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The use of EBUS-TBNA and ROSE in the diagnosis of lung cancer

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Abstract

Endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) is a minimally invasive method for diagnosing and staging of lung cancer. EBUS-TBNA obtained small specimens. Rapid on-site examination (ROSE) is a rapid, real-time examination method. The aim of our study is to evaluate the impact of ROSE on adequate specimen sampling, rapid results and high diagnostic rate. We present the experience of the Department of Bronchology, "Leon Daniello" Clinic of Pulmonology, Cluj-Napoca, Romania, with EBUS-TBNA as a tool for diagnostic of adenopathies of unknown etiology. We evaluated the diagnostic capacity of ROSE for malignant tumors, by considering the histopathological examination as the diagnostic "gold standard". In our retrospective and descriptive study, we analyze the data of 147 EBUS-TBNA examinations with ROSE and histopathological exam, performed for diagnostic purposes for hilar and mediastinal adenopathies of unknown origin. The age of the patients varied from 21 to 80 years, with an average age of 54.36 years. There were 98 male patients, representing 66.66% of the group. From the total of 90 cases of malignancy, 72 (80%) cases were identified as a primary lung tumor, 13 (14.44%) cases were identified as lymphoma, and five cases as malignant tumor of extrapulmonary origin. The sensitivity of the ROSE is 85.71%. By the introduction of this method, EBUS-TBNA with ROSE, in our country, we can diagnose patients with lung and mediastinal tumors, which cannot be diagnosed by traditional bronchoscopy. This brings a valuable contribution to the improvement of lung cancer diagnostic.

Keywords: EBUS, TBNA, ROSE, lung cancer.

Introduction

Endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) is a recently developed technique, which allows the physician a real-time fine-needle aspiration of the exobronchial tumors and lymph nodes under echographic guidance. It is a minimally invasive procedure for diagnosing and staging non-small cell lung cancer, as well as for diagnostic puncture of hilar and mediastinal lymph nodes of unknown etiology.

Indications of EBUS are:

- diagnosis and staging of lung cancer (visualization and biopsy of the following lymph node stations: 2, 3, 4, 7, 10, 11);
- diagnostic of hilar or mediastinal adenopathies of unknown etiology, discovered by computed tomography (CT) or positron emission tomography (PET)-CT examinations;
- diagnostic of mediastinal tumors, which have direct contact to the tracheal or bronchial wall [1].

ROSE is a rapid, on site examination of the tissue obtained by EBUS-TBNA, allowing a proper specimen examination with an increased diagnostic yield. With the help of the rapid results, ROSE can contribute to the reduction of the examination time, giving the physician a valuable feedback and orientation about the number of passages needed for a complete evaluation of the lymph node stations. The utility of cytology represents an important debate subject especially in the context of the standardized small biopsy specimens and cytological specimens' interpretation recommendations used for lung cancer staging purposes [2–4]. Although subjected to sampling errors cytology in the context of advanced tumors' examination is considered by some comparable to histology [2, 5]. Using ROSE provides better sampling, reduces overall number of passages and may serve as a control for the pathology examination [2, 5]. When performed by an experienced cytologist ROSE can provide a control on the cellularity (useful for example in highly necrotic tumors or to certify that sampling has been performed from lymph nodes), thereby increasing the sensitivity of EBUS-TBNA [6]. A high agreement between ROSE and the final pathological evaluation have been observed, making ROSE an important adjunct technique to lung cancer staging using EBUS-TBNA [7].

Aim

The aim of our study is to evaluate the impact of ROSE on adequate specimen sampling and rapid results with EBUS. We want to compare the diagnostic yield of EBUS-TBNA with ROSE and the yield of EBUS-TBNA with histopathological examination, in a group of patients with a clinical and radiological suspicion of lung cancer, where the traditional bronchoscopic examination could not lead to a diagnosis, as well as in the case of the patients with hilar or mediastinal adenopathies of unknown etiology.

Patients, Materials and Methods

Specimen collection by EBUS-TBNA

We selected for this retrospective study 147 consecutive patients, referred to the Department of Bronchology, "Leon Daniello" Clinic of Pulmonology, Cluj-Napoca, Romania, between August 2014–December 2015, that benefited from EBUS examinations. All the patients had thoracic CT before EBUS examination, which showed enlarged hilar or mediastinal lymph nodes or mediastinal tumors of unknown etiology. Each patient was aware of the indications, technique and possible complications of EBUS and an informed consent for the EBUS was signed before the procedure. All examinations were performed under general anesthesia with laryngeal mask airway, using a Fuji echoendoscope. Before EBUS, a routine traditional bronchoscopic examination was performed in all the patients. We used a 21 G needle for lymph node punction. After the standard assessment of the hilar and mediastinal lymph nodes, and their measurement, transbronchial fine-needle aspiration has been performed, an average of four punctures/lymph node station was done. The examination lasted on average between 30 minutes–1 hour. No immediate or post-procedural complications were noted. All the examinations were performed by an experienced bronchologist, who have been performed bronchoscopies for 20 years and she was trained in many EBUS workshops abroad under the supervision of the well-known bronchologists. This is the first study about EBUS results from Romania.

For each patient, we recorded the following: the lymph node stations, the size of the lymph nodes, the puncture site, the number of passages/station, the on-site diagnostic and the final diagnostic after histopathological examination.

On-site examination of the EBUS-TBNA specimens

The smears were air dried before Diff-Quik staining is used.

The composition of the staining kit and the step-by-step process are presented in Table 1.

Table 1 – ***The composition of the staining kit for Diff-Quik staining and the step-by-step work process***

Diff-Quik staining		
Reagents	***Composition***	***Work process***
Solution A	Triarylmethane, methanol	Dipping 5× = 1 minute
		Removing the excess of reagent
Solution B	Eosin G, phosphate buffer	Dipping 5× = 1 minute
		Removing the excess of reagent
Solution C	Thiazine, phosphate buffer	Dipping 5× = 1 minute
		Removing the excess of reagent
Distilled water		The smear is rinsed
Drying		Air drying

For ROSE, 3–5 cytological specimens were examined on site. The rapid on-site examination of the specimens has been performed by an experienced cytologist. Her feedback immediately confirmed that the sample is adequate, and drew attention to the presence of tumor cells. Whenever it was possible, a diagnostic suspicion has been established based on the ROSE.

The remaining unstained specimens were fixed in 95% alcohol and sent to a laboratory, where Periodic Acid–Schiff (PAS) and Babeş–Papanicolau stainings were performed. The "core" fragments were fixed in 10% formalin and sent to the pathologist for histopathological examination. The liquid remaining after the washing of the punction needle was centrifuged and cellblocks were made. Pathologists from two reference laboratories examined the specimens, two pathologists with experience in general pathology from the first institution and an experienced pathologist, in general pathology in the other. All histopathological examinations included immunohistochemical stainings.

ROSE interpretation algorithm

The preliminary on-site diagnostic was noted as "negative", in case of absence of tumor cells in the stained smears and as "positive", when the examined specimens contained tumor cells or inflammation. A specimen was noted as representative for lymph node tissue, if the lymphocytes ratio was more than 30% of all nucleated elements.

The final on-site diagnosis was defined as: malignant, lymphoma, negative, chronic granulomatous inflammation and inflammatory smear.

The cases where no diagnostic could be obtained underwent mediastinoscopy or exploratory thoracotomy. After the diagnosis, the patients were referred to the oncologists or pulmonologists.

Statistical methods

Microsoft Excel 2010 was used for statistical analysis. Descriptive statistics included patient distribution by gender and age with the calculation of the mean and median. Contingency tables were used for the determination of ROSE parameters, by considering the histopathological examination as the "gold standard". Sensitivity was defined as the percentage of ROSE positive for neoplasia cases, using the formula: sensitivity = true positive × 100/(true positive + false negative). Specificity was defined as the percentage of ROSE negative for neoplasia cases: specificity = true negative × 100/(true negative + false positive). Positive predictive value (PPV) was calculated by the formula: PPV = true positive × 100/(true positive + false positive). Negative predictive value (NPV) was calculated by the formula: NPV = true negative × 100/(true negative + false negative).

Results

The study includes 147 consecutive patients who underwent EBUS-TBNA, in the period between August 2014–December 2015, in the Department of Bronchology, "Leon Daniello" Clinic of Pulmonology, Cluj-Napoca, Romania. The age of the patients varied from 21 to 80 years, with an average age of 54.36 years, and median age of 57 years.

There were 98 male patients, representing 66.66% of the group. Ninety-four patients (representing 64% of the total number) came from an urban background.

The clinical diagnostics of the patients at the moment of admission is represented in Table 2. The majority of the examined patients were admitted for adenopathies suggesting malignancy from suspected primary lung tumors – 53.74% (79 cases).

Table 2 – ***Summary of the clinical diagnosis at admission***

Clinical diagnosis	No. of cases (%)
Enlarged lymph nodes	25 (17.01)
Adenopathy suggesting malignancy (primary – suspicion of lung tumor)	79 (53.74)
Sarcoidosis	27 (18.37)
Lymphoma	2 (1.36)
Adenopathy suggesting malignancy (secondary – patient without oncological history)	6 (4.08)
Adenopathy suggesting malignancy (secondary – patient with oncological history)	8 (5.44)
Total	147 (100)

ROSE has been performed in all cases by an experienced cytopathologist. Cytological and pathological examinations followed in a reference laboratory. All specimens were representative for lymph node tissue (by a lymphocyte ratio greater than 30% from all nucleated elements). The results of the on site examination ROSE have shown malignant cells in 75 (51.02%) patients and for 62 (42.18%) patients the results were negative. For 85 patients, we could establish a diagnosis on the spot. The results of ROSE are presented in Table 3.

Table 3 – ***Summary of the ROSE results***

ROSE results	No. of cases (%)
No malignant cells	62 (42.18)
Malignant cells	75 (51.02)
Suspicion of lymphoma	1 (0.68)
Chronic granulomatous inflammation	6 (4.08)
Inflammatory smear	3 (2.04)
Total	147 (100)

ROSE: Rapid on-site examination.

The final pathological examination revealed in 90 (61.62%) cases a diagnosis of malignancy and in 26 (17.69%) cases chronic granulomatous inflammation. The summary of the results of the final pathological examination is presented in Table 4.

Table 4 – ***Summary of histopathological results***

Results of histological examination	No. of cases (%)
Malignant	90 (61.62)
No histological signs of malignancy	26 (17.69)
Acute inflammation	5 (3.4)
Chronic granulomatous inflammation	26 (17.69)
Total	147 (100)

From the total of 90 cases of malignancy, 72 (80%) cases were identified as a primary lung tumor, epithelial and/or neuroendocrine differentiation, as represented in Table 5. The most common malignancy diagnosed in our series was adenocarcinoma, representing 30% of all tumors. The next histological type was small cell carcinoma – 21.11%. Thirteen (14.44%) cases were identified as lymphoma and five cases as malignant epithelial tumor of extrapulmonary origin.

Table 5 – ***Summary of the histology of the malignant cases***

Malignancies (detailed histology)	No. of cases (%)
Adenocarcinoma	27 (30)
Small cell lung cancer	19 (21.11)
Squamous cell carcinoma	8 (8.89)
Non-small cell carcinoma (NOS)	18 (20)
Lymphoma	13 (14.44)
Metastasis	–
Renal cell carcinoma	2 (2.22)
Melanoma	1 (1.11)
Prostatic carcinoma	1 (1.11)
Colon carcinoma	1 (1.11)
Total	90 (100)

NOS: Not otherwise specified.

We evaluated the diagnostic capacity of ROSE for malignant tumors, by considering the histopathological examination as the diagnostic "gold standard".

A summary of the descriptive parameters of the on-site examinations is presented in Table 6. Sensitivity of ROSE was 77.78%, specificity 91.23% with negative predictive value of 72.22% and very high positive predictive value 93.33%. If we exclude the 13 cases of lymphoma, the sensitivity of the on-site examination rises to 85.71%, specificity 91.23%, with very high predictive negative and positive value 82.54% and 92.96%.

In Table 7, we present the same values for the rest of the malignancies other than lymphoma.

Table 6 – ***Descriptive parameters of ROSE (all cases)***

Sensitivity (%)	**77.78**
Specificity (%)	91.23
False negatives (%)	22.22
False positives (%)	8.77
Negative predictive value (%)	72.22
Positive predictive value (%)	93.33

ROSE: Rapid on-site examination.

Table 7 – ***Descriptive parameters of ROSE (cases other than lymphoma)***

Sensitivity (%)	**85.71**
Specificity (%)	91.23
False negatives (%)	14.29
False positives (%)	8.77
Negative predictive value (%)	82.54
Positive predictive value (%)	92.96

ROSE: Rapid on-site examination.

Morphology of EBUS specimens and the use of immunohistochemical stains

As EBUS specimen size is much reduced, any morphological arguments are extremely valuable. Inflammatory conditions usually lack cells suspicious of malignancy, but sometimes "contaminant" represented by bronchial epithelial cells are observed, some with

reactive/degenerative changes. Careful examination of nuclear morphology, the presence of visible, but not prominent nucleoli, the lack of mitotic activity and the presence of ciliated cells should allow the distinction. Granulomatous inflammation is characterized by the presence of more or less nodular aggregates of epithelioid histiocytes, with inconstant presence of giant cells with or without central necrosis. Usually, a rim of lymphocytes is visible at the periphery of granulomas. However, when issuing a diagnosis of granulomatous inflammation one should take into consideration that the specimen is collected from lymph nodes in general and histiocytic aggregates are common findings. Furthermore, because several malignant tumors can associate the presence of granulomatous inflammation one should always exclude, as possible, a malignant proliferation. Morphological evidence of glandular differentiation (formation of acinar, papillary, micropapillary or solid patterns) are in our experience rare with this type of samples and one should also refer to cytological criteria when examining specimens (homogenous or foamy cytoplasm, eccentrically situated nuclei with finely granular or coarse, irregular distributed chromatin and the presence of macronucleoli (Figures 1 and 2). Squamous differentiation is based on the observation of keratinization, pearls formation and the presence of intercellular bridges. Small cell carcinoma is characterized by presence of small cells, less that the diameter of three small resting lymphocytes with little cytoplasm and fine granular chromatin and no nucleoli. "Molding" of cells is sometimes visible in some specimens and helps to distinguish form other neoplastic process that enters the differential diagnosis (including lymphoma). Ancillary immunohistochemistry (IHC) studies are very helpful in the differential diagnosis. Adenocarcinoma markers thyroid transcription factor-1 (TTF-1) and napsin-A are sensitive and specific, but have to be interpreted in the appropriate context of a primary lung neoplasia. P63 and p40 are very useful markers that argue in favor of squamous differentiation (Figure 3). When faced with small cell lung cancer (SCLC) chromogranin A, synaptophysin and CD56 markers positivity are used as diagnosis arguments for neuroendocrine differentiation and have to be interpreted in the appropriate morphological context (Figure 4). Also, the use of Ki-67 is recommended to highlight the high proliferation index of SCLC. With problematic cases, epithelial differentiation can be assessed by using several cytokeratin cocktails. When melanoma is suspected the use of Melan-A, HMB45 and S100 are used for confirmation.

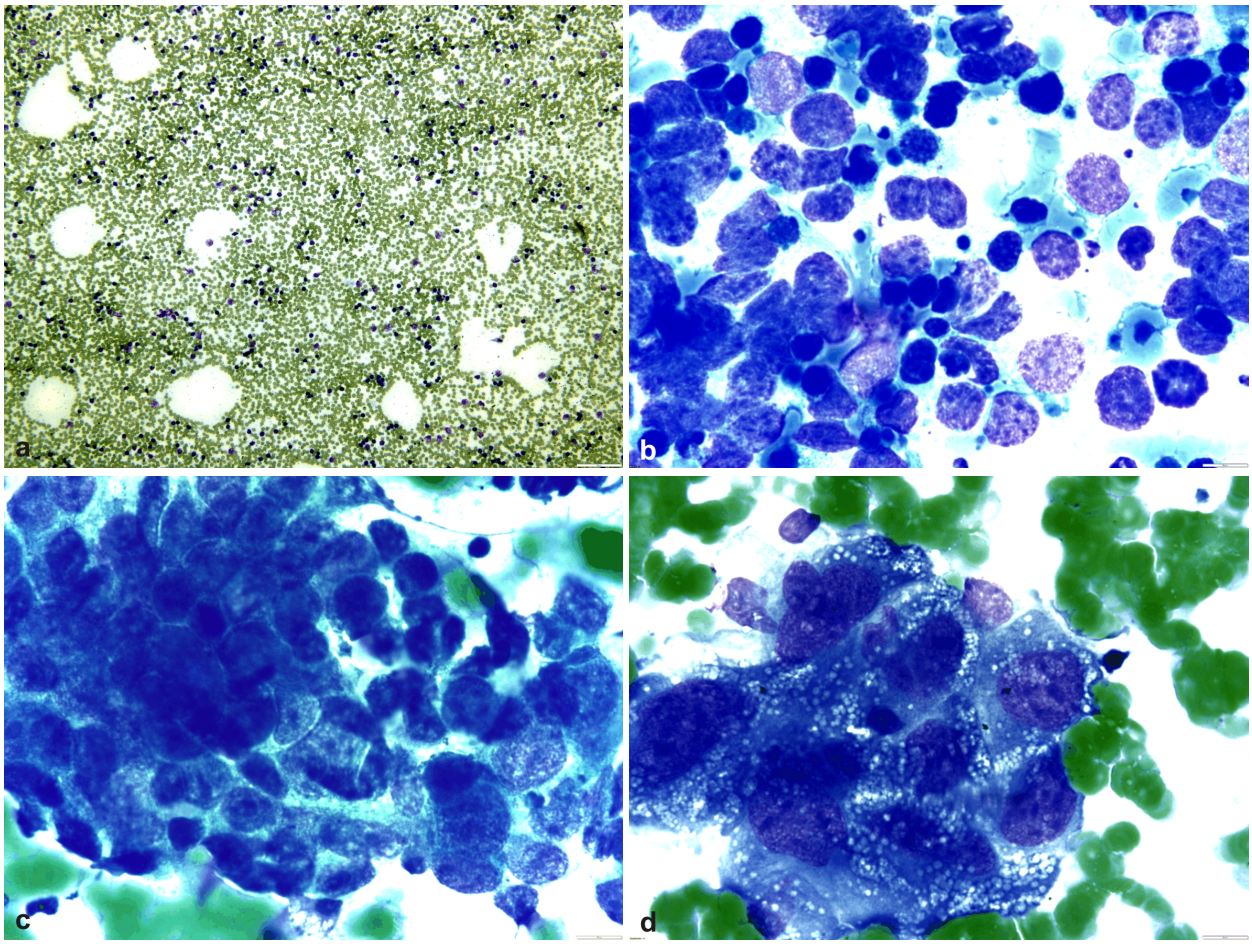

Figure 1 – ***(a) Representative specimen for lymph node tissue (Diff-Quik staining, ×100); (b) Small cell carcinoma – mononucleated tumor cells, small size, fine condensed chromatin, no nucleoli, visible mitosis (Diff-Quik staining, ×100); (c) Adenocarcinoma – tumor cells arranged in placards with amorular appearance with eccentric nucleus, vacuolated cytoplasm and anisokaryosis (Diff-Quik staining, ×100); (d) Squamous cell carcinoma – elongated tumor cells, with multiple nucleoli and vacuolated cytoplasm (Diff-Quik staining, ×100).***

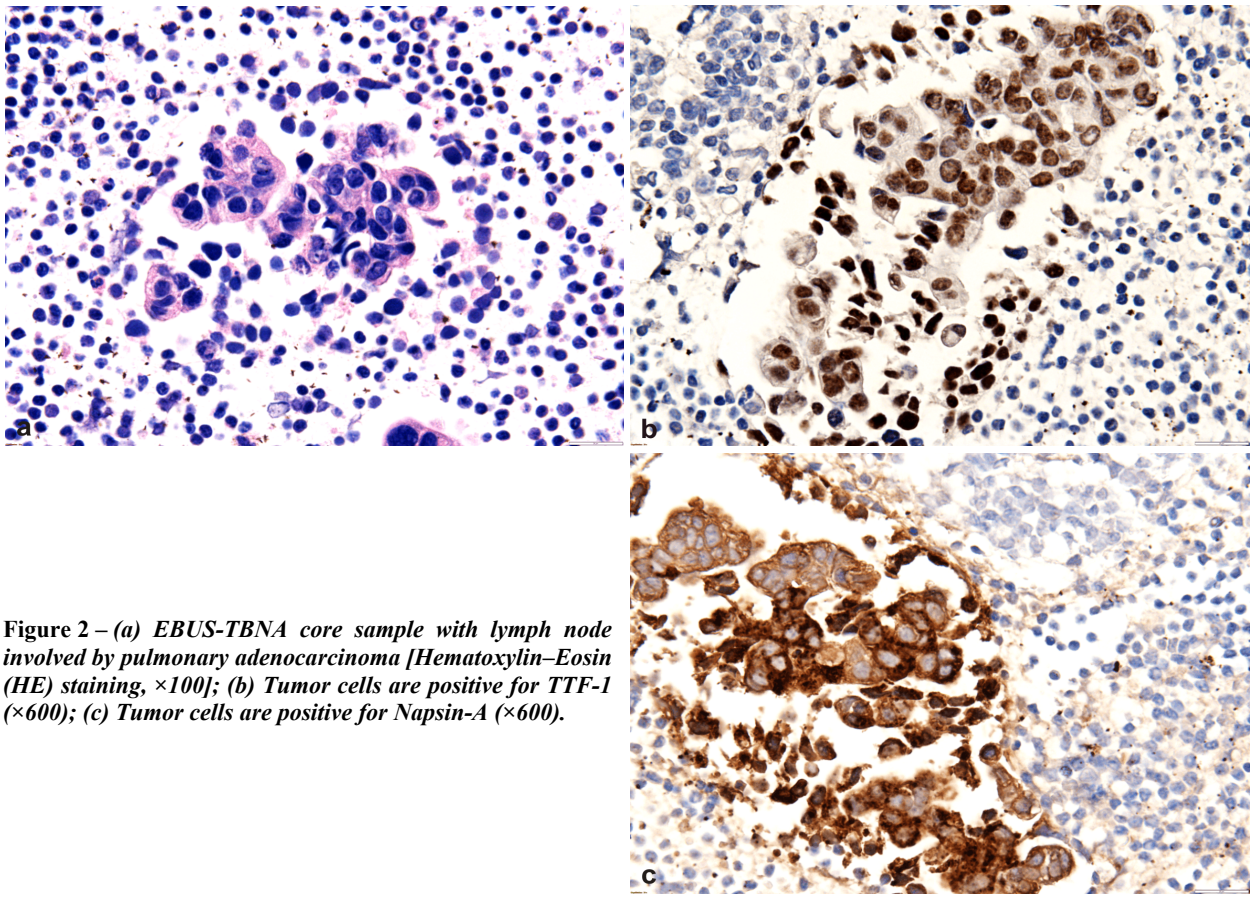

Figure 2 – *(a) EBUS-TBNA core sample with lymph node involved by pulmonary adenocarcinoma [Hematoxylin–Eosin (HE) staining, ×100]; (b) Tumor cells are positive for TTF-1 (×600); (c) Tumor cells are positive for Napsin-A (×600).*

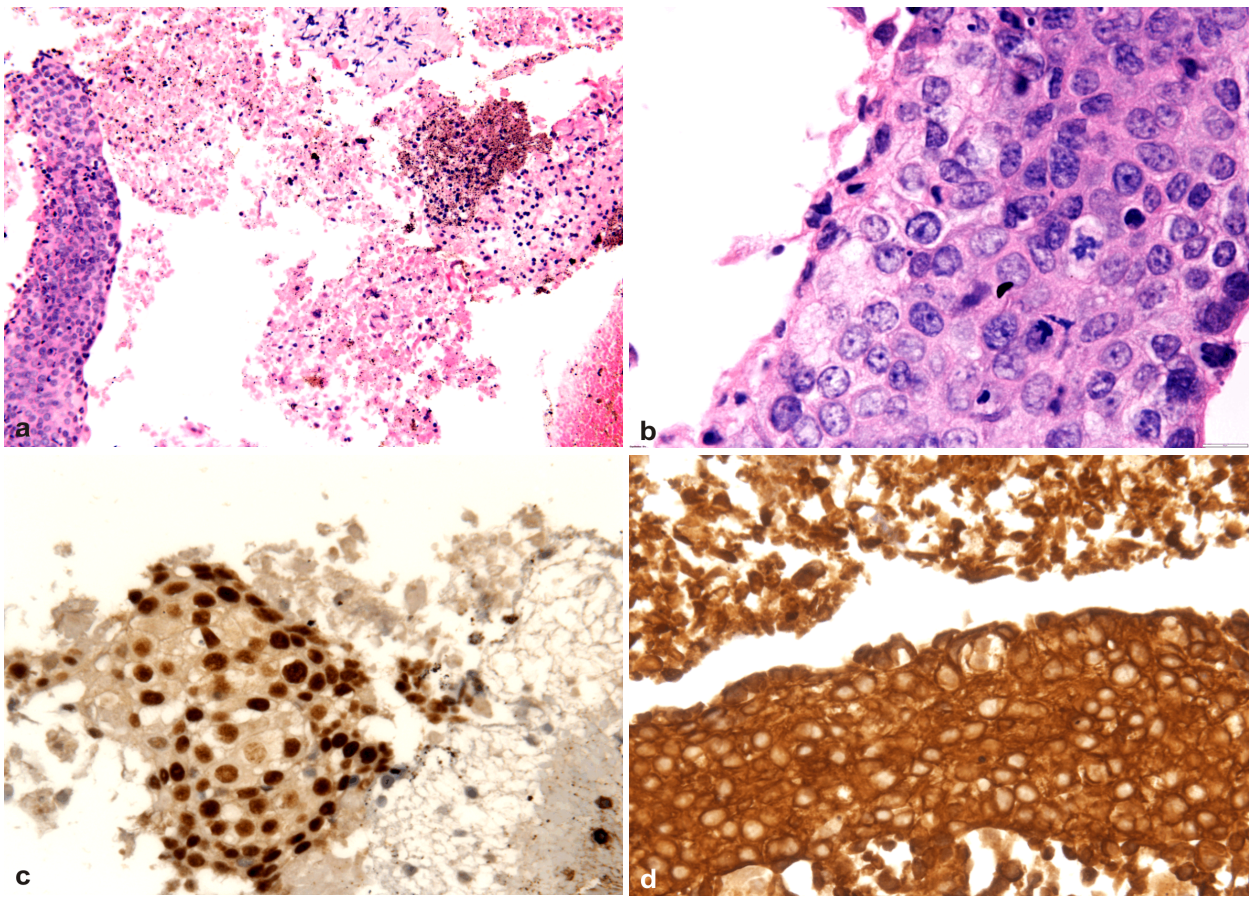

Figure 3 – *(a) EBUS-TBNA core specimen from a mediastinal lymph node in a patient with a history of cervical invasive non-keratinizing squamous cell carcinoma – note the necrotic background (HE staining, ×200); (b) Detail view of a squamous nest – there is no evidence of keratinization (HE staining, ×100); (c) Tumor cells express p40 (×600); (d) Cytokeratin 5 expression is noted also in necrotic areas (×600).*

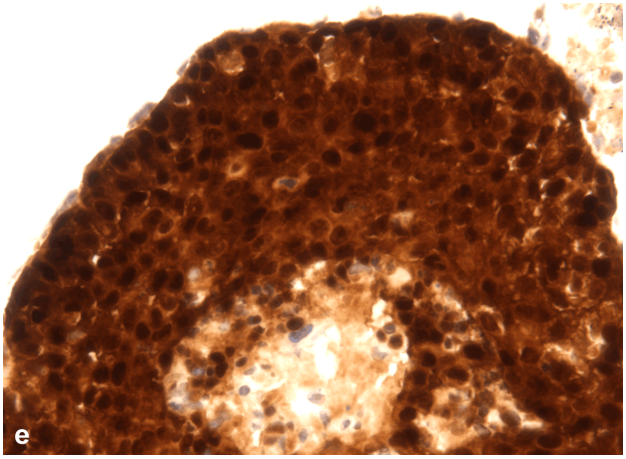

Figure 3 (continued) – ***(e) Tumor nests are positive for p16 (×600).***

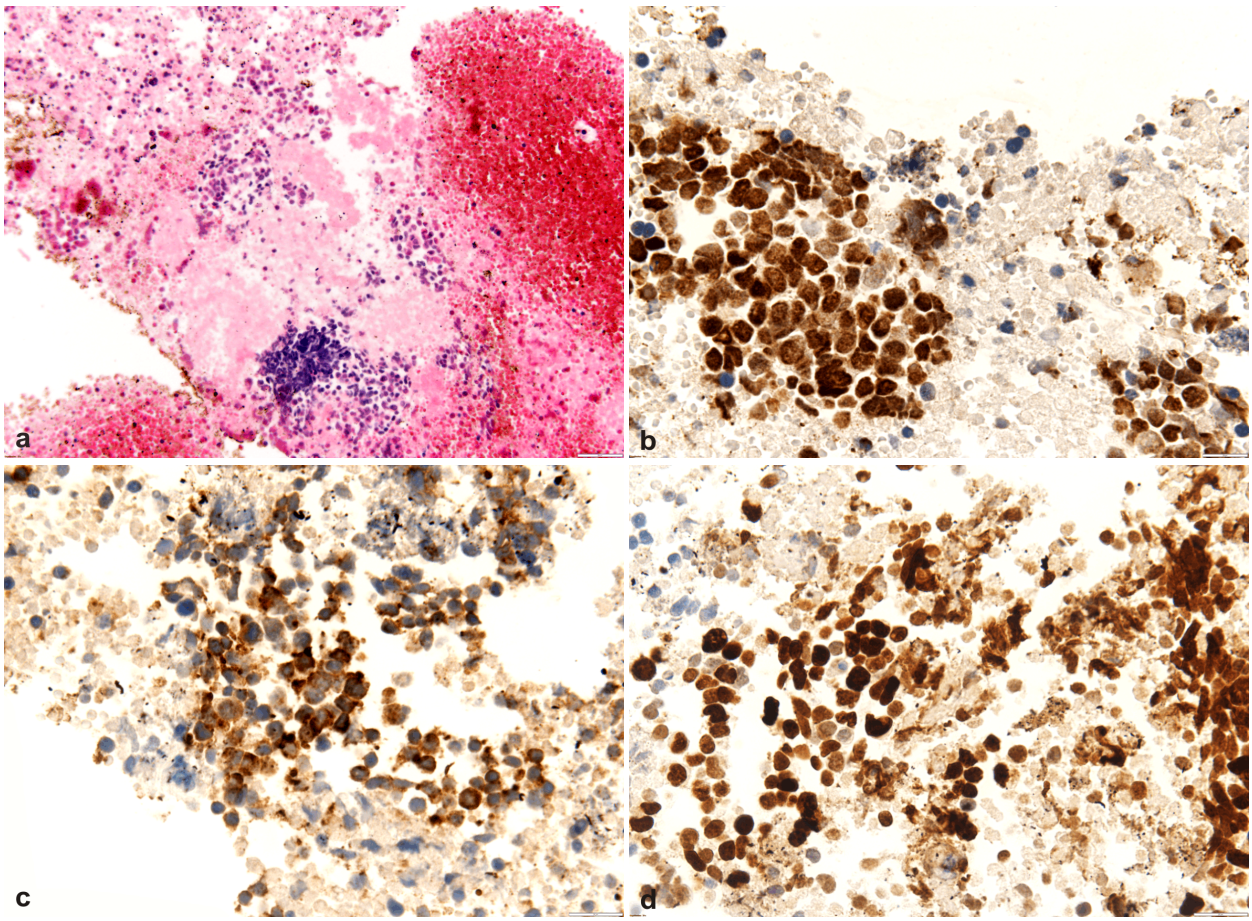

Figure 4 – ***(a) EBUS-TBNA core specimen showing a highly necrotic tumor pulmonary small-cell carcinoma. Tumor cells are small blue, monotonous, with no nucleoli – note the crush artifact and necrotic cells (HE staining, ×200); (b) Expression of TTF-1 in tumor cells (×600); (c) Chromogranin A expression argues in favor of neuroendocrine differentiation (×600); (d) High Ki-67 expression (×600).***

Discussion

The current study retrospectively analyses the efficiency of the ROSE examination results on samples obtained using EBUS-TBNA, by comparing the results with the final pathologic result. We retrospectively assessed 147 cases that benefited both ROSE and histopathology. The patient median age was of 57 years and the male to female ratio was of 2. The final pathological examination revealed in 90 (61.62%) cases a diagnosis of malignancy. Five cases (5.55% of the total of malignant cases) were represented by metastasis, from an extrapulmonary primary malignant tumor (four epithelial, one melanoma) and 13 cases (8.84% of the total of malignant cases) were represented by lymphomas.

The most common malignancy diagnosed in our series was adenocarcinoma, representing 30% of all tumors. The next histological type was small cell carcinoma. The low number of squamous cell carcinomas can be explained by the fact that it develops lymph node metastases later

than the first two. Considering the histopathological examination as the "gold standard" the overall sensitivity and specificity of the ROSE examination (benign *vs.* malignant) was of 77.78% respectively of 91.23%. We excluded from the statistical analysis the lymphoma cases and the sensitivity of the ROSE examination reached 85.71% (Table 7). Most studies report a ROSE sensitivity from 86% to 100% [2, 5]. These values are usually obtained in the context of primary malignant epithelial/ neuroendocrine tumors. These high values represent a strong argument in favor of using ROSE. As a rapid screening method and due to its high specificity and high positive predictive value, ROSE ensures adequate and representative samples.

The most common advantages of using ROSE, cited in the literature, are:

- it diminishes the number of passages required to obtain adequate samples;
- it obtains a higher sample adequacy rate;
- it provides a rapid response in terms of positive or negative for tumor cells in a lymph node station.

Like this, ROSE reduces requirement for supplementary sampling thus lowering the chance of procedural complication [7–10].

On the other hand, there are other studies that found no significant differences between the results obtained by EBUS-TBNA with ROSE, and the ones obtained by EBUS-TBNA without ROSE [11–13].

Oki *et al.* [11] reported the data from a controlled randomized trial of EBUS-TBNA with and without ROSE, used to diagnose primary pulmonary tumors and found that ROSE reduces the number of passages per lymph node, it has a significant influence in the number of stations required to be sampled for staging purposes and reduces the number of additional bronchoscopy procedures. Also, the work group reported a negative predictive value of 40% in the group which benefited from ROSE and a value of 65% in the group without ROSE [11, 14], which constitutes a surprising result.

Navani *et al.* [15] concluded that, when considering the need for a molecular diagnosis there are not enough data in favor of a higher sensitivity, efficiency and specificity of the on-site examination The authors also emphasized the high cost of having a cytopathologist in the procedural room [14, 15].

The available material for the final histopathological examination in biopsies/aspirates is much reduced, hence the increased number of non-small cell carcinoma, NOS (not otherwise specified) cases. The use of immunohistochemistry reduces the percentage of cases that are classified in the NOS-category, but the challenge is pushed forward in our days, by the need for molecular diagnosis. EBUS-TBNA can provide material for immunohistochemical and genetic studies. A comment is warranted here regarding the use of immunohistochemical stainings. In the case of primary pulmonary neoplasms and/or in the case of metastatic tumor with a known primary site and histology, the use of a limited amount of immunohistochemical stains is possible, but in the case of metastasis from a poorly differentiated primary tumor with an unknown point of origin the pathology examination most likely will require a large amount of immunohistochemical stainings and the amount of material available for further studies will be reduced.

One study found that in 90% of patients with adenocarcinomas EBUS-TBNA without ROSE provides sufficient material for the determination of the epidermal growth factor receptor (EGFR) mutation status [15]. In the case of a positive result on ROSE, some authors recommend the continuation of the sampling procedure, because of the need for additional material for genetic studies. Also, the stopping of the EBUS procedure in case of a positive result on ROSE, might reduce the rate of histopathological confirmations [13].

A study by Joseph *et al.* reported a number of cases, which were negative on ROSE and positive on final pathology examination [13]. Also, the study of Trisolini *et al.* did not reveal differences in the final diagnosis of patients with lymphadenopaties in the hilum or the mediastinum when using ROSE compared to the groups that benefited only from the histopathology examination [16]. Sampling method was for both groups EBUS-TBNA and TBNA. On the other hand, Cardoso *et al.* concluded that ROSE is important in increasing the diagnosis performance and the quality of the samples [8]. EBUS-TBNA diagnostic accuracy reported by the work group was of 91% in the ROSE group and 83% in the group without ROSE [10, 17].

In terms of staging purposes, Nakajima *et al.* reported a 94.3% concordance between ROSE and the final histopathological diagnosis. The sensitivity, specificity, negative predictive value and accuracy of EBUS-TBNA for diagnosis of bronchopulmonary neoplasia were 96.5%, 100%, 89.8% and 98.2% [7].

The on-site examination has to take into account several challenges: the very rich cellularity in the lymph nodes, that can be interpreted in several ways (infection, inflammation, neoplasia) and the variety of the mediastinal pathology, with a high variety of cytological features [18, 19]. It goes without saying that perhaps the most important factor in determining the results of ROSE is the cytopathologists experience. The experience of the cytologist is essential in guiding the final pathological examination, as the amount of tissue available is very limited. The cytologist can orientate the pathologist on the presence of tumor cells, can orientate towards the origin of the tumor cells (epithelial tumor, lymphoma, melanoma) or to orientate towards a specific histological type (adenocarcinoma, squamous cell carcinoma, small cell carcinoma – Figure 1) and can aid in deciding the approach for ancillary studies.

The use of IHC is recommended to be as limited as possible, in order to preserve material for cytogenetic and molecular studies, but the small sample size and other features sometimes make this goal difficult to achieve, especially for newly diagnosed cases. When interpreting a histopathological result from such limited samples clinicians should take into considerations the limitations of the method and the heterogeneity of pulmonary tumors as combined tumors are not infrequent [20].

The number of passages (aspirations) for EBUS-TBNA is considered sufficient when three aspirations per nodal station with EBUS-TBNA are made [21]. Regarding the experience of the bronchologist in EBUS, the learning curve is slower, but our bronchologist has good skills

in conventional TBNA and she participated in a lot of EBUS courses and workshops for improving her experience in EBUS techniques. The *American College of Chest Physicians* guidelines for interventional pulmonary procedures states that trainees should be supervised for 50 EBUS procedures and a chest physician should perform 5 to 10 procedures per year to maintain competency. The *European Respiratory Society*/*American Thoracic Society* joint statement on interventional pulmonology recommends that the initial training consist of 40 supervised procedures, and that 25 procedures should be done per year to maintain competency [22].

Further research would be required to define the ultrasonic lymph node characteristics, which will predict the malignancy.

The limitations of the study are related to the selection of the patients, the follow-up and the lack of the correlation with the results of the mediastinoscopy.

Conclusions

EBUS is a minimally invasive method for evaluating the mediastinum, as well as for diagnosing and staging lung cancer. EBUS-TBNA combined with ROSE-transbronchial fine-needle aspiration of the hilar and mediastinal lymph nodes with on-site cytological examination is a rapid diagnostic method. A preliminary diagnosis is obtained in 10–30 minutes. The on-site examination of the small TBNA specimens can evaluate the quality of the specimens (is it representative for a lymph node?) and it can tell whether there is enough material for further histopathological examinations. The diagnostic yield of EBUS-TBNA is increased by the use of ROSE – it can rapidly point towards a negative result and infirm the tumor suspicion. EBUS-TBNA with ROSE provides a rapid diagnosis and staging method, way shorter than EBUS-TBNA without ROSE or more invasive methods as mediastinoscopy or thoracotomy. By the introduction of this method EBUS-TBNA with ROSE in our country, we can diagnose patients with lung and mediastinal tumors, which cannot be diagnosed by traditional bronchoscopy. This brings a valuable contribution to the improvement of lung cancer staging and diagnostic. The success of the EBUS-TBNA examination highly depends on the good teamwork and collaboration between the members of a multidisciplinary team consisting of pulmonologist, bronchoscopist, cytopathologist, anesthetist and medical nurses.

Conflict of interests

The authors declare that they have no conflict of interests.

Consent information

Each patient was aware of the indications, technique and possible complications of EBUS and an informed consent was signed before the procedure.

For this type of study (retrospective study), formal consent for using the data is not required.

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