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Rotation aiding technique for endobronchial ultrasound-guided transbronchial needle aspiration biopsy of intrathoracic lymph nodes: A complementary approach to the conventional jabbing method

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Abstract

Background: This study aimed to compare the lymph node core tissue lengths obtained via mediastinal or hilar lymphadenopathy using the complementary "rotation aiding" and conventional Jab technique.

Methods: We prospectively measured the lymph node core tissue length in patients who sequentially underwent the Jab and rotation aiding (RA) techniques between October 2012 and December 2014. Wilcoxon signed-rank test was used to compare the core tissue length and grade of diagnostic cells obtained by each technique. McNemar's test was used to compare the proportion of adequate cellularity ($\geq$grade 2) between the aspiration techniques.

Results: The core tissue length of 61 lymph nodes from 43 patients (mean age: 63 years, range: 16–86 years) was analyzed. Pathological findings were consistent with malignant lesions in 25 (41%) patients and benign lesions in 36 (59%). The most common diagnosis in benign lymph nodes was reactive, followed by tuberculosis and sarcoidosis. We obtained longer core tissue with RA technique than with the Jab technique (83.2 ± 12.7 vs. 60.1 ± 10.1 mm; $p = 0.02$). There was a significant increase in cellularity grade and proportion of $\geq$grade 2 cells with the RA technique than with the Jab technique (2.39 ± 1.08 vs. 1.84 ± 1.14; $p < 0.001$, 78.7% vs. 52.5%; $p = 0.002$), regardless of the pathological diagnosis.

Conclusions: RA technique facilitated more lymph node samples in terms of core tissue length and cellularity than the Jab technique.

KEYWORDS
endobronchial ultrasound, mediastinal or hilar lymphadenopathy, rotation, transbronchial needle aspiration

INTRODUCTION

Mediastinal lymph node (LN) sampling is a key step in the staging of lung cancer and diagnosis of non-neoplastic disease, such as granulomatous diseases. Noninvasive methods rely on imaging studies, which are convenient and feasible but lack pathologic confirmation. Therefore, significant mediastinal LNs on computed tomography (CT) images or F-18-fluorodeoxyglucose (FDG) uptake on positron emission tomography–CT (PET-CT) scans require further assessment by invasive methods, in which tissue acquisition is performed either by surgical techniques or other minimally invasive needle aspiration techniques.

However, acquiring appropriate and sufficient intrathoracic lymphadenopathy tissue is a great diagnostic challenge and is highly important for the pathological examination and subsequent immunohistochemistry. Characteristic features of mediastinal LNs to be considered include anatomic

location of the lesion; amount of tissue needed; cost, safety, and risks, and expected diagnostic yield/accuracy.

Mediastinoscopy is the "gold standard" for invasive mediastinal staging in lung cancer[1]. Entire LNs or large tissue samples can be obtained with this method; large tissue samples may be especially useful to exclude malignancy, for minimizing sampling errors. However, the areas of possible biopsy using mediastinoscopy are limited and include only the upper and lower paratracheal and subcarinal regions. This procedure requires general anesthesia and may have some complications, such as left recurrent laryngeal nerve injury, pneumothorax, and bleeding, but a very low morbidity rate (<0.5%). Additionally, there are some relative contraindications for the procedure, such as previous mediastinoscopy and radiation therapy, severe cervical arthritis leading to limitations in hyperextension of the neck, and cutaneous tracheostomy[2]. Moreover, the cost of the procedure prevents its routine use.

Traditionally, conventional transbronchial needle aspiration (TBNA) is used for diagnosis, but the pathologic results are sometimes insufficient; thus, a more invasive mediastinoscopic biopsy is required. Recently, conventional TBNA and mediastinoscopic biopsy have been replaced by convex endobronchial ultrasound (EBUS) probe and a dedicated biopsy needle, which provides safe, real-time EBUS-guided TBNA[3]. EBUS-TBNA is a minimally invasive, widely accepted alternative to mediastinoscopy used to sample mediastinal or hilar LNs.

A previous study using an in vitro experimental system evaluated the effect of a rotational maneuver during simulated coaxial cutting needle biopsies of tissue cores using agarose gel showed that core tissues obtained by changing the rotational orientation of the cutting needle between passes were generally longer and thicker[4]. Some experts place the EBUS scope at different angles on each biopsy pass (i.e., a 15 degree turn for each pass) based on their personal anecdotal experience; however, needle movement within the LN tissue in patients undergoing intrathoracic lymphadenopathy has not been studied. The direction of the needle can be changed within the target lesion to cover a wider area. Instead of using the rotational orientation of the needle, we developed an innovative procedure, in which the rotation of the needle itself along with negative aspiration was used to obtain more tissue. Although the EBUS needle does not as yet have a rotation function, we aimed to examine the effect of this manual rotation for adequate sampling in the mediastinal lymphadenopathy. The mechanism of this conceived maneuver, using forward and helical movements, is to obtain more tissue compared to the previous procedure that uses a straight jabbing motion as the biopsy needle passes through the LN. We hypothesized that the rotation aiding (RA) technique could obtain more LN tissue(s) than the conventional "Jab" method, regardless of the LN pathology. Consequently, the objective of this study was to show complementary value of the RA technique over the conventional Jab technique when obtaining an intrathoracic LN sample.

METHODS

Patient population

Between October 2012 and December 2014, we enrolled 43 patients for whom chest CT showed enlarged or growing intrathoracic LN(s), regardless of their underlying disease. A total of 61 LN samples from the 43 patients were obtained by EBUS-TBNA using both the Jab and RA techniques, and the data regarding the tissue core length and cellularity of the LNs were prospectively collected. We then retrospectively analyzed medical records of surgical specimen pathology, treatment responses, and follow-up chest CTs to confirm a diagnosis of malignant or benign LN(s). The short-axis diameter of the targeted node was also recorded. Written informed consent was obtained from all patients prior to the procedure. The study was approved by the Ethics Committee on Human Experimentation of Ulsan University Hospital, University of Ulsan College of Medicine (Institutional Review Board [IRB] No. UUH 2017-11-006).

Device

These procedures were performed with an ultrasonic bronchoscope (CP-EBUS, BF-UC260F-OL8; Olympus) via the transbronchial route. A dedicated ultrasound scanner (EU-C2000; Olympus) was used as the image processor. After a 22-gauge needle (NA-202C; Olympus) was inserted into the LN, a sample was obtained under negative pressure using a 20-ml VacLoc syringe (Merit Medical Systems, Inc) attached to the needle end to provide suction. The needle was moved in and out of the target lesion approximately 10–20 times. Suction was then released, the needle was withdrawn, and the sample was processed. For histological examination, the stylet was used to expel the sample into formalin. Sterile saline solution was then used to wash any remaining tissue or cells into the formalin. The "core" biopsies were then embedded in paraffin and stained with hematoxylin and eosin. Cytological preparations were made by using the stylet to expel the sample from the needle onto a slide. If this yielded minimal to no sample, air was used to push the sample out and then smeared onto a glass slide fixed with 95% alcohol.

Procedure

The Jab method uses a back-and-forth movement, performed between 10 and 20 times for each pass, whereas the RA technique manually rotates a needle without locking while jabbing with the same number of back-and-forth movements. In detail, the operator applies 10 to 20 jabbing motions for each pass, while the assistant rotates the needle 360°. When manually rotated, the needle showed helical movement as indicated in the virtual video (Video S1–S3). If an adequate sample could not be obtained after the conventional Jab method was applied twice to the LN, RA technique was performed once or

twice inside the same LN depending on the obtained tissue amount. Specifically, when the first pass of RA technique did not obtain enough tissue, the LN was targeted a second time as it was when the jabbing method was performed. We then measured and compared the core tissue length obtained by each method and subjectively assessed cellularity on smear slides to prove our hypothesis and subsequently analyze whether the tissue amount or cytology was influenced by histological diagnoses.

The aspirated cells from each TBNA procedure were assessed for adequacy. The presence of malignant cells, benign lymphoid cells, or other specific non-neoplastic cells was considered to be an adequate sample. The adequate specimen was then categorized into one of three grades according to the number of acquired diagnostic cells: grade 1, minimal (rarely seen, less than 5% of the area within the slide); grade 2, moderate (occasionally seen, approximately 5%–50% of the area within the slide); and grade 3, numerous (frequently seen, over 50% of the area within the slide)[5]. A specimen containing only bronchial epithelial cells or blood cells was categorized as inadequate or grade 0.

Statistical analysis

Estimation of the sample size necessary to detect a difference in the lengths of core LN tissue samples between the two techniques using a paired t-test was performed using G*Power version software 3.1 (Franz, Universitat Kiel, Germany)[6]. With a power of 90%, 0.05 level of statistical significance and effect size of 0.4 (assuming a mean difference of 20 mm and standard deviation of 50 mm for the core tissue length), the sample size (LNs sample number) was calculated as 68. The assumed mean difference and standard deviation originated from our pilot study using samples from 10 patients. To identify how core tissue lengths are distributed according to each technique, the Kolmogorov–Smirnov test was used. Depending on the normality of data distribution, applicable tests were planned to compare the difference in core tissue length obtained by both techniques; a paired t-test was used to analyze normally distributed data, while the Wilcoxon signed-rank was used to analyze skewed data. Pearson's correlation coefficient (r) was calculated to identify a linear correlation between the core tissue length and targeted LN size. Finally, Wilcoxon signed-rank test and McNemar's tests were used to determine any significant association between the aspiration techniques and the grade of diagnostic cells. SPSS version 21.0 for Windows (IBM Corp.) was used for statistical analysis. All tests were two-tailed, and statistical significance was set at $p < 0.05$.

RESULTS

Baseline characteristics

The baseline characteristics of study participants are shown in Table 1. In 43 patients who showed intrathoracic

TABLE 1 Characteristics of study participants and LNs

Characteristics	Data
Patients, No.	43
Male/female sex, No.	23/20
Median age (range), year	63 (16–86)
LN biopsied, No.	61
Pathological diagnosis	
Malignant LAP	25 (41.0)
Benign LAP	36 (59.0)
Reactive	18 (50.0)
Tuberculosis	11 (30.5)
Sarcoidosis	7 (19.5)
Median LN size on CT image (range), mm	14.5 (7.0–42.5)
Location of LN station	
2R	6 (9.8)
4R	23 (37.7)
4 L	4 (6.5)
7	18 (29.6)
10R	4 (6.5)
10 L	2 (3.3)
11R	2 (3.3)
11 L	2 (3.3)
EBUS-TBNA related complications	None

Note: Data are presented as absolute number (%) unless otherwise indicated.
Abbreviations: CT, computed tomography; EBUS-TBNA, endobronchial ultrasonography-transbronchial needle aspiration; L, left; LAP, lymphadenopathy; LN, lymph node; R, right.
[a]Average diameter of short and long axis.

lymphadenopathy on their chest CT, the sex ratio (male: female) was 23:20, and the median age was 63 years (range, 16–86 years). Among the 61 LN biopsies obtained, 25 (41%) were malignant lymphadenopathy (LAP), and 36 (59%) were benign LAP. Of the 36 benign LAP, 18 (50%) were reactive, 11 (31%) were related tuberculosis LAP, and 7 (19%) were diagnosed as sarcoidosis. The median size of the targeted enlarged LNs was 14.5 mm (range, 7–42.5 mm). The locations of the sampled LNs are shown in Table 1; the right lower paratracheal lymph node station (4R LN) was the most common location for EBUS-guided sampling (38% of the sampled node), followed by the subcarinal LN station (7 LN) (30% of the sampled node).

Comparison of core tissue length between jab and RA techniques

Using the RA technique, we were able to extract longer core tissue samples than using the conventional Jab method (Figure 1, 83.2 mm vs. 60.1 mm; $p = 0.021$). In subgroup analysis, the RA technique allowed us to obtain longer tissue samples, specifically in 25 malignant LNs (Figure 2a, 111.0 mm vs. 75.2 mm; $p = 0.033$), whereas in 36 benign
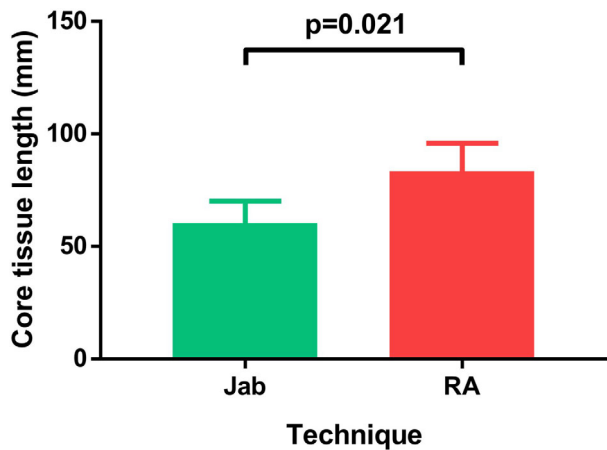
FIGURE 1 Wilcoxon signed-rank test was used to compare the core tissue lengths obtained by the rotation aiding (RA) technique and Jab method. Core tissue length was statistically longer when using RA technique ($p = 0.021$)
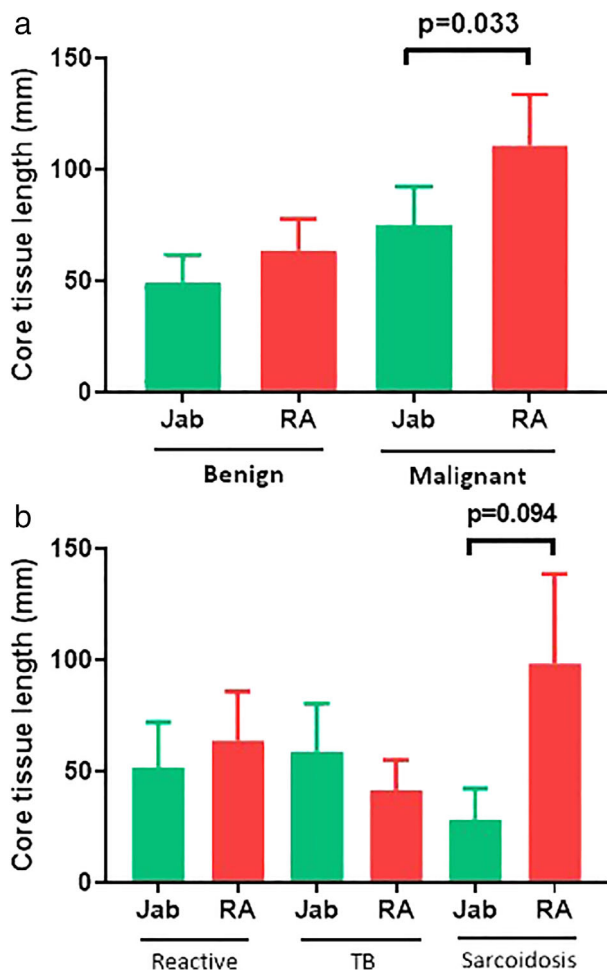

FIGURE 2 (a) In subgroup analysis, the rotation aiding (RA) technique enabled longer tissue samples, specifically in malignant lymph nodes ($p = 0.033$), whereas in benign lymph nodes, there was no statistically significant difference between the techniques. (b) There was no statistically significant difference in core tissue length obtained using Jab and RA techniques in benign lymphadenopathy ($p > 0.1$), except in the case of sarcoidosis ($p = 0.094$)
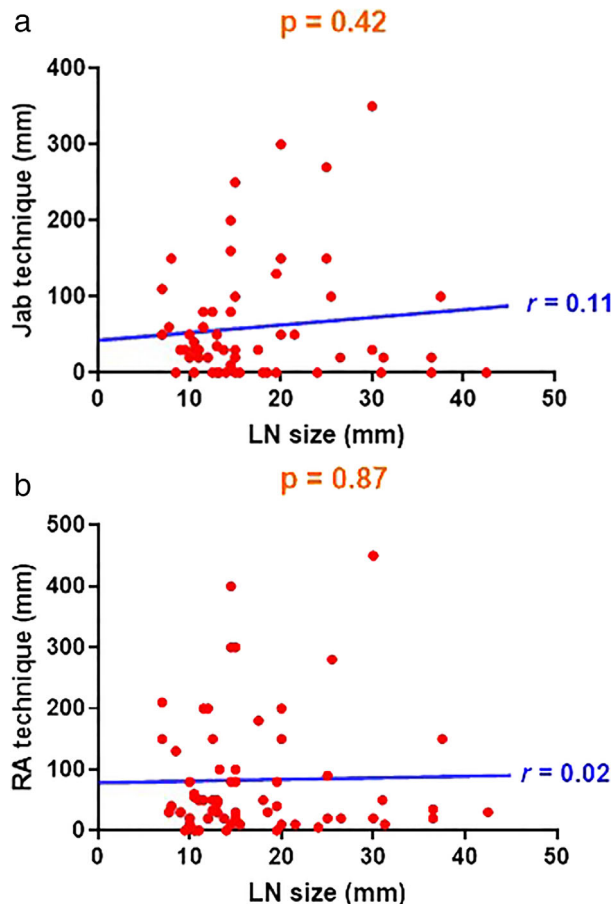

FIGURE 3 (a) The results of the Pearson's correlation test revealed that longer core tissues were not correlated with the targeted lymph node size when the Jab technique was used. (b) The results of the Pearson's correlation test revealed that longer core tissues were not correlated with the targeted lymph node size when the rotation aiding technique is used

LNs, there was a trend showing a numerical difference between the two techniques (Figure 2a, 63.8 mm vs. 49.6 mm; $p = 0.241$). Among the benign LNs, the most prominent difference was observed in the seven samples that were taken from patients with sarcoidosis (Figure 2b, 98.6 mm vs. 28.6 mm; $p = 0.075$). The results of the bivariate correlation using Pearson's coefficient r revealed that core tissue length was not significantly correlated with the targeted LN size, regardless of whether the Jab technique (Figure 3a, $r = 0.11$; $p = 0.42$) or RA technique (Figure 3b, $r = 0.02$; $p = 0.87$) was utilized. Therefore, the yield of core tissue was assumed to be related to the tissue pathology rather than LN size or technique used.

Comparison of proportion of adequate cells found on the smear slides

Of the 61 LNs, six inadequate LN samples (grade 0) were obtained (9.8%). Five (8.2%) grade 0 slides were obtained using the Jab technique, and one (1.6%) grade 0 slide was obtained using RA technique. Regarding the numbers of
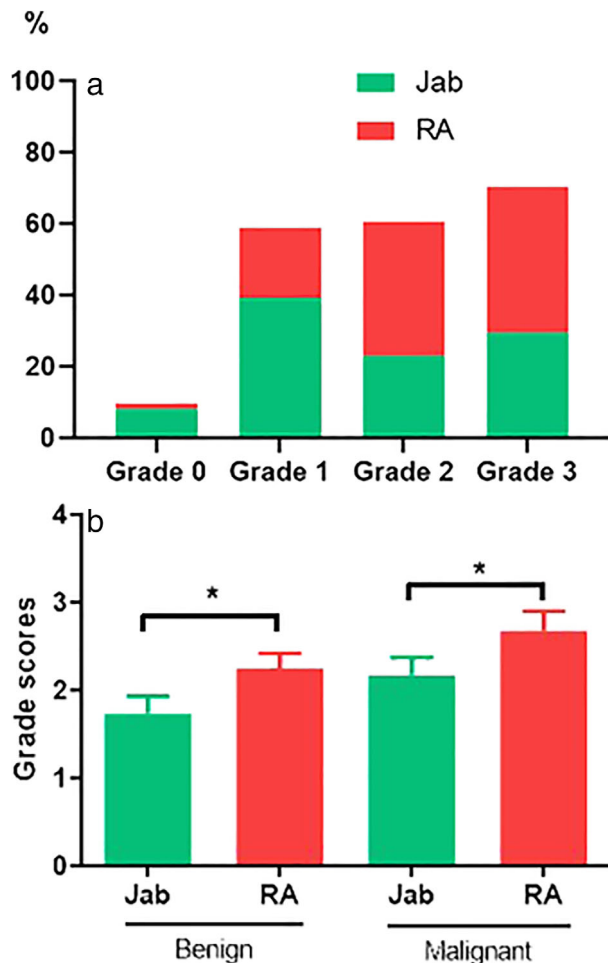
FIGURE 4 Comparison of subjective cytological grading obtained by the Jab and rotation aiding (RA) technique. (a) Significantly, inadequate samples (grade 0) were obtained from the Jab rather than RA technique. Considering the proportion of grades 2 and 3 samples, use of RA technique provided a statistically significant number of cytological samples (78.7% vs. 52.5%; $p < 0.001$ by McNemar's test). (b) Both in benign and malignant lymphadenopathies, the RA technique is superior to the Jab technique in slide grading score (*$p = 0.007$)

adequate cells in the total slides, the RA technique was associated with significantly better grading scores (2.39 ± 1.08 vs. 1.84 ± 1.14; $p < 0.001$ by Wilcoxon signed-rank test) and a higher proportion of slides with numerous significant cells (≥grade 2) than the Jab technique (Figure 4. 78.7% vs. 52.5%; $p = 0.002$ by McNemar's test) regardless of the anatomical site, etiology (benign or malignant), or size of the LN.

DISCUSSION

The length of samples obtained using our novel RA technique was the primary outcome of this study, and quality of the samples could be guaranteed, regardless of the LN size. While the RA method produced more samples in total, the difference was mostly due to the difference of

malignant lesions. Moreover, the cytological quality was better in samples obtained using RA technique, regardless of pathology.

The tissues obtained from EBUS-TBNA can be used for pathological diagnosis, including immunohistochemistry[7]. Larger tissue samples can be obtained via EBUS-TBNA than that with conventional TBNA. Furthermore, EBUS-TBNA can have a predictive value in the sonographic appearance of lymphadenopathy, such as the characteristics of malignant LN patterns.[3] EBUS-TBNA and mediastinoscopy reportedly achieved similar results for mediastinal staging of non-small cell lung cancer (diagnostic accuracy of 93% for both procedures)[8]. Additionally, both procedures showed high negative predictive values (EBUS-TBNA, 91%; mediastinoscopy, 90%). However, EBUS-TBNA has certain drawbacks: the yield of core tissue is smaller than that when using surgical biopsy. Additionally, the tissue sample size is associated with the number of aspirations[9], number of needle passes, needle size, and the experience of operators[10]; thus, the yield of EBUS-TBNA has several limitations, such as architectural assessment, performance of immunohistochemistry, and molecular analysis[11,12]. It requires multiple needle passes, and a rapid onsite cytopathology assessment is needed to improve its diagnostic accuracy[13]. Eventually, despite the significant results of the diagnostic EBUS-TBNA yield, the possibility of LN micrometastasis mandates that all negative results revealed by EBUS-TBNA are either followed up clinically or subjected to further evaluation, such as mediastinoscopy or surgical LN dissection, to confirm that the results are in fact true negatives[14]. The limitations may be attributed to an inadequate amount of the tissue in EBUS-TBNA samples. Consequently, we devised a way to obtain a larger amount of tissue with EBUS-TBNA.

The samples obtained with RA technique were longer, and these longer samples showed a statistically greater amount in malignant lesions ($p = 0.033$). Conversely, the pathological diagnosis of benign lymphadenopathy was difficult because the key cells required for confirming a diagnosis form a very small proportion of the enlarged LN(s), which might be the reason for the difficult diagnosis of benign lymphadenopathy using RA technique. Additionally, this result may be due to the small number of benign lymphadenopathy ($n = 36$), making it difficult to achieve a statistical difference. However, presumably, there would be no difference between the two techniques, even if more samples with tuberculous or reactive lymphadenopathy were collected, which was estimated with our statistical data (Figure 2b). Unlike other benign lymphadenopathy, although the number of patients with sarcoidosis was small, more samples of LNs affected by sarcoidosis were obtained from RA technique. Because of the small number of sarcoidosis patients ($n = 7$), statistical significance could not be reached ($p = 0.094$). If more samples with sarcoidosis were collected, it would have been clear if the diagnostic rate is higher with RA technique because the cells that form noncaseous granuloma in sarcoidosis are compacted together[15,16].

Finally, whether it was diagnosed or not, the RA technique showed more statistically significant results than the Jab technique in terms of adequate cells on the slide. These results were barely affected by the location, size, and etiology of the LN. Therefore, we carefully propose that the RA technique has superiority in the cytological evaluation of mediastinal lymphadenopathy. Additionally, there was a trend for obtaining more tissues in malignant lymphadenopathy with both techniques, suggesting that the yield of diagnostic cells was affected not by the size of the LN but by its etiology.

There were several limitations in our study. First, the sample size was small. However, it was calculated in advance as a pilot study since no other similar study could be found. Second, we admit that it was not possible to obtain the clear diagnostic yield for the LN of each method. To obtain a diagnostic yield for each method, the Jab and RA methods should be compared in large-scale randomized controlled trials with the primary outcome of accuracy in diagnosing malignant and benign lesions. Since the RA method is not an accepted tissue gain method yet, it could not be used as a tool to obtain tissue independently. Therefore, we employed the RA method only when the Jab method failed and a single arm study design was inevitable, as results of the LN biopsy using only the RA method were not available. Nevertheless, our study results could be valuable as the safety of RA technique was shown and this could provoke further large-scale studies. Although many studies evaluated the utility of newly introduced 19-gauge EBUS-TBNA needle, in the past 7–8 years, similar diagnostic yield has been reported for 19- and 22-gauge needles[17,18]. These results are evidence that the diagnostic yield does not increase with the needle caliber size. Therefore, we concluded that further innovative RA technique is needed to enhance tissue acquisition, which may be helpful for accurate diagnosis with more sufficient tissue samples than the conventional method. We hope our study results promote further studies to prove whether the RA method is a superior method for tissue acquisition.

Third, a relatively small number of patients with benign etiology were included, and the suggested benign lymphadenopathy was not pathologically diagnosed even though they did not undergo diagnostic surgery. On follow-up CT, the size of the enlarged LN either remained the same without any treatment or dramatically decreased after treatment with antituberculosis or systemic steroids. Lastly, this study was conducted from 2012 to 2014. EBUS biopsies were performed using an additional RA technique on 61 LNs from which sufficient samples could not be obtained with the traditional jab technique. To eliminate the operator dependent bias, only LNs obtained by one pulmonologist were enrolled. With the launch of improved EBUS needles by the Olympus company after the completion of our study, we decided not to report our results because we questioned the merit of the adjuvant RA technique. However, after several years, we are still finding some cases in which the EBUS needles do not obtain satisfactory LN biopsy results, and in these cases, the RA technique is helpful. Consequently, this study was revived and the data for this report reanalyzed. We believe our study will provoke future research into the RA technique and the technology around rotating needle devices.

In conclusion, the RA technique showed a valuable complementary function to a higher yield of sample tissues and cells in intrathoracic malignant lymphadenopathies obtained using the conventional Jab method. A future large-scale prospective, randomized controlled study is warranted to confirm our findings.

CONFLICT OF INTEREST
None of the authors have any conflicts of interest to disclose.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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