Original Article

Novel ProCore 25-gauge needle for endobronchial ultrasound-guided transbronchial needle aspiration reduces the puncture time and frequency, with comparable diagnostic rate for mediastinal and hilar lymphadenopathy

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Keywords
Endobronchial ultrasound; hilar lymphadenopathy; mRNA extraction; RNA concentration; transbronchial needle aspiration.

Abstract
Background: The ProCore 25-gauge needle is a novel specifically designed puncture needle for endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA), which may improve the puncture efficiency of the procedure while ensuring the diagnostic rate. The aim of the present study was to evaluate the diagnostic accuracy, mRNA yield, and complication rate of 25-gauge needles compared to those of 22-gauge needles in the evaluation of mediastinal and hilar lymphadenopathy.

Methods: A total of 39 patients undergoing EBUS-TBNA at our institution were evaluated. All the procedures were performed by an experienced endoscopist formally trained in interventional pulmonology. Both the traditional 22-gauge and ProCore 25-gauge needles were used at each lymph node station. For all specimens obtained via EBUS-TBNA, 50 μL was used to extract mRNA and detect the RNA concentration, whereas the other part was sent to the pathological evaluation. χ² test and t-test were performed to determine the differences between the two types of the needles. A P-value of <0.05 was considered significant.

Results: A total of 88 lymph nodes were punctured by the two needles separately. The diagnostic accuracy for each puncture between the two needles did not show any significant difference (P > 0.05). No serious procedure-related complications were reported. In addition, the mRNA concentration did not differ between the two types of needles (P > 0.05).

Conclusion: The ProCore 25-gauge needle gained a similar diagnostic yield with less puncture time and frequency compared with the 22-gauge needle.

Introduction
Initially, endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) was performed mainly for lung cancer staging, but it is now used as a standard of care for the diagnosis of mediastinal and hilar lymphadenopathy from various causes.1–3 It is possible to sample multiple mediastinal and hilar lymph nodes (LNs) in the same procedure under moderate sedation in outpatient settings with high accuracy and minimal complications using EBUS-TBNA.4 It has been reported as a minimally invasive and safe procedure in multiple systematic reviews and meta-analyses.5,6

EBUS-TBNA has been used to diagnose lung cancer, sarcoidosis, tuberculosis, and lymphoma, and has a significant impact on their subsequent management.3 It guides
 treatment in patients with lung cancer by diagnosing, subtyping, staging, molecular subtyping, and also restaging when indicated. Although computed tomography (CT) and positron emission tomography (PET) scan can show mediastinal lymphadenopathy, pathological confirmation is still required to complete the staging evaluation. Compared to mediastinoscopy, EBUS-TBNA is a minimally invasive technique with similar diagnostic rate, less postoperative pain and decreased complication rates. 

The quality of the samples punctured by EBUS-TBNA is very crucial for a precise diagnosis. There may be several potential factors that impact the quality of the samples, such as the size and type of the puncture needle. In order to identify the affect, several studies have been carried out. There are many types of puncture needles for this procedure. Traditionally, the 22-gauge needle (Olympus, Tokyo, Japan) has been the most commonly used needle for EBUS-TBNA due to its ease of use and better sampling. Recently, a new type of 25-gauge puncture needle has been released. Compared with the 22-gauge needle, it is more slender and the unique design of the side-cutting groove allows tissue to enter the needle lumen and aids in procuring a complete tissue specimen. However, it is uncertain if this novel core needle achieves comparable diagnostic accuracy with a shorter puncture time, lesser frequency, and lower complication rate.

The aim of the present study was to evaluate the diagnostic accuracy, mRNA yield, and complication rate of the 25-gauge needle compared to that of the 22-gauge needle in the evaluation of mediastinal and hilar lymphadenopathy.

Methods

Patients

Patients with clinical and radiological features of mediastinal or hilar lymphadenopathy were enrolled in the study. According to their doctor’s advice, the patients underwent EBUS-TBNA for tissue confirmation. Chest computed tomography (CT) was performed prior to bronchoscopic examination. Based on CT findings, at least one enlarged mediastinal or hilar lymphadenopathy >10 mm in the short axis was observed in all the patients. Patients were usually given local anesthesia unless they were unable to tolerate local anesthesia or required general anesthesia before surgery. Written informed consent was obtained from all patients included in this study. The study protocol was approved by the Institutional Review Board of Shanghai Pulmonary Hospital.

Procedural technique

The target LNs were determined before the operation based on the chest CT scan. If there were multiple target LNs for the same patient, we followed the order of N3-N2-N1 and puncture from the farthest to the nearest. Each LN was punctured three times with a 22-gauge needle (NA-201SX-4022; Olympus) and once with a 25-gauge needle (ECHO-HD-25-EBUS-O-C; Cook Medical, Bloomington, IN, USA). Under sedation, aspiration of LNs was performed 15 times for each puncture. The order of the puncture needles was randomly arranged to exclude the bias which was caused by the different order of puncture needles. The operation process was carried out according to the standard procedure by the same experienced endoscopist, using an Olympus EBUS-TBNA system (BF-UC260FW endoscope and CV-260SL main engine, Olympus).

Pathological diagnosis and RNA concentration detection

The specimens obtained with each needle pass were extracted to the slides by pushing the stylet and flushing the syringe with air.

For all the specimens obtained via EBUS-TBNA, 50 μL was immediately placed in an RNase-free Eppendorf tube containing 500 μL of RNA later (Cat No.AM 7021, Life Technologies) and stored at −20°C. Once the specimen was confirmed by our pathologists to have more than 50 tumor cells, the RNA later preserved part was removed from storage to extract mRNA. RNeasy Mini Kit (Qiagen, Hilden, Germany) was used to extract the mRNA, which was subsequently quantitated by the NanoDrop 2000 Spectrophotometer (Thermo Scientific, Waltham, MA, USA).

The other specimens were sent for pathological evaluation. If material such as core tissue was found on the slide, it was removed for histological analysis. It was formalin fixed, processed, paraffin embedded, and subsequently stained with hematoxylin and eosin. Immunohistochemical staining was performed only by a pathologist. The remaining slides were smeared and fixed in 100% ethanol for off-site cytological analysis. They were prepared with staining. Cytological and histological evaluations were performed by a single pathologist who was blinded to the types of the needles. The pathologists also assessed the quality of the specimen. The experimental process is shown in Fig 1.

Statistical analysis

Categorical data were analyzed by the χ² test as appropriate. Continuous data were analyzed by the t-test. All statistical analyses were performed using SPSS 20.0 (SPSS Inc., Chicago, IL, USA) and a P-value of <0.05 was considered significant.
Results

A total of 39 patients were enrolled in the study including 20 men and 19 women. The average age was 63 years (range, 43–73 years). During the EBUS-TBNA procedure, general anesthesia and local anesthesia were given to nine and 30 patients, respectively. Of the 39 patients, 74% underwent puncture of two target LNs and 26% underwent puncture of three target LNs (Table 1).

A total of 88 LNs were punctured separately by the two needles. The images of chest CT and ultrasonography during puncture from one case are shown in Fig 2. There was no significant difference in the pathological diagnosis between the two groups by McNemar’s test ($P = 0.21$, Table 2).

The most common complications were bleeding and hypoxia during puncture. There were 12 cases of bleeding at the puncture site by the novel needle, and only one patient was given further treatment. A total of 15 bleeding cases occurred using the 22-gauge needle, and four patients were given further treatment. A few patients developed mild hypoxia during the examination but improved after symptomatic treatment. No serious procedure-related complications were observed. In addition, the mean time cost per puncture was not different between the two needles (five minutes vs. four minutes) (Table 3).

The extracted mRNA underwent quality testing to ensure that it was not degraded during the procedure. The mRNA concentration did not differ between the two types of needles ($P > 0.05$, Fig 3).

Discussion

This study demonstrated that the diagnostic outcomes, including accuracy of diagnosis and adequacy of sample, were comparable between the novel 25-gauge and traditional 22-gauge needles. The ProCore needle reduced the puncture time and frequency while achieving the same diagnostic rate as that of the traditional needle.

EchoTip ProCore is designed to acquire cytological and histological samples (cells) during endobronchial ultrasonography. The Menghini tip was used for the ProCore needles, which is much more safe and sharper than the traditional tip. Further, we observed that the ProCore needle more easily reached the LNs of each station, especially stations 10 LNs. This is probably because the ProCore needle has better elasticity and greater puncture force. In addition, the unique design of the CoreTrap allows tissue to enter the needle lumen and aids in procuring a complete tissue specimen (Fig 4).

Our study showed that the novel 25-gauge needle could obtain enough samples for diagnosis though a single-pass puncture. The mean time of each puncture was similar between the two needles. Therefore, the novel needle could greatly reduce the total puncture time, risk of puncture, and patient’s discomfort.

In recent years, progress has been made in the development of molecular targeted drugs and immune therapy for lung cancer.11–19 Detection of gene mutations is important

Table 1 The demographic and clinical characteristics of the patients

| Characteristics                              | N (%)   |
|---------------------------------------------|---------|
| Age, years, median (range)                  | 63 (43–73) |
| Sex                                         |         |
| Male                                        | 20 (51%) |
| Female                                      | 19 (49%) |
| Anesthesia                                  |         |
| General anesthesia                          | 9 (23%)  |
| Local anesthesia                            | 30 (77%) |
| Number of sampled LNs for each patient      |         |
| 2                                           | 29 (74%) |
| 3                                           | 10 (26%) |
| The stations of sampled LNs                 |         |
| 2                                           | 1 (1%)   |
| 4                                           | 37 (42%) |
| 7                                           | 23 (26%) |
| 10                                          | 9 (11%)  |
| 11                                          | 18 (20%) |

LNs, lymph nodes.
for lung cancer, especially NSCLC. Identification of the tumor’s molecular characteristics is crucial for subsequent management of the disease. Several studies showed that EBUS-TBNA sampling is a feasible approach in detecting EGFR mutations, ALK rearrangements, and other gene alterations due to sequencing, fluorescence in situ hybridization, or polymerase chain reaction analyses. Both DNA and RNA can be used to detect mutations by different methods. The detection frequency of mutation is much higher in RNA than in DNA because genes with driver mutations have high transcriptional activity. However, RNA is more susceptible to degradation than DNA during sample collection and RNA extraction. Since RNA is more susceptible to degradation and more sensitive to the process of obtaining tissue specimens, we chose to detect the RNA concentration of the tissue in order to indirectly reflect the time of the puncture, and simultaneously prove that the amount of tissue obtained using the new puncture needle is sufficient for pathological examination.

Table 2 Pathological diagnostic results of all sampled lymph nodes by the traditional 22-gauge needle and novel 25-gauge needle

| Traditional 22-gauge needle | Novel 25-gauge needle |
|----------------------------|-----------------------|
| Adenocarcinoma             | 3                     | 2                     |
| Squamous cells             | 2                     | 0                     |
| NSCLC                      | 6                     | 2                     |
| SCLC                       | 5                     | 1                     |
| Malignant tumor cells      | 1                     | 0                     |
| Negative                   | 5                     | 6                     |
| Total                      | 22                    | 6                     |

Table 3 Complications and puncture time

| Complications                  | Novel 25-gauge needle | Traditional 22-gauge needle |
|--------------------------------|-----------------------|----------------------------|
| Bleeding                       | 12 (31%)              | 15 (38%)                   |
| No intervention               | 11 (28%)              | 11 (28%)                   |
| necessary                      | 1 (3%)                | 4 (10%)                    |
| Local hemostasis with thrombin |                      |                            |
| SO₂ decrease                   | 2 (5%)                | 3 (8%)                     |
| >80%                           | 2 (5%)                | 3 (8%)                     |
| Mean time per puncture (minutes) | 5                    | 4                           |

NSCLC, non-small cell lung cancer; SCLC, small cell lung cancer.

In conclusion, with less puncture time and frequency, the novel 25-gauge needle exhibited a similar diagnostic yield compared with the traditional 22-gauge needle. This may improve operational efficiency and expand the clinical applications. Further studies need to be carried out to identify the various possible applications of this procedure.
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Disclosure

The authors have no conflicts of interest to declare.

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