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Technical Factors and Lesion Characteristics Affecting Cytology Positivity in Conventional TBNA for Mediastinal Lymphadenopathy without ROSE

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ABSTRACT

Conventional transbronchial needle aspiration (cTBNA) for mediastinal lymphadenopathy without rapid on-site cytological evaluation (ROSE) remains an essential diagnostic modality, especially in resource-limited settings. This study aims to determine the technical factors and lesion characteristics that influence positive cTBNA cytology. This is an observational study that utilized the primary data of station 4R and station 7 mediastinal lymphadenopathy patients based on chest CT scans at the Persahabatan Hospital, Jakarta, Indonesia, from November 2019 to February 2020. An analysis of lymph node sites, lymph node size, needle size, needle puncture method, TBNA sampling sets, and the number of needles passed with positive cytology results were performed. A total of 33 subjects underwent 33 cTBNA procedures on lymph nodes stations 7 and 4R. There were 20 positive TBNA results (60.6%) consisting of 18 malignant and two *M. Tuberculosis* infection cases. Station 7 lymph nodes had greater positive TBNA results than station 4R lymph nodes (75% and 47.1%, respectively). Lymph node sizes ≥30 mm had more positive TBNA results than lymph node size <30 mm (53.8% vs. 36.4%). 21G needles showed more positive cTBNA results than 19G needles (68.2% and 45.5%, respectively). The TBNA sampling 1-2 set group showed 55.6% positive cytology, while the TBNA sampling 3-4 sets group showed 66.7% positive cytology results. A total of 10-14 passes showed 70% positive cTBNA results, while 15-20 needles passed showed 56.5% positive TBNA results. 21G TBNA needle was associated with positive cTBNA cytology without ROSE.

1. INTRODUCTION

Transbronchial needle aspiration (TBNA) is a minimally invasive modality that can be used as a diagnostic modality for mediastinal and hilar lymphadenopathy. Transbronchial needle aspiration allows for sampling of parabronchial and paratracheal lymph nodes and hilar and mediastinal lymph nodes [1]. Transbronchial needle aspiration can reduce the need for a more invasive and costly diagnostic procedure such as

mediastinoscopy. Transbronchial needle aspiration has high sensitivity and diagnostic accuracy for subcarinal and paratracheal lymph nodes in patients with a high probability of malignancy. Transbronchial needle aspiration aids in determining the required therapy for patients with cancer [2].

Endobronchial ultrasound-guided TBNA is superior to conventional TBNA (cTBNA). Rapid on-site cytopathological evaluation service (ROSE) also increases the accuracy of TBNA by providing results immediately so that it is easy to know whether sufficient TBNA samples have been obtained. If the obtained specimens are insufficient, a repeat TBNA procedure must be performed [3]. However, EBUS-TBNA is more expensive than cTBNA and is not widely available. ROSE could not also be done in every center. Therefore, cTBNA, precisely without ROSE, still has a role in diagnosing and staging lung cancer and mediastinal tumors, especially in resource-limited settings [2], [4]. The characteristics of technical factors that influence cTBNA cytology's results need to be studied and explored to increase the diagnostic yield.

2. Methods

This is an observational, prospective, single-center study at the bronchoscopy unit, Persahabatan Hospital, Jakarta, Indonesia, from November 2019 to February 2020. This study was given ethical approval (Protocol number 19-10-1240) from the Faculty of Medicine, Universitas Indonesia, and written informed consent was obtained from all participants.

2.1 Patient Selection and Study Procedures

Consecutive patients with lung cancer or mediastinal tumor aged >18 years with a minimal of station 7 and 4R lymphadenopathy >15mm (short axis) on chest CT undergone cTBNA at station 7 and/or 4R lymph nodes in patients with pro-diagnostic lung and/or mediastinum tumors in Persahabatan Hospital, Jakarta were included in this study. We gathered chest CT data performed within the last month. We excluded patients with severe acute respiratory failure, severe heart failure, unstable hemodynamics, severe coagulopathy disorders, and those who did not provide informed consent. TBNA was performed by four pulmonary intervention consultant operators with at least five years' experience of performing cTBNA in the bronchoscopy suite with local anesthesia (2% lidocaine) and mild sedation (intravenous Fentanyl 0.5 mg and Midazolam 2.5 mg) or under general anesthesia. The scope used was the Fuji Video Bronchoscope EB-580S Standard Type with a 2.2 mm working channel. The needle used was the eXcelon^{TM} Transbronchial Aspiration Needle with a 19- or 21-Gauge needle and sheath with an outer diameter of 1.8 mm. After puncturing the needle, continuous suction was applied to the catheter by an assistant through the syringe. When the blood return was confirmed to be absent, the needle was moved back and forth through the tracheal wall during the suctioning.

The aspirated cytology specimen was pushed out of the TBNA needle using a stylet and placed on a specimen slide which was then smeared uniformly using another specimen slide, followed by fixation with 96% ethanol. After that, the residual specimens were sprayed with air through the syringe onto a new slide, evenly smeared using another slide, and fixed with 96% ethanol. The TBNA cytology specimens were sent to the Anatomical Pathology laboratory according to the procedures performed at the Persahabatan Hospital.

Adequate lymph node specimens were defined as those able to demonstrate lymphoid tissue on cytological specimens. Other bronchoscopy diagnostic procedures, including bronchial lavage, bronchial brush, transbronchial biopsy, and endobronchial biopsy procedures, were performed if clinically indicated.

2.2 Data measurements and analysis

The data recorded for analysis were age, gender, tumor location, lymph node site, lymph node size, additional

diagnostic bronchoscopy procedures, needle size, needle puncture method, TBNA sampling set, number of needles passed, and pathology subtype. Lymph node sizes recorded were only for lymph nodes that underwent TBNA and measured on short-axis chest CT (Figure 1). The needle size used was determined through consecutive randomization. The needle puncture techniques were grouped into jabbing, piggyback, hub against the wall, and cough. A TBNA sampling set was a one-time cytology specimen collection. The number of needles passed was the number of punctures in a cytology specimen collection.

Statistical analyzes were performed using the Statistical Package Program for Social Sciences (SPSS for Windows 10.0; SPSS 20). A comparative bivariate analysis of categorical variables was performed on positive TBNA cytology using the chi-square test or Fisher exact test, where appropriate. P-value <0.05 was considered statistically significant.

3. Results

Adequate lymph node samples were obtained in all 33 patients. All TBNA procedures were performed in one lymph node site at every procedure. TBNAs were performed for diagnostic in 25 (75.8%) advanced-stage lung cancer patients and eight (24.2%) mediastinal tumor patients. The mean size of station 4 lymph nodes was slightly greater than station 7 lymph nodes (37.9±13.2 mm and 30.66±8.10 mm, respectively). Other diagnostic bronchoscopy procedures were performed in all patients, among which endobronchial biopsy was the most common procedure (Table 1).

Twenty (60.6%) TBNA cytology results were positive, while 13 (39.4%) were negative. There were no insufficient TBNA samples in this study. The greatest number of positive TBNA cytology were adenocarcinoma, which was 12 cases, followed by *M. Tuberculosis* infection in two cases, and the remainder with one case each were small cell carcinoma, non-Hodgkin's lymphoma, germ cells, yolk sac tumor, high-grade neuroendocrine carcinoma, and small cell carcinoma (Table 2). The cytopathological results of cTBNA preparations were stained with Papanicolaou staining under 40x magnification 4 (Figure 2).

In this study, a more significant number of positive TBNA was found in lymph node site 7, lymph node size ≥30 mm, 21G TBNA needle, needle puncture with the hub against the wall technique, collecting 3-4 sets of TBNA cytology specimens and 10-14 needles passed. A total of 17 and 16 TBNA procedures were performed at station 4R and station 7 lymph nodes. Station 7 lymph nodes yielded more positive results than station 4R lymph nodes (75% vs. 47.1%). The usage of 21G needles also showed greater positive cytology compared to 19G needles (36.4% vs. 72.7%). The needle puncture method most often used is the hub against the wall method (45.6%), while the coughing method was not used. The hub against the wall method gave 73.3% positive TBNA diagnostic results higher than other methods. The most common TBNA sampling set was 1-2 sets of TBNA (54.5%) with a positive cytology result of 55.6%. The most frequent number of needles passed, which was performed by bronchoscopy operators, was 15-20 punctures (69.7%) for each TBNA sampling set. However, 10-14 punctures resulted in a 70% positive cytology result (Table 3). We encountered immediate complications in the form of intrabronchial hemorrhage or pneumothorax within 24 hours of the procedure in all procedures.

4. Discussion

This study was an observational study that directly assessed the association between the characteristics of lymph node lesion and cTBNA technique without ROSE on positive cytology. We received a 100% adequate sample and a positive cytology rate of 60.6%. Based on the assessment of lymph node location and size, needle size, needle puncture method, TBNA sampling set, and the number of needles passed, we reported that 21G TBNA needles yielded more positive cytology results than 19G needles. The subjects of this study

were patients with advanced-stage lung cancer and mediastinal tumors, which required cTBNA [5]. A systematic review stated that localized lymph node metastases were a predictor of positive TBNA; meanwhile, lymph node staging did not affect the TBNA results. The tumor location, either central or peripheral, also did not significantly influence positive TBNA results. The types of small cell cancer were reported in several studies to be the best predictors of positive TBNA [6]. Station 7 and 4R lymph nodes are the most common sites for cTBNA, with a 65-90% diagnostic yield [7], [8]. These lymph nodes showed higher diagnostic results than lymph nodes of the same size in the other stations [9]. Previous studies reported adequate samples of cTBNA without ROSE to be as high as 73%, and more than half were diagnostic samples [10]. cTBNA with ROSE was also reported to have similar proportions of diagnostic samples in other studies [11]. Based on the Papanicolaou classification, most TBNA cytology was categorized as malignant because tumor cells are found on TBNA cytology specimens to establish a definite diagnosis of neoplasms. At the same time, only a small portion is benign. The benign category, also known as specific benign lesions, includes all benign neoplasms, inflammatory processes, and infections such as fungi, mycobacterium, and bacteria. A total of 13 TBNA cytology results were included in the non-diagnostic category or also called non-diagnostic specimens. In the non-diagnostic category, cytological preparations do not contain cellular components of the specimens. The cellular components can be distorted due to blood, artifacts, poor preservation, or poor handling of the specimen, resulting in difficulty making a pathological diagnosis. Specimens included in this category are specimens composed of benign cellular components such as airway epithelium, macrophages, and inflammatory cells; thus, they do not adequately describe the lesions present in the airway [12].

There is no robust clinical evidence reporting the ideal needle puncture method since all methods can be performed with caution [13]. The size of the needle provides different cytological positivity. Stated that using the 19G TBNA needle showed a higher sensitivity than the 22G TBNA needle (85.5% vs. 52.7%) [14]. Stated that the 19G TBNA needle resulted in 80% positive results than the 22G TBNA needle, which yielded 66% positive results; however, the study did not use 19G TBNA needles for small (<1 cm) sized lymph nodes. The 19G and 22G TBNA needles had the same diagnostic results in large lymph nodes (>2 cm), 82% and 77%, respectively [15]. Another study that utilized 19G needles with ROSE in a small number of samples yielded 89% positive results without significant complications [16]. Experts have suggested that before using a 19G needle, one must have performed at least 25 TBNAs using a cytology needle.

The TBNA sampling set and the number of needles passed can also influence TBNA cytology. Reported that, in malignant cases, the mean number of TBNA aspirations required until a positive TBNA result was obtained was 2.5 ± 2.0 with ROSE and 3.7 ± 1.6 without ROSE [7]. Reported that the first TBNA needle puncture passing through the lymph node contributes to providing the highest proportion of positive TBNA results. TBNA results increased by almost 50% from the previous results when the next TBNA needle puncture showed 88% of the positive TBNA results became plateau after the total number of TBNA punctures that passed through the lymph nodes were three times and 94% positive TBNA results became plateau after the number of TBNA punctures that passed through the lymph nodes were four times [14].

This study did not assess the relationship between the operators and positive TBNA cytology. The four operators were considered to have sufficient experience in performing cTBNA. Reported that operators with experience in performing TBNA procedures could improve the TBNA diagnostic results and more frequently perform TBNA procedures to aid with diagnosis. Concluded that an experience of at least 50 TBNA procedures is required to achieve excellent TBNA diagnostic results [18]. In this study, there were no serious or life-threatening complications associated with the TBNA procedure. On bronchoscopy reports, complications of severe bleeding, pneumothorax, or pneumomediastinum associated with cTBNA were

absent, including the risk of bleeding associated with damage to the vascular due to the use of a histology needle [19]. The risk of complications from cTBNA reported in various studies ranged between 0% to 8% [20]. Complications due to infections developed in 0.19% [21]. Therefore, cTBNA is a relatively safe procedure with a low incidence of complications. This study has several limitations; among others, the number of research subjects included in the final analysis was short of the minimum sample size, affecting the relationship between the variables assessed and the outcome. This was caused by the COVID-19 pandemic, which stopped the operators from performing bronchoscopy. Both positive and negative TBNA results could not be confirmed by biopsy because all patients were in the advanced stage, and surgical resection could not be performed.

5. Conclusion

Lymph node cTBNA without ROSE is a feasible, useful, and safe diagnostic technique for lung or mediastinal tumors, especially in resource-limited settings. cTBNA procedures need to be routinely performed on stations 4R and 7 lymph nodes, especially those with a diameter of ≥30 mm, to help increase the diagnostic level and improve the operator skills. Positive cytology results were associated with 21G TBNA needles. Further studies on predictor factors of positive cytology results from more robust and large-scale studies are recommended.

6. References

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Table 1. Baseline characteristics of the study population

Variable	Total n (%) (n = 33)
Mean age ± SD, years	49.51±17.19
Gender	
Male	23 (69.7)
Female	10 (30.3)

Tumor location	
Lung	25 (75.8)
Mediastinum	8 (24.2)
Lymph node site	
4R	15 (45.5)
7	18 (54.5)
Mean lymph node size ±SD, mm	
4R	37,9±13,2
7	30,66±8,10
Additional diagnostic bronchoscopic procedures	
Endobronchial biopsy	14 (42.4)
Transbronchial biopsy	2 (6)
Bronchial brush	10 (30.3)
Bronchial lavage	33 (30.3)

Table 2. Cytologic results of TBNA

Pathology subtype	Total n (%)
Malignant	
Adenocarcinoma	12 (36.4)
Small cell carcinoma	1 (3)
Non-small cell lung cancer	1 (3)
Neuroendocrine	1 (3)
Non-Hodgkin Lymphoma	1 (3)
Germinal cell tumor	1 (3)
Yolk sac tumor	1 (3)
Benign	
Tuberculosis	2 (6)
No specific malignant/benign findings	13 (39.4)

Table 3. Factors influencing positive TBNA cytology

Variable	Total (n = 33)	Positive (n = 20)	Negative (n = 13)	*p*-value
Lymph node site				0.101
4R	17 (51.5)	8 (47.1)	9 (52.9)	
7	16 (48.5)	12 (75)	4 (25)	
Lymph node size				0.392
< 30 mm	16 (48.5)	6 (37.5)	10 (62.5)	
≥ 30 mm	17 (51.5)	9 (53)	8 (47)	
Needle size				0.048
19 G	11 (33.3)	4 (36.4)	7 (63.4)	
21 G	22 (66,7)	16 (72.7)	6 (27.3)	
Needle puncture method				0.619
hub against the wall	15 (45.6)	11 (73.3)	4 (26.7)	
jabbing	12 (36.3)	5 (41.7)	7 (58.3)	
piggyback	6 (18.1)	4 (66.7)	2 (33.3)	
TBNA sampling set				0.386
1-2 set	18 (54.5)	10 (55.6)	8 (44.4)	
3-4 set	15 (45.5)	10 (66.7)	5 (33.3)	

Number of Needle Passes				0.701
10-14	10 (30.3)	7 (70)	3 (30)	
15-20	23 (69.7)	13 (56.5)	10 (43.5)	

TBNA: Transbronchial needle aspiration

Figure Legend

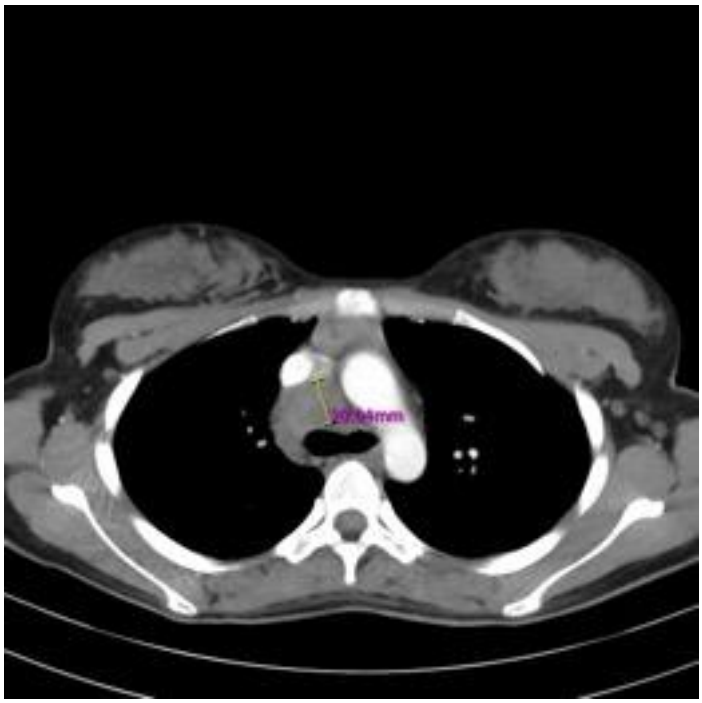

Figure 1. Axial computed tomography (CT) image showing measurements of a right lower paratracheal lymph node (station 4R)

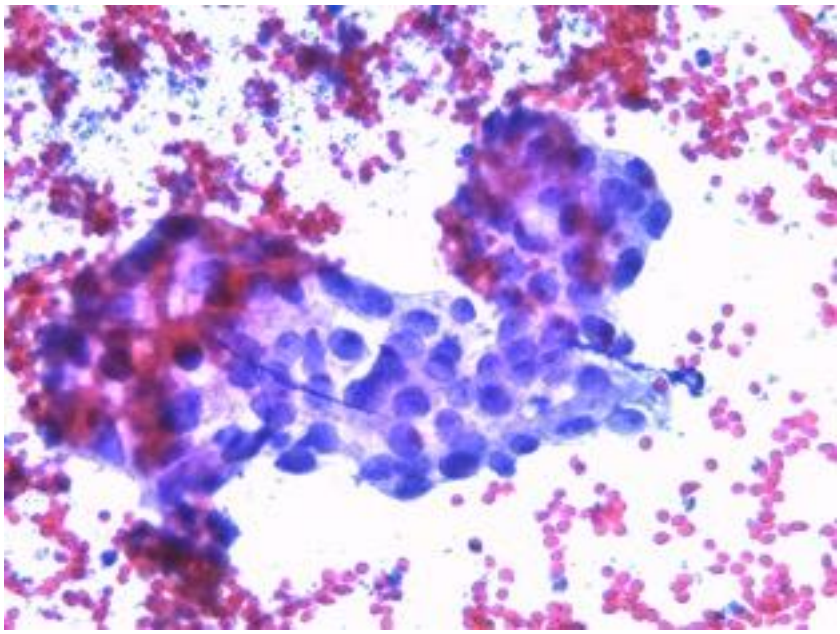

Figure 2. Cytologic image of cTBNA specimen showing malignant tumor cells, non-small cell adenocarcinoma type.