meta-analysis of 18 studies which included a total of 1,201 LC patients was performed for assessment of sensitivity and specificity of ultrasound-guided fine needle aspiration in mediastinal staging of lung cancer. Authors showed sensitivity of 83% (range 45-100%) and specificity of 97% (range 88-100%) of these methods. [10] In eight studies limited to patients with enlarged mediastinal lymph nodes seen on CT, sensitivity was 90% (95% CI: 84 to 94%) and specificity was 97% (95% CI: 95 to 98%). In patients without enlarged mediastinal lymph nodes visible on CT, the overall sensitivity was 58% (95% CI: 39-75%). Therefore, the use of EBUS-TBNA increases the accuracy in the estimation of the stage of lung cancer and may radically influence the further treatment of the patient and the selection of the treatment method. Staging N2/N3 is a premise for the use of radiochemotherapy and/or immunotherapy and allows the patient to be protected against the burden of surgical procedure. EUS-FNA enables confirming the presence of distant metastases, which has a decisive influence on therapeutic decisions. However, all false negative results delay cancer diagnosis and force the repetition of diagnostic procedures including surgery. Earlier detection of the lung cancer gives patients the chance for better treatment. [11]

Aim

The objective of our study was to assess the yield of bronchoscopy in the diagnosis of lung cancer. Especially, we devoted special attention to the possibility of diagnosis of individual pathomorphological types of LC with various techniques use for collecting materials during bronchoscopy.

Material and methods

In our observational cross-sectional study, we analyzed the results of pathomorphological examination carried out on the material obtained by the 5279 bronchoscopies performed in 2016-2018. Those bronchoscopies were performed in three Polish pulmonology departments. The study included 1892 women and 3387 men with median age of 65 years.

Patient and Public Involvement

No patient involved. It was a retrospective study that relied fully on the documents gathered, thus eliminating the need for collaboration between patients and researchers.

Various diseases of the respiratory system were indication for bronchoscopy: 3127 (59.2%) patients had suspicion of chest tumor in computed tomography, 882 (16.7%) patients demonstrated hilar lymphadenopathy, 205 (3.9%) patients had suspicion of sarcoidosis and 20 (0.4%) patients had suspicion of pulmonary fibrosis. Other indications for bronchoscopy occurred in 1045 (19.8%) patients (e.g. suspicion of tuberculosis, chronic cough, hemoptysis, etc.). In patients with suspected cancer, samples of the tissue were taken through the bronchoscopy and technique was chosen in compliance with tumor or metastatic lymph node's location as it is described in the introduction. Forceps biopsies were performed using Olympus BF-1T180 and Pentax EB-1970K bronchoscopes, EBUS-TBNA – using Olympus BF-UC180F and Pentax EB-1970UK bronchoscopes (22-gauge needles), and EUS-FNA – using Olympus GF-UCT180 endoscope. Premedication for bronchoscopy was under local or general anesthesia, depending on the situation.

Samples underwent pathomorphological examination which included hematoxylin-eosin (H&E) staining, mucicarmine staining and immunohistochemistry (IHC) examination such as staining of TTF-1 (thyroid transcription factor 1) and p63/p40. Samples diagnosed with non-squamous NSCLC were in-depth reported and underwent molecular testing for the presence of EGFR mutation by real-time PCR technique (RT-PCR), ALK gene rearrangement and PD-L1 expression by IHC. PD-L1 expression was also assessed in SCC patients. Large cell carcinoma (LCC) of the lung according to The 2015 World Health Organization (WHO) Classification of Tumors of the Lung, Pleura, Thymus and Heart cannot be diagnosed in small

specimens and aspiration biopsy materials. The diagnosis of LCC can only be made in the postoperative material. Therefore, there were no patients diagnosed with LCC in our study. Such patients were included in the group of patients diagnosed with NSCLC NOS. Chromogranin and synaptophysin was used in IHC examination of neuroendocrine tumors (small cell lung cancer or NSCLC NOS). All the centers participating in the study used these same procedures described above.

After receiving the diagnosis, we selected a population of patients with lung cancer and divided them to groups of patients with different cancer types (squamous cell lung cancer, adenocarcinoma, large cell carcinoma, not otherwise specified NSCLC, and small cell lung cancer). Then, we assessed the prevalence of different types of LC in the materials obtained with various bronchoscopic procedures.

Clinical and demographic factors were analyzed using the Pearson's Chi-square test. P-values below 0.05 were considered significant. The percentages reflect the relative number of all lung cancer patients diagnosed with a particular procedure. We only analyzed the results of the first-time bronchoscopy, that could be nondiagnostic. The evaluation of relative diagnostic yield (sensitivity) of different bronchoscopic procedures could not be done because, in our study, it was not possible to verify the final diagnosis of patient in the materials collected during the next bronchoscopy or another procedures (this applies mainly to patients with lung tumor or hilar lymphadenopathy). The following diagnostic procedures were carried out in various clinical centers throughout Poland. Therefore, we were unable to verify the diagnoses obtained later.

The protocol of the study was approved by the Committee of Ethics and Research at the Medical University of Lublin (KE-0254/5/2018).

Results

In 3565 (67.5%) patients, EBUS-TBNA and transesophageal EUS-FNA was performed and cytological material archived in a cytoblock was obtained. In 1346 (25.5%) patients, EBUS-TBNA (without EUS-FNA) was the only diagnostic procedure. In the remaining patients, EBUS-TBNA was supplemented by EUS-FNA. There were no patients in whom EUS-FNA would be the only diagnostic method. 1714 (32.5%) patients had non-ultrasound-guided bronchoscopy with the forceps biopsy of endobronchial lesions allowing to obtain a small histological specimen.

Lung cancer was confirmed in group of 1923 (36.42%) patients, including 1280 men and 643 women. Only reactive lymph nodes were found in 16.06% of the patients, sarcoidosis

was diagnosed in 4.13% of the patients, idiopathic pulmonary fibrosis – in 0.19% of the patients, metastases to the lungs from other organs – in 2.39% of the patients. 40.81% of the patients had no definitive diagnosis (Figure 1). Lung cancer was confirmed in 51% of patients with suspected tumor in computed tomography. While, in the group of patients with hilar lymphadenopathy, lung cancer was diagnosed in only 10.1% of cases.

Among patients with lung cancer, squamous cell carcinoma (32.07%) was most often diagnosed, then adenocarcinoma (30.61%), small cell lung cancer (25.83%) and NSCLC-NOS (11.49%) (Figure 2). SCLC and AC were significantly more frequent ($\chi^2 = 8.649$, $p = 0.0033$ and $\chi^2 = 21.128$, $p < 0.000005$, respectively) in women (29.97% and 37.42% of women with lung cancer) than in men (23.75% and 27.19% of male patients with lung cancer). Squamous cell carcinoma appeared significantly more often ($\chi^2 = 41.881$, $p < 0.000001$) among male (36.95%) than among female (22.36%) patients with lung cancer (Figure 3). SCC was significantly more often ($\chi^2 = 4.17$, $p = 0.041$) diagnosed in the group of patients older than 65 years than in younger patients. Other pathomorphological types of lung cancer occurred with similar frequency in these two age groups.

Endobronchial biopsies significantly more often ($\chi^2 = 7.566$, $p = 0.0059$) provided material for the diagnosis of lung cancer than the EBUS-TBNA and EUS-FNA procedures. 42.35% of endobronchial biopsies and 33.6% of TBNA and FNA provided material sufficient to diagnose lung cancer. Fine needle biopsy of lymph nodes enabled the diagnosis of lung cancer in 29.2% of cases, fine needle biopsy of lung tumor – in 66.6% of cases, and forceps biopsy of bronchial mucosa lesions – in 48.2% of cases. These differences were statistically significant.

Among patients with lung cancer, TBNA or FNA compared to endobronchial biopsies were similarly effective ($\chi^2 = 0.656$, $p = 0.418$) in detecting SCLC (26.5% vs. 24.8%). On the other hand, the diagnosis of AC and NSCLC-NOS was obtained significantly more frequently ($\chi^2 = 20.394$, $p = 0.000006$ and $\chi^2 = 3.902$, $p = 0.0482$) in EBUS-TBNA and EUS-FNA compared to endobronchial biopsies (34.3% vs. 24.52% and 12.6% vs. 9.6%, respectively). SCC was significantly more often ($\chi^2 = 43.143$, $p < 0.000001$) diagnosed in material obtained from forceps biopsy than in material from EBUS-TBNA and EUS-FNA (41.09% vs 26.62%) which was directly related to the more frequent central location and bronchial infiltration of this type of tumor. Computed tomography showed that in 79% of SCC patients the tumor was centrally located in the large bronchi. The analysis of bronchoscopic images showed that tumor deformed the bronchial mucosa or showed endobronchial growth in 67% of SCC patients. (Figure 4). Table 1 shows the effectiveness of bronchoscopy in the diagnosis of

individual pathomorphological types of lung cancer depending on place of collecting the material.

Discussion

Our study on the yield of EBUS-TBNA and forceps biopsy in obtaining material for the diagnosis of various lung diseases is among the largest worldwide. The study points to numerous problems arising from the use of these techniques in routine clinical practice. We are aware of the many limitations of our study. First, we cannot determine the sensitivity and specificity of our methods, because it was not possible to determine the final diagnosis in this large group of patients (5 279 cases). In addition, we could not distinguish between material collected by EBUS-TBNA and EUS-FNA. Thirdly, diagnosis of large cell carcinoma was not possible in small specimens (LCC was probably qualified to the NSCLC-NOS group). A limitation of our study was also the lack of detailed clinical and radiological characteristics of all patients who underwent the bronchoscopy procedures.

However, we found that advanced small cell lung cancer may be more common in Poland than previously thought. This tumor is characterized by rapid growth and metastases, therefore, more often it could be diagnosed in advanced stages using bronchoscopic techniques. EBUS-TBNA/EUS-FNA of lymph nodes and endobronchial biopsy had similar efficiency in SCLC detection. Furthermore, the difference in the percentage of patients with squamous cell carcinoma and adenocarcinoma diagnosed with endobronchial biopsies and TBNA or FNA is noteworthy. In our study, most patients with adenocarcinoma were diagnosed with EBUS-TBNA or EUS-FNA of lymph nodes, while patients with squamous cell carcinoma were diagnosed more often based on examination of material from endobronchial biopsy (forceps biopsy). Patients in cohort with squamous cell carcinoma were more likely to have endobronchial disease accessible by forceps. Therefore we could not ascertnained that endobronchial biopsy is more effective for diagnosis of squamous cell lung cancer as there was no comparison to EBUS-TBNA and EUS-FNA for those patients. Thus, we point to the problem that effective bronchoscopic procedures depends on the location of the primary tumor and the presence of metastases in the lymph nodes.

In addition to pathological diagnosis, molecular tests were also performed (*ALK* and *ROS1* gene rearrangement, *EGFR* mutation, PD-L1 expression), which are necessary for further qualification for treatment. These studies are made simultaneously from the same cytoblock. The method of sampling during bronchoscopy may affect the diagnostic value of the sample. Schmid-Bindert et al. performed prospective study in group of 106 patients with

NSCLC diagnosis. Authors used bronchoscopy methods to provide highest RNA yield for multiple biomarker. They also compared diagnostic value of bronchoscopic samples in molecular tests. By the way, the authors showed how often they detected different pathomorphological types of lung cancer using various bronchoscopic methods. Small biopsies were collected by three different methods: forceps biopsy (44.6%), EBUS-TBNA (32.7%), and CT-guided core biopsy (22.8%). 38% of adenocarcinoma, 51% of squamous cell carcinoma and 11% of NSCLC NOS were diagnosed using forceps biopsy. EBUS-TBNA results were as follows: 45% of adenocarcinoma, 30% of squamous cell carcinoma and 24% of NSCLC-NOS. The study showed the sufficient effectiveness of all three methods in the diagnosis of lung cancer. However, it was indicated that the largest amount of genetic material was collected using EBUS-TBNA and this method showed the highest diagnostic value for molecular tests. [12]

Many authors emphasize that the diagnosis of NSCLC NOS is the most common in the case of material obtained from EBUS-TBNA. Esterbrook et al. found that NSCLC-NOS rate was 20.8% in EBUS-TBNA samples. Similar results were received by Navani et al. In group of 774 patients with known or suspected lung cancer, 23% of patients had a final diagnosis of NSCLC NOS. [13, 14] Our study confirmed the high percentage of NSCLC NOS patients diagnosed with EBUS-TBNA procedures. Endobronchial biopsy was less likely to provide a diagnosis of NOS NSCLC.

Chin et al. reported that EBUS-TBNA is the most sensitive diagnostic methods for SCLC, because it allows to sample of specimens from mediastinal as well as submucosal lesions. They also mentioned that the quality of specimens obtained by needle aspiration is better than by forceps biopsies, which may contain crush artifacts. [15] In addition, other studies noticed that the sensitivity of EBUS-TBNA for SCLC detection was higher than for NSCLC diagnosis. [16, 17] Our findings confirmed above statements. The majority of SCLC cases were diagnosed with EBUS-TBNA. Three SCLC patients were diagnosed from metastatic lesions in the adrenal gland using transesophageal EUS-FNA.

Many authors raise the problem that forceps biopsy has low sensitivity in the diagnosis of lung cancer. Forceps biopsy has a diagnostic yield ranging between 65-82%. In our preliminary study conducted in 212 patients with lung cancer suspicion, we compared sensitivity and accuracy of routine bronchoscopy with endobronchial biopsy, EBUS-TBNA, and combination of EBUS-TBNA and EUS-FNA techniques. Sensitivity and accuracy of endobronchial biopsy vs EBUS-TBNA vs combination of transbronchial biopsies were 43% vs 44.3% vs 93.7% and 93.8% vs 94.7% and 94.8%, respectively. This demonstrates high

usefulness of the combination of EBUS-TBNA and EUS-FNA in the diagnosis of lung cancer [18]. Verma et al. demonstrated sensitivity of EBUS-TBNA in cancer diagnosis of 91.4% in small group of 37 patients with lesions located adjacent to the trachea or lesions located adjacent to the main bronchi. [19] Tournoy et al. indicated that EBUS-TBNA has a sensitivity of 82% and low negative predictive value (23%). [20] The similar results received Zhao et al. for lesions located near the central airways. [21] Oki et al. showed that the combined endoscopic method with EBUS-TBNA and EUS-FNA with a single bronchoscope gives better results in staging of NSCLC than each technique alone. However, they mentioned that significant number of patients had false-negative EBUS-TBNA and EUS-FNA results. Moreover Oki et al. suggested that very important issue is bronchoscopists experience, which may cause differences in the results. [22] On the other hand, Wallace et al. showed a EBUS-TBNA sensitivity of only 69% in a group of 150 patients with lung cancer suspicion. [23]

Despite relatively low negative predictive value of EBUS-TBNA, there is an indication to perform other procedures (e.g., surgical procedures) for final diagnosis in a significant group of patients. In our study, 56.9% of patients who underwent bronchoscopy did not receive a definitive diagnosis of the diseases and they have been subjected to other diagnostic procedures or observations. We showed the results of all performed bronchoscopies and three different method of material collection (endobronchial biopsy and combination EBUS-TBNA and EUS-FNA). Moreover, unselected and heterogeneous patient population were recruited in three different hospitals that employ a total of 8 bronchoscopists. In most studies, the evaluation of the usefulness of EBUS-TBNA and EUS-FNA for detecting malignancy was conducted in selected patients with high clinical suspicion of the tumor. Small, preselected groups of patients with high risk of lung cancer could be the reason of the low negative predictive value of bronchoscopic procedures in these studies. Thus, these observations may not reflected the real clinical situation.

In studies where population was heterogenic regarding the disease (lung cancer, sarcoidosis, tuberculosis), EBUS-TBNA had diagnostic value only in 60-75% of patients. [24] Lange et al. showed diagnostic results of EBUS-TBNA in only 61.4% of unselected patients undergoing routine diagnostic procedures. [25] Fournier et al. examined 185 patients with extrathoracic malignancy and mediastinal lymphadenopathy in real life practice. Pathomorfological types of malignancy were successfully identified using EBUS-TBNA in only 93 patients (50.3%). The diagnostic sensitivity, specificity, negative predictive value, and positive predictive value were 68.4%, 100%, 53.3%, and 100%, respectively. [26] Murthi et al conducted research compare the accuracy of EBUS-TBNA to surgery in diagnosing hilar

and mediastinal pathologies. EBUS-TBNA for all pathologies had an accuracy of 81.2% and sensitivity of 55.1%. [27]

Conclusions

Comparing all these data we could conclude that bronchoscopy is vital but not an ideal technique in the routine diagnosis of respiratory diseases. Our study showed that 41% bronchoscopy material was insufficient to perform reliable pathomorphological examination. This mainly concerned patients with suspected lung tumor or lymphadenopathy. Especially, here is a problem in the diagnosis of advanced lung tumors (mainly adenocarcinomas) if only one procedure was performed during bronchoscopy. It is advisable to use several different techniques of collecting specimens from both the tumor and the mediastinal lymph nodes during bronchoscopy. The use of brusch biopsy, forceps biopsy, bronchoaspirate analysis EBUS-TBNA and EUS-FNA simultaneously or if desired was of the highest diagnostic value. However, sometimes this fails and bronchoscopy must be repeated, or thoracic procedures (e.g. mediastinoscopy or thoracoscopy) must be performed. We found that the EBUS-TBNA value in daily clinical practice differed from that in clinical trials. Therefore, precise estimation of the frequency of individual pathomorphological types of lung cancer based on material obtained bronchoscopically is not possible. However, EBUS-TBNA play an essential role in staging of invasive lung cancer. Therefore, its value in the diagnosis of lung cancer is not limited to demonstrating the presence of cancer and its type, but above all to determining the extent of the disease and qualification for appropriate treatment. It seems that the incidence of lung cancer with a typical peripheral localization (adenocarcinoma) may be underestimated in comparison to the incidence of lung cancer with a typical central localization (squamous cell carcinoma, SCLC).

Authorship contribution statement:

Conception and design of the study: JB, MF, PK, JP. Administrative support: PK, JP, AP, JB, AS, JS, MM, PK, RK, JM. Provision of study material or patients: JP, AP, JB, AS, JS, MM, PK, RK, JM. Collection and assembly of data: JB, MF, PK, JP, AP, JB, AS, JS, MM, PK, RK. Data analysis and interpretation: JB, MF, PK. Manuscript writing: JB, MF, PK. All authors gave final approval of the published manuscript.

Data Availability Statement:

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest:
The authors declare no potential conflicts of interest.

Foundings:
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Table 1. The effectiveness of varies techniques during bronchoscopy in the diagnosis of individual pathomorphological types of lung cancer depending on place of collecting the material and nodal station.

Material	**SCLC**	**Adenocarcinoma**	**Squamous cell**	**NOS**	**Total lung**

			carcinoma		**cancer**
EBUS-TBNA/EUS-FNA of lymph nodes	250 (27.7%)	331 (36.7%)	209 (23.1%)	114 (12.5%)	904 (100%)
EBUS-TBNA/EUS-FNA of tumor	88 (24.7%)	94 (25.8%)	135 (37.2%)	44 (12.3%)	361 (100%)
EBUS-TBNA/EUS-FNA metastases to adrenal gland	3 (42.9%)	1 (14.3%)	2 (28.6%)	1 (14.3%)	7 (100%)
Forceps biopsy of tumor	156 (22%)	163 (25%)	270 (41.5%)	62 (9.5%)	651 (100%)

Figure 1. Results of pathomorphological examination carried out on material obtained from 5279 bronchoscopies (entire study population)

Figure 2. The incidence of individual pathomorofological types of LC in the entire study group of lung cancer patients

Figure 3. The incidence of individual pathomorofological types of LC according to the gender of patients with lung cancer

Figure 4. Percentage of patients with different types of lung cancer detected in materials collected with different bronchoscopic techniques. Frequency of different types of lung cancer was calculated in the whole group of patients undergoing a given bronchoscopic procedure (100%), which resulted in the diagnosis of lung cancer

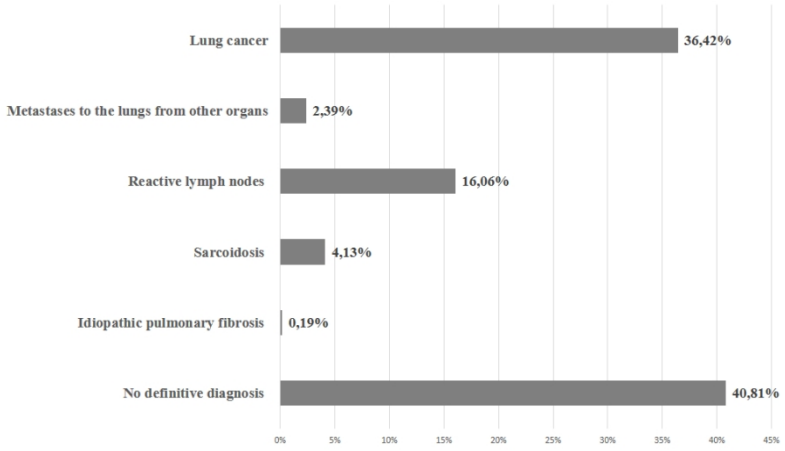

Figure 1. Results of pathomorphological examination carried out on material obtained from 5279 bronchoscopies (entire study population)

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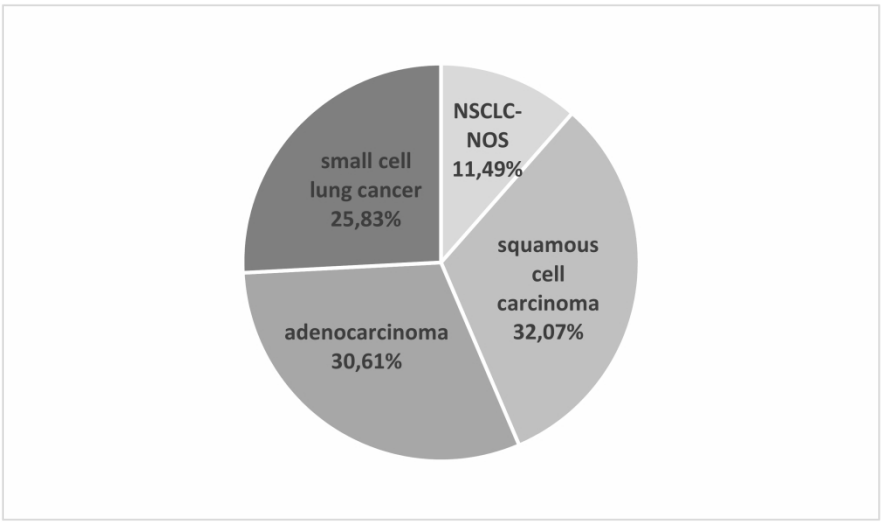

Figure 2. The incidence of individual pathomorofological types of LC in the entire study group of lung cancer patients

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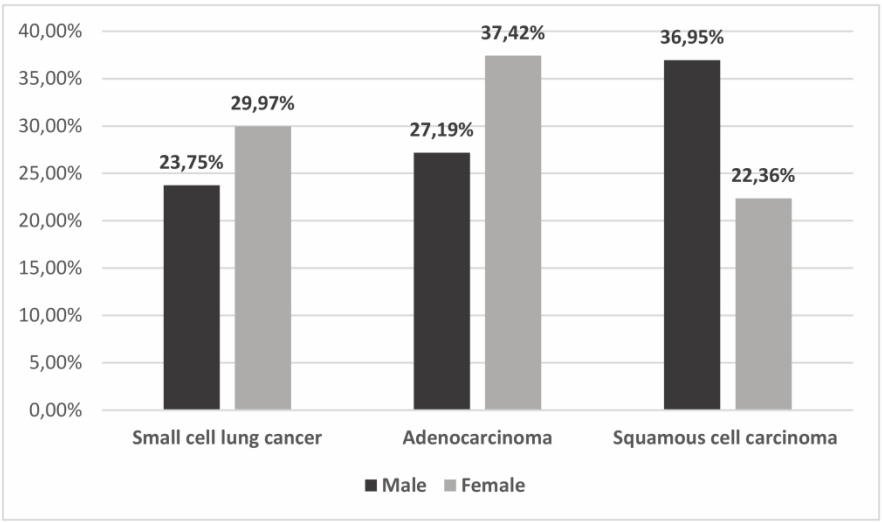

Figure 3. The incidence of individual pathomorofological types of LC according to the gender of patients with lung cancer

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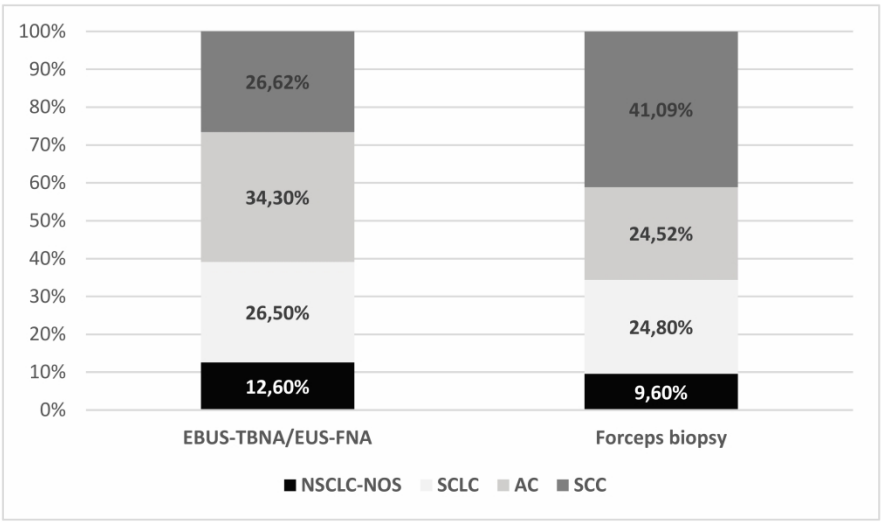

Figure 4. Percentage of patients with different types of lung cancer detected in materials collected with different bronchoscopic techniques. Frequency of different types of lung cancer was calculated in the whole group of patients undergoing a given bronchoscopic procedure (100%), which resulted in the diagnosis of lung cancer

296x209mm (300 x 300 DPI)

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4-5
Objectives	3	State specific objectives, including any prespecified hypotheses	6
Methods			
Study design	4	Present key elements of study design early in the paper	6-7
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6-7
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	6-7
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	6-7
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6-7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	6-7
Bias	9	Describe any efforts to address potential sources of bias	6-7
Study size	10	Explain how the study size was arrived at	6-7
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6-7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	6-7
		(*b*) Describe any methods used to examine subgroups and interactions	
		(*c*) Explain how missing data were addressed	
		(*d*) If applicable, explain how loss to follow-up was addressed	
		(*e*) Describe any sensitivity analyses	
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	8
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	8
		(b) Indicate number of participants with missing data for each variable of interest	
		(c) Summarise follow-up time (eg, average and total amount)	
Outcome data	15*	Report numbers of outcome events or summary measures over time	8

Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (*b*) Report category boundaries when continuous variables were categorized (*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	8
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	-
Discussion			
Key results	18	Summarise key results with reference to study objectives	9
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	9
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	10-11
Generalisability	21	Discuss the generalisability (external validity) of the study results	12
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	13

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

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Observational cross-sectional study of 5279 bronchoscopy results for the practical effectiveness of various biopsy techniques in the diagnosis of lung diseases with particular emphasis on lung cancer

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Observational cross-sectional study of 5279 bronchoscopy results for the practical effectiveness of various biopsy techniques in the diagnosis of lung diseases with particular emphasis on lung cancer

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Short title: **The yield of bronchoscopy in the diagnosis of lung cancer**

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Abstract

Introduction

Bronchoscopy is the main method in the diagnosis of various lung diseases. Endobronchial ultrasound transbronchial needle aspiration (EBUS-TBNA) is the most modern bronchoscopic technique useful in diagnosis and staging of lung cancer.

Objectives

The aim of the study was to assess the yield of bronchoscopy in patients with suspected various respiratory diseases including lung cancer. In particular, we examined the efficiency of different biopsy techniques in the diagnosis of lung cancer in correlation with its localization and pathomorphological type.

Patients and Methods

The results of pathomorphological examinations from 5279 bronchoscopies performed in 2016-2018 were analyzed. The material was collected with endobronchial ultrasound transbronchial needle aspiration (EBUS-TBNA), endoscopic ultrasound guided fine needle aspiration (EUS-FNA) and endobronchial forceps biopsy. Clinical and demographic factors were analyzed using the Fisher χ^2 test.

Results

5279 patients were diagnosed due to various respiratory symptoms. Lung cancer was confirmed in 36.42% of patients. 40.81% of patients had no definitive patomorphological diagnosis. Among patients with lung cancer, the most frequent diagnosis was non-small cell lung cancer: squamos cell lung cancer (SCC) - 32.07% and adenocarcinoma (AC) - 30.61%, then small cell lung cancer (SCLC) - 25.83% and not otherwise specified non-small cell lung cancer (NSCLC NOS) - 11.49%. Diagnosis of SCC was obtained significantly more often (χ^2=43.143, $p<0.000001$) by forceps biopsy (41.09%) than by EBUS-TBNA/EUS-FNA (26.62%). On the contrary, diagnosis of AC or NSCLC NOS was significantly more often (χ^2=20.394, $p<0.000007$ and χ^2=3.902, $p<0.05$, respectively) observed in EBUS-TBNA/EUS-FNA (34.31% and 12.6%) than in endobronchial biopsies (24.52% and 9.64%).

Conclusions

The use of bronchoscopy in the diagnosis of various lung diseases is vital but also has many limitations. Effectiveness of EBUS-TBNA and endobronchial forceps biopsy in diagnosis of lung cancer is strongly affected by tumor localization and type of cancer.

Key words: lung cancer; bronchoscopy; endobronchial biopsy; EBUS-TBNA; EUS-FNA

Article summary

Strengths and limitations of this study

1. 5 279 patients were enrolled to the study group which makes it one of the largest studies in the world with assement of bronchoscopy effectiveness in routine clinical practice.
2. We analyzed bronchoscopies performed in 2016-2018 in a few polish medical centers across the country, in a diversified population which ensures a high level of generalisability.
3. The study is an important contribution to the epidemiological data of advanced lung cancer in Poland.
4. Bronchoscopies were performed by 8 different bronchoscopists. This fact may cause human-dependent variation in results.
5. Results of pathomorphological assessment of the material obtained during single bronchoscopy was analyzed. Patients without diagnosed disease in the bronchoscopic material underwent further diagnostics using other methods or in other centers. Therefore, we were unable to report a definitive diagnosis in patients with inconclusive results of diagnostic prcedures.

Introduction

Epidemiological analyses indicate that lung cancer (LC) is the most common cause of cancer-related deaths. It is usually diagnosed in an unresectable, advanced stage. [1, 2] Lung cancer is the most common cancer in men and the third most common in women. In 2018, there were more than 2 million new cases of lung cancer worldwide. [3]

In the United States from 2004 to 2009, a total of 1,096,276 lung cancer cases were diagnosed and reported. This American study investigated the histologic type of lung cancer and demographic characteristics of the patients. The incidence of individual types of LC was as follows: small cell lung cancer (SCLC) – 14.9%, squamous cell carcinoma (SCC) – 21.9%, adenocarcinoma (AC) – 37.1% and large cell carcinoma (LCC) – 3.2% of cases. [4]

It is difficult to obtain accurate epidemiological data on the occurrence of individual pathomorphological types of advanced LC in Poland. Up to date, no epidemiological studies have been conducted on a sufficiently large group of patients with advanced lung cancer to obtain reliable results. Statistics on pathomorphological diagnoses of lung cancer in material from bronchoscopy have not been conducted so far. In Poland, the main source of such data is the National Lung Cancer Registry which only contains details about patients undergoing surgery in earlier stages of the disease. There where 17,783 patients diagnosed and operated in Polish thoracic surgery centers and registered in the National Lung Cancer Registry in the years

2014-2018. This group includes: 48.7% of patients with AC, 40.8% of patients with SCC, 6.45% of patients with LCC, 2.1% of patients with adenosquamous cell carcinoma, 1.05% of patients with SCLC and 0.9% of patients with non-otherwise specified (NOS) non-small cell lung cancer (NSCLC). This material included low percentage of patients with SCLC (usually an inoperable type of lung cancer) and patients with NSCLC-NOS (large surgical material is easier for pathomorphological examination which is a factor of reducing misdiagnosis). According to IASLC (International Association for the Study of Lung Cancer) recommendations, large cell carcinoma should be diagnosed only in surgical materials extracted from the entire resected tumor. [5] Therefore, this type of cancer was recorded in surgical pathology in non-advanced NSCLC patients and almost absent in patients with advanced lung cancer diagnosed with tumor biopsy. [2]

Due to different locations of tumor types in the lungs or metastatic lymph nodes, varied approaches are required to cancer diagnosis. The peripheral location is characteristic for AC, while squamous and small cell carcinomas most often are located centrally. Bronchoscopy is an appropriate method for detecting LC and the endobronchial ultrasound-guided with transbronchial needle aspiration (EBUS-TBNA) procedure has an essential role in the invastigation of lung cancer. If the tumor is centrally located and infiltrated the bronchus, the most optimal procedure seems to be the endobronchial biopsy using a brush or forceps. In contrast, adenocarcinoma frequently metastasizes to the mediastinal lymph nodes, which may be available on EBUS-TBNA or EUS-FNA procedures.

Metastasis to mediastinal lymph nodes is typical for each of types of lung cancer. We distinguish compartments of the mediastinal lymph nodes: superior mediastinal nodes (stations 2, 3 and 4), aortic nodes (stations 5 and 6), inferior mediastinal nodes (stations 7, 8, 9), hilar and interlobar lymph nodes (stations 10 and 11) and peripheral lymph nodes (station 12 for lobar nodes, station 13 for segmental nodes and station 14 for subsegmental nodes). EBUS-TBNA is most often used in diagnosis of superior mediastinal nodes, station 7 of inferior mediastinal nodes and stations 10, 11, 12 lymph nodes. EUS-FNA is preffered in diagnosis of superior mediastinal nodes and stations 7, 8 and 9 of inferior mediastinal nodes. Moreover EUS-FNA is used for sampling subphrenic lymph nodes and metastates in liver and left adrenal gland. Both methods – EBUS-TBNA and EUS-FNA are preferred for mediastinal lymph nodes assessment for evaluation of N-stage in NSCLC patients. [6] These two methods could be used in sampling visible tumors localized in central airways but also peripherally to the main bronchi, i.e. in lobar or even segmental bronchi.

Lung cancer staging is initially assessed through imaging studies. Normal mediastinum lymph nodes are defined below 10 mm in computed tomography (CT). Such a size of these lymph nodes suggests the N0 clinical stage. Currently, sampling for N0 nodes is not recommended, while surgery is the primary treatment method for N0/N1stage of lung cancer. However, everyday practice shows that it is worth collecting non-enlarged nodes for pathomorphological examination, because cancer cells are often found in such nodes. [7,8]

The sensitivity of detecting lung cancer using different bronchoscopy methods varies from 34% to 88% depending on the size and location of the tumor and preliminary diagnosis of the patients. [9] Meta-analysis of 18 studies which included a total of 1,201 LC patients was performed for assessment of sensitivity and specificity of ultrasound-guided fine needle aspiration in mediastinal staging of lung cancer. Authors showed sensitivity of 83% (range 45-100%) and specificity of 97% (range 88-100%) of these methods. [10] In eight studies limited to patients with enlarged mediastinal lymph nodes seen on CT, sensitivity was 90% (95% CI: 84 to 94%) and specificity was 97% (95% CI: 95 to 98%). In patients without enlarged mediastinal lymph nodes visible on CT, the overall sensitivity was 58% (95% CI: 39-75%). Therefore, the use of EBUS-TBNA increases the accuracy in the estimation of the stage of lung cancer and may radically influence the further treatment of the patient and the selection of the treatment methods. EUS-FNA enables confirming the presence of distant metastases, which has a decisive impact on therapeutic decisions. However, all false negative results delay cancer diagnosis and force the repetition of diagnostic procedures including surgery. Earlier detection of lung cancer gives patients the chance for better treatment. [11]

Aim

The objective of our study was a descriptive analysis of lung diseases diagnoses, especially lung cancer, established by various bronchoscopic procedures. We devoted special attention to the possibility of diagnosis of individual pathomorphological types of LC with various techniques used for collecting materials during bronchoscopy.

Material and methods

In our observational cross-sectional study, we analyzed the results of pathomorphological examination carried out on the material obtained during 5279 bronchoscopies performed in 2016-2018. Those bronchoscopies were performed in three Polish

pulmonology departments. The study included 1892 women and 3387 men, with median age of 65 years.

The study was retrospective and relied fully on the analysis of documents gathered, thus eliminating the need for collaboration between patients and researchers. No patients were enrolled specifically to carry out this study.

Various diseases of the respiratory system were indication for bronchoscopy: 3127 (59.2%) patients had suspicion of chest tumor in computed tomography, 882 (16.7%) patients demonstrated hilar lymphadenopathy, 205 (3.9%) patients had suspicion of sarcoidosis and 20 (0.4%) patients had suspicion of pulmonary fibrosis. Other indications for bronchoscopy occurred in 1045 (19.8%) patients (e.g. suspicion of tuberculosis, chronic cough, hemoptysis, etc.). In patients with suspected cancer, samples of the tissue were acquired through bronchoscopy and the technique was chosen in compliance with tumor or metastatic lymph node's location as it is described in the introduction. Forceps biopsies were performed using Olympus BF-1T180 and Pentax EB-1970K bronchoscopes, EBUS-TBNA – using Olympus BF-UC180F and Pentax EB-1970UK bronchoscopes (22-gauge needles), and EUS-FNA – using Olympus GF-UCT180 endoscope. Premedication for bronchoscopy was under local or general anesthesia, depending on the situation.

Samples underwent pathomorphological examination which included hematoxylin-eosin (H&E) staining, mucicarmine staining and immunohistochemistry (IHC) examination, such as staining of TTF-1 (thyroid transcription factor 1) and p63/p40. Samples diagnosed with non-squamous NSCLC were in-depth reported and underwent molecular testing for the presence of *EGFR* gene mutation by real-time PCR technique (RT-PCR), *ALK* gene rearrangement and PD-L1 expression by IHC. PD-L1 expression was also assessed in SCC patients. Large cell carcinoma (LCC) of the lung according to the 2015 World Health Organization (WHO) Classification of Tumors of the Lung, Pleura, Thymus and Heart cannot be diagnosed in small specimens and aspiration biopsy materials. The diagnosis of LCC can only be made in the postoperative material. Therefore, there were no patients diagnosed with LCC in our study. Such patients were included in the group of patients diagnosed with NSCLC NOS. Chromogranin and synaptophysin was used in IHC examination of neuroendocrine tumors (small cell lung cancer or NSCLC NOS). All the centers participating in the study used these same procedures described above.

After receiving the diagnosis, we selected a population of patients with lung cancer and divided them into groups of patients with different cancer types detectable with bronchoscopic procedure (squamous cell lung cancer, adenocarcinoma, large cell carcinoma, not otherwise

specified NSCLC, and small cell lung cancer). Then, we assessed the prevalence of different types of LC in the materials obtained with various bronchoscopic procedures.

Clinical and demographic factors were analyzed using the Pearson's Chi-square test. P-values below 0.05 were considered significant. The percentages reflect the relative number of all lung cancer patients diagnosed with a particular procedure. We only analyzed the results of the first-time bronchoscopy, that could be nondiagnostic. The evaluation of relative diagnostic yield (sensitivity) of different bronchoscopic procedures could not be done because, in our study, it was not possible to verify the final diagnosis of patients in the materials collected during the next bronchoscopy or another procedures (this applies mainly to patients with lung tumor or hilar lymphadenopathy). The following diagnostic procedures were carried out in various clinical centers throughout Poland. Therefore, we were unable to verify the diagnoses obtained later.

The protocol of the study was approved by the Committee of Ethics and Research at the Medical University of Lublin (KE-0254/5/2018).

Results

In 3565 (67.5%) patients, EBUS-TBNA and transesophageal EUS-FNA was performed and cytological material was archived in a cellblock. In 1346 (25.5%) patients, EBUS-TBNA (without EUS-FNA) was the only diagnostic procedure. In the remaining patients, EBUS-TBNA was supplemented by EUS-FNA. There were no patients in whom EUS-FNA would be the only diagnostic method. 1714 (32.5%) patients had non-ultrasound-guided bronchoscopy with the forceps biopsy of endobronchial lesions allowing to obtain a small histological specimen.

Lung cancer was confirmed in a group of 1923 (36.42%) patients, including 1280 men and 643 women. Reactive lymph nodes were found in 16.06% of the patients, sarcoidosis was diagnosed in 4.13%, idiopathic pulmonary fibrosis – in 0.19%, metastases to the lungs from other organs – in 2.39% of the patients. 40.81% of the patients had no definitive diagnosis (Figure 1). Lung cancer was confirmed in 51% of patients with suspected tumor in computed tomography. While, in the group of patients with hilar lymphadenopathy, lung cancer was diagnosed in only 10.1% of cases.

Among those with lung cancer, squamous cell carcinoma (32.07%) was most often diagnosed, then adenocarcinoma (30.61%), small cell lung cancer (25.83%) and NSCLC-NOS (11.49%) (Figure 2). SCLC and AC were significantly more frequent ($\chi^2 = 8.649$, $p = 0.0033$ and $\chi^2 = 21.128$, $p < 0.000005$, respectively) in women (29.97% and 37.42% of women with

lung cancer) than in men (23.75% and 27.19% of male patients with lung cancer). Squamous cell carcinoma appeared significantly more often ($\chi^2 = 41.881$, $p < 0.000001$) among male (36.95%) than among female (22.36%) patients with lung cancer (Figure 3). SCC was significantly more often ($\chi^2 = 4.17$, $p = 0.041$) diagnosed in the group of patients older than 65 years than in younger patients. Other pathomorphological types of lung cancer occurred with similar frequency in these two age groups.

Endobronchial biopsies significantly more often ($\chi^2 = 7.566$, $p = 0.0059$) provided material for the diagnosis of lung cancer than the EBUS-TBNA and EUS-FNA procedures. 42.35% of endobronchial biopsies and 33.6% of TBNA and FNA provided material sufficient to diagnose lung cancer. Fine needle biopsy of lymph nodes enabled the diagnosis of lung cancer in 29.2% of cases, fine needle biopsy of lung tumor – in 66.6% of cases, and forceps biopsy of bronchial mucosa lesions – in 48.2% of cases. These differences were statistically significant.

Among patients with lung cancer, TBNA or FNA compared to endobronchial biopsies gave a similar result ($\chi^2 = 0.656$, $p = 0.418$) in detection of SCLC (26.5% vs. 24.8%). On the other hand, the diagnosis of AC and NSCLC-NOS was obtained significantly more frequently ($\chi^2 = 20.394$, $p = 0.000006$ and $\chi^2 = 3.902$, $p = 0.0482$) in EBUS-TBNA and EUS-FNA compared to endobronchial biopsies (34.3% vs. 24.52% and 12.6% vs. 9.6%, respectively). SCC, among other lung cancer types detected by bronchosopy, was diagnosed in 41.77% in materials obtained by forceps biopsy and only in 26.62% in materials with EBUS-TBNA or EUS-FNA ($\chi^2 = 43.143$, $p < 0.000001$), which was directly related to the more frequent central location and bronchial infiltration of this type of tumor. Computed tomography showed that in 79% of SCC patients the tumor was centrally located in the large bronchi. The analysis of bronchoscopic images showed that tumor deformed the bronchial mucosa or showed endobronchial growth in 67% of SCC patients. (Figure 4). Table 1 shows the results of bronchoscopy procedures in the diagnosis of individual pathomorphological types of lung cancer depending on place of collecting the material.

Discussion

Our study on the results of EBUS-TBNA/EUS-FNA and forceps biopsy in obtaining materials for the diagnosis of various lung diseases is among the largest worldwide. The study points to numerous problems arising from the use of these techniques in routine clinical practice. We are aware of the many limitations of our study. First, we cannot determine the sensitivity and specificity of our methods, because it was not possible to determine the final

diagnosis in such a large group of patients (5 279 cases). In addition, we could not distinguish between material collected by EBUS-TBNA and EUS-FNA. We do not know how many lung cancer patients were diagnosed only in the material from EBUS-TBNA or only in the material from EUS-FNA, or in both types of these materials. We also do not know the number of biopsies performed during one bronchoscopy. This data is missing from the results of the pathomorphological examination that we analyzed. Thirdly, diagnosis of large cell carcinoma was not possible in small specimens (LCC was probably qualified to the NSCLC-NOS group). A limitation of our study was also the lack of detailed clinical and radiological characteristics of all patients who underwent bronchoscopy procedures.

However, we found that advanced small cell lung cancer may be more common in Poland than previously thought. This tumor is characterized by rapid growth and metastases, therefore, more often it could be diagnosed in advanced stages using bronchoscopic techniques. SCLC diagnosis in EBUS-TBNA/EUS-FNA of lymph nodes and endobronchial biopsy occurs at the same frequency. Furthermore, the difference in the percentage of patients with squamous cell carcinoma and adenocarcinoma diagnosed with endobronchial biopsies and TBNA or FNA is noteworthy. In our study, most patients with adenocarcinoma were diagnosed with EBUS-TBNA or EUS-FNA of lymph nodes, while patients with squamous cell carcinoma were diagnosed more often based on examination of material from endobronchial biopsy (forceps biopsy). Patients in cohort with squamous cell carcinoma were more likely to have endobronchial disease accessible by forceps. Therefore we could not ascertnain that endobronchial biopsy is more effective for diagnosis of squamous cell lung cancer as there was no comparison to EBUS-TBNA and EUS-FNA for those patients. Thus, we point to the problem that results of bronchoscopic procedures depends on the location of the primary tumor and the presence of metastases in the lymph nodes.

Schmid-Bindert et al. showed how often they detected different pathomorphological types of lung cancer using various bronchoscopic methods. Small biopsies were collected by three different methods: forceps biopsy (44.6%), EBUS-TBNA (32.7%), and CT-guided core biopsy (22.8%). 38% of adenocarcinoma, 51% of squamous cell carcinoma and 11% of NSCLC NOS were diagnosed using forceps biopsy. EBUS-TBNA results were as follows: 45% of adenocarcinoma, 30% of squamous cell carcinoma and 24% of NSCLC NOS. [12]

Many authors emphasize that the diagnosis of NSCLC NOS is the most common in the case of material obtained from EBUS-TBNA. Esterbrook et al. found that NSCLC-NOS rate was 20.8% in EBUS-TBNA samples. Similar results were achieved by Navani et al. In group of 774 patients with known or suspected lung cancer, 23% of patients had a final diagnosis of

NSCLC NOS. [13, 14] Our study confirmed the high percentage of NSCLC NOS patients diagnosed with EBUS-TBNA procedures. Endobronchial biopsy was less likely to provide a diagnosis of NSCLC NOS.

Chin et al. reported that EBUS-TBNA is the most sensitive diagnostic method for SCLC detection, because it allows to sample specimens from mediastinal as well as submucosal lesions. They also mentioned that the quality of specimens obtained by needle aspiration is better than by forceps biopsies, which may contain crushed artifacts. [15] In addition, other studies noticed that the sensitivity of EBUS-TBNA for SCLC detection was higher than for NSCLC diagnosis. [16, 17] Our findings confirmed the above statements. The majority of SCLC cases were diagnosed with EBUS-TBNA. Three SCLC patients were diagnosed from metastatic lesions in the adrenal gland using transesophageal EUS-FNA.

Many authors raise the problem that forceps biopsy has low sensitivity in the diagnosis of lung cancer. Forceps biopsy has a diagnostic yield ranging between 65-82%. In preliminary study by Pasko et al. conducted in 212 patients with lung cancer suspicion, authors compared sensitivity and accuracy of routine bronchoscopy techniques: endobronchial biopsy, EBUS-TBNA, and combination of EBUS-TBNA and EUS-FNA. Sensitivity and accuracy of endobronchial biopsy vs EBUS-TBNA vs combination of transbronchial biopsies were 43% vs 44.3% vs 93.7% and 93.8% vs 94.7% and 94.8%, respectively. This demonstrates high usefulness of the combination of EBUS-TBNA and EUS-FNA in the diagnosis of lung cancer [18]. Verma et al. demonstrated sensitivity of EBUS-TBNA in cancer diagnosis of 91.4% in small group of 37 patients with lesions located adjacent to the trachea or lesions located adjacent to the main bronchi. [19] Tournoy et al. indicated that EBUS-TBNA has a sensitivity of 82% and low negative predictive value (23%). [20] The similar results were received by Zhao et al. for lesions located near the central airways. [21] Oki et al. showed that the combined endoscopic method with EBUS-TBNA and EUS-FNA with a single bronchoscope gives better results in staging of NSCLC, than each technique alone. However, they mentioned that significant number of patients had false-negative EBUS-TBNA and EUS-FNA results. Moreover Oki et al. suggested that very important issue is bronchoscopists experience, which may cause differences in the results. [22] On the other hand, Wallace et al. showed a EBUS-TBNA sensitivity of only 69% in a group of 150 patients with lung cancer suspicion. [23]

Despite relatively low negative predictive value of EBUS-TBNA, there is an indication to perform other procedures (e.g., surgical procedures) for final diagnosis in a significant group of patients. In our study, 56.9% of patients who underwent bronchoscopy did not receive a definitive diagnosis of the diseases and they have been subjected to other diagnostic procedures

or observations. We showed the results of all performed bronchoscopies and three different methods of material collection (endobronchial biopsy and combination EBUS-TBNA and EUS-FNA). Moreover, unselected and heterogeneous patients were recruited in three different hospitals that employ a total of 8 bronchoscopists. In most studies, the evaluation of the usefulness of EBUS-TBNA and EUS-FNA for detecting malignancy was conducted in selected patients with high clinical suspicion of the tumor. Small, preselected groups of patients with high risk of lung cancer could be the reason of the low negative predictive value of bronchoscopic procedures in these studies. Thus, these observations may not reflected the real clinical situation.

In studies where population was heterogenic regarding the disease (lung cancer, sarcoidosis, tuberculosis), EBUS-TBNA had diagnostic value only in 60-75% of patients. [24] Lange et al. showed diagnostic results of EBUS-TBNA in only 61.4% of unselected patients undergoing routine diagnostic procedures. [25] Fournier et al. examined 185 patients with extrathoracic malignancy and mediastinal lymphadenopathy in real life practice. Pathomorfological types of malignancy were successfully identified using EBUS-TBNA in only 93 patients (50.3%). The diagnostic sensitivity, specificity, negative predictive value, and positive predictive value were 68.4%, 100%, 53.3%, and 100%, respectively. [26] Murthi et al conducted research comparing the accuracy of EBUS-TBNA to surgery in diagnosis of hilar and mediastinal pathologies. EBUS-TBNA for all pathologies had an accuracy of 81.2% and sensitivity of 55.1%. [27]

Conclusions

Comparing all these data, we could conclude that bronchoscopy is vital but not an ideal technique in the routine diagnosis of respiratory diseases. Our study showed that 41% of bronchoscopy materials were insufficient to perform reliable pathomorphological examination. This mainly concerned patients with suspected lung tumor or lymphadenopathy. The use of brush biopsy, forceps biopsy, bronchoaspirate analysis EBUS-TBNA and EUS-FNA simultaneously, if desired, was of the highest diagnostic value. However, sometimes this fails and bronchoscopy must be repeated, or thoracic procedures (e.g. mediastinoscopy or thoracoscopy) must be performed. We found that the EBUS-TBNA value in daily clinical practice differed from that in clinical trials. Therefore, precise estimation of the frequency of individual pathomorphological types of lung cancer, based on material obtained bronchoscopically, is not possible. However, EBUS-TBNA plays an essential role in staging of invasive lung cancer. Therefore, its value in the diagnosis of lung cancer is not limited to

demonstrating the presence of cancer type, but, above all, to determining the extent of the disease and qualification for appropriate treatment. It seems that the incidence of lung cancer with a typical peripheral localization (adenocarcinoma) may be underestimated in comparison to the incidence of lung cancer with a typical central localization (squamous cell carcinoma, SCLC), when bronchoscopy is used as the primary diagnostic method.

Authorship contribution statement:

Conception and design of the study: JB, MF, PK, JP. Administrative support: PK, JP, AP, JB, AS, JS, MM, PK, RK, JM. Provision of study material or patients: JP, AP, JB, AS, JS, MM, PK, RK, JM. Collection and assembly of data: JB, MF, PK, JP, AP, JB, AS, JS, MM, PK, RK. Data analysis and interpretation: JB, MF, PK. Manuscript writing: JB, MF, PK. All authors gave final approval of the published manuscript.

Data Availability Statement:

The data that supports the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest:

The authors declare no potential conflicts of interest.

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Table 1. The results of varies techniques during bronchoscopy in the diagnosis of individual pathomorphological types of lung cancer depending on place of collecting the material and nodal station.

Material	**SCLC**	**Adenocarcinoma**	**Squamous cell carcinoma**	**NOS**	**Total lung cancer**
EBUS-TBNA/EUS-FNA of lymph nodes	250 (27.7%)	331 (36.7%)	209 (23.1%)	114 (12.5%)	904 (100%)
EBUS-TBNA/EUS-FNA of tumor	88 (24.7%)	94 (25.8%)	135 (37.2%)	44 (12.3%)	361 (100%)
EBUS-TBNA/EUS-FNA metastases to adrenal gland	3 (42.9%)	1 (14.3%)	2 (28.6%)	1 (14.3%)	7 (100%)

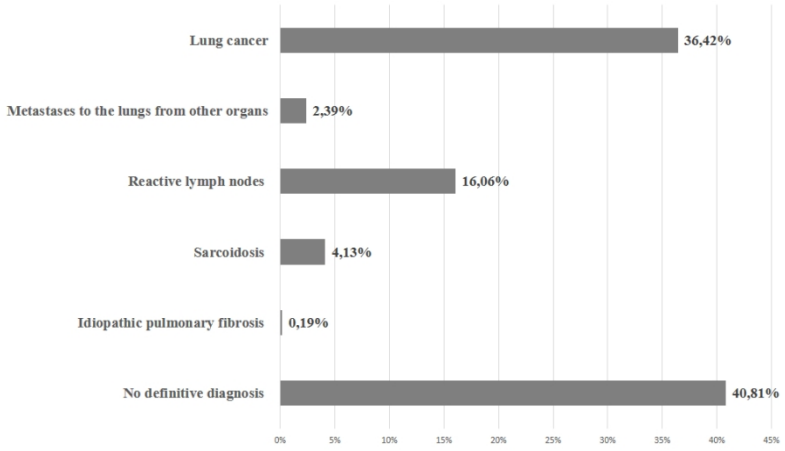
Forceps biopsy of tumor	156 (22%)	163 (25%)	270 (41.5%)	62 (9.5%)	651 (100%)

Figure 1. Results of pathomorphological examination carried out on material obtained from 5279 bronchoscopies (entire study population)

Figure 2. The incidence of individual pathomorofological types of LC in the entire study group of lung cancer patients

Figure 3. The incidence of individual pathomorofological types of LC according to the gender of patients with lung cancer

Figure 4. Percentage of patients with different types of lung cancer detected in materials collected with different bronchoscopic techniques. Frequency of different types of lung cancer was calculated in the whole group of patients undergoing a given bronchoscopic procedure (100%), which resulted in the diagnosis of lung cancer



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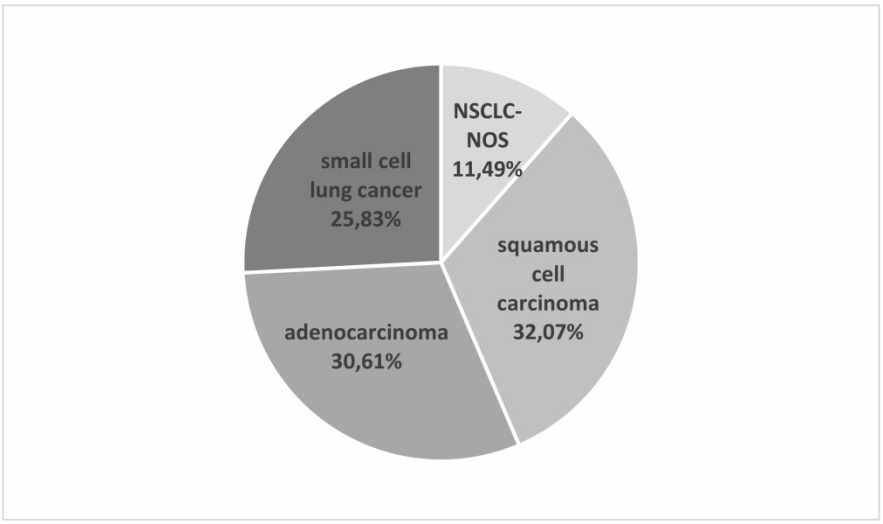

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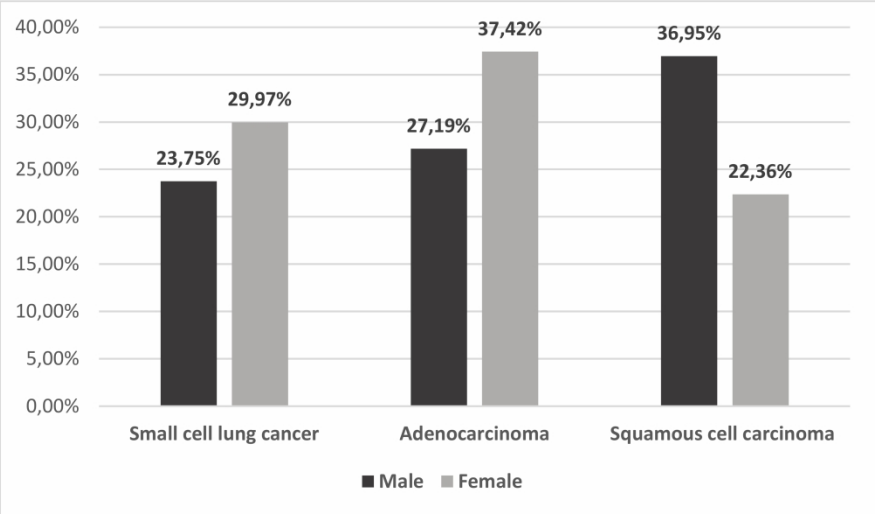

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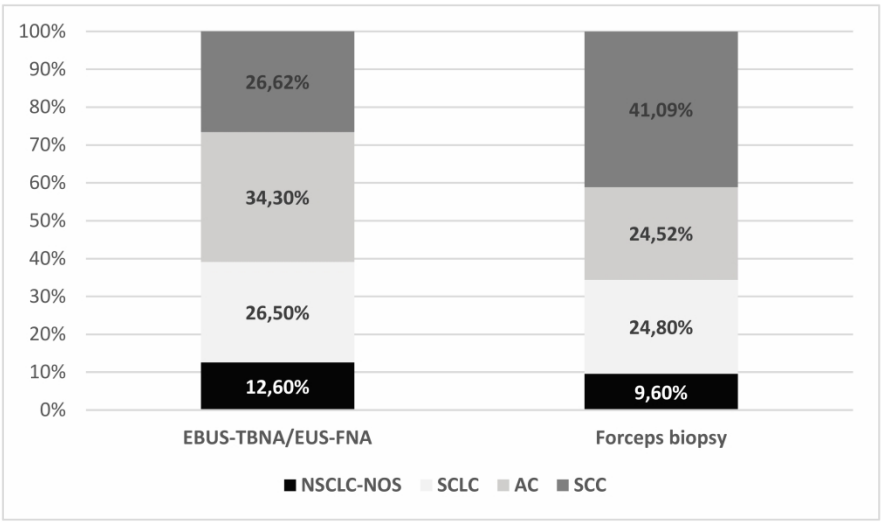

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STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4-5
Objectives	3	State specific objectives, including any prespecified hypotheses	6
Methods			
Study design	4	Present key elements of study design early in the paper	6-7
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6-7
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	6-7
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	6-7
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6-7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	6-7
Bias	9	Describe any efforts to address potential sources of bias	6-7
Study size	10	Explain how the study size was arrived at	6-7
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6-7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	6-7
		(*b*) Describe any methods used to examine subgroups and interactions	
		(*c*) Explain how missing data were addressed	
		(*d*) If applicable, explain how loss to follow-up was addressed	
		(*e*) Describe any sensitivity analyses	
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	8
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	8
		(b) Indicate number of participants with missing data for each variable of interest	
		(c) Summarise follow-up time (eg, average and total amount)	
Outcome data	15*	Report numbers of outcome events or summary measures over time	8

Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (*b*) Report category boundaries when continuous variables were categorized (*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	8
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	-
Discussion			
Key results	18	Summarise key results with reference to study objectives	9
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	9
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	10-11
Generalisability	21	Discuss the generalisability (external validity) of the study results	12
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	13

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

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Observational cross-sectional study of 5279 bronchoscopy results for the practical effectiveness of various biopsy techniques in the diagnosis of lung diseases with particular emphasis on lung cancer

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Observational cross-sectional study of 5279 bronchoscopy results for the practical effectiveness of various biopsy techniques in the diagnosis of lung diseases with particular emphasis on lung cancer

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Short title: **The yield of bronchoscopy in the diagnosis of lung cancer**

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Abstract

Introduction

Bronchoscopy is the main method in the diagnosis of various lung diseases. Endobronchial ultrasound transbronchial needle aspiration (EBUS-TBNA) is the most modern bronchoscopic technique useful in diagnosis and staging of lung cancer.

Objectives

The aim of the study was to assess the yield of bronchoscopy in patients with suspected various respiratory diseases including lung cancer. In particular, we examined the efficiency of different biopsy techniques in the diagnosis of lung cancer in correlation with its localization and pathomorphological type.

Patients and Methods

The results of pathomorphological examinations from 5279 bronchoscopies performed in 2016-2018 were analyzed. The material was collected with endobronchial ultrasound transbronchial needle aspiration (EBUS-TBNA), endoscopic ultrasound guided fine needle aspiration (EUS-FNA) and endobronchial forceps biopsy. Clinical and demographic factors were analyzed using the Fisher χ^2 test.

Results

5279 patients were diagnosed due to various respiratory symptoms. Lung cancer was confirmed in 36.42% of patients. 40.81% of patients had no definitive patomorphological diagnosis. Among patients with lung cancer, the most frequent diagnosis was non-small cell lung cancer: squamos cell lung cancer (SCC) - 32.07% and adenocarcinoma (AC) - 30.61%, then small cell lung cancer (SCLC) - 25.83% and not otherwise specified non-small cell lung cancer (NSCLC NOS) - 11.49%. Diagnosis of SCC was obtained significantly more often (χ^2=43.143, $p<0.000001$) by forceps biopsy (41.09%) than by EBUS-TBNA/EUS-FNA (26.62%). On the contrary, diagnosis of AC or NSCLC NOS was significantly more often (χ^2=20.394, $p<0.000007$ and χ^2=3.902, $p<0.05$, respectively) observed in EBUS-TBNA/EUS-FNA (34.31% and 12.6%) than in endobronchial biopsies (24.52% and 9.64%).

Conclusions

The use of bronchoscopy in the diagnosis of various lung diseases is vital but also has many limitations. Effectiveness of EBUS-TBNA and endobronchial forceps biopsy in diagnosis of lung cancer is strongly affected by tumor localization and type of cancer.

Key words: lung cancer; bronchoscopy; endobronchial biopsy; EBUS-TBNA; EUS-FNA

Article summary

Strengths and limitations of this study

1. 5 279 patients were enrolled to the study group which makes it one of the largest studies in the world with assement of bronchoscopy effectiveness in routine clinical practice.
2. We analyzed bronchoscopies performed in 2016-2018 in a few polish medical centers across the country, in a diversified population which ensures a high level of generalisability.
3. The study is an important contribution to the epidemiological data of advanced lung cancer in Poland.
4. Bronchoscopies were performed by 8 different bronchoscopists. This fact may cause human-dependent variation in results.
5. Results of pathomorphological assessment of the material obtained during single bronchoscopy was analyzed. Patients without diagnosed disease in the bronchoscopic material underwent further diagnostics using other methods or in other centers. Therefore, we were unable to report a definitive diagnosis in patients with inconclusive results of diagnostic prcedures.

Introduction

Epidemiological analyses indicate that lung cancer (LC) is the most common cause of cancer-related deaths. It is usually diagnosed in an unresectable, advanced stage. [1, 2] Lung cancer is the most common cancer in men and the third most common in women. In 2018, there were more than 2 million new cases of lung cancer worldwide. [3]

In the United States from 2004 to 2009, a total of 1,096,276 lung cancer cases were diagnosed and reported. This American study investigated the histologic type of lung cancer and demographic characteristics of the patients. The incidence of individual types of LC was as follows: small cell lung cancer (SCLC) – 14.9%, squamous cell carcinoma (SCC) – 21.9%, adenocarcinoma (AC) – 37.1% and large cell carcinoma (LCC) – 3.2% of cases. [4]

It is difficult to obtain accurate epidemiological data on the occurrence of individual pathomorphological types of advanced LC in Poland. Up to date, no epidemiological studies have been conducted on a sufficiently large group of patients with advanced lung cancer to obtain reliable results. Statistics on pathomorphological diagnoses of lung cancer in material from bronchoscopy have not been conducted so far. In Poland, the main source of such data is the National Lung Cancer Registry which only contains details about patients undergoing surgery in earlier stages of the disease. There where 17,783 patients diagnosed and operated in Polish thoracic surgery centers and registered in the National Lung Cancer Registry in the years

2014-2018. This group includes: 48.7% of patients with AC, 40.8% of patients with SCC, 6.45% of patients with LCC, 2.1% of patients with adenosquamous cell carcinoma, 1.05% of patients with SCLC and 0.9% of patients with non-otherwise specified (NOS) non-small cell lung cancer (NSCLC). This material included low percentage of patients with SCLC (usually an inoperable type of lung cancer) and patients with NSCLC-NOS (large surgical material is easier for pathomorphological examination which is a factor of reducing misdiagnosis). According to IASLC (International Association for the Study of Lung Cancer) recommendations, large cell carcinoma should be diagnosed only in surgical materials extracted from the entire resected tumor. [5] Therefore, this type of cancer was recorded in surgical pathology in non-advanced NSCLC patients and almost absent in patients with advanced lung cancer diagnosed with tumor biopsy. [2]

Due to different locations of tumor types in the lungs or metastatic lymph nodes, varied approaches are required to cancer diagnosis. The peripheral location is characteristic for AC, while squamous and small cell carcinomas most often are located centrally. Bronchoscopy is an appropriate method for detecting LC and the endobronchial ultrasound-guided with transbronchial needle aspiration (EBUS-TBNA) procedure has an essential role in the invastigation of lung cancer. If the tumor is centrally located and infiltrated the bronchus, the most optimal procedure seems to be the endobronchial biopsy using a brush or forceps. In contrast, adenocarcinoma frequently metastasizes to the mediastinal lymph nodes, which may be available on EBUS-TBNA or EUS-FNA procedures.

Metastasis to mediastinal lymph nodes is typical for each of types of lung cancer. We distinguish compartments of the mediastinal lymph nodes: superior mediastinal nodes (stations 2, 3 and 4), aortic nodes (stations 5 and 6), inferior mediastinal nodes (stations 7, 8, 9), hilar and interlobar lymph nodes (stations 10 and 11) and peripheral lymph nodes (station 12 for lobar nodes, station 13 for segmental nodes and station 14 for subsegmental nodes). EBUS-TBNA is most often used in diagnosis of superior mediastinal nodes, station 7 of inferior mediastinal nodes and stations 10, 11, 12 lymph nodes. EUS-FNA is preffered in diagnosis of superior mediastinal nodes and stations 7, 8 and 9 of inferior mediastinal nodes. Moreover EUS-FNA is used for sampling subphrenic lymph nodes and metastates in liver and left adrenal gland. Both methods – EBUS-TBNA and EUS-FNA are preferred for mediastinal lymph nodes assessment for evaluation of N-stage in NSCLC patients. [6] These two methods could be used in sampling visible tumors localized in central airways but also peripherally to the main bronchi, i.e. in lobar or even segmental bronchi.

Lung cancer staging is initially assessed through imaging studies. Normal mediastinum lymph nodes are defined below 10 mm in computed tomography (CT). Such a size of these lymph nodes suggests the N0 clinical stage. Currently, sampling for N0 nodes is not recommended, while surgery is the primary treatment method for N0/N1stage of lung cancer. However, everyday practice shows that it is worth collecting non-enlarged nodes for pathomorphological examination, because cancer cells are often found in such nodes. [7,8]

The sensitivity of detecting lung cancer using different bronchoscopy methods varies from 34% to 88% depending on the size and location of the tumor and preliminary diagnosis of the patients. [9] Meta-analysis of 18 studies which included a total of 1,201 LC patients was performed for assessment of sensitivity and specificity of ultrasound-guided fine needle aspiration in mediastinal staging of lung cancer. Authors showed sensitivity of 83% (range 45-100%) and specificity of 97% (range 88-100%) of these methods. [10] In eight studies limited to patients with enlarged mediastinal lymph nodes seen on CT, sensitivity was 90% (95% CI: 84 to 94%) and specificity was 97% (95% CI: 95 to 98%). In patients without enlarged mediastinal lymph nodes visible on CT, the overall sensitivity was 58% (95% CI: 39-75%). Therefore, the use of EBUS-TBNA increases the accuracy in the estimation of the stage of lung cancer and may radically influence the further treatment of the patient and the selection of the treatment methods. EUS-FNA enables confirming the presence of distant metastases, which has a decisive impact on therapeutic decisions. However, all false negative results delay cancer diagnosis and force the repetition of diagnostic procedures including surgery. Earlier detection of lung cancer gives patients the chance for better treatment. [11]

Aim

The objective of our study was a descriptive analysis of lung diseases diagnoses, especially lung cancer, established by various bronchoscopic procedures. We devoted special attention to the possibility of diagnosis of individual pathomorphological types of LC with various techniques used for collecting materials during bronchoscopy.

Material and methods

In our observational cross-sectional study, we analyzed the results of pathomorphological examination carried out on the material obtained during 5279 bronchoscopies performed in 2016-2018. Those bronchoscopies were performed in three Polish

pulmonology departments. The study included 1892 women and 3387 men, with median age of 65 years.

The study was retrospective and relied fully on the analysis of documents gathered, thus eliminating the need for collaboration between patients and researchers. No patients were enrolled specifically to carry out this study.

Various diseases of the respiratory system were indication for bronchoscopy: 3127 (59.2%) patients had suspicion of chest tumor in computed tomography, 882 (16.7%) patients demonstrated hilar lymphadenopathy, 205 (3.9%) patients had suspicion of sarcoidosis and 20 (0.4%) patients had suspicion of pulmonary fibrosis. Other indications for bronchoscopy occurred in 1045 (19.8%) patients (e.g. suspicion of tuberculosis, chronic cough, hemoptysis, etc.). In patients with suspected cancer, samples of the tissue were acquired through bronchoscopy and the technique was chosen in compliance with tumor or metastatic lymph node's location as it is described in the introduction. Forceps biopsies were performed using Olympus BF-1T180 and Pentax EB-1970K bronchoscopes, EBUS-TBNA – using Olympus BF-UC180F and Pentax EB-1970UK bronchoscopes (22-gauge needles), and EUS-FNA – using Olympus GF-UCT180 endoscope. Premedication for bronchoscopy was under local or general anesthesia, depending on the situation.

Samples underwent pathomorphological examination which included hematoxylin-eosin (H&E) staining, mucicarmine staining and immunohistochemistry (IHC) examination, such as staining of TTF-1 (thyroid transcription factor 1) and p63/p40. Samples diagnosed with non-squamous NSCLC were in-depth reported and underwent molecular testing for the presence of *EGFR* gene mutation by real-time PCR technique (RT-PCR), *ALK* gene rearrangement and PD-L1 expression by IHC. PD-L1 expression was also assessed in SCC patients. Large cell carcinoma (LCC) of the lung according to the 2015 World Health Organization (WHO) Classification of Tumors of the Lung, Pleura, Thymus and Heart cannot be diagnosed in small specimens and aspiration biopsy materials. The diagnosis of LCC can only be made in the postoperative material. Therefore, there were no patients diagnosed with LCC in our study. Such patients were included in the group of patients diagnosed with NSCLC NOS. Chromogranin and synaptophysin was used in IHC examination of neuroendocrine tumors (small cell lung cancer or NSCLC NOS). All the centers participating in the study used these same procedures described above.

After receiving the diagnosis, we selected a population of patients with lung cancer and divided them into groups of patients with different cancer types detectable with bronchoscopic procedure (squamous cell lung cancer, adenocarcinoma, large cell carcinoma, not otherwise

specified NSCLC, and small cell lung cancer). Then, we assessed the prevalence of different types of LC in the materials obtained with various bronchoscopic procedures.

Clinical and demographic factors were analyzed using the Pearson's Chi-square test. P-values below 0.05 were considered significant. The percentages reflect the relative number of all lung cancer patients diagnosed with a particular procedure. We only analyzed the results of the first-time bronchoscopy, that could be nondiagnostic. The evaluation of relative diagnostic yield (sensitivity) of different bronchoscopic procedures could not be done because, in our study, it was not possible to verify the final diagnosis of patients in the materials collected during the next bronchoscopy or another procedures (this applies mainly to patients with lung tumor or hilar lymphadenopathy). The following diagnostic procedures were carried out in various clinical centers throughout Poland. Therefore, we were unable to verify the diagnoses obtained later.

Patient and Public involvement statement: Patients were not involved in research.

The protocol of the study was approved by the Committee of Ethics and Research at the Medical University of Lublin (KE-0254/5/2018).

Results

In 3565 (67.5%) patients, EBUS-TBNA and transesophageal EUS-FNA was performed and cytological material was archived in a cellblock. In 1346 (25.5%) patients, EBUS-TBNA (without EUS-FNA) was the only diagnostic procedure. In the remaining patients, EBUS-TBNA was supplemented by EUS-FNA. There were no patients in whom EUS-FNA would be the only diagnostic method. 1714 (32.5%) patients had non-ultrasound-guided bronchoscopy with the forceps biopsy of endobronchial lesions allowing to obtain a small histological specimen.

Lung cancer was confirmed in a group of 1923 (36.42%) patients, including 1280 men and 643 women. Reactive lymph nodes were found in 16.06% of the patients, sarcoidosis was diagnosed in 4.13%, idiopathic pulmonary fibrosis – in 0.19%, metastases to the lungs from other organs – in 2.39% of the patients. 40.81% of the patients had no definitive diagnosis (Figure 1). Lung cancer was confirmed in 51% of patients with suspected tumor in computed tomography. While, in the group of patients with hilar lymphadenopathy, lung cancer was diagnosed in only 10.1% of cases.

Among those with lung cancer, squamous cell carcinoma (32.07%) was most often diagnosed, then adenocarcinoma (30.61%), small cell lung cancer (25.83%) and NSCLC-NOS

(11.49%) (Figure 2). SCLC and AC were significantly more frequent ($\chi^2 = 8.649$, $p = 0.0033$ and $\chi^2 = 21.128$, $p < 0.000005$, respectively) in women (29.97% and 37.42% of women with lung cancer) than in men (23.75% and 27.19% of male patients with lung cancer). Squamous cell carcinoma appeared significantly more often ($\chi^2 = 41.881$, $p < 0.000001$) among male (36.95%) than among female (22.36%) patients with lung cancer (Figure 3). SCC was significantly more often ($\chi^2 = 4.17$, $p = 0.041$) diagnosed in the group of patients older than 65 years than in younger patients. Other pathomorphological types of lung cancer occurred with similar frequency in these two age groups.

Endobronchial biopsies significantly more often ($\chi^2 = 7.566$, $p = 0.0059$) provided material for the diagnosis of lung cancer than the EBUS-TBNA and EUS-FNA procedures. 42.35% of endobronchial biopsies and 33.6% of TBNA and FNA provided material sufficient to diagnose lung cancer. Fine needle biopsy of lymph nodes enabled the diagnosis of lung cancer in 29.2% of cases, fine needle biopsy of lung tumor – in 66.6% of cases, and forceps biopsy of bronchial mucosa lesions – in 48.2% of cases. These differences were statistically significant.

Among patients with lung cancer, TBNA or FNA compared to endobronchial biopsies gave a similar result ($\chi^2 = 0.656$, $p = 0.418$) in detection of SCLC (26.5% vs. 24.8%). On the other hand, the diagnosis of AC and NSCLC-NOS was obtained significantly more frequently ($\chi^2 = 20.394$, $p = 0.000006$ and $\chi^2 = 3.902$, $p = 0.0482$) in EBUS-TBNA and EUS-FNA compared to endobronchial biopsies (34.3% vs. 24.52% and 12.6% vs. 9.6%, respectively). SCC, among other lung cancer types detected by bronchosopy, was diagnosed in 41.77% in materials obtained by forceps biopsy and only in 26.62% in materials with EBUS-TBNA or EUS-FNA ($\chi^2 = 43.143$, $p < 0.000001$), which was directly related to the more frequent central location and bronchial infiltration of this type of tumor. Computed tomography showed that in 79% of SCC patients the tumor was centrally located in the large bronchi. The analysis of bronchoscopic images showed that tumor deformed the bronchial mucosa or showed endobronchial growth in 67% of SCC patients. (Figure 4). Table 1 shows the results of bronchoscopy procedures in the diagnosis of individual pathomorphological types of lung cancer depending on place of collecting the material.

Discussion

Our study on the results of EBUS-TBNA/EUS-FNA and forceps biopsy in obtaining materials for the diagnosis of various lung diseases is among the largest worldwide. The study points to numerous problems arising from the use of these techniques in routine clinical

practice. We are aware of the many limitations of our study. First, we cannot determine the sensitivity and specificity of our methods, because it was not possible to determine the final diagnosis in such a large group of patients (5 279 cases). In addition, we could not distinguish between material collected by EBUS-TBNA and EUS-FNA. We do not know how many lung cancer patients were diagnosed only in the material from EBUS-TBNA or only in the material from EUS-FNA, or in both types of these materials. We also do not know the number of biopsies performed during one bronchoscopy. This data is missing from the results of the pathomorphological examination that we analyzed. Thirdly, diagnosis of large cell carcinoma was not possible in small specimens (LCC was probably qualified to the NSCLC-NOS group). A limitation of our study was also the lack of detailed clinical and radiological characteristics of all patients who underwent bronchoscopy procedures.

However, we found that advanced small cell lung cancer may be more common in Poland than previously thought. This tumor is characterized by rapid growth and metastases, therefore, more often it could be diagnosed in advanced stages using bronchoscopic techniques. SCLC diagnosis in EBUS-TBNA/EUS-FNA of lymph nodes and endobronchial biopsy occurs at the same frequency. Furthermore, the difference in the percentage of patients with squamous cell carcinoma and adenocarcinoma diagnosed with endobronchial biopsies and TBNA or FNA is noteworthy. In our study, most patients with adenocarcinoma were diagnosed with EBUS-TBNA or EUS-FNA of lymph nodes, while patients with squamous cell carcinoma were diagnosed more often based on examination of material from endobronchial biopsy (forceps biopsy). Patients in cohort with squamous cell carcinoma were more likely to have endobronchial disease accessible by forceps. Therefore we could not ascertnain that endobronchial biopsy is more effective for diagnosis of squamous cell lung cancer as there was no comparison to EBUS-TBNA and EUS-FNA for those patients. Thus, we point to the problem that results of bronchoscopic procedures depends on the location of the primary tumor and the presence of metastases in the lymph nodes.

Schmid-Bindert et al. showed how often they detected different pathomorphological types of lung cancer using various bronchoscopic methods. Small biopsies were collected by three different methods: forceps biopsy (44.6%), EBUS-TBNA (32.7%), and CT-guided core biopsy (22.8%). 38% of adenocarcinoma, 51% of squamous cell carcinoma and 11% of NSCLC NOS were diagnosed using forceps biopsy. EBUS-TBNA results were as follows: 45% of adenocarcinoma, 30% of squamous cell carcinoma and 24% of NSCLC NOS. [12]

Many authors emphasize that the diagnosis of NSCLC NOS is the most common in the case of material obtained from EBUS-TBNA. Esterbrook et al. found that NSCLC-NOS rate

was 20.8% in EBUS-TBNA samples. Similar results were achieved by Navani et al. In group of 774 patients with known or suspected lung cancer, 23% of patients had a final diagnosis of NSCLC NOS. [13, 14] Our study confirmed the high percentage of NSCLC NOS patients diagnosed with EBUS-TBNA procedures. Endobronchial biopsy was less likely to provide a diagnosis of NSCLC NOS.

Chin et al. reported that EBUS-TBNA is the most sensitive diagnostic method for SCLC detection, because it allows to sample specimens from mediastinal as well as submucosal lesions. They also mentioned that the quality of specimens obtained by needle aspiration is better than by forceps biopsies, which may contain crushed artifacts. [15] In addition, other studies noticed that the sensitivity of EBUS-TBNA for SCLC detection was higher than for NSCLC diagnosis. [16, 17] Our findings confirmed the above statements. The majority of SCLC cases were diagnosed with EBUS-TBNA. Three SCLC patients were diagnosed from metastatic lesions in the adrenal gland using transesophageal EUS-FNA.

Many authors raise the problem that forceps biopsy has low sensitivity in the diagnosis of lung cancer. Forceps biopsy has a diagnostic yield ranging between 65-82%. In preliminary study by Pasko et al. conducted in 212 patients with lung cancer suspicion, authors compared sensitivity and accuracy of routine bronchoscopy techniques: endobronchial biopsy, EBUS-TBNA, and combination of EBUS-TBNA and EUS-FNA. Sensitivity and accuracy of endobronchial biopsy vs EBUS-TBNA vs combination of transbronchial biopsies were 43% vs 44.3% vs 93.7% and 93.8% vs 94.7% and 94.8%, respectively. This demonstrates high usefulness of the combination of EBUS-TBNA and EUS-FNA in the diagnosis of lung cancer [18]. Verma et al. demonstrated sensitivity of EBUS-TBNA in cancer diagnosis of 91.4% in small group of 37 patients with lesions located adjacent to the trachea or lesions located adjacent to the main bronchi. [19] Tournoy et al. indicated that EBUS-TBNA has a sensitivity of 82% and low negative predictive value (23%). [20] The similar results were received by Zhao et al. for lesions located near the central airways. [21] Oki et al. showed that the combined endoscopic method with EBUS-TBNA and EUS-FNA with a single bronchoscope gives better results in staging of NSCLC, than each technique alone. However, they mentioned that significant number of patients had false-negative EBUS-TBNA and EUS-FNA results. Moreover Oki et al. suggested that very important issue is bronchoscopists experience, which may cause differences in the results. [22] On the other hand, Wallace et al. showed a EBUS-TBNA sensitivity of only 69% in a group of 150 patients with lung cancer suspicion. [23]

Despite relatively low negative predictive value of EBUS-TBNA, there is an indication to perform other procedures (e.g., surgical procedures) for final diagnosis in a significant group

of patients. In our study, 56.9% of patients who underwent bronchoscopy did not receive a definitive diagnosis of the diseases and they have been subjected to other diagnostic procedures or observations. We showed the results of all performed bronchoscopies and three different methods of material collection (endobronchial biopsy and combination EBUS-TBNA and EUS-FNA). Moreover, unselected and heterogeneous patients were recruited in three different hospitals that employ a total of 8 bronchoscopists. In most studies, the evaluation of the usefulness of EBUS-TBNA and EUS-FNA for detecting malignancy was conducted in selected patients with high clinical suspicion of the tumor. Small, preselected groups of patients with high risk of lung cancer could be the reason of the low negative predictive value of bronchoscopic procedures in these studies. Thus, these observations may not reflected the real clinical situation.

In studies where population was heterogenic regarding the disease (lung cancer, sarcoidosis, tuberculosis), EBUS-TBNA had diagnostic value only in 60-75% of patients. [24] Lange et al. showed diagnostic results of EBUS-TBNA in only 61.4% of unselected patients undergoing routine diagnostic procedures. [25] Fournier et al. examined 185 patients with extrathoracic malignancy and mediastinal lymphadenopathy in real life practice. Pathomorfological types of malignancy were successfully identified using EBUS-TBNA in only 93 patients (50.3%). The diagnostic sensitivity, specificity, negative predictive value, and positive predictive value were 68.4%, 100%, 53.3%, and 100%, respectively. [26] Murthi et al conducted research comparing the accuracy of EBUS-TBNA to surgery in diagnosis of hilar and mediastinal pathologies. EBUS-TBNA for all pathologies had an accuracy of 81.2% and sensitivity of 55.1%. [27]

Conclusions

Comparing all these data, we could conclude that bronchoscopy is vital but not an ideal technique in the routine diagnosis of respiratory diseases. Our study showed that 41% of bronchoscopy materials were insufficient to perform reliable pathomorphological examination. This mainly concerned patients with suspected lung tumor or lymphadenopathy. The use of brush biopsy, forceps biopsy, bronchoaspirate analysis EBUS-TBNA and EUS-FNA simultaneously, if desired, was of the highest diagnostic value. However, sometimes this fails and bronchoscopy must be repeated, or thoracic procedures (e.g. mediastinoscopy or thoracoscopy) must be performed. We found that the EBUS-TBNA value in daily clinical practice differed from that in clinical trials. Therefore, precise estimation of the frequency of individual pathomorphological types of lung cancer, based on material obtained

bronchoscopically, is not possible. However, EBUS-TBNA plays an essential role in staging of invasive lung cancer. Therefore, its value in the diagnosis of lung cancer is not limited to demonstrating the presence of cancer type, but, above all, to determining the extent of the disease and qualification for appropriate treatment. It seems that the incidence of lung cancer with a typical peripheral localization (adenocarcinoma) may be underestimated in comparison to the incidence of lung cancer with a typical central localization (squamous cell carcinoma, SCLC), when bronchoscopy is used as the primary diagnostic method.

Authorship contribution statement:
Conception and design of the study: JB, MF, PK, JP. Administrative support: PK, JP, AP, JB, AS, JS, MM, PK, RK, JM. Provision of study material or patients: JP, AP, JB, AS, JS, MM, PK, RK, JM. Collection and assembly of data: JB, MF, PK, JP, AP, JB, AS, JS, MM, PK, RK. Data analysis and interpretation: JB, MF, PK. Manuscript writing: JB, MF, PK. All authors gave final approval of the published manuscript.

Data Availability Statement:
The data that supports the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest:
The authors declare no potential conflicts of interest.

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Table 1. The results of varies techniques during bronchoscopy in the diagnosis of individual pathomorphological types of lung cancer depending on place of collecting the material and nodal station.

Material	**SCLC**	**Adenocarcinoma**	**Squamous cell carcinoma**	**NOS**	**Total lung cancer**
EBUS-TBNA/EUS-FNA of lymph nodes	250 (27.7%)	331 (36.7%)	209 (23.1%)	114 (12.5%)	904 (100%)
EBUS-TBNA/EUS-FNA of tumor	88 (24.7%)	94 (25.8%)	135 (37.2%)	44 (12.3%)	361 (100%)
EBUS-TBNA/EUS-FNA metastases to adrenal gland	3 (42.9%)	1 (14.3%)	2 (28.6%)	1 (14.3%)	7 (100%)

Forceps biopsy of tumor	156 (22%)	163 (25%)	270 (41.5%)	62 (9.5%)	651 (100%)

Figure 1. Results of pathomorphological examination carried out on material obtained from 5279 bronchoscopies (entire study population)

Figure 2. The incidence of individual pathomorofological types of LC in the entire study group of lung cancer patients

Figure 3. The incidence of individual pathomorofological types of LC according to the gender of patients with lung cancer

Figure 4. Percentage of patients with different types of lung cancer detected in materials collected with different bronchoscopic techniques. Frequency of different types of lung cancer was calculated in the whole group of patients undergoing a given bronchoscopic procedure (100%), which resulted in the diagnosis of lung cancer

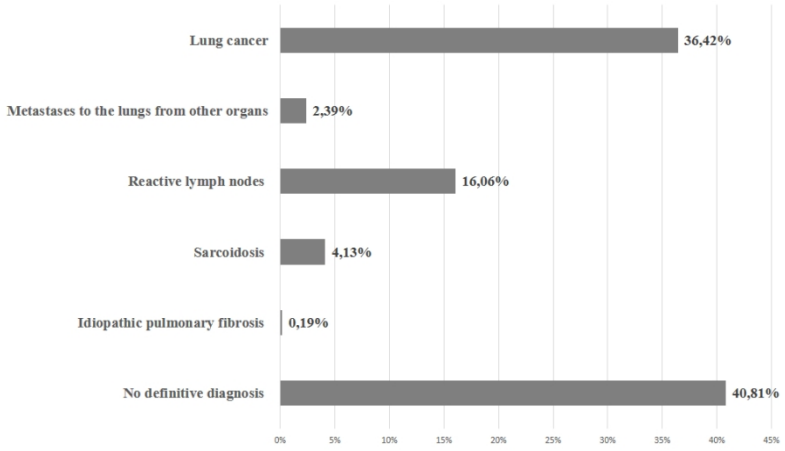

Figure 1. Results of pathomorphological examination carried out on material obtained from 5279 bronchoscopies (entire study population)

108x60mm (300 x 300 DPI)

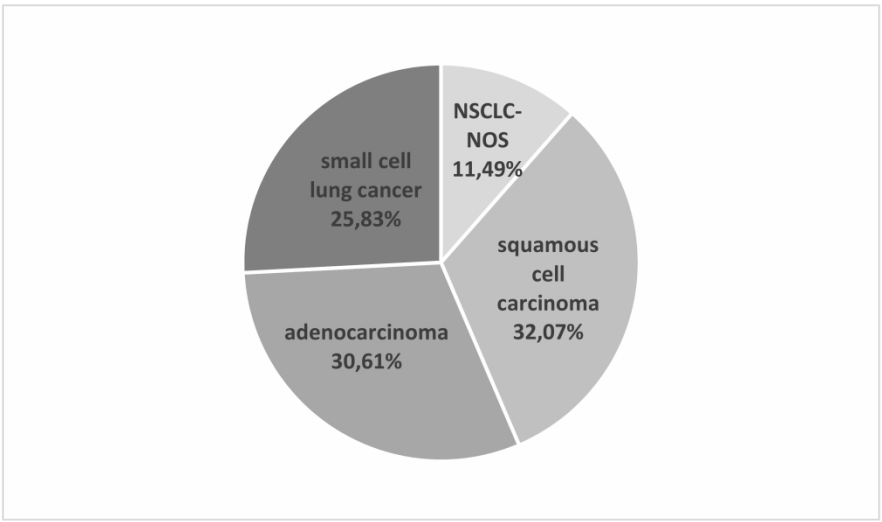

Figure 2. The incidence of individual pathomorofological types of LC in the entire study group of lung cancer patients

296x209mm (300 x 300 DPI)

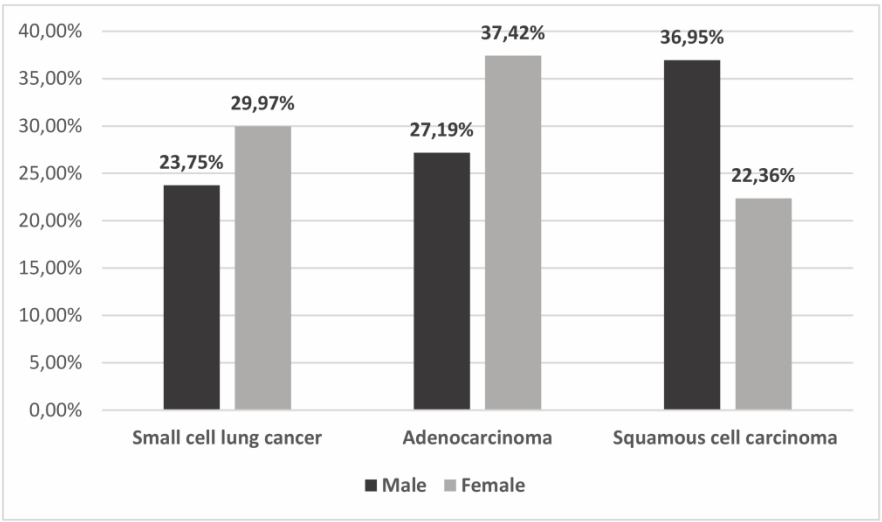

Figure 3. The incidence of individual pathomorofological types of LC according to the gender of patients with lung cancer

296x209mm (300 x 300 DPI)

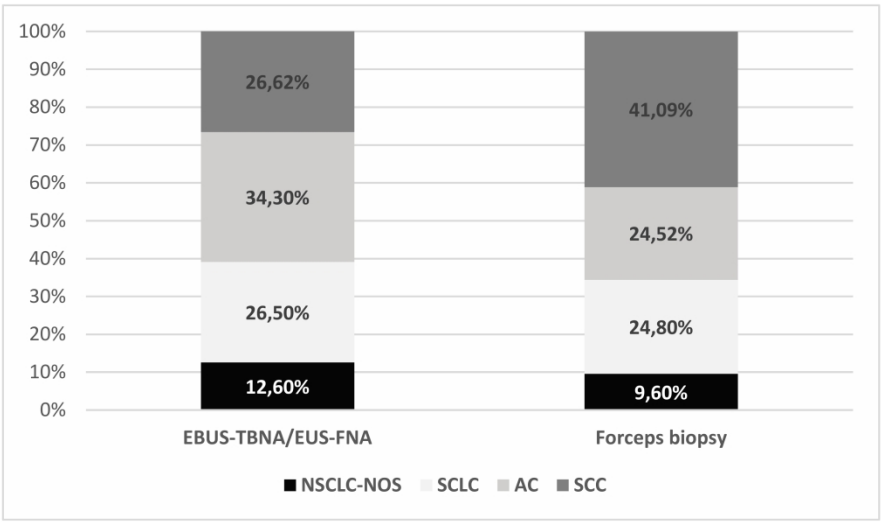

Figure 4. Percentage of patients with different types of lung cancer detected in materials collected with different bronchoscopic techniques. Frequency of different types of lung cancer was calculated in the whole group of patients undergoing a given bronchoscopic procedure (100%), which resulted in the diagnosis of lung cancer

296x209mm (300 x 300 DPI)

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4-5
Objectives	3	State specific objectives, including any prespecified hypotheses	6
Methods			
Study design	4	Present key elements of study design early in the paper	6-7
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6-7
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	6-7
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	6-7
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6-7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	6-7
Bias	9	Describe any efforts to address potential sources of bias	6-7
Study size	10	Explain how the study size was arrived at	6-7
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6-7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	6-7
		(*b*) Describe any methods used to examine subgroups and interactions	
		(*c*) Explain how missing data were addressed	
		(*d*) If applicable, explain how loss to follow-up was addressed	
		(*e*) Describe any sensitivity analyses	
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	8
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	8
		(b) Indicate number of participants with missing data for each variable of interest	
		(c) Summarise follow-up time (eg, average and total amount)	
Outcome data	15*	Report numbers of outcome events or summary measures over time	8

Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (*b*) Report category boundaries when continuous variables were categorized (*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	8
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	-
Discussion			
Key results	18	Summarise key results with reference to study objectives	9
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	9
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	10-11
Generalisability	21	Discuss the generalisability (external validity) of the study results	12
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	13

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.