Thorac Res Pract. **DOI:** 10.4274/ThoracResPract.2026.2025-11-1

Original Article

Endobronchial Ultrasonography (EBUS) with Lymph Node Aspiration: On-site Evaluation and Final Pathology Correlation

Ferhan Karataş[1], Makbule Çisel Aydın Meriçöz[2], Işıl Uzel[1], Tuğçe Yakut[3], Emine Karabul[1], Öner Dikensoy[1]

[1]Department of Pulmonology, Koç University Hospital, İstanbul, Türkiye
[2]Department of Pathology, Koç University Hospital, İstanbul, Türkiye
[3]Department of Allergy and Immunology, Koç University Hospital, İstanbul, Türkiye

Cite this article as: Karataş F, Aydın Meriçöz MÇ, Uzel I, Yakut T, Karabul E, Dikensoy Ö. Endobronchial ultrasonography (EBUS) with lymph node aspiration: on-site evaluation and final pathology correlation. *Thorac Res Pract.* [Epub Ahead of Print]

ABSTRACT

OBJECTIVE: Rapid on-site evaluation (ROSE) during endobronchial ultrasound (EBUS)-guided transbronchial needle aspiration (TBNA) may improve sample adequacy and expedite clinical decision-making; however, its impact on diagnostic performance and concordance with final pathology remains unclear.

MATERIAL AND METHODS: We retrospectively analyzed consecutive adult patients who underwent EBUS-TBNA between February 2023 and January 2024. ROSE adequacy, final diagnostic adequacy, and ROSE-final pathology concordance were calculated. Factors associated with these outcomes were assessed using logistic regression analysis.

RESULTS: Ninety-five patients (mean age 65±13.8 years; 62.2% male) and 203 targets were sampled with 683 aspirations (3.4±1.8 passes/station). Based on lymph node (LN) level ROSE sensitivity, specificity, positive predictive value, negative predictive value, and accuracy were 76.6%, 100.0%, 100.0%, 85.4%, and 90.1%, respectively. The adequacy rates were 89.7% for ROSE and 90.6% for final pathology, and the ROSE-final pathology concordance was 88.2%. Diagnostic discordance occurred in 11.8% of the nodes, most of which were related to limitations in tumor subtyping. A greater number of needle passes was positively associated with ROSE adequacy ($P = 0.038$) and final adequacy ($P = 0.021$), whereas a larger LN diameter favored final adequacy ($P = 0.045$). Larger LNs and greater experience of the final-diagnosis pathologist were associated with lower ROSE-final diagnosis concordance ($P = 0.026$ and $P = 0.015$, respectively).

CONCLUSION: ROSE during EBUS-TBNA yielded high sensitivity and overall accuracy, demonstrated strong sample adequacy, and showed substantial concordance with the final pathology. Optimizing the number of passes and considering LN size can enhance adequacy, and recognizing the limitations of cytological subtyping and potential observer variability is essential for multidisciplinary decision-making.

KEYWORDS: Endobronchial ultrasonography, lymph node aspiration, rapid on-site evaluation, final pathology correlation

Received: 03.11.2025 ***Revision Requested:*** 10.02.2026 ***Last Revision Received:*** 25.02.2026

Accepted: 12.03.2026 ***Epub:*** 07.04.2026

INTRODUCTION

Endobronchial ultrasound (EBUS) is a minimally invasive diagnostic modality that enables real-time evaluation of the bronchial mucosa, mediastinal and hilar lymph nodes (LN), and major vessels, as well as nodal tissue sampling in both malignant and benign diseases with mediastinal LN involvement.[1] EBUS-guided transbronchial needle aspiration (TBNA), which is used when a mediastinal and/or hilar LN biopsy is required for diagnostic purposes, staging, or molecular testing, has high sensitivity.[1,2] In recent years, EBUS has significantly reduced the need for mediastinoscopy for mediastinal LN sampling and has become the preferred initial procedure. Furthermore, tissue samples obtained using EBUS-TBNA are suitable for both pathological tumor subtyping and genotyping.[2]

Corresponding author: Ferhan Karataş, MD, e-mail: fkaratas19@hotmail.com

Rapid on-site evaluation (ROSE), an assessment technique used in interventional pulmonology, involves rapid specimen preparation, staining, and cytological examination to ensure safe access to the target lesion during the procedure.[3] Providing immediate feedback on sample quality facilitates decision-making regarding the need for additional sampling or termination of the procedure.[4] The impact of evaluating EBUS-TBNA specimens under ROSE guidance on the diagnostic yield remains controversial. While some studies[5,6] have reported that unnecessary biopsies are prevented, procedure-related complications are reduced, the number of sampled LNs decreases, the likelihood of encountering suspicious material declines, and diagnostic performance improves, other studies[7] have indicated that similar benefits could not be achieved.

Accurate and rapid diagnosis of primary tumors or metastases in lung cancer and other malignancies is critical for treatment planning and prognosis.[8] Therefore, concordance between ROSE and the final pathological diagnosis is of particular significance.

In this study, we aimed to evaluate ROSE, the final pathological diagnostic adequacy, diagnostic concordance, and factors influencing these outcomes in patients undergoing LN aspiration with EBUS.

MATERIAL AND METHODS

Study Design and Patient Population

This study was designed retrospectively in accordance with the Declaration of Helsinki and approved by the Koç University Ethics Committee (decision/protocol number: 2024.222.IRB2.103, date: 07.06.2024). All patients aged >18 years who underwent EBUS in the department of chest diseases between February 2023 and January 2024 for either benign or malignant disease were included in the study. Data on procedures and cases were obtained through retrospective review of the hospital's electronic medical records. There were no missing data for the primary outcome or key procedural variables used in the diagnostic performance and regression analyses. However, some morphological variables (e.g., LN margin, echogenicity, and shape) had missing values owing to incomplete procedural documentation. These variables were excluded from the regression analyses, and available-case analyses were used for descriptive statistics. No imputation method was used.

Main Points

- Rapid on-site evaluation (ROSE) during endobronchial ultrasound (EBUS)-guided transbronchial needle aspiration (TBNA) provided high diagnostic performance (accuracy 90.1%; sensitivity 76.6%). ROSE yielded robust adequacy rates (89.7%) and substantial concordance with final pathology (88.2%).
- Number of needle passes and lymph node (LN) diameter improved ROSE and final diagnostic adequacy.
- Diagnostic discordance occurred in 11.8% of nodes, related to subtyping limitations and observer variability.
- Larger LNs and greater pathologist experience were associated with lower ROSE-final diagnosis concordance.
- ROSE enhances EBUS-TBNA efficiency and yield while acknowledging cytologic subtyping constraints. Results can guide pass number targets and node selection strategies in routine practice.

Endobronchial Ultrasound Procedure

In our clinic, patients undergoing EBUS-TBNA are routinely informed of the procedure, including its indications and potential complications. Before the procedure, the patients are evaluated for bleeding diathesis, hypoxemia, cardiac, renal, or hepatic failure, and neurological disorders. Consultation and approval from relevant specialists are obtained when deemed necessary. Written informed consent was obtained from all patients prior to the procedure.

General anesthesia is administered by an anesthesiologist for all procedures. A 7.5 MHz convex probe bronchoscope (BF-UC180F, Olympus Optical Co., Tokyo, Japan) and an EU-C2000 processor (Olympus, Tokyo, Japan) are used for EBUS procedures. Standard bronchoscopic examination was performed first, followed by EBUS. During EBUS, LN stations are visualized according to the Mountain-Dresler LN map,[9] and systematic sampling is performed using an Olympus ViziShot NA-SX 22G EBUS needle. The decision to terminate the procedure is made jointly by the chest physician and a pathologist.

During EBUS-TBNA, morphological features of LNs, such as margins, echogenicity, short-axis diameter, number of needle passes per station, and ROSE-based diagnosis, were recorded.

Rapid on-Site Evaluation and Final Pathological Diagnosis

In our institution, materials obtained by EBUS-TBNA are routinely evaluated for bedside adequacy. After macroscopic assessment, selected portions of the aspirated material are smeared onto slides and stained using the Diff-Quik method, while the remaining material is fixed in formaldehyde to prepare a cell block. Diff-Quik-stained smears are examined microscopically in the procedure room by a pathologist. Samples containing >30% lymphocytes or anthracotic macrophages are considered representative and classified as "adequate." When malignant cells are identified, tumor subtyping is performed whenever possible; otherwise, the sample is recorded as malignant. In the absence of malignant cells, samples are categorized as benign if adequate, or as hypocellular if not fully representative. In the present study, the final pathological diagnoses were established by re-evaluating smear preparations and cell blocks in the pathology laboratory.

Statistical Analysis

All statistical analyses were conducted using the Statistical Package for the Social Sciences for Windows (SPSS), version 28.0 (IBM Corp., Armonk, NY, USA). Categorical variables were summarized as frequencies and percentages, while continuous variables were reported as mean ± standard deviation.

Diagnostic performance parameters, including sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and overall diagnostic accuracy, were calculated

with corresponding 95% confidence intervals (CI) using the exact binomial (Clopper–Pearson) method.

Associations between categorical variables were evaluated using the chi-square test or Fisher's exact test, as appropriate. The distribution of continuous variables was assessed using the Shapiro–Wilk test and confirmed by visual inspection of histograms and quantile–quantile plots. Normally distributed variables were compared using parametric tests, including the independent samples t-test or one-way analysis of variance (ANOVA), as appropriate. Non-normally distributed variables were analyzed using the Mann–Whitney U test or Kruskal–Wallis test. When ANOVA demonstrated statistically significant differences, Scheffé post-hoc analysis was performed to identify pairwise group differences.

Multivariable logistic regression analysis was performed to identify independent predictors of ROSE adequacy, final diagnostic adequacy, and ROSE-final diagnostic concordance. Model calibration was assessed using the Hosmer–Lemeshow goodness-of-fit test, and model discrimination was quantified using the area under the receiver operating characteristic curve (AUC) and Nagelkerke R^2. Multicollinearity was assessed using variance inflation factor, and no significant multicollinearity was detected.

Cases with missing data for outcome variables were excluded from the respective analyses, and complete-case analysis was performed without imputation. All statistical tests were two-sided, and a P value < 0.05 was considered statistically significant.

RESULTS

A total of 95 cases, with a mean age of 65±13.8 years, including 59 males (62.2%), were included in the study. EBUS-TBNA was performed at 203 stations (197 LN and 6 masses). All procedures were performed under general anesthesia, with 77 aspirations (37.9%) performed using an endotracheal tube and 126 (62.1%) performed using a laryngeal mask airway. A total of 683 aspirations were conducted, with a mean number of needle passes of 3.4±1.8 per station.

Of the LNs, 125 (61.5%) were in the mediastinum, 143 (88.3%) had a well-defined border, 139 (73.9%) exhibited heterogeneous echogenicity, and 65 (56.5%) were round in shape, with a mean diameter of 16.61±11.12 mm. No missing data were present for the primary outcome variables. Some morphological variables had missing values owing to incomplete documentation, and analyses were performed using the available case data (Table 1).

The most frequently identified diagnosis was "lymphoid tissue," which was observed in 107 cases of ROSE (52.7%) and in 95 cases on final pathological examination (46.8%). Detailed ROSE and final diagnostic results are shown in Figure 1.

Based on LN-level analysis, ROSE demonstrated a sensitivity of 76.6% (95% CI: 65.6–85.5), specificity of 100.0% (95% CI: 96.6–100.0), PPV of 100.0% (95% CI: 93.9–100.0), NPV of 85.4% (95% CI: 79.6–89.7), and an overall accuracy of 90.1% (95% CI: 84.8–94.0).

Table 1. Localization and morphological features of sampled targets

Localization n (%)		
Mediastinal		**125 (61.5)**
	7	56 (27.6)
	4R	48 (23.6)
	4L	17 (8.4)
	2R	4 (2)
Hilar		**78 (38.5)**
	11L	26 (12.8)
	11R	26 (12.8)
	10R	10 (4.9)
	10L	9 (4.4)
	Mass	6 (3)
	12L	1 (0.5)
Morphological features n (%)		
Edge features		
	Smooth	143 (88.3)
	Uncertain	17 (10.5)
	Lobulated	2 (1.2)
Echogenicity features		
	Heterogeneous	139 (73.9)
	Hypoechoic	38 (20.2)
	Isoechoic	11 (5.9)
Shape features		
	Round	65 (56.5)
	Conglomerate	40 (34.8)
	Oval	10 (8.7)
LN diameter, mm (mean ± SD)		16.6±11.1

LN: lymph node, SD: standard deviation

Diagnostic discordance was observed in 24 LNs (11.8%). The ROSE and final pathological results of discordant cases are presented in Table 2. Additionally, in 2 of the 9 LNs diagnosed as non-small cell lung cancer (NSCLC) by ROSE, and in 8 of the 18 LNs diagnosed as "carcinoma" (5 "NSCLC", 3 "carcinoma"), specific tumor subtyping could not be determined on final pathological analysis.

The mean number of needle passes in EBUS-TBNA procedures with ROSE adequacy (3.5±1.7) was significantly higher than that in those without adequacy (2.6±1.8) (P = 0.008). Similarly, the mean diameter of LN in ROSE-adequate EBUS-TBNA cases (15.7±9.3 mm) was larger than in those inadequate for ROSE (P = 0.028). In EBUS-TBNA procedures with final diagnostic adequacy, the mean number of needle passes was also higher (3.5±1.7; P = 0.002). Moreover, the mean LN diameter (15.8±9.5 mm) was significantly larger than that in cases without final diagnostic adequacy (10.9±6.8 mm) (P = 0.022). In EBUS-TBNA cases with ROSE-final diagnosis concordance, the mean LN diameter (14.9±9.1 mm) was lower than that in discordant cases (18.6±10.5 mm), although this difference was not statistically significant (P = 0.056). In contrast, the

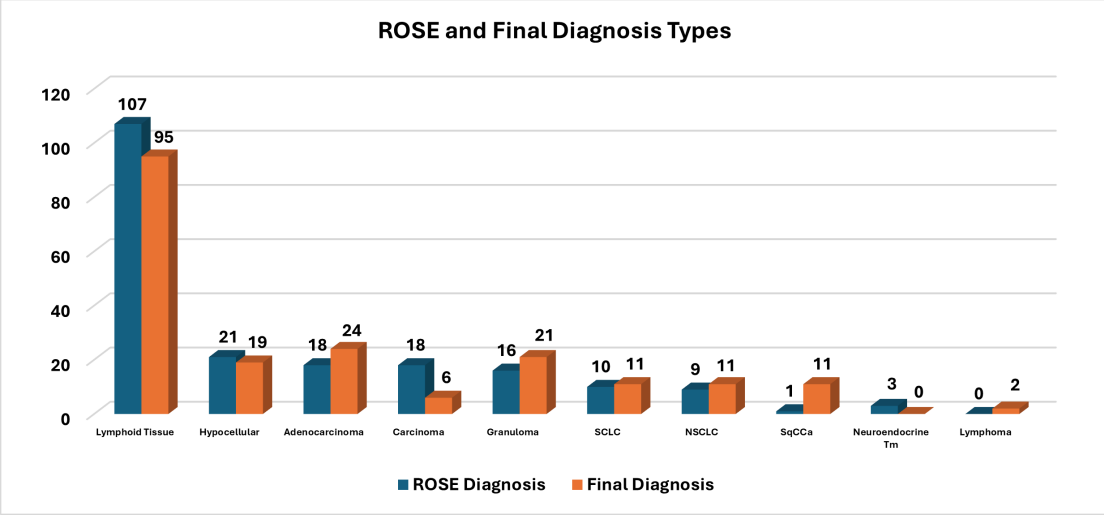

Figure 1. Comparison of ROSE and final pathological diagnosis categories in lymph nodes sampled by EBUS-TBNA. This figure illustrates the distribution of diagnostic categories obtained by ROSE and the corresponding final pathological diagnosis. Lymphoid tissue was the most frequently identified category in both ROSE and the final diagnosis. Differences between ROSE and the final diagnosis were observed in specific pathological subtypes, including carcinoma, adenocarcinoma, granuloma, squamous cell carcinoma, and lymphoma, reflecting cases of diagnostic discordance and refinement after definitive pathological evaluation.

ROSE: rapid on-site evaluation, EBUS: endobronchial ultrasound, TBNA: transbronchial needle aspiration, SqCCa: squamous cell carcinoma, SCLC: small cell lung cancer, NSCLC: non-small cell lung cancer

Table 2. ROSE and final pathology results in cases with diagnostic discordance

		ROSE diagnoses (n)			
		Lymphoid tissue	**Hypocellular**	**Granuloma**	**Total**
Final diagnoses (n)	Lymphoid tissue	-	4	1	5
	Hypocellular	4	-	-	4
	Granuloma	6	-	-	6
	RCC	3	-	-	3
	SqCCa	1	1	-	2
	SCLC	1	-	-	1
	NSCLC	1	-	-	1
	Lymphoma	1	1	-	2
	Total	17	6	1	24

ROSE: rapid on-site evaluation, RCC: renal cell carcinoma, SqCCa: squamous cell carcinoma, SCLC: small cell lung cancer, NSCLC: non-small cell lung cancer

professional experience of the pathologists evaluating the final diagnosis was significantly lower in concordant cases (18.2±11.3 years) compared with discordant cases (23.4±11.4 years) (P = 0.044) (Table 3).

Multivariate logistic regression analysis demonstrated that the number of needle passes was independently associated with both ROSE diagnostic adequacy [odds ratio (OR): 1.433, P = 0.038] and final diagnostic adequacy (OR: 1.593, P = 0.021). LN diameter was independently associated with final diagnostic adequacy (OR: 1.078, P = 0.045) and with diagnostic concordance (OR: 0.951, P = 0.026). Final pathologist experience was independently associated with diagnostic concordance (OR: 0.951, P = 0.015), but did not significantly affect diagnostic adequacy (Table 4).

The final diagnostic adequacy model demonstrated excellent calibration (Hosmer–Lemeshow P = 0.902), good discrimination (AUC: 0.758), and acceptable explanatory power (Nagelkerke R^2: 0.164). The ROSE adequacy model showed acceptable calibration (P = 0.073) and moderate discrimination, while the concordance model demonstrated near-acceptable discrimination with modest explanatory power (Nagelkerke R^2: 0.108); both models had AUC: 0.697. No evidence of multicollinearity was detected among the independent variables (Table 5).

DISCUSSION

In this study of LNs sampled using EBUS-TBNA, the diagnostic concordance rate between ROSE and final pathology was 88.2%. The sample adequacy rates were 89.7% for ROSE and 90.6% for the final pathology. Multivariate logistic regression analysis showed that the number of needle passes was independently associated with both ROSE and final diagnostic adequacy; LN diameter was associated with diagnostic adequacy; and greater final pathologist experience was independently associated with lower ROSE-final diagnostic concordance.

Table 3. Factors associated with ROSE adequacy, final diagnostic adequacy, and ROSE-final diagnostic concordance

LN features		ROSE adequacy			Final diagnostic adequacy			Diagnostic concordance		
		No	Yes	*P*	No	Yes	*P*	No	Yes	*P*
Gender n (%)	Male	15 (11.7)	113 (88.3)	0.401	12 (9.4)	116 (90.6)	0.992	18 (14.1)	110 (85.9)	0.197
	Female	6 (8.0)	69 (92.0)		7 (9.3)	68 (90.7)		6 (8.0)	69 (92.0)	
Type of intubation n (%)	ETT	8 (10.4)	69 (89.6)	0.986	8 (10.4)	69 (89.6)	0.693	7 (9.1)	70 (90.9)	0.346
	LMA	13 (10.3)	113 (89.7)		11 (8.7)	115 (91.3)		17 (13.5)	109 (86.5)	
Location n (%)	Hilar	11 (14.7)	64 (85.3)	0.121	9 (12.0)	66 (88.0)	0.322	12 (16.0)	63 (84.0)	0.158
	Mediastinal	10 (7.8)	118 (92.2)		10 (7.8)	118 (92.2)		12 (9.4)	116 (90.6)	
Age (mean ± SD)		65.2±14.3	66.5±12.9	0.843	65.9±15.5	66.4±12.8	0.736	65.5±12.4	66.4±13.1	0.823
LN diameter (mean ± SD)		11.9±9.1	15.7±9.3	**0.028**	10.9±6.8	15.8±9.5	**0.022**	18.6±10.5	14.9±9.1	0.056
Number of passes (mean ± SD)		2.6±1.8	3.5±1.7	**0.008**	2.5±1.7	3.5±1.7	**0.002**	3.6±2.0	3.3±1.7	0.438
ROSE pathologist experience (years) (mean ± SD)		13.5±7.9	12.7±7.1	0.327	13.7±8.3	12.6±7.1	0.43	11.3±4.8	12.9±7.5	0.814
Final diagnostic pathologist experience (years) (mean ± SD)		24.3±11.1	18.1±11.2	**0.023**	23.5±11.4	18.3±11.3	0.067	23.4±11.4	18.2±11.3	**0.044**

ROSE: rapid on-site evaluation, SD: standard deviation, LN: lymph node, ETT: endotracheal tube, LMA: laryngeal mask airway

Table 4. Multivariate binary logistic regression analysis identifying independent predictors of ROSE diagnostic adequacy, final diagnostic adequacy, and diagnostic concordance

	ROSE diagnostic adequacy		Final diagnostic adequacy		Diagnostic concordance	
Independent variable	**OR (95% CI)**	***P* value**	**OR (95% CI)**	***P* value**	**OR (95% CI)**	***P* value**
LN diameter	1.058 (0.995–1.125)	0.073	1.078 (1.002–1.161)	**0.045**	0.951 (0.909–0.994)	**0.026**
Number of passes	1.433 (1.020–2.015)	**0.038**	1.593 (1.074–2.361)	**0.021**	0.911 (0.717–1.157)	0.444
ROSE pathologist experience	0.982 (0.924–1.043)	0.550	-	-	1.058 (0.975–1.147)	0.175
Final diagnosis pathologist experience	-	-	0.960 (0.918–1.003)	0.069	0.951 (0.914–0.990)	**0.015**

Multivariate binary logistic regression analyses were performed using the enter method. Statistically significant results are shown in bold
ROSE: rapid on-site evaluation, OR: odds ratio, CI: confidence interval, LN: lymph node,

Table 5. Calibration, discrimination, and explanatory power of multivariate logistic regression models

Model	Hosmer–Lemeshow *P* value	Nagelkerke R^2	AUC
ROSE diagnostic adequacy	0.073	0.091	0.697
Final diagnostic adequacy	0.902	0.164	0.758
Diagnostic concordance	0.021	0.108	0.697

Hosmer–Lemeshow test was used to assess model calibration. Nagelkerke R^2 was used to evaluate explanatory power. Model discrimination was assessed using the area under the receiver operating characteristic curve (AUC). AUC values ≥0.7 indicate acceptable discrimination
ROSE: rapid on-site evaluation

Previous studies have demonstrated that ROSE significantly enhances the diagnostic performance of EBUS-TBNA, reporting sensitivity of 88–97%, specificity of 93–99%, PPV of 94–98%, and NPV of 87–98%.[5,6,10] These findings indicate that ROSE strengthens sample adequacy and concordance with final pathology. In a recent study, Magdy et al.[11] emphasized the superiority of this technique by reporting that the diagnostic accuracy reached 100% in the ROSE-applied group. In our study, ROSE demonstrated a sensitivity of 76.6%, a specificity of 100.0%, a PPV of 100.0%, an NPV of 85.4%, and an overall diagnostic accuracy of 90.1%. Consistent with the high sensitivity and diagnostic accuracy reported in the literature, these results confirm the value of ROSE in providing a rapid and reliable diagnosis. These results support the potential of ROSE to significantly increase the effectiveness of EBUS-TBNA and reduce the need for invasive surgical intervention.

In another study conducted during the severe acute respiratory syndrome coronavirus 2 pandemic, EBUS-TBNA was performed without ROSE, using only the cell block technique to reduce the risk of coronavirus disease-2019 transmission.[12] A total of 1,097 and 806 procedures were included in the ROSE and non-ROSE groups, respectively. In this study, sample adequacy from stations 4R and 7 was higher, likely because of their more accessible anatomical locations. The number of biopsy sites per patient was 2.4±2.3 in the ROSE group and 2.9±2.7 in the non-ROSE group, with a statistically significant difference between the groups ($P < 0.001$). While no difference in overall sample adequacy was observed between the two groups ($P = 0.785$), the malignancy detection rate was significantly higher in the ROSE group ($P = 0.036$). These findings may have been influenced by the triage strategy during the pandemic, in which cases with

lower clinical suspicion were followed up, whereas cases with higher clinical suspicion were referred for EBUS-TBNA. In this retrospective analysis, sample adequacy was similar between the ROSE and non-ROSE groups; nevertheless, ROSE increased the malignancy detection rate, and EBUS-TBNA should not be delayed in highly suspicious cases.

The diagnostic yield of EBUS-TBNA is influenced by factors such as lesion size, number of samples, needle gauge, and number of passes; reported accuracy rates range from 71% to 99%.[13] Current guidelines recommend at least three passes for diagnosis and four to six passes for molecular analysis.[1] Kim et al.[14] reported that increasing the number of passes improved the success rates from 52.6% to 100%, with a significant improvement observed after the third pass. In contrast, Hu et al.[15] indicated that the use of general anesthesia and ROSE reduced the number of punctures but did not improve diagnostic efficacy. In our study, the number of needle passes and the LN diameter were significantly higher in cases with ROSE adequacy; similar findings were observed in cases with final diagnostic adequacy. These results align with the literature that supports optimal pass numbers and LN diameters as determinants of diagnostic performance. Fielding et al.[16] also demonstrated that three agitations provided a cell and DNA yield equivalent to ten agitations, reduced blood contamination, and shortened the procedure.

Although ROSE provides high diagnostic accuracy for malignancy, limitations in subtype characterization have been reported.[17] Cytological smears are typically adequate; however, histological biopsies may be required for molecular analysis.[13] In our study, tumor subtyping could not be determined on final pathology in two of nine nodes diagnosed as "NSCLC" by ROSE and in 8 of 18 nodes diagnosed as "carcinoma" by ROSE, which illustrates this limitation. The literature attributes this issue to the lack of preserved tissue architecture and the presence of reactive cells that mimic malignant features.[4,11] Despite its lower NPV (85.4%), the relatively high specificity (100.0%) may be explained by cytological limitations and sample heterogeneity. Technical parameters are also important, as Fielding et al.[16] highlighted the advantages of reduced agitation, and Sakaguchi et al.[18] reported lower blood contamination with 25G needles. In the study by Caupena et al.,[10] the ROSE-final pathology concordance was 96.1% with a false-negative rate of 1.2% and a false-positive rate of 0.2%. In our cohort, the discordance rate was 11.8% with most discrepancies observed in NSCLC and carcinoma cases owing to difficulties in subtyping.

We found that LN diameter was negatively associated with concordance between ROSE and final pathology, with larger LNs being more frequently observed in discordant cases. This finding may be due to necrosis or cystic degeneration, which are more common in larger LNs and may impair cytological evaluation. Additionally, greater professional experience among pathologists who evaluated the final diagnosis (23.4 years vs. 18.2 years; $P = 0.044$) was associated with lower concordance. Previous studies have documented that inter-observer variability and more meticulous assessments by highly experienced pathologists in complex cases may increase the risk of discordance.[11,12] These findings emphasize the crucial roles of morphological features and observer-dependent factors in diagnostic concordance and support the need for multidisciplinary evaluation in challenging cases.

In the study by So et al.,[19] one of the causes of EBUS-TBNA failure in mediastinal LN staging was failure to sample other nodes, owing to false-positive ROSE interpretation. Although the final pathology is rarely positive, this risk exists during ROSE interpretation. The similarity between reactive epithelial cells and malignant cells, particularly for cytopathologists, may complicate their differentiation. As similar issues may occur in granulomatous disease, the ROSE results must be carefully interpreted and confirmed during staging. A systematic review reported a pooled sensitivity of 89.6%, a specificity of 95.9%, and a false-positive rate of 4.1% for ROSE performed by pulmonologists.[2] Pulmonologists have achieved high diagnostic accuracy in malignancy detection, with most errors arising from misinterpretation of bronchial epithelial cells. The concordance rate between pulmonologists and pathologists was 90%, suggesting that pulmonologists can serve as reliable alternatives when a pathologist is not available. A recent meta-analysis by Chen et al.[20] further confirmed the high diagnostic accuracy of ROSE for lung cancer during EBUS-TBNA. When used for mediastinal or hilar LN staging, diagnostic performance improved, with subgroup analyses showing a sensitivity of 90.1% and a specificity of 96.9%. ROSE increases sample adequacy, reduces the need for repeated procedures, shortens diagnostic time, ensures sufficient material for molecular testing, and enhances patient safety by enabling high diagnostic accuracy with fewer needle passes. These findings confirm that ROSE is a feasible, cost-effective technique that enhances the diagnostic yield and specificity of EBUS-TBNA and can be safely performed by trained pulmonologists in the absence of pathology support. Furthermore, the integration of artificial intelligence-assisted digital ROSE systems is expected to reduce processing time and improve standardization.

Study Limitations

This study has several limitations. First, its retrospective, single-center design may have introduced selection bias and limited the generalizability of the findings. The relatively small sample size may have reduced the statistical power, particularly in the subgroup analyses. ROSE and the final diagnosis were evaluated by different pathologists, introducing inter-observer variability that could affect diagnostic concordance. Although the final pathology is considered to be the gold standard, false-negative or false-positive results remain possible. Although no a priori power calculation was performed because of the retrospective design, a post hoc power analysis indicated that the study had approximately 82% power to detect moderate effect sizes in the concordance analysis. However, smaller subgroup sizes may limit the ability to detect weaker associations. Cytological limitations, particularly the lack of architectural preservation and sample heterogeneity, may hinder the subtype classification. Technical parameters (needle gauge, number of agitations, and vacuum use) were not standardized, which could have influenced diagnostic performance. Additionally, the lack of multidisciplinary evaluation and the absence of methods (e.g., cryobiopsy or molecular testing) in complex cases are additional limitations.

CONCLUSION

This study demonstrates that ROSE during EBUS-TBNA provides high diagnostic accuracy and sensitivity in evaluating mediastinal and hilar LNs. Our findings show that ROSE enhances sample adequacy, ensures strong concordance with final pathology, and expedites clinical decision-making. Nevertheless, limitations in sensitivity and negative predictive value, along with challenges in tumor subtypingassociated with cytological interpretation and sample heterogeneity underscore the importance of multidisciplinary assessment and supplementary pathological methods. Implementation of ROSE offer significant clinical advantages by reducing the need for invasive surgical interventions.

Ethics

Ethics Committee Approval: This study was designed retrospectively in accordance with the Declaration of Helsinki and approved by the Koç University Ethics Committee (decision/protocol number: 2024.222.IRB2.103, date: 07.06.2024).

Informed Consent: Written informed consent was obtained from all patients prior to the procedure.

Footnotes

Authorship Contributions

Surgical and Medical Practices: F.K., M.Ç.A.M., I.U., Ö.D., Concept: F.K., Design: F.K., Data Collection or Processing: F.K., M.Ç.A.M., I.U.,T.Y., E.K., Analysis or Interpretation: F.K., Literature Search: F.K., Writing: F.K., M.Ç.A.M.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

REFERENCES

1. Gilbert CR, Dust C, Argento AC, et al. Acquisition and handling of endobronchial ultrasound transbronchial needle samples: an American College of Chest Physicians Clinical Practice Guideline. *Chest.* 2025;167(3):899-909. **[Crossref]**
2. Ehab A, Elgenidy A, Abobakr OA, et al. The accuracy and adequacy of rapid on-site evaluation conducted by trained pulmonologist on transbronchial needle aspiration samples: a systemic review and meta-analysis. *Respir Med.* 2025;236:107903. **[Crossref]**
3. Li C, Xie W, Cao J, Feng J. Detailed procedure and clinical application overview of rapid on-site evaluation in diagnostic interventional pulmonology. *J Res Med Sci.* 2020;25:35. **[Crossref]**
4. Vasugi GA, Mathivanan KM, Rajendiran S, Sundaram S, Ayub II. Traveling with ROSE in EBUS-TBNA – experience from a Tertiary Care Hospital in South India. *J Cytol.* 2021;38(4):175-179. **[Crossref]**
5. Al-Najjar H, Almadhi M, Rajai A, et al. Comparing diagnostic performance across two high-volume endobronchial ultrasound services in greater manchester with and without rapid on-site evaluation. *Respiration.* 2025;104(8):586-596. **[Crossref]**
6. Silav ZK, Çıkrıkçıoğlu M, Aydemir B, Yıldırım M. EBUS ROSE-guided cytological evaluations of intrathoracic lesions. Assessments of the pathological material adequacy and the diagnostic efficacy. *Diagn Cytopathol.* 2023;51(7):423-433. **[Crossref]**
7. Mercer C, Brackenborough K, Davidson C, et al. Rapid onsite evaluation: a prospective observational study of endobronchial ultrasound-guided transbronchial needle aspirates to expedite diagnosis. *Br J Biomed Sci.* 2024;81:12938. **[Crossref]**
8. Jaiyesimi IA, Leighl NB, Ismaila N, et al. Therapy for stage IV non-small cell lung cancer with driver alterations: ASCO living guideline, version 2023.3. *J Clin Oncol.* 2024;42(11):1-22. **[Crossref]**
9. Mountain CF, Dresler CM. Regional lymph node classification for lung cancer staging. *Chest.* 1997;111:1718-1723. **[Crossref]**
10. Caupena C, Esteban L, Jaen A, et al. Concordance between rapid on-site evaluation and final cytologic diagnosis in patients undergoing endobronchial ultrasound-guided transbronchial needle aspiration for non-small cell lung cancer staging. *Am J Clin Pathol.* 2020;153:190-197. **[Crossref]**
11. Magdy O, AbdelDayem A, ELMaraghi A, Ali M, Hafez F. The value of rapid on-site evaluation during conventional and endobronchial ultrasound needle aspiration in the diagnosis of mediastinal lymphadenopathy and lung cancer. *The Egyptian Journal of Bronchology.* 2024;18:70. **[Crossref]**
12. Zhao X, Boothe P, Hussnain Naqvi SM, et al. Assessing ROSE for adequacy of EBUS-TBNA compared with a direct-to-cell block approach as a response to the COVID-19 pandemic. *J Am Soc Cytopathol.* 2022;11(6):368-374. **[Crossref]**
13. Bandiera A, Arrigoni G. The impact of pathological analysis on endobronchial ultrasound diagnostic accuracy. *Mediastinum.* 2020;4:19. **[Crossref]**
14. Kim BK, Choi H, Kim CY. Factors associated with increased diagnostic yield of endobronchial ultrasound-guided transbronchial needle aspiration: an observational single center study. *J Thorac Dis.* 2024;16(1):439-449. **[Crossref]**
15. Hu YH, Xu WH, Li YY. The impact of anesthesia methods and rapid on-site evaluation on the diagnosis of lung cancer using endobronchial ultrasound-guided transbronchial needle aspiration: a retrospective study. *J Thorac Dis.* 2024;16(12):8493-8502. **[Crossref]**
16. Fielding D, Dalley AJ, Singh M, et al. Prospective optimization of endobronchial ultrasound-guided transbronchial needle aspiration lymph node assessment for lung cancer: three needle agitations are noninferior to 10 agitations for adequate tumor cell and DNA yield. *JTO Clin Res Rep.* 2022;3(10):100403. **[Crossref]**
17. Murthi M, Donna E, Arias S, et al. Diagnostic accuracy of endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) in real life. *Front Med (Lausanne).* 2020;7:118. **[Crossref]**
18. Sakaguchi T, Inoue T, Miyazawa T, Mineshita M. Comparison of the 22-gauge and 25-gauge needles for endobronchial ultrasound-guided transbronchial needle aspiration. *Respir Investig.* 2021;59(2):235-239. **[Crossref]**
19. So C, Matsumoto Y, Imabayashi T, et al. Identifying factors causing failure of nodal staging by endobronchial ultrasound-guided transbronchial needle aspiration in non-small cell lung cancer. *Transl Lung Cancer Res.* 2023;12:2169-2180. **[Crossref]**
20. Chen CC, Lu SC, Chang YK, et al. Diagnostic performance of rapid on-site evaluation during bronchoscopy for lung cancer: a comprehensive meta-analysis. *Cancer Cytopathol.* 2025;133(1):22908. **[Crossref]**