Rapid On-Site Evaluation of Endobronchial Ultrasound–Guided Transbronchial Needle Aspirations for the Diagnosis of Lung Cancer

A Perspective From Members of the Pulmonary Pathology Society

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• ***Context.***—**Endobronchial ultrasound–guided transbronchial needle aspiration (EBUS-TBNA) has emerged as a very useful tool in the field of diagnostic respiratory cytology. Rapid on-site evaluation (ROSE) of EBUS-TBNA not only has the potential to improve diagnostic yield of the procedure but also to triage samples for predictive molecular testing to guide personalized treatments for lung cancer.**

Objective.—**To provide an overview of the current status of the literature regarding ROSE of EBUS-TBNA in the diagnosis of lung cancer.**

Data Sources.—**An electronic literature search in PubMed and Google databases was performed using the following key words: cytology, lung cancer, on-site evaluation, rapid on-site evaluation, and ROSE EBUS-TBNA. Only articles published in English were included in this review.**

Conclusions.—**Rapid on-site evaluation can ensure that the targeted lesion is being sampled and can enable appropriate specimen triage. If available, it should be used with EBUS-TBNA in the diagnosis of lung cancer because it can minimize repeat procedures for additional desired testing (ie, molecular studies). Some studies have shown that ROSE does not adversely affect the number of aspirations, total procedure time of EBUS-TBNA, or the rate of postprocedure complications; it is also helpful in providing a preliminary diagnosis that can reduce the number of additional invasive procedures, such as mediastinoscopy. As EBUS technology continues to evolve, our knowledge of the role of ROSE in EBUS-TBNA for the diagnosis of lung cancer will also continue to grow and evolve.**

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Endobronchial ultrasound (EBUS) has emerged as an alternative minimally invasive method for surgical mediastinal staging of lung cancer.[1,2] It allows real-time EBUS-guided transbronchial needle aspiration (EBUS-TBNA) of mediastinal and hilar lymph nodes (LNs) by providing access to the paratracheal LN stations (levels 2R, 2L, 4R, and 4L), the subcarinal LN (level 7), and the hilar, interlobar, and lobar LNs (levels 10, 11, and 12; Figure 1).[3] The main indications of EBUS-TBNA include primary

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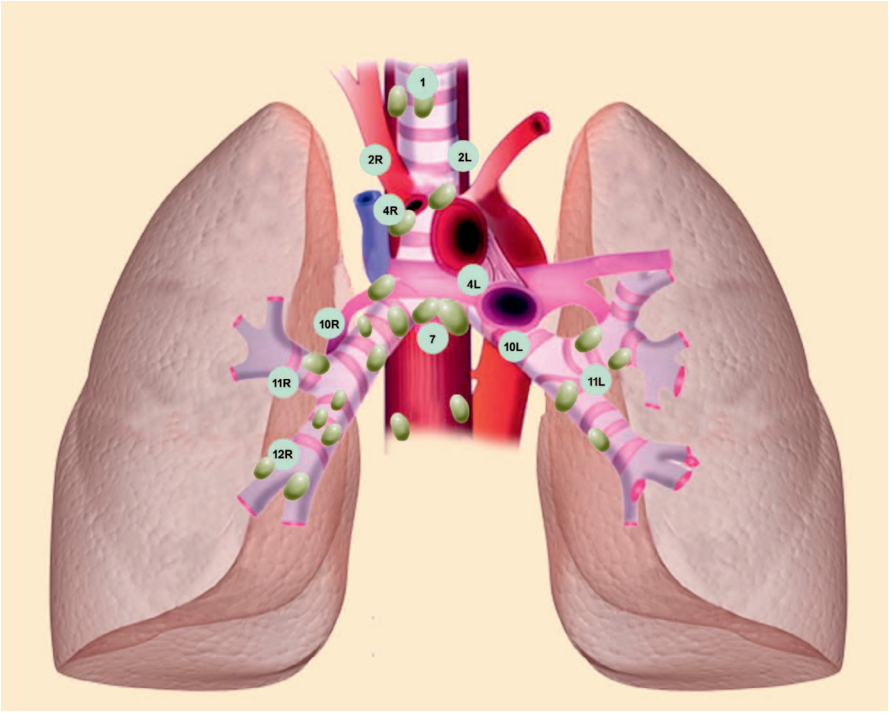

Figure 1. *Lymph node stations accessible by endobronchial ultrasound–guided transbronchial needle aspirate, which include paratracheal lymph node stations (levels 2R, 2L, 4R, and 4L), the subcarinal lymph node (level 7), and the hilar, interlobar, and lobar lymph nodes (levels 10, 11, and 12).*

staging and restaging of lung cancer, staging of extrathoracic malignancies, and the diagnosis of granulomatous disease (eg, sarcoidosis and mycobacterial infection), in addition to the obtaining of tissue for diagnosis and/or ancillary testing for almost any centrally located mediastinal mass of undetermined etiology.[4,5]

Rapid on-site evaluation (ROSE) of EBUS-TBNA specimens aims to check sample adequacy and establish a preliminary diagnosis by performing a rapid stain in the bronchoscopy suite or operating room, with evaluation by a cytopathologist or a trained cytotechnologist.[6,7] The basic purpose of ROSE is to increase the adequacy rate, diagnostic yield, and accuracy of the procedure. In addition, when performed in the operating room, ROSE of EBUS-TBNA is akin to frozen section evaluation by providing a preliminary diagnosis that directly impacts the management of the patient in deciding whether to proceed to mediastinoscopy. More importantly, in an era of targeted therapy, ROSE is performed to ensure collection of adequate and sufficient material for ancillary studies, including immunohistochemistry (IHC) for subtyping of lung cancer and potential molecular testing in these patients.[8–12]

PURPOSE OF ROSE

1. To evaluate sampling adequacy of mediastinal LNs as evidenced by the presence of representative normal tissue (lymphoid tissue or anthracotic pigment-laden macrophages) and/or other lesional material (eg, granulomatous inflammation, malignancy).
2. To evaluate the diagnostic yield for neoplastic or nonneoplastic disease.
3. To ensure sampling of adequate material for appropriate triage of the sample for ancillary studies, including immunohistochemistry, microbiology studies, flow cytometry analysis, and molecular assays.
4. To provide a preliminary diagnosis to direct immediate patient care, akin to a frozen section evaluation.

WORKFLOW OF ROSE

Rapid on-site evaluation of EBUS-TBNA guides the interventional pulmonologist or thoracic surgeon in real time and allows termination of sampling after the appropriate acquisition of diagnostic material and sufficient material for ancillary testing. Multiple targeted sites are usually sampled, and the TBNAs from each pass are triaged appropriately by ROSE (Figure 2).

Type of Needles

There are 3 types of EBUS needles available, which include different sizes (21, 22, and 25 gauge) and material (stainless steel and nitinol). The most commonly used sizes are 21- and 22-gauge needles, with limited data available for 25-gauge needles.[13] Studies indicate that there is no difference in sample adequacy and diagnostic yield between 21- and 22-gauge needles, although 21-gauge needles in combination with ROSE have been reported to be associated with fewer number of passes per LN station, as well as better histology.[14] Because of the larger bore size of the 21-gauge needle, on the other hand, the samples tend to be more hemorrhagic than the samples obtained using the 22-gauge needle, and a trend toward more inadequate samples have been noted in some studies.[14,15] However, there is no evidence that indicates superiority of one needle size compared with the other.[16,17]

A small pilot study using ProCore ultrasound biopsy needles (Cook Medical, Bloomington, Indiana) found that they did not provide additional value in comparison with conventional fine-needle aspiration (FNA) needles.[18]

Current literature recommends the use of either a 21- or a 22-gauge needle, and the choice of needle size is usually determined by the operator based on the station of the LNs, vascularity of the node, and the type of specimen processing

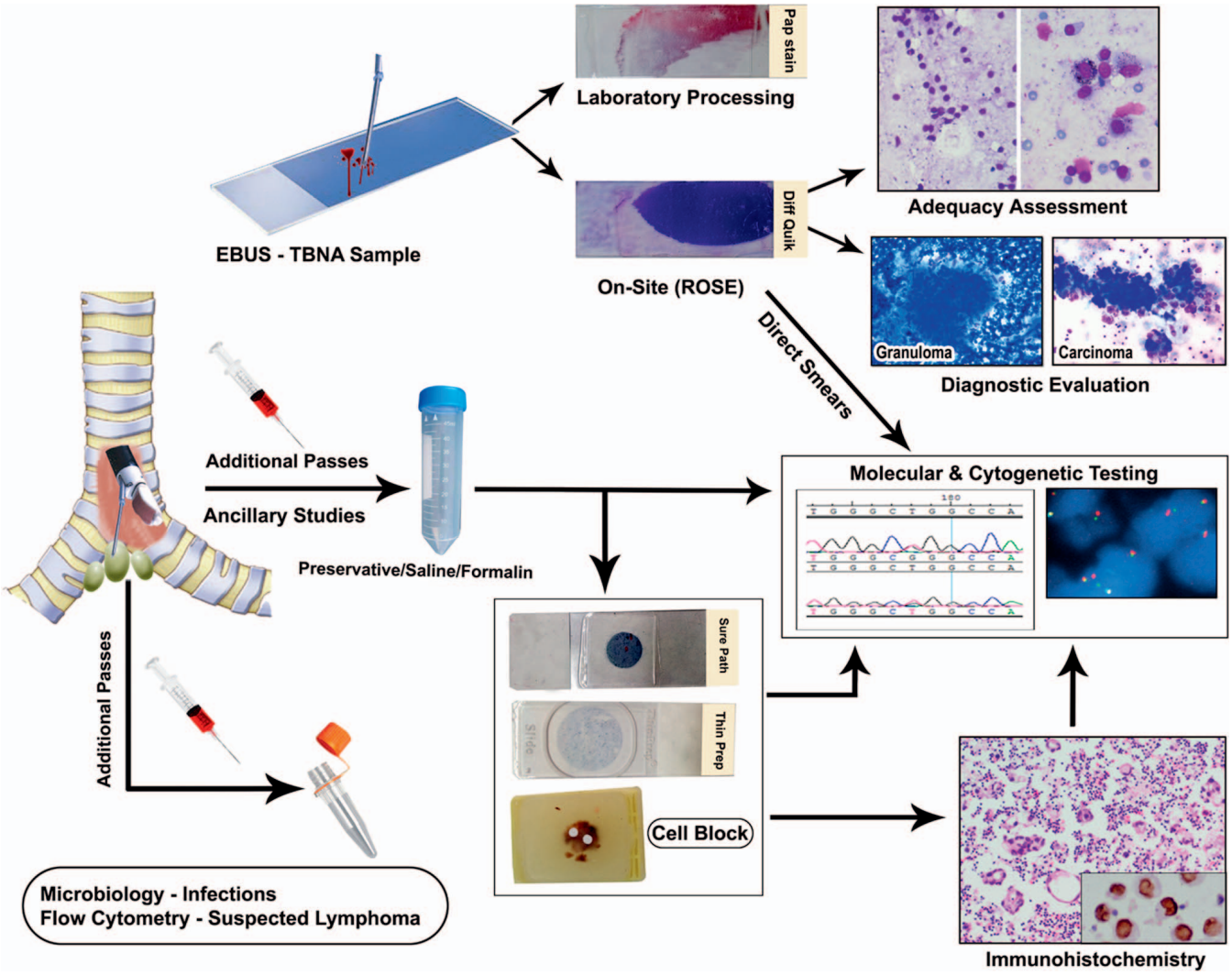

Figure 2. *Schematic diagram to show purpose and algorithmic flow of rapid on-site evaluation (ROSE) for endobronchial ultrasound–guided transbronchial needle aspirate (EBUS-TBNA) specimens. Abbreviation: Pap, Papanicolaou.*

(cytology versus histology).[13] In general, the larger needles have a tendency to be less flexible, and this may impact a proceduralist's choice when trying to access more difficult LN stations, such as the hilar nodes, that require more flexibility for sampling. On the other hand, larger needles might help in obtaining tissue fragments for histologic evaluation of lymphoproliferative disorders.

Number of Passes

A single needle pass encompasses a single insertion of the aspiration needle, from entry to exit, through the airway wall into the target site, and includes 5 to 15 excursions of the needle within the target lesion. Studies indicate that in lung cancer diagnosis and staging, optimal diagnostic yield can be obtained after a minimum of 3 passes with EBUS-TBNA.[19] For lung cancer staging, it is recommended to have a minimum of 3 aspiration passes at each LN station.[19] Only small improvements in diagnostic yield after 4 or more passes have been seen, because the diagnostic yield tends to plateau after 3 passes. Rapid on-site evaluation of lung cancer specimens may also be needed to determine adequacy for molecular analysis[20,21]; however, in this setting, a single study showed that a median of 4 passes along with ROSE is required to obtain adequate material.[20]Anesthesia time is usually sufficient for multiple passes to increase sampling, allowing triage and support for the technician for ROSE.

There are many variables that impact the adequacy of material for ancillary testing. An advantage of ROSE is that it allows gross inspection of the material collected in the needle rinse for producing cell blocks and preparation of smears. Those steps can be crucial in optimizing these small samples for molecular testing.

Specimen Type

The EBUS-TBNA is essentially FNA performed through the bronchial wall using a bronchoscope and real-time ultrasound guidance. Although there is some controversy in the literature regarding the use of core needle biopsy versus FNA in other organs, inferior results of FNA are frequently due to a lack of experience and expertise of the operator, and the absence of proper adequacy assessment.[22,23]A meta-analysis of 20 studies found no significant difference in sensitivity and specificity of lung cancer diagnosis between core needle biopsy and transthoracic FNA samples.[24]

Table 1. Comparison of Cytologic Stains Frequently Used for Rapid On-Site Evaluation

	May-Grünwald Giemsa	Diff-Quik	Wright-Giemsa	Papanicolaou	Hematoxylin-Eosin
Smear	Air dried	Air dried	Air dried	Wet	Wet
Fixative	Unfixed	Unfixed	Unfixed	Alcohol fixed	Alcohol fixed
No. of steps	5	3	3	15	10
Time	15 s	15–20 s	45 s	3–5 min	3 min
Assistance	Not required	Not required	Not required	May need laboratory assistant or extra technician	May need laboratory assistant or extra technician
Morphologic features	Good for cytoplasmic features and background, eg, mucin	Good for demonstration of lymphocytes	Good for cytoplasmic features and background, eg, mucin	Better nuclear details	Good for nuclear features
Cost	Relatively expensive	Relatively expensive	Cheaper	Costly	Relatively expensive

The FNA cell blocks and direct FNA smears show comparable results with core needle biopsy samples for molecular testing.[25,26] Recently published commentary by van Zante and Ljung[22] discuss the importance of correct technique and application of ROSE in FNAs for obtaining an adequate sample that is superior to that of a core needle biopsy sample.

Similarly, in mediastinal lesions, EBUS with ROSE is preferred to more invasive mediastinoscopy and video-assisted thoracic surgery–guided biopsies for the diagnosis and staging of lung cancer, because of the increased diagnostic yield of the technique.[27–29]

Studies have shown that EBUS with ROSE can diagnose metastatic lung cancers with a sensitivity and positive predictive value of greater than 90%. The negative predictive value, however, varies from 61% to 97%, which may be due to a combination of factors, such as limited time for ROSE, lack of diagnostic material in an otherwise adequate aspirate, type of mediastinal station, and suboptimal staining quality of the smear.[30–32]

Interestingly it has been seen that the vast majority of LNs sampled during EBUS-FNA with a diagnosis of negative and unsatisfactory for evaluation are likely to be truly negative on follow-up.[33]

METHODOLOGY OF ROSE

The EBUS-TBNA material is immediately expressed over labeled glass slides for direct smears. The smears are stained by rapid stains (Romanowsky stains on unfixed air-dried [eg, Diff-Quik or Giemsa stains] and rapid Papanicolaou or hematoxylin-eosin stains on wet alcohol-fixed smears) and evaluated under light microscopy by the cytopathologist/cytotechnologist for sample adequacy and a preliminary diagnosis. The needle rinses from the FNA passes are typically collected in saline, RPMI, Hanks solution, formalin, or a preservative solution, like Cytolyt, and processed as a cell block, liquid-based cytology, or cytospin preparation. If a diagnosis of lung cancer is established on-site, additional passes may be requested to yield additional sample material to assess predictive biomarkers.[34] If a sample looks concerning for a lymphoproliferative process, material may also be collected and submitted for flow cytometry.

Staining Methods for ROSE

A wide variety of cytologic stains are employed across laboratories, and the choice depends on the infrastructure of the hospital for on-site services, budget allocation, and the availability of trained cytology staff. There are various commercial staining kits available (Table 1), among which Diff-Quik is the most commonly employed stain because of the rapidity of the procedure, the ability to perform the stain on air-dried slides, and its superiority over the Papanicolaou stain in evaluating lymphoid samples. The staining method employed during ROSE, however, does not influence the final diagnosis.[35]

Adequacy Assessment and Performing ROSE

The availability of an on-site experienced cytopathologist for ROSE is a considerable issue at most institutions and depends largely on the institutional infrastructure, the location of the EBUS-TBNA (eg, operating room with the ability to immediately convert to mediastinoscopy versus bronchoscopy without the ability to convert to mediastinoscopy), and the case volume. To alleviate the costs and time associated with ROSE and encourage its use in clinical practice, several institutions have implemented the use of ROSE by trained interventional pulmonologists and cytotechnologists.[36,37] Although a single study has shown satisfactory interobserver agreement (greater than 90%) between cytopathologists and interventional pulmonologists or trained cytotechnologists,[38] those professionals cannot render a preliminary diagnosis and can only assess adequacy. Another option is the use of telecytology to provide ROSE services to a remote location without physically having the cytopathologist on-site. The telecytology system can serve as a valid substitute for on-site assessment of EBUS-TBNA using a digital camera attached to the microscope to transmit stained slide images via a secure Ethernet connection to a cytopathologist who can perform ROSE remotely, with the results communicated by a voice communication system to the proceduralist.[39,40]

However, resources are still needed for this approach, including personnel trained to generate the immediately stained slides, and information technology infrastructure to ensure a reliable and secure transmission of the slide imaging.

PITFALLS AND CHALLENGES OF INTERPRETATION OF ROSE

While evaluating EBUS-TBNA specimens, it is important to be aware of airway elements that may be present in the sample, because the EBUS-TBNA needle passes via the upper aerodigestive tract through the wall of the bronchus into the target lesion. These elements can include squamous cells with bacteria from the oral cavity, ciliated respiratory columnar epithelial cells, histiocytes with anthracotic pigment, chondromyxoid fragments of cartilage, mesothelial cells, mucus, and cuboidal glandular cells of submucosal glands.

Table 2. Adequacy Criteria of Rapid On-Site Evaluation Specimens of Endobronchial Ultrasound (EBUS)–Guided Transbronchial Needle Aspirate Samples of Lymph Node

	Alsharif et al,[44] 2008	Nayak et al,[45] 2012	Jeffus et al,[11] 2015	Choi et al,[46] 2016
Overview	Lymphocytes in the most cellular areas on ×40 magnification	Scanning the slide at the low power	Scanning the slide at the low power	Procedure-related parameters and microscopic findings, number of punctures (>3 per node), length of core tissue (>2 cm), the gross appearance of aspirates (puslike or anthracotic), and microscopic findings
Criteria	Score 0: <40 lymphocytes per HPF Score 1: 41–200 lymphocytes per HPF Score 2: >200 lymphocytes (nonconfluent) per HPF Score 3: >200 lymphocytes per HPF (confluent) or germinal center fragments Any score >1 is adequate Or Pigment-laden macrophages Or Diagnostic material (cancer cells or granulomas) Airway contamination has no bearing on adequacy	>5 fields with at least 100 lymphocytes per low-power field (×100) in a smear Plus <2 groups of bronchial cells per low-power field (×100) Or Germinal fragments present	Presence of diagnostic material, germinal center fragments, >5 fields at ×100 magnification with at least 100 lymphocytes per field, and <2 groups of contaminating bronchial cells per field	Tissue core in EBUS needle ≥2 cm Or malignant cells Or microscopic anthracotic pigment Or lymphocyte density >40 per 10 high-power fields, at ×40 magnification
Assigned categories	a. Nondiagnostic b. Negative for malignancy c. Atypical d. Suspicious for malignancy e. Positive for malignancy	a. Nondiagnostic b. Negative for disease c. Granulomatous d. Suspicious for malignancy e. Positive for malignancy	a. Unsatisfactory b. Adequate, negative c. Adequate, benign d. Adequate, atypical e. Adequate, suspicious f. Adequate, positive	Objective algorithm proposed for clinicians

Abbreviation: HPF, high-power field.

Reactive bronchial epithelial cells can often be challenging to interpret in ROSE when lung cancer is suspected clinically, particularly in the setting of smoking exposure, prior radiation therapy, and especially on air-dried smears because of the enlarged size of cells and nuclei. Attention should be focused on the periphery of cell clusters, where bronchial epithelial cells are thinly spread, for the presence of cilia, terminal bars, and columnar morphology that indicates the benign nature of these reactive cells. Familiarity with the spectrum of reactive respiratory epithelial changes and identification of ciliated cells are crucial in the setting of florid goblet cell metaplasia, which may mimic low-grade invasive mucinous adenocarcinoma. In addition, attention should be given to cellular dyscohesion and background necrosis, which are usually seen in neoplastic lesions, especially if cells are pleomorphic and show anaplastic features. However, caution should be used because dyscohesive lymphocytes and epithelioid histiocytes should not be misdiagnosed as tumor cells. On the other hand, signet ring cells of adenocarcinoma can sometimes resemble macrophages. The key features for a correct diagnosis are the presence of anthracotic pigment within macrophages and nuclear atypia within tumor cells.[41] A detailed clinical history and meticulous evaluation of the morphology can help at the time of ROSE to diagnose less common diagnostic possibilities,[42] such as granulomatous disease and lymphoproliferative disorders.

Sometimes lymphohistiocytic aggregates or germinal center fragments can mimic nonnecrotizing granulomas during ROSE. The presence of epithelioid histiocytes in clusters can be indicative of granulomatous lymphadenitis, which can help in triaging the specimen for microbial cultures and special stains. Although the diagnostic yield of EBUS-TBNA in sarcoidosis is good, fibrotic LNs may pose a challenge for yielding adequate tissue for diagnosis.[43] A careful inspection for malignant cells is always important at the time of ROSE, even in the presence of granulomas, because granulomas may coexist with malignancy (eg, germ cell tumors, squamous cell carcinoma, lymphoma, etc). Other diagnostic pitfalls include the cytomorphologic overlap between reserve cell hyperplasia and small cell carcinoma, in addition to goblet cell or squamous metaplasia and non–small cell lung carcinoma at the time of ROSE. Given that cell block and immunohistochemistry slides are not available at the time of ROSE, the experience and knowledge of the cytopathologist evaluating the cytomorphology in EBUS-TBNA specimens are crucial to maximize the concordance of preliminary and final diagnoses, minimize indeterminate diagnoses, and appropriately direct patient care.

The evaluation of EBUS-TBNA aspirates for lymphoproliferative disorders can be particularly problematic for small cell lymphomas, especially in the absence of appropriate clinical history or clinical suspicion. However, if a lymphoid sample is obtained, consideration of a possible lymphoproliferative process may prompt allocation of material for flow cytometry and/or clonality or gene rearrangement studies in order to increase the likelihood of a definitive and accurate

Table 3. Studies Analyzing the Role of Rapid On-Site Evaluation (ROSE) in Endobronchial Ultrasound–Guided Transbronchial Needle Aspirate (EBUS-TBNA) in the Diagnosis of Lung Cancer

Source, y	Total EBUS Specimens With ROSE	Site	Place of Procedure/ROSE Performer	Purpose of ROSE	Utility of ROSE	Remarks
Griffin et al,[48] 2011	140	LN lung	BR/CP	Diagnostic yield and clinical decision-making	No difference in diagnostic yield, number of sites sampled, and clinical decision-making compared with non-ROSE	This study challenges the utility of ROSE in EBUS-TBNA
Nakajima et al,[6] 2013	438	LN	BR/NA	Correlation with final pathologic diagnosis	95% concordance; false negativity 5.7%	ROSE is important for diagnosis and staging of lung cancer
Joseph et al,[49] 2013	131	LN	OR/CP, CT	Comparison with the final pathology	ROSE does not impact clinical decision	Despite inadequate sample on ROSE, final diagnosis was possible
Oki et al,[50] 2013	55	LN tumor adjacent to central airway	BR/NA	Efficacy testing	Less need for additional bronchoscopic procedures and fewer punctures needed	No difference in mean procedure time, sensitivity, and accuracy compared with non-ROSE group
Collins et al,[51] 2013	340	LN lung	BR/CP	Impact on EBUS procedure and laboratory resource use	Improved laboratory resource use and patient care	Number of biopsy sites and number of slides per patient reduced
Yarmus et al,[20] 2013	85	LN	BR/CT	Number of passes for molecular analysis	A median of 4 passes with a 21-gauge needle, resulting in adequate sample acquisition for molecular profiling in 95.3% of cases	*EGFR*, *KRAS*, and *ALK* testing was done
Murakami et al,[52] 2014	77	LN	BR/CT	Diagnosis of small cell carcinoma	No impact on diagnostic yield	Fewer additional aspirates due to ROSE
Jeffus et al,[11] 2015	118	LN	BR/CP	Comparison of adequacy criteria versus unstructured approach	Sensitivity of ROSE is better if structured criteria are used	Authors propose a ROSE reporting system
Trisolini et al,[53] 2015	126	LN lung nodules/ masses	BR/CP	Multigene molecular analysis in lung cancer	Yield material for molecular profiling with fewer needle passes	*EGFR*, *KRAS*, and *ALK* testing was done
Cardoso et al,[54] 2015	41	Hilar-mediastinal lesions and lung cancer staging	BR/NA	Adequacy and diagnostic accuracy	No difference in lung carcinoma staging compared with non-ROSE	Increased adequacy and accuracy in diagnosis of mediastinal lesions
Dyhdalo et al,[55] 2014	575	LN lung	BR/CP	Concordance with final diagnosis	89% concordance	Discordance due to sampling error
Guo et al,[7] 2016	122	LN	OR/NA	Diagnostic yield	Increased diagnostic yield compared with non-ROSE	ROSE can reduce suspicious category specimens

Abbreviations: BR, bronchoscopy room; CP, cytopathologist; CT, cytotechnician; LN, lymph node; NA, not available; OR, operating room.

diagnosis. The typical findings include a monomorphic population of lymphoid cells (eg, monotony in cell size and chromatin pattern) without germinal center fragments or tingible body macrophages.

ADEQUACY CRITERIA FOR ROSE

Adequacy for Peribronchial/Peritracheal LNs

An adequate LN sample should possess lymphocytes and/or lymphohistiocytic aggregates or germinal center fragments. However, if the lymphoid sampling is limited, qualitative criteria must be applied when evaluating sampling adequacy. Although there are no universally accepted criteria for EBUS LN adequacy, structured semiquantitative scoring schemes for ROSE and diagnostic category assignments have been proposed in previous publications (Table 2).[11,44–46]

A sample is considered to be adequate if there is sufficient diagnostic lesional material (eg, tumor or granulomatous pathology) even in the absence of lymphoid tissue, or sufficient benign lymphoid tissue to suggest adequate sampling of a benign/reactive LN.

Adequacy for Parenchymal Lung Lesions

Adequacy assessment of parenchymal lesions is more challenging because of the distinction between nondiagnostic and negative categories. Caution should be exercised when assigning the negative category in the presence of a mass lesion by imaging studies, especially if smears are sparsely cellular (containing only benign elements, pneumocytes, alveolar macrophages, and respiratory columnar cells).

UTILITY OF ROSE IN THE DIAGNOSIS OF LUNG CANCER

Sample Adequacy Assessment

This is likely the most important component of performing ROSE. It reduces sampling error and controls the diagnostic yield of the procedure by directing the interventional pulmonologist or thoracic surgeon with real-time ultrasound guidance regarding sampling adequacy and the exact timing of termination of the procedure.[47]

There are few observational studies and comparative randomized trials that assess the performance of ROSE in EBUS-TBNA for lung cancer diagnosis, staging, and molecular profiling (Table 3).[48–55]

Morphologic Diagnosis

Subtyping lung cancer by ROSE is feasible when performed by an experienced cytopathologist or cytotechnologist. The cytomorphologic features for subtyping non–small cell carcinoma have been classically described. Morphologic criteria used for a cytologic diagnosis of adenocarcinoma include small to medium-size cell clusters (often 3-dimensional) with enlarged irregular nuclei, nuclear crowding/overlapping, prominent nucleoli, and wispy vacuolated cytoplasm and/or intracytoplasmic mucin. Sheets of cells with dense cytoplasm, distinct cytoplasmic borders, and irregular nuclei with multiple small nucleoli are more characteristic of squamous cell carcinoma. Often, background necrosis and hyperchromatic nuclei are seen. Typical findings of small cell carcinoma include predominantly singly dispersed cells with scant cytoplasm, nuclear molding, readily identifiable mitotic figures, basophilic cytoplasm with paranuclear blue bodies, and moderate necrosis/apoptosis.

Acquisition of Material for Ancillary Studies

After acquisition of a diagnostic sample on ROSE, the EBUS-TBNA could be terminated to minimize complications, or, if safe, additional passes can be obtained for ancillary testing and/or additional sampling of other stations for further staging information.[50,53]

For instance, if lymphoma is suspected on ROSE, additional passes can be obtained for immunophenotyping by flow cytometry. If granulomatous inflammation is seen on ROSE, additional passes can be sent for microbiologic culture and special stains. To increase the sensitivity of the diagnostic bronchoscopy, EBUS-TBNA can be combined with bronchoalveolar lavage and bronchial brushing for sending specimens for microbiologic culture studies.

Extra passes (in formalin, saline, RPMI, or Hanks solution) can be requested for ancillary studies after a ROSE diagnosis of carcinoma. Cytology smears provide excellent-quality DNA and consistent genotyping results; however, the current molecular testing guidelines for non–small cell lung carcinoma, which recommend the use of cell blocks instead of conventional cytologic preparations, may reflect the preference of most molecular pathologists used to working with preparations that are similar to histology samples.[56] Given that a variety of different cytology preparations can be used for molecular testing, institutional protocols should be used to allocate material in the appropriate manner (eg, additional unstained smears or cell block), because the choice depends on individual laboratory practice and validation of ancillary tests.[57]

ROLE OF ROSE IN MOLECULAR TESTING OF LUNG CANCER

Non–small cell lung carcinoma accounts for more than 80% of newly diagnosed lung cancer cases, and most patients receive a diagnosis of an advanced stage of the disease in which surgical resection is not possible.[58,59]A large fraction of the lung cancer patients receive a diagnosis by either cytology or small biopsy. Endobronchial ultrasound–TBNA has been incorporated as a first step in the diagnosis and staging of suspected lung cancer.[2] Often, TBNA is the only material available for both the diagnosis and the molecular testing that are required for patient care. Use of ROSE in combination with EBUS ensures adequate material for the diagnosis, staging, and molecular testing of lung cancer.

Molecular testing accuracy depends on multiple factors that include overall cellularity, method of fixation, tumor fraction of the sample (the ratio of tumor cells compared with all nucleated cells), and the analytic sensitivity of the molecular testing platform used for the analysis. Rapid on-site evaluation helps in triaging material for molecular and cytogenetic studies, including *EGFR* and *KRAS* mutations and *ALK* and *ROS1* rearrangement.[20,52,60–63] The material obtained by EBUS-TBNA is suitable for molecular analysis in more than 90% of the samples.[64,65] Specimens were satisfactory and adequate for molecular analysis in 95% of all cases in the study by Yarmus et al,[20] where they used material for *EGFR* and *KRAS* sequencing and *ALK* fluorescence in situ hybridization analysis.

It is noteworthy that ROSE is useful for the confirmation of the presence of tumor cells and judging tumor burden within the samples for molecular analysis.

Table 4. Advantages and Limitations of Rapid On-Site Evaluation (ROSE)	
Advantages	**Limitations**
Adequacy assessment of the specimen	Needs an experienced cytopathologist or a dedicated trained cytotechnician
Improved diagnostic yield	Cost may not be reimbursed
Reduction of additional procedures	Time-consuming process (35–56 min)[67]
Obtain additional passes for molecular testing, microbiology cultures, and flow cytometry	At present, no statistically significant results for ROSE and increased diagnostic yield, fewer aspirations, decreased procedure time, and reduced rate of complications
Better use of laboratory resources and reduced laboratory effort because of the lower number of total slides	
Improved patient care	

A randomized controlled trial by Trisolini et al[53] evaluated the role of ROSE in EBUS-TBNA samples for molecular testing. The results, although not statistically significant, were clinically relevant and showed a 10% increase in the success rate of EBUS-TBNA for optimal lung cancer genotyping.

Current data are insufficient to know exactly how many passes are needed to obtain adequate material for molecular analysis, but it is strongly recommended to obtain additional material for molecular analysis after acquiring a diagnostic sample obtained using ROSE.

Of note, cytopathologists must understand the overall cellularity and tumor fraction required by the molecular laboratory to confidently assess adequacy for downstream testing, and conversely, to know when to request additional material. Thus, close correspondence or workshops with the molecular laboratory can assist cytopathologists in recognizing the lower limits of adequacy for molecular testing. Additionally, in situations in which the molecular laboratory accepts smeared slides for testing, ROSE can assist in allocating materials for distribution to the molecular testing facility.

ADVANTAGES AND LIMITATIONS OF ROSE

Two task force guidelines on the role of EBUS-TBNA in the diagnosis and molecular testing of lung cancer have critically examined the utility of ROSE based on the literature.[13,66] The guidelines found that ROSE is effective in reducing additional procedures. Interestingly, no statistically significant differences were seen in terms of the number of aspirations, diagnostic yield, procedure time, and rate of complications in procedures with or without ROSE. The expert panel recommended tissue sampling with or without ROSE in patients undergoing EBUS-TBNA for diagnostic evaluation of lung cancer (grade 1C).[13]

Rapid on-site evaluation was used initially with conventional TBNA to increase the diagnostic yield of the procedure. Some argue that because EBUS is a real-time technique where needle and target can be seen during the procedure to check for the procedure's accuracy, ROSE is theoretically not needed. Liquid-based cytology further militates against ROSE and in fact ultimately eliminates the need for ROSE entirely. In liquid-based cytology, the sample is directly transferred to the preservative fluid for processing in the laboratory without making any direct smear on-site. Other arguments indicate the need for an experienced dedicated cytopathologist as a limitation of ROSE, because it may not be possible to accommodate in all hospitals or institutes. However, the increasing use of telecytology for ROSE may allow both an adequacy assessment and an immediate diagnosis if performed by a cytopathologist from a remote location. Other criticisms of ROSE include the uncertainty of the diagnostic material in a cell block despite the use of ROSE services, the time spent in performing the evaluation, and the cost. Rapid on-site evaluation is a time-consuming process because of repeated staining and examination of the samples, and the cost of ROSE may not be fully recoverable.[67]

Because procedures can take a long time, the on-site presence of a pathologist is considered to be prohibitive by some laboratories. However, overall cost analysis shows significant benefit in the global patient care.[68] The cost of ROSE depends on local factors, such as the geographic location of a hospital with physical proximity to laboratories and bronchoscopy suites, staff availability, hospital infrastructure, and access to appropriate follow-up health care. ROSE is cost effective; it reduces the need for additional procedures, and it reduces the number of cytology slides examined. The ability to better triage the sample for ancillary studies can save both time and money, and reduce complications in terms of the lower number of repeat procedures for ancillary studies.

In addition, as stated earlier, ROSE quickly guides appropriate sampling, which includes sending a sample to microbiology in cases of granulomatous pathology, and triage for flow cytometry/cytogenetics/molecular biology if a lymphoproliferative lesion or lung cancer is suspected.

It is recommended that patients with a negative diagnosis on EBUS-TBNA should undergo a more invasive procedure, such as mediastinoscopy, for confirmation of diagnosis.[69]

In these situations, ROSE has evolved as a very useful tool to reduce the percentage of inadequate specimens, and consequently contributes in a big way to patient care.[51]

Table 4 briefly summarizes the advantages and limitations of ROSE.

CONCLUSIONS

The ROSE of EBUS-TBNA is a well-established tool to improve yield of the procedure if performed by a cytopathologist or an experienced cytotechnologist. Rapid on-site evaluation used in combination with TBNA ultimately reduces the workload of the cytopathology laboratory through a substantial reduction in the total number of slides sent by pulmonologists compared with samples without ROSE. Most importantly, improved patient care is achieved with ROSE. Different clinical trials and large series have reported lower complications rates when ROSE is performed.[13,51,70,71] The evidence supports inclusion of ROSE in every EBUS-TBNA procedure, whenever feasible, in patients with suspected lung cancer and enlarged mediastinal or hilar LN and/or centrally located tumors.[48,50] The decision of whether or not to provide ROSE for EBUS-TBNA procedures is largely institution dependent, and it is impacted by where the procedure is occurring (eg,

bronchoscopy versus operating room), if a preliminary diagnosis is needed for immediate patient care, and if special triage is desired. The ability to provide ROSE for EBUS-TBNA and prevent a more invasive procedure like mediastinoscopy in lung cancer patients with advanced disease is an important advantage. Additional studies are needed to compare efficacy of ROSE for optimal performance of EBUS-TBNA in the diagnosis and workup of lung cancer patients.

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