Endobronchial ultrasound-transbronchial needle aspiration for mediastinal staging of non-small cell lung cancer: variability of results and perspectives

Elisa Nardecchia, Maria Cattoni, Lorenzo Dominioni

Center for Thoracic Surgery, Department of Medicine and Surgery, University of Insubria, Ospedale di Circolo, Varese, Italy
Correspondence to: Lorenzo Dominioni, MD. Center for Thoracic Surgery, Department of Medicine and Surgery, University of Insubria, Ospedale di Circolo, Via Guicciardini, 9, Varese 21100, Italy. Email: lorenzo.dominioni@uninsubria.it.

Abstract: The remarkable value of endobronchial ultrasound-transbronchial needle aspiration (EBUS-TBNA) for mediastinal staging of non-small cell lung cancer (NSCLC) is recognized worldwide. Reports from different centers however show considerable variation of EBUS-TBNA performance in terms of diagnostic yield, sensitivity and negative predictive value (NPV). Interpretation of EBUS-TBNA diagnostic efficacy requires clarifying whether the technique is used for purely diagnostic purpose or mediastinal staging, recognizing that different study groups may be inherently heterogeneous and that numerous factors may impact on the procedure outcomes. Review of these factors indicates that the prevalence of N2/N3 disease, the thoroughness of mediastinal sampling and >3 needle passes per target lymph node (LN) [in the absence of rapid on-site evaluation (ROSE)] influence the procedure outcomes, while many details in the sample preparation technique are unlikely to impact on the results and should be left to the proceduralists' preference. Generalized use of a standardized database for prospective collection of relevant EBUS-TBNA data would allow reporting institutional results by sub-groups of N2/N3 disease prevalence and thoroughness of staging, and would help establishing quality standards for the procedure.

Keywords: Endobronchial ultrasound-transbronchial needle aspiration (EBUS-TBNA); lung cancer; mediastinal staging; diagnostic performance

Introduction

Over the last 15 years endobronchial ultrasound-transbronchial needle aspiration (EBUS-TBNA) has emerged as the technique of first choice for biopsy of mediastinal lymph nodes (LN). In patients with suspect/known non-small cell lung cancer (NSCLC), the primary indications of EBUS-TBNA are tissue confirmation of computed tomography (CT)-enlarged or positron emission tomography (PET)-positive mediastinal LN, and systematic mediastinal LN staging, a major determinant of tumor resectability and prognosis. In selected patients with suspect lung cancer the EBUS-TBNA technique may be used as first-line tissue sampling procedure; if

this shows N2/N3 invasion and identifies the lung cancer subtype, simultaneous diagnosis and mediastinal staging of the disease can be obtained, and the duration and cost of the diagnostic process are reduced. Moreover, it may be helpful averting lung tumor biopsy when this is technically difficult, or high-risk (e.g., emphysematous patients), or inappropriate (e.g., patient unfit for surgery).

EBUS-TBNA for diagnosis of malignancy in mediastinal nodes is a highly effective technique, with a low (about 1%) rate of complications, almost exclusively minor (1-5). In the context of NSCLC diagnosis and staging, reports from different centers show considerable variation of EBUS-TBNA diagnostic yield (71–99%) (5-9), and of

Table 1 Factors possibly affecting the efficacy of EBUS-TBNA for mediastinal staging of NSCLC

Factors
Prevalence of N2/N3 disease
Patient sedation method
Sample acquisition and preparation technique
Thoroughness of mediastinal staging
Learning curve and volume

EBUS-TBNA, endobronchial ultrasound-transbronchial needle aspiration; NSCLC, non-small cell lung cancer.

key performance indices, namely sensitivity (46–97%) and negative predictive value (NPV) (60–99%) (10). Discrepancies in the reported efficacy of the procedure and lack of generally accepted benchmarks for EBUS-TBNA outcomes complicate the comparison of findings across institutions. The performance indices of EBUS-TNBA mediastinal staging may be influenced by numerous factors (*Table 1*), the most relevant of which are the N2/N3 disease prevalence and the thoroughness of staging (10-12). We will focus on these factors, as they are important for interpreting differences in the procedure efficacy among institutions and for establishing quality standards of EBUS-TBNA practice.

Prevalence of N2/N3 disease

Prevalence of N2/N3 disease in the tested population of NSCLC patients impacts on the diagnostic performance of EBUS-TBNA (12-14). As indicated by the American College of Chest Physicians (ACCP) meta-analysis of EBUS-TBNA mediastinal staging, which included a total of 2,756 procedures (10), the prevalence of N2/N3 disease directly correlates with diagnostic sensitivity and inversely correlates with NPV: with <20% prevalence of mediastinal lymph nodal metastases, EBUS-TBNA showed 78% sensitivity and 96% NPV; with ≥80% prevalence, 96% sensitivity and 83% NPV (12). Because prevalence of mediastinal nodal metastasis is a major bias, it is not surprising that EBUS-TBNA diagnostic performance varies among series with different N2/N3 disease prevalence, and staging results are difficult to translate from one study to another (15). For meaningful assessment of the quality of EBUS-TBNA outcomes, it is necessary to know the prevalence of mediastinal nodal metastasis in the examined population (12).

Patient sedation method

Patient sedation for conducting EBUS-TBNA can be obtained by conscious sedation (usually with fentanyl and midazolam) or by general anesthesia (with propofol and oro-tracheal tube/laryngeal mask). Proponents of general anesthesia maintain that abolishing respiratory variation and cough facilitates sampling when target nodes are small and the patient is scarcely collaborating. Whether the type of sedation makes a difference in the procedure performance has been debated. There are no randomized studies documenting a superior diagnostic performance of EBUS-TBNA with either sedation method. In a retrospective multicentric study of EBUS-TBNA that we recently carried out, sampling adequacy rate was similar with general anesthesia and with conscious sedation (87% and 92%; P=0.09) (16), in agreement with the conclusion of current systematic literature reviews indicating that the choice of sedation method can be left to the operator preference (17,18).

Sample acquisition and preparation techniques

EBUS-TBNA procedures are generally performed with 21-Gauge (G) or 22-G needle. After reviewing the evidence from studies comparing the diagnostic yield of EBUS-TBNA with these two needle sizes, Wahidi *et al.* concluded that either size is an acceptable option (18). Some authors prefer the larger-bore needle (21-G) because it provides more tissue material, which can be used for histology and for molecular studies. In the context of potentially operable NSCLC, for thorough assessment of mediastinal node involvement it is generally agreed that 3 needle passes per target LN should be obtained, if rapid on-site evaluation (ROSE) of samples is not used (17-20). Each pass should include 5 to 15 needle agitations within the target node (18), with or without suction (21). The important indication that three is the optimal number of punctures per target LN was provided by the study of Lee *et al.* who evaluated by EBUS-TBNA 163 LN stations in 102 NSCLC patients (19). In that study each target LN was punctured four times, however after three passes the sample adequacy was 100%; the sensitivity for differentiating malignant from benign LN stations was 95.3% and did not increase with four passes (19).

The most frequently used techniques for EBUS-TBNA specimen acquisition and processing are cytology slides, cell-block, core-tissue, combination of cytology slides and

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Table 2 Mean number of lymph node stations biopsied per patient, diagnostic yield and sensitivity in recent series of EBUS-TBNA procedures for mediastinal staging of lung cancer

Authors	Year	Patients (n)	LN stations biopsied per patient (n)	Diagnostic yield (%)	Sensitivity (%)
Yasufuku *et al.* (6)	2011	153	2.8	71	94
Lee *et al.* (5)	2012	73	1.9	99	81
Tian *et al.* (7)	2013	185	–	99	95
Nakajima *et al.* (8)	2013	438	2.2	91	97
Figueiredo *et al.* (9)	2015	149	2.7	87	96
Median	–	–	2.5	95	95

EBUS-TBNA, endobronchial ultrasound-transbronchial needle aspiration; LN, lymph nodes.

core-tissue, combination of cytology slides and cell-block. Only few studies comparing these techniques have been published and there is no consensus on the optimal method of specimen preparation (17). In our institute a study was carried out to identify the best performing technique among those currently available for EBUS-TBNA specimen acquisition and processing; we found that the diagnostic yield with cytology smear and with core-tissue were high and similar (81% *vs.* 87%; P=0.44) (22). In the literature, like in our study, the comparison of cytology smear, core-tissue and cell-block methods showed that no single method is superior to the others (23,24). Notably, we attained the highest diagnostic yield (100%) by combining two methods (cytology slides and core-tissue, or cytology slides and cell-block); however the use of two methods in combination is expensive and time-consuming (22). Considering that familiarity and expertise with a technique impacts on the final results, choice of needle size to use for EBUS-TBNA and of specimen processing method should be left to the operator experience and preference (17).

The influence of ROSE on quantity, quality and yield of EBUS-TBNA samples in patients with known or suspect lung cancer has been the object of a systematic literature review by Van der Heijden *et al.* (17). These authors provided guidelines for specimen acquisition and preparation, indicating that ROSE does not modify EBUS-TBNA diagnostic yield, nor does it affect the number of needle passes, the duration of the procedure, and the complication rate. However, when EBUS-TBNA was the first diagnostic procedure in patients with suspect lung cancer, ROSE was found to reduce the number of additional procedures (25,26).

In conclusion, the sample acquisition and preparation technique are unlikely to impact on EBUS-TBNA diagnostic yield, provided that at least three needle passes per target LN are done (in the absence of ROSE of samples).

Thoroughness of mediastinal staging

Mediastinal nodal staging is a critical step for determining the best treatment of NSCLC. For this purpose, until recently mediastinoscopy was the generally accepted gold standard, with about 80% sensitivity and about 90% NPV in confirming N2/N3 disease (10). Pretreatment mediastinal staging of NSCLC has been revolutioned by the advent of EBUS-TBNA, a procedure characterized by sensitivity equivalent to that of mediastinoscopy (*Table 2*), but less invasive, less risky, and obviating the need for general anesthesia (10). As a results, in recent years EBUS-TBNA has almost replaced surgical staging by mediastinoscopy in many centers (6,12).

It has been remarked that the reliability of mediastinal staging with EBUS-TBNA, as with other staging techniques, largely depends on the thoroughness of the procedure (10,11,13,19). Thorough mediastinal staging by mediastinoscopy or EBUS-TBNA is best performed with systematic sampling, which requires biopsy of representative LNs in stations 2R, 2L, 4R, 4L, 7 (10). Current guidelines recommend aiming to target at least 3 LN stations (typically 4R, 4L and 7), including those LNs with CT/PET features suggestive of metastasis (6,10,12,20).

Despite these recommendations, in real life the EBUS-TBNA practice is generally characterized by <3 sampled mediastinal nodal stations per patient (27,28), for multiple reasons that include difficult punture of LNs, bleeding, restless patient, longer procedure, ROSE showing metastasis early in the procedure. In early reports of EBUS-TBNA the median number of mediastinal LN stations

sampled per patient was <2 (5), a result that has improved in more recent series (*Table 2*) and that needs further improvement (12). Limited thoroughness of mediastinal staging likely contributes to the variability of EBUS-TBNA staging accuracy in different studies.

Whether mediastinoscopy should be routinely done after negative EBUS-TBNA remains controversial (6,29-31). The prospective randomized trial of EBUS-TBNA *vs.* mediastinoscopy for staging of NSCLC performed by Yasufuku *et al.* showed equivalent effectiveness of the two techniques in determining the true pathologic N stage (6). However, for EBUS-TBNA negative cases at high risk of lung cancer metastases, the current ACCP and British Thoracic Society (BTS) guidelines recommend mediastinoscopy or other surgical approaches to obtain mediastinal nodes' biopsy (10,32).

Learning curve and volume

The skill and experience of the operator, usually an interventional pulmonologist or a thoracic surgeon, influence EBUS-TBNA results. For acquisition of the EBUS-TBNA technique the ACCP, the European Respiratory Society and the American Thoracic Society recommend an initial training of 40–50 supervised procedures. In addition, 20 procedures per year are suggested for maintaining competency (33,34). The speed of learning the EBUS-TBNA procedure varies for different operators, either highly experienced or trainee bronchoscopist (35,36). Furthermore, improvements in performance are reported even after doing 200 procedures, indicating that volume affects the quality of EBUS-TBNA outcomes (36,37). In a recent EBUS-TBNA workshop held in October 2016 in Varese, Italy, a multicentric study was presented that included 485 EBUS-TBNA mediastinal staging procedures in NSCLC patients and compared the outcomes of the five participating units. In all those centers the prevalence of N2 disease was >70%, but wide variation was found in the rate of inadequate sampling (0–29%) and in the rate of false-negative (FN) EBUS-TBNA results (4–11% of all adequate samples); interestingly, the center with the largest EBUS volume (50 procedures/year) showed the lowest FN rate (4%), and the FN rate inversely correlated (P<0.009) with the volume of procedures in the individual centers (38).

To date, the length of the learning curve for EBUS-TBNA proficiency is unclear and is probably different for each operator; no diagnostic yield cut-off has yet been established to define the standard capability of performing EBUS-TBNA.

Perspectives

Molecular testing

Improvement of target therapy makes tumor subtyping and genotyping increasingly necessary for management of lung cancer; obtaining from EBUS-TBNA a specimen suitable for molecular analysis is therefore important. The success in performing molecular tests on TBNA samples depends on the absolute number and percentage of malignant cells present in the sampled material, on quality of cell preservation and on type and sensitivity of the test itself (17,39). Recent reports from high-volume centers indicate that 72–97% of EBUS-TBNA samples are appropriate for testing the most frequently used prognostic markers of lung cancer, namely EGFR, ALK and KRAS (17,25,39,40). Both smear and cell block preparation, or core tissue, can be utilized for molecular testing (17,23); however, while EGFR and KRAS status can be determined using all three specimen preparation techniques, the ALK translocation is best assessed using cell block and core tissue (17). Cell block and core tissue are currently judged to be the best material for molecular analysis, suggesting to privilege these two processing methods if possible.

In advanced lung cancer patients whose treatment may vary on the molecular results, a recent randomized trial showed that ROSE increased by 10% (although not significantly) the success rate of EBUS-TBNA for genotyping, and reduced the number of redo procedures and of further investigations (25).

No randomized studies are available on the influence of needle size, use of suction and type of sedation on the adequacy of samples for molecular testing. In patients undergoing EBUS-TBNA for diagnosis and/or staging of suspect/known NSCLC, more than three needle passes may be necessary to obtain sufficient material for molecular testing (18,30); it is therefore recommended that additional samples be obtained for EGFR and ALK testing (41).

Quality standards

The vast majority of published EBUS-TBNA studies consists of retrospective cohort reports without pre-defined standards and represents real life practice. Efforts should be made to shift from targeted sampling of enlarged

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or PET positive nodes to systematic mediastinal node sampling (12); this will likely improve the thoroughness of staging, the accuracy of EBUS-TBNA and the interpretation of local performance data. EBUS-TBNA guidelines have been made available from expert centers (17,18) but quality standards for assessment of EBUS-TBNA performance have not yet been established, with the exception of the 2014 BTS Quality Standard that states a minimum of 88% sensitivity for mediastinal staging of suspected NSCLC (42). EBUS-TBNA practice needs to be standardized to ensure the best outcomes in all institutions (12). To this effect the performance of staging could be measured by the sensitivity and NPV metrics, stratifying the population by prevalence of N2/N3 disease, because the latter influences both sensitivity and NPV. The ACCP guidelines on NSCLC staging classify patients into four groups (A, B, C, D), according to the index staging CT scan of the chest (10); this classification correlates with N2/N3 disease prevalence and may be used to define standards for nodal staging and for comparison of EBUS-TBNA outcomes across centers (12). Importantly, for meaningful comparison of EBUS-TBNA results, the data should be gathered prospectively, because those retrospectively collected are frequently uncertain as to the indication to perform EBUS-TBNA (diagnosis *vs.* mediastinal staging), the prevalence of N2/N3 disease, and the exact LN stations successfully sampled. An important step forward will be made when the use of a standardized database for prospective collection of relevant EBUS-TBNA data will be generalized (12).

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Footnote

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