



The impact of pathological analysis on endobronchial ultrasound diagnostic accuracy

Alessandro Bandiera^{1^}, Gianluigi Arrigoni²

¹Department of Thoracic Surgery, ²Department of Pathology, IRCCS San Raffaele Scientific Institute, Milan, Italy

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Correspondence to: Alessandro Bandiera, MD. Department of Thoracic Surgery, San Raffaele Scientific Institute, Via Olgettina, 60, 20132 Milan, Italy. Email: bandiera.alessandro@hsr.it.

Abstract: Endobronchial ultrasound transbronchial needle aspiration (EBUS-TBNA) is a minimally invasive procedure used for lung cancer diagnosis and staging. Several aspects, including pathological analysis, may impact on its diagnostic accuracy. Differences in diagnostic accuracy between the different specimen processing techniques have not been demonstrated. Cytological slides are generally adequate for diagnosis, subtyping and genotyping. However, some pathological laboratories may require cell blocks or histological core biopsies for a complete molecular profiling. Rapid on-site evaluation (ROSE) is a technique for immediate evaluation of samples obtained with EBUS-TBNA. The aims of ROSE are to increase sampling adequacy, improving diagnostic yield of EBUS-TBNA and ensuring collection of adequate material for ancillary studies. However, the reported data on the impact of ROSE in the diagnostic yield of EBUS-TBNA and in lung cancer diagnosis and staging are controversial. Some series reported a valuable contribution of ROSE to diagnosis and staging of lung cancer and a high concordance between ROSE and the final diagnosis. However, randomized trials failed in finding differences in diagnostic yield between EBUS-TBNA performed with and without ROSE. The yield of EBUS-TBNA for molecular analyses varies between 72% and 98%, and ROSE may warrant the collection of adequate material for molecular profiling. In lung cancer diagnosis and staging a recommended number of three to four passes during EBUS-TBNA at each target is a minimum requirement to obtain enough material for molecular analysis. The use of ROSE may reduce the number of passes for molecular profiling and the number of additional invasive diagnostic procedures. EBUS-TBNA is a procedure with a high accuracy rate and ROSE may contribute to a further improvement of the results. The possibility to avoid additional invasive procedure is an important advantage leading to an overall improvement of patient care.

Keywords: Lung cancer; endobronchial ultrasound transbronchial needle aspiration (EBUS-TBNA); rapid on-site evaluation (ROSE); diagnostic yield

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[^], ORCID: 0000-0002-1962-0045

Introduction

Lung cancer is the leading cause of tumor related mortality (1). Early diagnosis, assessment of nodal status and acquisition of adequate tissue for cytological and histological subtyping and genotyping are essential in lung cancer workup (2). Endobronchial ultrasound transbronchial needle aspiration (EBUS-TBNA) is a minimally invasive procedure, which has emerged as a technique for hilar and mediastinal lymph node diagnosis and staging (3,4). It provides real time access under echographic guidance to paratracheal, subcarinal, hilar, interlobar and lobar lymph nodes and allows the collection of specimens for diagnosis and ancillary testing (5,6). EBUS-TBNA is an accurate, cost-effective and safe procedure with a low morbidity rate, and therefore the American College of Chest Physicians and the European Society of Thoracic Surgeons recommend it as the procedure of choice for invasive mediastinal staging (7-11). Reported data concerning diagnostic yield show heterogeneous results, varying from 71% to 99%, influenced by several factors as lesion size, number of lesions sampled, needle size and number of needle passes (12-16). Another issue that may influence diagnostic accuracy of EBUS-TBNA is pathological analysis. There are almost three methods of specimen preparation: cytological slides, cell-blocks and core biopsies. The choice of one processing method instead of another or their combination differs according to the single Institute preference (17,18). The use of rapid on-site evaluation (ROSE) may also influence the diagnostic yield of EBUS-TBNA. ROSE is a technique for immediate evaluation of samples obtained with EBUS-TBNA. A cytopathologist performs a rapid stain of the collected samples for an immediate evaluation, with the purpose of defining adequacy and when possible a preliminary diagnosis. ROSE can consequently guide EBUS-TBNA suggesting if the collection of further material is required. The aims of ROSE are to increase sampling adequacy, improve diagnostic yield of EBUS-TBNA and ensure sampling of adequate material for ancillary studies (5).

Specimen collection and preparation

Advances in lung cancer treatment and the possibility of tailored therapies according to specific biomarkers in the tumor cells or tissue has increased the number of minimally invasive procedures, which have progressively become more challenging both concerning the sites of the biopsies and the

amount of tissue to be collected to obtain primary diagnosis, immunohistochemistry and molecular studies (19).

The material collected with EBUS-TBNA may be sampled and processed by several techniques: cytological smears, cell blocks or core biopsies. The EBUS-TBNA sample is immediately transferred over a glass slide and smeared with other glass slides for cytological examination. The material may be processed by air drying and wet fixation. The dried slides are stained for ROSE while wet fixed slides are stained in the cytology laboratory. The material collected and needle rinses may also be prepared as cell blocks. Tissue fragments are generally collected and fixed in 10% formalin for histological examination. As discussed by van der Heijden and colleagues, there is no consensus in the literature on the processing method associated with the best diagnostic yield (17). In fact, the studies which compared cytology smears, cell blocks and core biopsies did not find differences in terms of diagnostic accuracy (18,20,21). The specimen preparation may therefore vary between Institutions according to the expertise of the operator and the pathologist. *Figure 1* depicts the cytological and histological samples of a lymph nodal localization of adenocarcinoma obtained by means of EBUS-TBNA.

Another point to be discussed concerns the role of ROSE during EBUS-TBNA. During ROSE several smears are processed with rapid staining. The type of rapid stain methods does not seem to change the results of EBUS-TBNA in terms of final diagnosis (22). After each pass a cytopathologist examines the specimen under light microscopy and evaluates its adequacy for diagnosis and ancillary studies. In case of suspicion or diagnosis of lung cancer the cytopathologist may guide the procedure requiring other passes for further sampling.

ROSE requires the availability of a cytopathologist in the endoscopic suite or in the operating room during EBUS-TBNA, which is not always possible in all Institutions because of costs, time associated to the procedure and workload for the pathology laboratories. In order to overcome these limitations in several institutions ROSE may be performed by other trained professionals like biomedical scientists or through a telecytology service in which the cytopathologist is not on site and evaluates from a remote location slide images transmitted through a digital camera connected to the microscope (23,24). However, at present these options are not commonly used since they require the training of a specific staff and the use of expensive technologies.

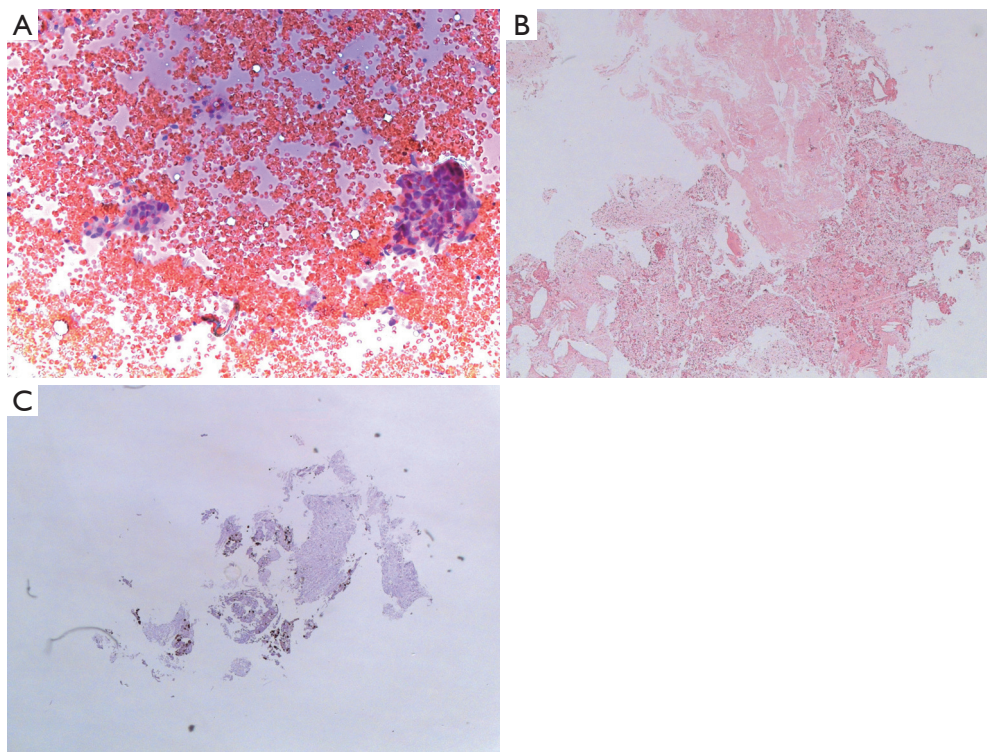


Figure 1 EBUS-TBNA samples showing lymph node localization of adenocarcinoma. (A) Cytological smear, hematoxylin-eosin (HE) staining, $\times 200$. (B) Core biopsy, HE staining, $\times 40$. (C) Immunohistochemistry showed TTF-1 expression in tumor cells, $\times 40$. EBUS-TBNA, endobronchial ultrasound transbronchial needle aspiration.

Type of needles and number of passes

EBUS-TBNA allows sampling of cytological and histological specimens by the use of either 22-gauge or 21-gauge needles (25). Only few studies investigated the impact of needle size on diagnostic yield of EBUS-TBNA (Table 1) (26-30). These studies did not demonstrate a clear superiority of one needle in comparison with the other. In fact, no differences in diagnostic yield were observed, even if one of the studies demonstrated that fewer needle passes were required when the 21-gauge needle was associated with ROSE (29). Jeyabalan *et al.* also observed a better characterization of non-small cell lung cancer with the use of a 21-gauge rather than when the specimens were collected with 22-gauge needles (30). Conversely, a larger size needle may be associated with haemorrhagic contamination (26). The literature does not recommend therefore one needle instead of another and the operator may choose needle size according to site, size and vascularization of the target. Larger needles may be useful in collecting tissue fragments for histological evaluation but smaller needles may be more

flexible and consequently more maneuverable in case of sampling of difficult targets (5).

A single insertion of the needle in the target lymph node is defined as a pass, and each pass includes 5 to 15 needle excursions within the target lymph node (5). In lung cancer diagnosis and staging, the recommended number of passes at each target is a minimum of three (11,17,25,31). A study by Yarmus *et al.* indicates that a median of four passes is required to sample sufficient material for molecular analysis (32).

In a recent meta-analysis Sehgal *et al.* showed that the use of ROSE did not improve the diagnostic yield of EBUS-TBNA, and it did not reduce the procedure length (33). On the other hand, ROSE was associated with fewer number of passes and a lower number of additional bronchoscopic procedures to obtain a final diagnosis. These results are similar to those reported in the review by van der Heijden *et al.* in which ROSE was not associated with a better diagnostic yield of EBUS-TBNA and did not influence the duration of the procedure (17).

However, other studies showed that ROSE, when used

Table 1 Studies analyzing the impact of needle size on diagnostic yield of EBUS-TBNA

Author	Year	Number of patients	Main conclusions
Nakajima <i>et al.</i> (26)	2011	45	Better histological preservation but more blood contamination with 21 G needle
Oki <i>et al.</i> (27)	2011	60	No differences in diagnostic yield
Saji <i>et al.</i> (28)	2011	56	Better diagnostic accuracy with 21 G
Yarmus <i>et al.</i> (29)	2013	1,235	No differences in diagnostic yield. Fewer needle passes with 21 G needle associated with ROSE
Jeyabalan <i>et al.</i> (30)	2014	303	Better characterization of non-small cell lung cancer with the use of 21 G needles

EBUS-TBNA, endobronchial ultrasound transbronchial needle aspiration.

in cases of suspected lung cancer, may reduce the number of repeated diagnostic invasive procedures, especially those aimed at molecular profiling, increasing by 10% the success rate of the procedure for genotyping (34,35).

The impact of ROSE in diagnosis, staging and molecular profiling of lung cancer

The impact of ROSE during EBUS-TBNA for diagnosis and staging of lung cancer is still a matter for debate. The main advantages of ROSE consist in increasing the diagnostic yield of the collected samples, potentially reducing the number of needle passes required to ensure adequate material for molecular profiling (36). Some authors showed an increase in diagnostic yield when ROSE is associated to CT guided fine needle aspiration, or during esophageal endoscopic ultrasound (37,38). These results have not been definitively established when ROSE is used in association with EBUS-TBNA for lung cancer diagnosis and staging (39). In fact, several studies investigated the contribution of ROSE to the adequacy of sampling for lung cancer diagnosis during EBUS-TBNA, with contradictory results (2,6,15,35,40). Some of the trials reported a significant contribution of ROSE to the diagnosis and staging of lung cancer and a high concordance between ROSE and the final diagnosis (6,41). On the contrary, randomized trials failed in finding differences in diagnostic yield when EBUS-TBNA is performed with or without the use of ROSE (33,34,42).

In a study by Joseph *et al.* conducted on a series of 131 patients, ROSE was inadequate in 30 cases, but in 22 out of these 30 cases a diagnosis was obtained after the final histological analysis (40). The sensitivity and specificity of ROSE were 89.5% and 96.4%, while the sensitivity

and specificity of EBUS-TBNA were 92.1% and 100%, respectively. Analyzing these data the authors concluded that ROSE did not have an impact on clinical decision making after a complete mediastinal staging with EBUS-TBNA. On the other hand, several studies reported that ROSE reduces the number of passes per procedure (33,34,42,43). Oki *et al.* did not find differences in EBUS-TBNA duration between ROSE and non-ROSE procedures; procedure time was 22.3 minutes when ROSE was used and 22.1 without the support of ROSE (34). As discussed by Caupena *et al.*, the absence of differences in the duration of the procedures may be due to a reduction of the number of sampled nodes and passes per target associated with the use of ROSE. In fact, when following ROSE a diagnosis of positive N3 node is reached, no further sampling on N2/N1 sampling has necessarily to be performed (41). Moreover, without ROSE a minimum of three passes per target is advised (44), while the use of ROSE may allow to reduce the number of biopsies required to obtain diagnosis (35). In *Table 2* studies concerning the use of ROSE are reported.

Other studies showed that ROSE reduces the number of additional diagnostic invasive procedures (34,35). Avoiding repeated procedures is very attractive, and Diette *et al.* showed that the diagnostic yield of sampling could be increased when ROSE is used to guide the number of biopsies (45). Nevertheless, the results of a study by Joseph *et al.* did not show a role of ROSE in avoiding unnecessary procedures. These authors therefore concluded that ROSE is not of help in decision making when complete mediastinal staging is performed with EBUS-TBNA (40).

Another point of discussion is the role of ROSE in ancillary studies and molecular profiling of lung cancer (5). This issue is particularly relevant in a clinical scenario where most patients affected by lung cancer get a diagnosis

Table 2 Studies analyzing the impact of ROSE on diagnostic yield of EBUS-TBNA

Author	Year	Number of patients	Role of ROSE
Guo <i>et al.</i> (2)	2016	122 with ROSE; 114 without ROSE	Better diagnostic yield in the ROSE group than in the non-ROSE group (90.5% vs. 81.2%, P=0.003)
Simon <i>et al.</i> (6)	2017	147	The sensitivity of ROSE was 85.71%
Nakajima <i>et al.</i> (15)	2013	438	No false-positive results on ROSE; 5.7% false-negative on ROSE
Sehgal <i>et al.</i> (33)	2018	Meta-analysis with 618 subjects	ROSE did not improve the diagnostic yield. ROSE was associated with fewer number of needle passes and a lower number of additional procedures
Oki <i>et al.</i> (34)	2013	108	ROSE was associated with lower need for additional procedures and puncture number
Trisolini <i>et al.</i> (35)	2015	98 with ROSE; 99 without ROSE	ROSE reduced the number of repeated diagnostic invasive procedures aimed at molecular profiling and increased by 10% the success rate of the procedure for genotyping
Joseph <i>et al.</i> (40)	2013	131	The sensitivity and specificity of ROSE were 89.5% and 96.4%, while the sensitivity and specificity of EBUS-TBNA were 92.1% and 100%, respectively
Caupena <i>et al.</i> (41)	2020	64	ROSE diagnosis was concordant with final diagnosis in 96.1% of the cases
Yarmus <i>et al.</i> (42)	2011	34 with ROSE; 34 without ROSE	Differences in diagnostic yield were not observed with or without the use of ROSE
Murakami <i>et al.</i> (43)	2013	100	ROSE was associated with fewer number of passes per procedure

EBUS-TBNA, endobronchial ultrasound transbronchial needle aspiration; ROSE, rapid on-site evaluation.

in an advanced stage (46,47). The yield of EBUS-TBNA for molecular analyses varies between 72% and 98% (21,48-59). Molecular testing accuracy may be influenced by several factors, like cellularity and tumor burden in the sample, method of fixation and the molecular platform used for the evaluation (5). ROSE may ensure the collection of adequate material for molecular profiling, including EGFR and KRAS mutations and ALK and ROS1 rearrangement (21,32,35,52). In a randomized controlled trial by Trisolini *et al.*, molecular profiling was completed with EBUS-TBNA in more than 80% of cases (35). In this study the use of ROSE increased the success rate up to 90%. These data showed only a statistical significance trend, but the clinical impact of avoiding further invasive procedures may be considered relevant in a context where patients are often old, frail and affected by other comorbidities. In this series the use of ROSE also reduced the number of samples with a minimal tumor burden, adequate only for diagnosis and not for genotyping. If ROSE is available sampling strategy may be modified by proceeding with further sample collection when minimal tumor cells burden, necrosis or

contamination with blood are observed.

Available data are inadequate to establish the precise number of passes for complete genotyping, but ROSE may be useful to direct the recruitment of additional material. Yarmus *et al.* in a study evaluating the number of needle passes for analyzing EGFR, KRAS and ALK showed a 95% rate of success with a median of four passes using EBUS in association with ROSE (32). The randomized controlled trial by Trisolini *et al.* supported the observation that four passes may be sufficient for subtyping and genotyping lung cancer (35).

The minimal impact that ROSE has on diagnostic yield in EBUS-TBNA is probably related to the high rate of success of EBUS-TBNA. This statement is supported by a meta-analysis on influence of ROSE on the adequacy rate of fine needle cytology; the impact of ROSE was minimal in the procedures characterized by a high diagnostic yield (36).

Conclusions

Several factors may influence EBUS-TBNA accuracy in

lung cancer diagnosis and staging. Among these factors, pathological aspects may play a relevant role. Thanks to the improvement of lung cancer knowledge a higher number of minimally invasive diagnostic procedures has progressively become essential in diagnostic work-up with an increasing request of cytological and histological material for precise subtyping and genotyping. Considering that most patients have an advanced disease a fast, accurate and precise diagnosis is mandatory.

Concerning the pathological impact on EBUS-TBNA diagnostic accuracy, data in the literature are still controversial. Among the different techniques for processing the collected material, the superiority of one method rather than another has not been demonstrated. The guidelines for the acquisition and preparation of EBUS-TBNA specimens reflect the variability of the results and conclude that the specimen processing techniques may vary between Institutions according to the ability and expertise of the pathological staff (17).

No consensus has also been reached concerning the optimal size of the biopsy needles. The use of larger needles has been associated with a better characterization of cancer subtypes but also with a higher risk of blood contamination of the specimen (26-30). Thus, the type of needle should be chosen by the operator considering the site and the characteristics of the target.

ROSE associated with EBUS-TBNA is a valid diagnostic tool which may improve the diagnostic yield of the procedure. ROSE is effective in reducing the number of redo-procedures while does not significantly influence diagnostic yield, length of the procedure and morbidity (17).

Several advantages have been described with the use of ROSE. It warrants the adequacy of the sample and can improve the diagnostic yield, by reducing the rate of inadequate samples (60). Moreover, it can reduce the need of additional samples for molecular testing allowing an earlier conclusion of the procedure once adequate material for genotyping has been observed. Another advantage of ROSE is represented by the reduction of the total workload of the pathologic laboratory due to an overall reduction of slides to be examined.

Some authors do not consider ROSE a necessary tool during EBUS-TBNA, since during the procedure it is possible to perform a real-time check of the insertion of the needle in the target lesion, and since ROSE is a time consuming procedure. Moreover, ROSE requires a dedicated cytopathologist on site during the procedure (5). Moreover, several studies failed to demonstrate a significant

advantage with the use of ROSE in case of a high EBUS-TBNA diagnostic yield (33,34,42).

EBUS-TBNA is a procedure with a high accuracy rate. The choice of specimen processing techniques and of the type of needle and the availability of ROSE may contribute to a further improvement of its results. The possibility to avoid additional invasive procedure is an important advantage, reflecting an overall improvement of patients' care. Further studies are needed to investigate and strengthen the role of these issues and their impact in lung cancer work-up.

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