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Study Protocol Title: Endobronchial Ultrasound-guided Transbronchial Needle Aspiration (EBUS-TBNA) using a 22 vs 25-gauge needle; A Randomized Controlled Trial.

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AIM: To evaluate the 25-gauge needle for usability, sample adequacy and diagnostic yield and compare it to the currently used 22-gauge needle.

1. Background

EBUS-TBNA is a minimally invasive technique that has become standard of care for mediastinal staging of patients with Non Small Cell Lung Cancer[1]. It has been traditionally performed with 22-gauge needles with an excellent diagnostic accuracy and safety profile[2]. Although many have postulated that larger needle channels would increase diagnostic yield, the use of a 21 gauge needle has been shown to reduce sample quality due to excessive blood within the aspirate without effecting diagnostic yield[3]. In fact, a meta-analysis of Endoscopic Ultrasound guided biopsy in solid pancreatic lesions suggested that a smaller 25-gauge needle improves sensitivity for malignancy in comparison to a standard 22-gauge needle[4]. This phenomenon of improving sample adequacy with smaller needle biopsies has also been seen in ultrasonography-guided fine-needle biopsy of the thyroid[5]. Recently, a new 25-gauge EBUS needle (Boston Scientific, Natick, MA, USA) has become available, but no studies so far have compared the utility of this needle with the standard 22-gauge needle for EBUS-TBNA.

2. Objectives: To evaluate the utility of the 25-gauge needle relative to the traditionally used 22-gauge needle for sample adequacy and diagnostic yield during EBUS bronchoscopy.

2.1. Primary outcome: To determine the degree of concordance in determining sample adequacy and diagnostic yield between the 25-gauge and 22-gauge needle after using 2 passes with each needle size.

2.2. Secondary outcomes

- To assess the diagnostic yield obtained with each needle size
- To assess the concordance between each needle size based on diagnostic yield
- To evaluate EBUS-TBNA related complications with each needle size
- To evaluate differences in usability of the different needle sizes, and identify specific lymph node stations that might be more challenging as identified by Likert scale recorded by physicians.

3. Study Population: An estimated 120 patients with suspicion of either benign or malignant disease in mediastinal or hilar lymph nodes undergoing EBUS-TBNA for diagnostic purposes or mediastinal staging.

3.1. *Inclusion criteria:*

1) Age 18 years or older

2) Indication for EBUS-guided needle biopsy based on suspicion of either benign or malignant disease in mediastinal or hilar lymph nodes.

3.2. *Exclusion Criteria:*

1) Patients who are pregnant or lactating
2) Inability to give informed consent

3) Patients in which only one lymph node station is expected to be sampled by the performing clinician.

4. Research Plan and Methods:

4.1. Study Design: This is a prospective cohort study.

4.2. Patients with suspected or confirmed early stage lung cancer who require EBUS-TBNA as part of their staging process and patients with mediastinal or hilar adenopathy undergoing diagnostic EBUS-TBNA are eligible to participate.

4.3. EBUS-TBNA will be performed at MD Anderson Cancer Center as per our standard clinical protocol as follows:

4.3.1. Conventional flexible bronchoscopy will first be performed to examine the tracheobronchial tree, followed by examination of the intra-thoracic lymph nodes using a linear array ultrasound bronchoscope.

4.3.2. For patients undergoing EBUS-TBNA for evaluation of targeted radiographically abnormal lymph nodes, directed ultrasonographic evaluation of suspicious lymph nodes will be performed prior to biopsy.

4.3.3. In patients undergoing EBUS-TBNA for mediastinal staging of lung cancer a complete screening of mediastinal and hilar lymph nodes will be performed to identify those meeting criteria for sampling.

4.3.3.1. Ultrasound criteria for sampling: size> 0.5 mm OR a combination of features that are associated with malignancy (sharp margins, heterogeneity, central necrosis sign and rounded shape).

4.3.4. Nodal sampling will begin on the side opposite to the primary tumor to avoid the possibility of cross-contamination of specimens and resulting staging inaccuracies.

4.4. Once lymph nodes are identified which require sampling a minimum of five total passes will be performed with the following protocol: Note: concordance as described by the primary outcome will be assessed using needle passes 1, 2, 4 and 5, where the first needle size will correspond to passes 1 & 2 and the second needle size will correspond to passes 4 & 5. Because a total of 5 passes will be conducted:

4.4.1. (Passes 1, 2, & 3): Three initial needle passes from a lymph node will be performed with either the 22-G or 25-G needle depending on the order of the needle size used first determined by randomization. However, only needle passes 1 & 2 will be used for analysis purposes.

4.4.2. (Passes 4 & 5): Two final needle passes will be performed on the lymph node with the other needle, once all lymph node sampling with the first needle size is complete.

4.4.3. The order in which the needles will be used will be determined by randomization. The randomization will be carried out on a per patient basis.

4.5. Randomization**:** We will use the CORe system for randomization of needle size (25-G vs 22-G) order on a per patient basis.

4.6. For each specimen collected, as per our standard practice the stylet will be replaced through the needle lumen to push the sample in a slide. The slide will be smeared with a second slide. One of the slides will be stained with Diff quick method and the other with PAP. The remaining tissue will be pushed with a syringe and collected in a liquid medium containing RPMI.

4.7. For each station and each needle, the performing physician will rate their subjective experience on a five point Likert scale to assess the following characteristics; 1) ease of needle insertion through the scope, 2) bronchoscopic visibility of needle sheath, 3) scope flexibility following needle insertion into working

channel, 4) ultrasound image quality following needle insertion into working channel, 5) ease of needle puncture, 6) visibility of needle during puncture, and 7) ease of needle removal from the scope.

4.8. As per our standard practice, each needle pass will be submitted to cytology to assess the adequacy of the sample and diagnostic interpretation.

4.8.1. Cytology results will be categorized into one of the following five groups: inadequate material (defined as having a predominance of blood or bronchial epithelial cells), normal lymphoid tissue, granulomatous inflammation, necrosis, and malignancy.

4.8.2. Samples will be ranked from worst to best as inadequate material, necrosis, normal lymphoid tissue, granulomatous inflammation and malignancy. For every lymph node, we will select the best sample for each sampling technique.

4.8.3. For the outcome of sample adequacy and for determining concordance between the two needle sizes, we will dichotomize results as either "inadequate" (inadequate material) or "adequate" (normal lymphoid tissue, granulomatous inflammation, necrosis, or malignancy).

4.8.4. To determine the concordance between the two needle sizes on diagnosis, we will dichotomize results as either "diagnostic" (granulomatous inflammation, normal lymphoid tissue, or malignancy) or "nondiagnostic" (inadequate material or necrosis).

4.8.5. Each needle sample will also be assessed by cytologist for quality using the Mair's scoring system with values range from 0 to 10 and are classified as follows: 0 to 2= poor; 3 to 6=good; and 7 to 10= superior[6].

4.8.6. For study purposes, we will be computing concordance using the aggregate sample adequacy from the 2 needle passes pertaining to each (the 22 and 25-gauge) needle size.

5. Variables to collect:

- Demographic variables
- Conditions that might increase the risk of bleeding like aspirin or renal insufficiency.
- Number of Lymph nodes sampled per patient
- Lymph node size on CT and EBUS.
- Lymph node location.
- ROSE assessment for sample adequacy per each needle pass.
- ROSE assessment for diagnostic yield per each needle pass.
- Final diagnosis
- Physician's satisfaction
- Complications

6. Data Collection and Monitoring: Data will be collected prospectively using a data base specially constructed for the purpose.

6.1. Study data will be collected and managed using REDCap (Research Electronic Data Capture) electronic data capture tools hosted at MD Anderson. REDCap (www.project-redcap.org) is a secure, web-based application with controlled access designed to support data capture for research studies, providing: 1) an intuitive interface for validated data entry; 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless downloads to common statistical packages; and 4) procedures for importing data from external sources. In the case of multi-center studies REDCap uses Data Access Groups (DAGs) to ensure that personnel at each institution are blinded to the data from other institutions. REDCap (https://redcap.mdanderson.org) is hosted on a secure server by MD Anderson Cancer Center's Department of Research Information Systems & Technology Services. REDCap has undergone a Governance Risk & Compliance Assessment (May 2014) by MD Anderson's Information Security Office and found to be compliant with HIPAA, Texas Administrative Codes 202-203, University of Texas Policy 165, federal regulations outlined in 21CFR Part 11, and UTMDACC Institutional Policy #ADM0335.

Those having access to the data include the study PI and research team personnel. Users are authenticated against MDACC's Active Directory system. External collaborators are given access to the database once approved by the PI, with their access expiring in 6 months but renewable in 6 months increments at the request of the PI. The application is accessed through Secure Socket Layer (SSL). All protected health information (PHI) will be removed from the data when it is exported from REDCap for analysis. All dates for a given patient will be shifted by a randomly generated number between 0 and 364, thus preserving the distance between dates. Dates for each patient will be shifted by a different randomly generated number.
Following publication study data will be archived in REDCap. Since study data may be useful for future research studies performed under separate IRB approved protocols, study data will be archived indefinitely in REDCap. Since REDCap is a secure electronic database with controlled access, and because patient identifiers may be needed to link study data to data from other sources under future IRB approved protocols, patient identifying information will be retained in the archived database.

7. Sample size: A sample size of 200 lymph nodes is planned to provide a precise estimate of concordance between 22-G needle and 25-G needle in terms of adequacy and diagnosis. We expect the concordance (as indicated in Table 1) to be greater than 90%. Using the following hypothesis test: Ho: $p \leq 90\%$ versus Ha: $p > 90\%$ using a one-sample test.

Table 1. Summary of Concordance for 22 and 25-gauged needles

	22 gauge needle		
		Adequate	**Not adequate**
25 gauge needle	**Adequate**	**a**	**b**
	Not adequate	**c**	**d**

Concordance = (a + d)/N

We plan on assessing 200 lymph nodes with 3 interim looks. After the observance of every 50 lymph nodes sequentially, the final analysis plans to use all 200 lymph nodes. Using a one sample binomial test (East v5) to guide the trial, the test parameters considered were as follows: i) 2-Sided, 0.05 Significance Level, and Power of 88%. Assessments will be considered for both futility and superiority. The Lan and DeMets with O'Brien-Flemming type stopping boundaries were used to generate the p-values required to pause the trial for additional patient entry under the circumstances that a futility or superiority boundary is crossed. Note that the stopping boundaries are estimates to guide the trial because the sample size is relative to the number of lymph nodes (not patients), and it is highly expected that one patient will contribute multiple lymph nodes for analysis.

Table 2.

	Plan characteristics
Assumed Difference	96% versus 90%
2-sided Test	
Nominal Significance Level	0.05
Power	88%
1st Interim Analysis (N = 50)	
P-value to stop for superiority	< 0.001

P-value to stop for futility	0.998
2nd Interim Analysis (N = 100)	
P-value to stop for superiority	0.003
P-value to stop for futility	0.558
3rd Interim Analysis (N = 150)	
P-value to stop for superiority	0.018
P-value to stop for futility	0.159
Final Analysis (N =200) lymph nodes acquired	
P-value to reject Ho	0.044

We estimate an a priori concordance between the two techniques of 96% (or 192/200), which would yield a 95% CI with 2.25 percentage points as the distance from the observed proportion to the upper limit of the 95% CI, and 3.73 percentage points as the distance from the observed proportion to the lower limit of the 95% CI (noting that the Clopper-Pearson exact 95% CI is not symmetric).

8. Statistical analysis:

Descriptive statistics will be used to summarize the demographic and clinical characteristics of all patients in the study. The experimental unit is the lymph node, and all analyses will be performed on a per-Lymph node basis. Agreement with respect to the quality (poor, good, or superior) of samples obtained using 22-gauge and 25-gauge needle will be assessed using kappa statistic. Concordance between the 22-gauge and 25-gauge needles with regard to adequacy, diagnosis, and quality of samples will be estimated with exact 95% CIs. Regression modeling with sequence effect and period effect as a covariates will be used to determine the presence of a sequence or period effect. Moreover, we will compute the relative frequency of lymph nodes pertaining to a single patient. Hierarchical modeling will be used, if necessary, to account for within-patient correlation arising from more than one lymph node assessed per patient. Our analyses will be repeated for slides stained using the Romanowsky or Pap technique and for lymph nodes equal to or larger than 1 cm or smaller than 1 cm in short-axis diameter. We will use a two-sided P value of < .05 to define statistical significance. **Consent:** All patients will receive both verbal and written information about the study and will be asked to give informed consent using study-specific consent forms.

9. **Risk to participants:** The use of the new 25-gauge needle which has received FDA approval for use is not anticipated to be increased from the standard EBUS bronchoscopy with the standard 22-gauge needle.
10. **Funding**: The 25-gauge needles will be supplied through an unrestricted research grant from the manufacturer, Boston Scientific. Boston Scientific will have no input into study design, manuscript review or publication.
11. **Cost to participants**: The use of the 22-gauge needles is considered our current standard of care and therefore all expenses related to the bronchoscopy with the exclusion of the 25g-gauge needle will be accrued by the patient and-or insurance as standard patient care related expenses.

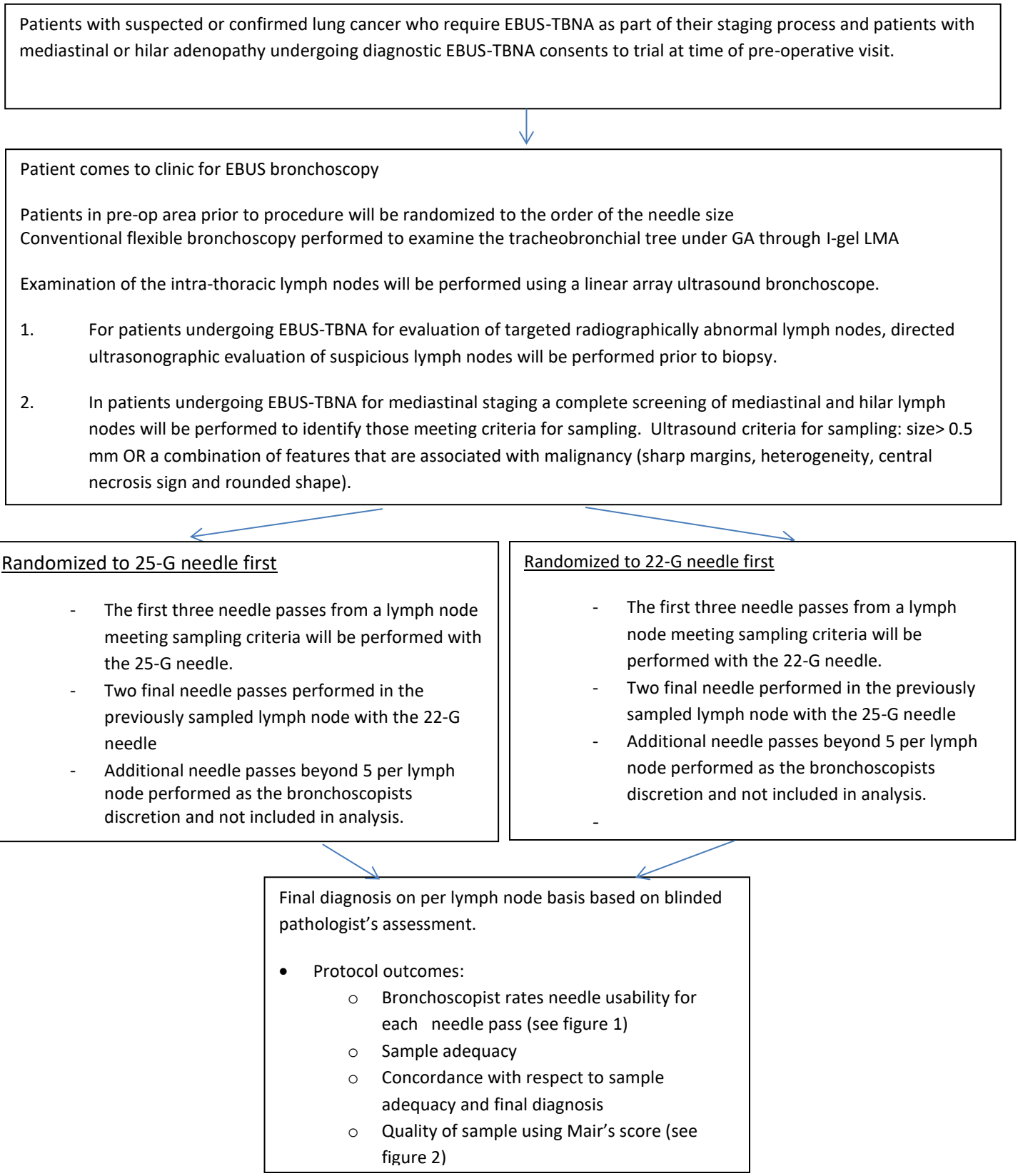
Fig 1: Bronchoscopists data collection sheet

LN station: Size on CT (mm) Size on EBUS (mm)					
Pass number:	1	2	3	4	5
Needle size: (22/25)					
Suction: (y/n)					
Bronchoscopist assessment items are scored from 1-5 as follows: 1= very poor, 2= poor, 3= adequate 4= good 5= excellent					
Ease of needle insertion					
visibility of needle sheath					
scope flexibility following needle insertion					
ultrasound image quality following needle insertion					
ease of needle puncture					
visibility of needle during puncture					
ease of needle removal					
Cytological Assessment: 1= inadequate material 2= normal lymphoid tissue 3= granulomatous inflammation 4= necrosis 5= malignancy					
Mair Score (See figure 2 below for reference)*					

Fig 2: Mair Scoring sheet template

Mair Score sheet		
Criterion	Quality Description	Point score
Background blood or clot		
	Large amount; great compromise to diagnosis	0
	Moderate amount; diagnosis possible	1
	Minimal diagnosis easy; specimen of 'textbook' quality	2
Amount of cellular material		
	Minimal to absent; diagnosis not possible	0
	Sufficient for diagnosis	1
	Abundant; diagnosis simple	2
Degree of cellular degeneration		
	Marked; diagnosis impossible	0
	Moderate; diagnosis possible	1
	Minimal; good preservation; diagnosis easy	2
Degree of cellular trauma		
	Marked; diagnosis impossible	0
	Moderate; diagnosis possible	1
	Minimal; diagnosis obvious	2
Retention of appropriate architecture		
	Minimal to absent; non-diagnostic	0
	Moderate; some preservation of, for example, follicles	1
	Excellent architectural display closely reflecting histology; diagnosis obvious	2

Figure 3. Study flow chart



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