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Review Article

Evolution of Transbronchial Biopsy and Development Prospects

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Abstract

The main source that allows for the final verification of the diagnosis of bronchopulmonary pathology, especially concerning the peripheral parts of the bronchopulmonary system, remains a multi-aspect analysis of its tissues, samples of which are obtained by biopsy. Traditional methods for obtaining such a biopsy are surgical biopsy, considered the gold standard, but associated with significant complications and mortality, and transbronchial forceps biopsy, which is a less invasive, but also significantly less informative method in terms of the quality of the material obtained. In recent years, the technology of transbronchial collection of biopsy material has undergone significant changes, allowing it to be obtained in a significantly higher quality state, both in volume and structure. The most informative of these is transbronchial needle cryobiopsy under endosonography control and various modifications of this method, which require a more detailed analysis. For this purpose, an analysis of 60 full-text literary sources was conducted, selected based on the results of a search in biomedical scientific information databases, including open sources on patents and the ClinicalTrials.gov website, as well as original studies and review papers found in the PubMed, Embase, Cochrane, and Index Medicus databases. As a result, an assessment of transbronchial needle cryobiopsy under endosonography control and various methods of its use was made, their capabilities were reflected in terms of efficiency and the presence of significant novelty, and the feasibility and prospects for use were determined. The conclusion notes the high information content of transbronchial needle cryobiopsy under endosonography control, its significant predominance over transbronchial forceps biopsy, the presence of positive technical solutions in some methods of using this method and the absence of such in others, the feasibility and prospects for its improvement, including through standardization.

Keywords: Endosonography; Fine-needle puncture biopsy; Transbronchial cryobiopsy; Transbronchial forceps biopsy; Surgical lung biopsy

Introduction

The main source that allows for the final verification of the diagnosis of bronchopulmonary pathology, especially concerning the peripheral parts of the bronchopulmonary system, remains a multi-aspect analysis of its tissues, samples of which are obtained by biopsy. Therefore, obtaining quality biomaterial, in particular biopsies of affected lung tissue and lymph nodes, is very important, especially when in recent years molecular studies have expanded our knowledge of the biological pathways of disseminated lung diseases, tumor processes, and have opened up opportunities for their precise genotyping and targeted therapy [1,2]. Usually, traditional methods of obtaining lung tissue biopsy are surgical biopsy, which allows for diagnosis in 90% of cases or more, and transbronchial forceps biopsy, which is a less invasive method, but is also significantly less informative in terms of the quality of the material obtained [3-6]. The latter method provides diagnostics in only 20-30% of cases of disseminated lung processes due to the small size of the biopsy samples, about 1-3 mm in the largest dimension, and frequent artifacts associated with forceps sampling of material. These methods are well known, so we will not dwell on them in detail. In recent years, methods have been developed that are also based on transbronchial collection of lung tissue biopsies, but which, thanks to modern technological capabilities, allow obtaining much higher-quality material for research,

both in terms of its volume and structure, which significantly increases diagnostic capabilities. The most informative of these is Transbronchial Needle Cryobiopsy Under Endosonography Control (TBNCBE) and various modifications of this method. However, their capabilities, advantages and disadvantages require a more detailed analysis.

Purpose of the Study

Analysis of the possibilities of obtaining lung and lymph node biopsies using TBNCBE, its various modifications and an assessment of the feasibility and prospects for their use.

Methodologically, literary data **were** used for the analysis, including open sources on patents and the ClinicalTrials.gov website, as well as original studies and review papers found in the PubMed, Embase, Cochrane, Index Medicus databases. The keywords used for the search were**:** transbronchial needle cryobiopsy, endosonography, endobronchial ultrasound. The analysis included 60 (Figure 1). Full-text literature sources were analyzed with narrowing at each stage, and then the selected data were structured for an analytical review article.

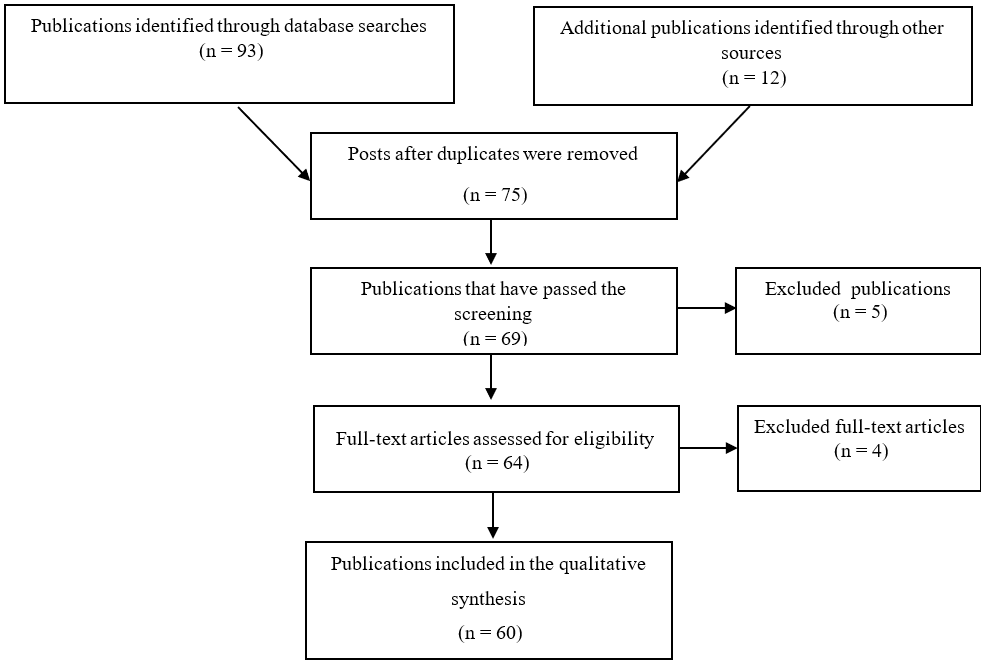

Figure 1: Publication selection scheme.

On the Evolution Of Biopsy Sampling in the Bronchopulmonary System. Basic Principles of TBNCBE +

A large heterogeneous group of more than 200 pulmonary diseases is diffuse parenchymatous lung diseases, which are very rare individually, but up to 15% in total, and are characterized by poor survival [7,8]. They contribute to diffuse and often bilateral focal damage to the lung parenchyma In the alveoli or small bronchi of the lung it can be located malignant tumor, projecting onto its peripheral parts, which is why it is called peripheral lung cancer. Its development can occur for a long time without symptoms, since the tumor does not block the large bronchi, and be accompanied by metastasis to the lymph nodes of the lung and mediastinum. Diagnosis in patients of these contingents is usually difficult and often the result of high-resolution computed tomography, as well as clinical and laboratory data, are insufficient. And the key diagnostic link in this case, allowing to establish a diagnosis, is a histological study of the tissue of the bronchopulmonary system affected by the pathological process. Therefore, ensuring high quality of biopsy material is fundamentally important. For many years, surgical lung biopsy was considered the gold standard, providing a high percentage of diagnosis of this group of diseases. However, not all patients safely tolerate this surgical procedure and for many of them the risk/benefit ratio determines its unacceptability and the use of surgical biopsy has significantly decreased since the 2000s due to an increase in mortality associated with this procedure [9-11]. In addition, it is necessary to take into account that surgical lung biopsy is unsafe in the presence of respiratory disorders and concomitant diseases [10,12]. Another traditional method of taking

a biopsy is transbronchial forceps biopsy of the lungs. However, its informativeness is not high, reaching up to 30%, and may even be lower, in particular in patients with idiopathic fibrosing alveolitis, which accounts for about 1/3 of the diseases in this group [13] or when peripheral lung cancer with a tumor size of up to 3 cm, limiting the accuracy of taking biopsy material and the resulting morphological verification to 50% or less [14-16].

Transbronchial forceps biopsy is reliable in bronchocentric or perilymphatic diseases and in diseases with distinctive histopathological features such as sarcoidosis, cratinomatous lymphangitis and organising pneumonia [17,18]. However, it is rarely of significant importance for establishing the diagnosis in diseases with a heterogeneous histological picture in diseases with the main histological abnormalities located at the periphery of the secondary lobule, such as usual interstitial pneumonia, most often associated with the clinical syndrome of pulmonary fibrosis [13,19]. In general, current expert opinion is against the use of this method for the diagnosis of interstitial pneumonia, as is especially true for surgical biopsy when there are relative contraindications. In this case, the unfavorable benefit/risk ratio is taken into account, in which the information content is low along with a high degree of complications, up to and including a fatal outcome, and with a characteristic unfavorable prognosis and reduced significance of the histological diagnosis due to the insufficient effectiveness of specific treatment with antifibrotic drugs [20-23]. It is emphasized, that such treatment makes sense in the early stages of the pathological process and in the contingent of patients who have a slow progression, which emphasizes the importance of a timely and adequate pathohistological examination, determined by the time of collection and the quality of the biopsy material [23]. This is due to the fact that the focal pattern of fibrosis is difficult to identify in small biopsies, often accompanied by artifacts, in particular crushing [13]. In the case of even a moderate number of foci of interstitial fibrosis and honeycomb changes, the information content of this method increases significantly, but the absence of histopathological changes does not exclude interstitial pneumonia due to unsuccessful collection of biopsy material [19]. The next step in the development of this direction was transbronchial biopsy using fine-needle puncture, carried out independently under the control of radiation diagnostic methods. Needle aspiration biopsy was first performed by puncturing the mediastinum through the tracheal spur and using a rigid bronchoscope back in 1949 and 1958, but it became widespread only in 1983 due to the development of a new needle and the use of a flexible bronchoscope, and an increase in the possibility of diagnosing lung cancer and determining its stage [24-27].

The development of the endobronchial ultrasound probe in 2002 opened up new prospects for real-time ultrasound-guided transbronchial needle aspiration (EBUS-TBNA), which ensures targeted biopsy taking, taking into account the most compromised area, significantly increasing the diagnostic accuracy [28]. It was found that three needle passes for each target area are sufficient to confirm the diagnosis, in particular for non-small cell lung cancer, and additional needle passes are required to obtain comprehensive molecular analysis [29-31]. This is consistent with the results of a large cohort study and a meta-analysis of 21 studies, indicating that a higher number of passes optimizes the obtained material and molecular studies [32,33]. The use of high-pressure suction of the collected material is indicated as an important factor influencing the size of the obtained sample [34]. However, convincing supporting data that this method reliably affected the diagnostic process have not been obtained. An additional technique that allows increasing the size of a biological sample, in particular a lymph node, is a biopsy with mini-forceps under ultrasound control, by inserting them through the initial hole made by the needle. This provided a higher diagnostic efficiency, by 1.5-2 times, but was significantly more often accompanied by complications [35]. The obtained material is recommended to be formed in the form of histological blocks after appropriate wiring, rather than the traditional smear method, which increases the amount of preserved material and expands the possibilities of its use for additional studies and in the future [36,37]. The next step in improving the collection of biopsy material, including from lymph nodes, was its collection using a cryoprobe [38]. In this case, the biopsy is obtained using the Joule-Thomson effect, in which the probe is cooled by releasing compressed gas with a high flow, for which carbon dioxide or nitric oxide is used. Due to the pressure difference relative to the atmosphere, the gas suddenly expands, causing a drop in temperature at the tip of the probe, and adjacent tissues adhere to the frozen probe [39,40]. This produces a biopsy of larger dimensions, up to 15 mm or more, compared to forceps. In this case, a biopsy of a larger size is obtained, up to 15 mm or more, compared to a forceps biopsy, which is usually, as already noted, no more than 3 mm [41,42]. The approach to this diagnostic procedure may vary depending on the place where it is carried out (in a room for endoscopic examination or an operating room), the type of premedication (moderate or deep), the method of bronchoscopy (using a flexible or rigid bronchoscope) and measures to prevent complications (in particular, hemoptysis), as well as the place of biopsy collection, the number of biopsies, etc., which we will discuss below. The most informative method seems to be TBNCBE , which, according to most authors, is the most optimal today.

Speaking about diagnostic methods based on TBNCBE, it can be noted that thanks to this technology, it is possible to obtain biopsy samples not only of better quality in terms of volume, but also in terms of the state of its morphological structures and compliance with the target site of concentration of the pathological process.

Comparative studies in patients have shown that the use of TBNCBE is a completely adequate alternative to surgical lung biopsy. Based on the above, it can be noted that TBNCBE is essentially based on two techniques: cryobiopsy and ultrasound control, the combination of which significantly increases the diagnostic value of the method. The first has proven itself well over a number of years, providing, through the use of cryotechnics, both a significantly larger volume of biopsy and good quality in the absence of artifacts, which are the talk of the town among morphologists. The second - due to the use of ultrasound in recent years in combination with the first, provides the ability to more accurately pre-determine and control the location of the pathology and the choice of the target site and the performance of a lung biopsy. The combined approach of this method allows not only to reliably increase the efficiency by 12% compared to the use of only transbronchial needle biopsy under endosonography control, but also provides a more accurate molecular and immunological analysis of lung cancer and a good safety profile [43]. Since its first report in 1992, TBNCBE has been incorporated into the diagnostic and staging algorithm for benign and malignant diseases of the gastrointestinal tract and adjacent organs, representing a breakthrough in the field of endoscopy. Perhaps a similar breakthrough may also occur in the field of bronchopulmonology. TBNCBE generally involves the use of low temperatures to freeze a tissue area and collect a biopsy under ultrasound control, which allows for increased accuracy of visual assessment of the location of the lesion, also previously performed using radiological diagnostic methods, including, for example, CT. In this case, TBNCBE is essentially performed in stages (Figure 2). First, fine-needle puncture is performed using endobronchial ultrasonography, which finally specifies the area for performing the puncture of the lesion, also previously outlined using radiological diagnostics. Then a cryoprobe is inserted into the formed puncture tract and the tissue of the organ being biopsied is locally frozen to -60 ˚C due to the effect of reduced gas, in particular CO2, which is supplied by the cryoprobe at a fixed time. Then the cryobiopsy is extracted in a block with a bronchoscope due to the fact that the size of the biopsy exceeds the diameter of its instrumental channel. Then the cryobiopsy is defrosted and processed accordingly for further studies.

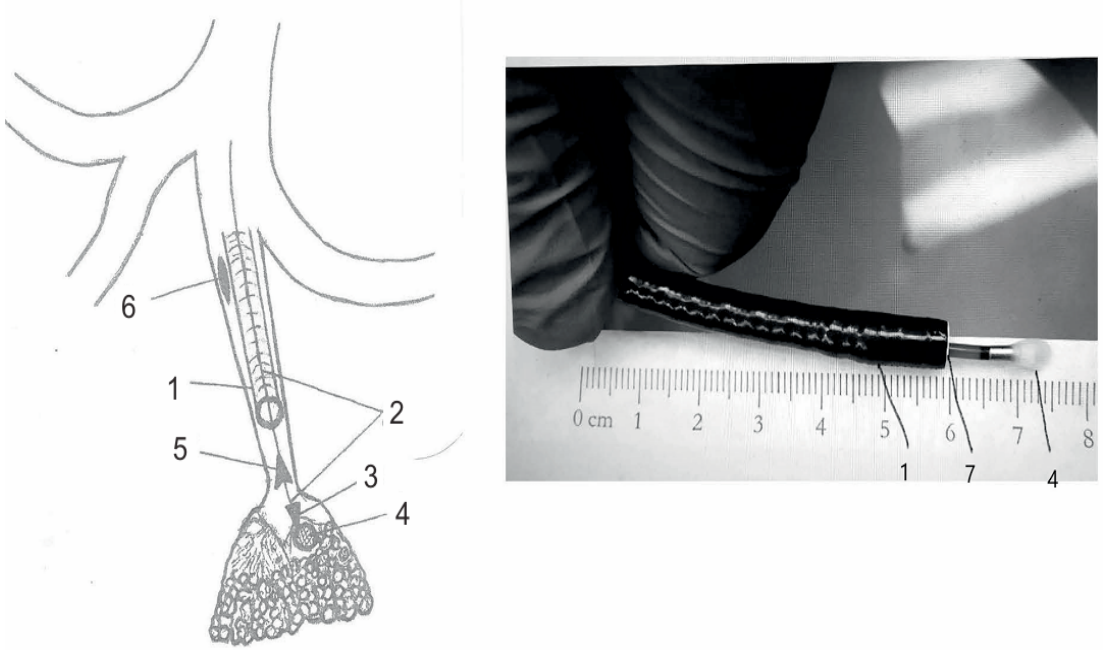

Figure 2: Transbronchial needle cryobiopsy under endosonography control (A - schematic diagram, B - a photo illustration of a cryobioptate specimen after its collection): 1 - bronchoscope tube, 2 - path of fine-needle puncture during ultrasonography and cryoprobe, 4 - biopsy sample, 5 - retraction of biopsy sample in a block with bronchoscope tube and cryoprobe, 6 - balloon catheter, 7 – cryobioptate.

TBNCBE, Modifications, Comparative Evaluation and Prospects

A number of studies, starting in 2017, have shown that transbronchial ultrasound-guided biopsy, due to the use of extremely low temperatures, is capable of providing a larger amount of structurally preserved biopsy tissue from the lung and mediastinal lymph nodes, taken purposefully, thereby improving the diagnostic efficiency not only in local pathology, rare tumors and benign diseases, but also in disseminated processes [38,44-46]. In one of them, the sample size was at least 73 patients per study group, which is necessary to obtain a power of 90%, provided that the type 1 error is 5% [45]. At the same time, the authors, among the first to use this approach, in 2021 switched from a micro-incision of the airways to conduct a cryoprobe to their fine-needle puncture, which was also used by other researchers, making this procedure for collecting biopsy material even less invasive, which in principle corresponds to modern TBNCBE technology. And only in subsequent years was this carried out by other researchers from a number of countries, including scientists from Russia and Spain [47,48].

Various modifications of this method, which is effective and safe and is used, including in children, can increase its diagnostic potential in pulmonology [49, 44]. At the same time, attempts are being made to improve TBNCBE, including in the field of pulmonary oncology by using different technical methods, their combinations at different stages of TBNCBE. For example, some authors use positron emission tomography, which significantly optimizes the planning and direction of the biopsy to the most active area of the tumor, where the cells undergo the greatest change, and their density is physically higher. Accordingly, this increases the probability of detection and clarification of the localization of malignant neoplasms in the patient's organs and tissues and ensures the targeted selection of the most informative area in the tumor focus for biopsy. Other researchers minimize the time of cryofixation or the number of a series of reciprocating movements of the needle (up to 4 times) when puncturing at one point in order to form a conductive channel for the cryoprobe, as well as the number of jerks (up to 3 times) after cryofixation with subsequent cryoextraction of the biopsy [39,49]. This ensures a decrease in the invasiveness of the procedure, both due to the actions themselves and a reduction in the time of obtaining biopsy material, thereby reducing the possibility of complications in the form of bleeding. However, it is important to choose the "golden mean", taking into account, on the one hand, the direct correlation between the size of the cryobiopsy, which is so important for the study, and on the other hand, the increased risk of bleeding with increasing freezing time. The optimal freezing time is 3-5 seconds, and only if necessary, it is increased to 6 seconds [50,51]. It should be emphasized that all the above parameters were determined as a result of numerous experimental and clinical studies. According to some researchers, the number of biopsies and taking them from different zones is important, which significantly increases the likelihood of detecting pathological changes and the diagnostic value of the biopsy. In this case, the optimal parameters are 4-fold biopsy sampling and taking it from at least two different lobes or from two different segments, if the biopsies are taken from one lobe [52-54]. Some authors consider it important to use one or another probe for transbronchial ultrasonography depending on the research objectives: using an echovideoscope with a convection sensor: linear, low-frequency (5-12 MHz) or radial, high-frequency (20-30 MHz). The first provides high resolution, but low penetration depth, and the second, on the contrary, lower resolution, but high penetration depth.

The quality of biopsy material was also significantly improved by using a certain type of needle and the corresponding cryoprobe size, which varied from 19 G to 22 G and from 1.9 to 2.4 mm, respectively. In this case, preference was given to the EBUS-TNBNA 22-G needle over the EBUS-TNBNA19-G needle, given that, despite the absence of a difference in the diagnostic value of samples (93% versus 95%, $P = 0.62$), the latter, despite a larger lumen caliber, yielded less adequate samples and more often with blood (55% and 19%, respectively) ($p < 0.001$) [55]. The puncture can be repeated with gradual withdrawal of the stylet and collection of tissue and/or subsequent cryofixation and cryoextraction of the biopsy, which is then thawed in air or in saline, but for a short time, since otherwise this can contribute to the degeneration of antigens and DNA, which complicates future immunohistochemical and molecular studies [45,53]. TBNCBE can also be optimized by using a certain size of cryoprobe. Thus, the use of a larger probe size yields a larger biopsy sample, but is more difficult to perform technically and is more often accompanied by complications, as a result of which the use of a 1.9 mm probe is preferable [4,5]. Moreover, the introduction of a cryoprobe of an even smaller diameter, 1.1 mm, further reduces the invasiveness of the method than when using a cryoprobe with a diameter of 1.9 mm or more. This not only improves the quality of the procedure when obtaining biopsy samples, but also preserves the quality of the biopsy samples and expands the possibilities for their collection, especially where there is a need to insert the probe at a sharper angle, which concerns more the upper lobes of the lung.

It is proposed to perform TBNCBE using a puncture needle with a side trap, which contributes to a significant increase in the volume and quality of the biopsy [49]. TBNCBE can also be modified using two techniques. This is by introducing the cryoprobe strictly at the same angle at which the previous punctures were performed, since if it is not inserted at the correct angle, the probe can bend and make the procedure difficult or even impossible. And also by monitoring the site of cryobiopsy collection using ultrasound

each time after obtaining a cryobiopsy to exclude bleeding [47]. The TBNCBE method has been declared relatively recently, which involves the use of a certain set of features, which, in the opinion of its authors, provides significant novelty, contributing to a significant increase in the diagnostic efficiency of TBNCBE [48]. However, a comparative analysis shows that this method does not contain any significant features that distinguish it from existing analogues. Without exception, all the essential features (actions) proposed in this method for obtaining a biopsy, as well as other information in it, are known from the state of the art and are publicly available prior to this announcement.

At the same time, the analysis showed that in the analogues, some of the features that take place in the declared method are either performed at a higher technical level or are absent relative to the declared method, which determines the advantage of the analogues. Thus, in the declared method, visualization of the volume, size, shape and localization of the lesion is carried out by the method of multispiral computed tomography, which in this case is inferior to the method of positron emission tomography used by other researchers. The implementation of TBNCBE is more invasive in relation to analogues due to a greater number of series of wire channel formation (up to 7 series), introduction of a larger diameter probe (up to 1.9 mm), as well as a longer implementation of cryofixations (up to 7 sec) and a greater number of jerks and cryoextractions (up to 7 times), which is due to the lack of preliminary experimental studies and the choice of optimal modes for these parameters, which was carried out by them only according to literary data, which a priori excluded novelty. At the same time, there was no, as in other authors. control of the angle of inclination of the cryoprobe, as well as ultrasound control after each biopsy, which reduces the quality of the procedure and increases the risks of complications from it. The set of signs, as well as their sequence, which is a priori predetermined by the logic of the procedure itself, which is identical in this case regardless of the method of TBNCBE, also did not have significant differences that give advantages in the declared method. Thus, the claimed method, based on the above analysis, is not original.

It should be emphasized that a significant part of the technical evolution of cryobiopsy as such is focused on improving safety, prevention and treatment in the event of major airway bleeding, which cryobiopsy is clearly capable of causing regardless of its technical features [45]. At the same time, according to a number of data, TBNCBE is generally safe and well tolerated, with relatively few adverse events [46]. Therefore, it is natural that analog technologies often provide for the use of preventive measures in this regard, which is not reflected in the declared method. Thus, there may be a prophylactic use of an occlusion balloon in a segmental or lobar bronchus in order to stop possible bleeding, for which different types of catheters were used, including the Fogarty obstetric balloon occlusion catheter or the percutaneous transluminal angioplasty balloon catheter [49-51]. It is probably difficult to achieve consensus on the unity in performing TBNCBE, since it depends on a number of component factors, their set. However, just as any surgical procedure can be performed using different techniques based on a common technical base, taking into account the safety and effectiveness of the procedure, TBNCBE can be performed safely and effectively using a variety of specific procedural elements that could, in principle, be standardized. At the same time, the goal of improving the outcome of TBNCBE remains, which is associated with a larger sample of material with fewer artifacts and a larger amount of alveolar tissue along with ensuring their subsequent fixation for a range of studies, including histological, immunohistochemical, genetic, etc. At the same time, it is necessary to be well aware of the risks and benefits of specific procedural decisions, and some procedural elements are probably necessary at least to ensure patient safety.

In conclusion, we emphasize that, undoubtedly, the expansion of the capabilities of bronchology methods allows us to solve the problem of bronchopulmonology, including issues of diagnosis, treatment and prevention of respiratory diseases. Therefore, a relatively new field of pulmonology, focusing on this symbiosis, or interventional bronchopulmonology, uses, among other things, endoscopy to improve the diagnosis and treatment of diseases of the lungs and airways. However, today this direction is only developing and the relationship between an endoscopist-bronchologist and a pulmonologist specialist leaves much to be desired. From the position of the above, it is important to understand that often for verification of a clinical diagnosis and even where it seems to be established, in the presence of any doubts, as well as for an adequate assessment of the dynamics of the pathological process against the background of treatment, a histological examination of the material is required. In these cases, the quality of the obtained biological material for research plays a particularly fundamental role, depending on the choice of the method for obtaining biopsy material based on knowledge of its capabilities and technology. Adequate interpretation of the results of the study of biopsy material in conjunction with the rest of the clinical data plays a decisive role in verifying the final diagnosis and assessing the effectiveness of treatment. It is desirable that the interpretation of the results of the study of biopsies in conjunction with the rest of the clinical data be carried out in an interdisciplinary discussion. The undoubted benefit of this was reflected earlier on the basis of our own experience back in the 2000s during the work of the so-called pulmonological differential diagnostic commissions, which were attended by specialists from various specialized institutions, usually research institutes, of various profiles: pulmonologists, surgeons, endoscopists, radiologists, phthisiologists [58]. Other researchers essentially adhere to a similar opinion [59,60]. Thus,

according to one of the reports, this benefit was obvious in the context of an interdisciplinary discussion. At the same time, a significant increase in the reliability of the diagnosis was noted with the presented biopsy data, and an important fact was established - there was no significant statistical difference between the two biopsy methods, including surgical and TBNCBE, which confirms the significant diagnostic role of the latter in a multidisciplinary group [60].

Conclusion

Thus, the TBNCBE method is a highly informative diagnostic method, prevailing in the quality of the material obtained for the study and its informativeness over transbronchial forceps biopsy of the lungs and is an alternative to surgical lung biopsy, but a less invasive and safe method. Its development and application, requiring standardization (in terms of the volume of the material taken, the technique of obtaining a biopsy, prevention of possible complications and other signs), is accompanied by its various modifications and continues to improve. One of the proposed methods for obtaining biopsies of lymph nodes and neoplasms of the lungs and mediastinum using TBNCBE considered in this paper is not characterized by significant novelty in comparison with the prototype method, since the prototype method was not adequately selected, thereby creating a difference that is not present with an adequate choice of the prototype method. All the techniques and procedural elements provided for in the implementation of the proposed method of TBNCBE are present in other methods of TBNCBE, which, according to some features, as the comparative analysis has shown, even have some advantage. There is an expediency of interpreting the results of TBNCBE in combination with other diagnostic results carried out using computed tomography or, better, positron emission tomography, clinical and laboratory studies, including histochemistry and immunogenetics.

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