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ORIGINAL ARTICLE

Conventional transbronchial needle aspiration is promising for identifying *EGFR* mutations in lung adenocarcinoma

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Abstract

Background: Conventional transbronchial needle aspiration (TBNA) is advantageous for the one-step diagnosis and staging of lung adenocarcinoma under topical anesthesia and conscious sedation. We examined its efficacy for identifying *EGFR* mutations.

Methods: Forty-seven patients with proven or suspected lung adenocarcinoma indicated for hilar-mediastinal lymph node (LN) staging between June 2011 and December 2017 were enrolled. The cellblock was prepared using the plasma-thrombin method. TaqMan PCR was used to detect mutations. Considering cost effectiveness, only the sample with the highest tumor cell fraction in the same patient was chosen for analysis.

Results: TBNA provided positive results of malignancy in 27 patients. Seventeen patients (63.0%) had cellblocks eligible for mutation testing. Bronchial biopsy (n = 6), neck LN fine needle aspiration (n = 1), and brushing (n = 1), provided higher tumor cell fractions for analysis in eight patients. TBNA was the exclusive method used in nine patients (19.1%). For patients with an inadequate TBNA cellblock, bronchial biopsy (n = 5), neck LN fine needle aspiration (n = 3), computed tomography-guided transthoracic needle biopsy (n = 1), and brushing (n = 1) were used for analysis. Modification to specimen processing to prevent exhaustion by cytology after June 2016 improved the adequacy of cellblock samples (9/10, 90% vs. 8/17, 47.1%; P = 0.042).

Conclusions: These findings suggest the promising role of conventional TBNA and highlight the challenges of doing more with less in an era of precision medicine.

Introduction

The presence of mutations in *EGFR* predicts the effectiveness of EGFR-tyrosine kinase inhibitors (TKIs).[1–3] Taiwan's National Health Insurance (NHI) approved EGFR-TKIs as first-line treatment according to *EGFR* mutation status in June 2011. We have previously reported the learning curve and safety of conventional transbronchial needle aspiration (TBNA), its use after the introduction of positron emission tomography-computed tomography (PET-CT) staging, and the practice of using larger 19 G needles.[4–7] Horiike *et al.* revealed the suitability of samples obtained through TBNA, not limited to lymph nodes (LNs), to analyze *EGFR* mutation in non-small cell lung cancer patients.[8] Herein, we try to better understand the contribution of conventional TBNA toward the determination of *EGFR* status in patients with lung adenocarcinoma compared to other diagnostic techniques.

Methods

Patients and conventional transbronchial needle aspiration (TBNA) sampling

We followed National Institute for Health and Care Excellence (NICE) guidelines with minimal staging and the principle of cost-effectiveness.[9] A hilar-mediastinal LN with a short axis diameter of > 10 mm on CT or ^{18}F-fluorodeoxyglucose uptake with > 2.5 standardized uptake value on PET-CT was classified as positive. Preliminary analysis of the suitability of conventional TBNA was conducted by assessing the patient's condition, including performance and comorbidities, LN size, and location. In selected cases with LN < 5 mm, an unusual location, or lacking an endobronchial landmark (e.g. upper paratracheal #2), endobronchial ultrasonography-guided TBNA (EBUS-TBNA) or mediastinoscopy is a more adequate diagnostic modality.[6]

Patients with either proven or suspected lung adenocarcinoma with a hilar-mediastinal LN indicated for conventional TBNA staging between June 2011 and December 2017 at the Sun Yat-Sen Cancer Center, a 200-bed hospital, were enrolled. Bronchoscopy was performed under conscious sedation and topical anesthesia. TBNA was performed in the order of contralateral, midline, and ipsilateral LNs. LNs were aspirated according to International Association for the Study of Lung Cancer (IASLC) LN station and the Wang TBNA staging system.[10,11] Correlation was indicated, as the latter is more specific and descriptive from the perspective of endobronchial biopsy.[12]

Processing of TBNA specimens

Specimens in the TBNA needle were flushed by air, air dried, and fixed in alcohol. The air-dried smears were stained with Liu's stain and evaluated by an onsite cytopathologist to confirm the "adequate" cell material, which was defined as material sufficient for a specific diagnosis or the presence of lymphocytes on the specimen. A Papanicolaou stain was used for the alcohol fixed slides. The needles were then rinsed in 15 mL of sterile saline. The needle rinse was processed by cellblock slide production. Incidental histology specimens were fixed in formalin for subsequent examination.

The specimen processing method in our institute was modified in June 2016. To avoid exhausting the specimens in cytology analysis, the material flushed and rinsed from the needle was all used to process the cellblock, except for the first needle pass, which was prepared with Liu's stain for onsite examination.

DNA extraction and EGFR mutation testing

The cellblock obtained by TBNA was prepared using the plasma-thrombin method (ThinPrep) (Fig 1).[13,14] DNA was extracted from the paraffin-embedded samples. TaqMan PCR and amplification-refractory mutation system PCR (Thermo Fisher Scientific, Waltham, MA, USA) were used to detect the activating and resistant mutations in exons 18–21 of the *EGFR* gene. Specifically, this assay detects mutations in codons 719, 768, 858, and 861. Detection tests for exon 19 deletions and exon 20 insertions are based on using PCR with high-resolution gel-electrophoresis.

Considering cost effectiveness, only the sample of various diagnostic techniques with the highest tumor cell fraction in the same patient was chosen for analysis.

Endpoints

A retrospective chart review was performed, and the following clinical and laboratory data were analyzed: (i) the mean LN short-axis diameter and needle passes, and the sensitivity and accuracy for malignancy by conventional TBNA; (ii) the percentage of the cellblock that could be used for *EGFR* mutation testing, the detection rate of *EGFR* mutation by conventional TBNA, and comparison with other diagnostic techniques (e.g. bronchial biopsy including transbronchial lung biopsy [TBLB], bronchial brushing, CT-guided transthoracic needle biopsy [TTNB],

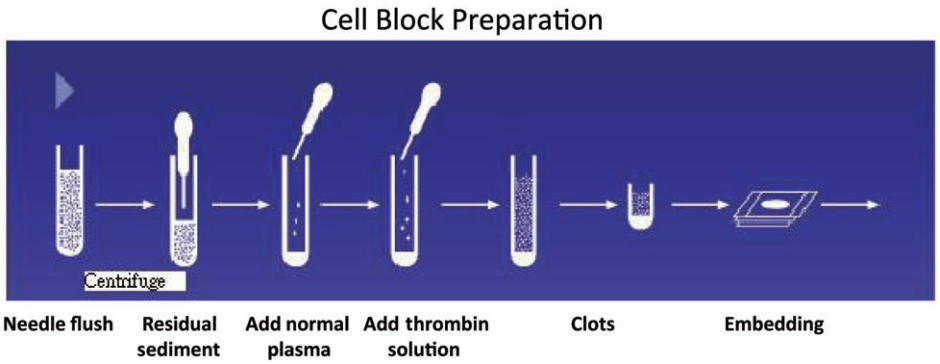

Figure 1 The plasma-thrombin method (ThinPrep) for cellblock preparation.

or neck LN fine needle aspiration [FNA] in the same patient); (iii) the overall response rate (partial response [PR]%), and the disease control rate (PR% + stable disease [SD]%), evaluated using Response Evaluation Criteria in Solid Tumors[15] in patients with *EGFR* mutations that favored the use of EGFR-TKIs; and (iv) the percentage of the cellblock that could be used for *EGFR* mutation testing after modifications to specimen processing methods in June 2016.

Ethical committee approval

All patients signed informed consent to undergo any procedures and for the retrospective review of their data. The Institutional Review Board of the Sun Yat-Sen Cancer Center approved this study (No. 20180129A). The study was also approved by the Ethics Committee of the hospital, and was conducted in accordance with the ethical principles stated in the Declaration of Helsinki or the guidelines on good clinical practice.

Statistical analysis

Patient demographics and disease characteristics were summarized using descriptive statistics. Continuous variables were compared using the two-sample Student's *t*-test, whereas categorical variables were compared using chi-square or Fisher's exact tests. A P value of < 0.05 for comparisons was considered to represent statistical significance. All analyses were performed using SAS version 9.4.

Results

During the study period, 130 patients underwent conventional TBNA and a total of 167 LN stations were sampled. TBNA provided positive results of malignancy in 69 patients. Sarcoidosis and tuberculosis were identified in seven and two patients, respectively. A true negative result was confirmed in 15 patients. The sensitivity and accuracy were 67.8%, and 71.5%, respectively.

Among these patients, TBNA was requested for the diagnosis and staging of lung adenocarcinoma in 47 patients (mean age 58.7 years, range 32–83; 17 women). A total of 56 LNs (right paratracheal #4R, 28; anterior carina #4R, 10; right upper hilar #11R, 4; subcarina #7, 4; right main bronchus #4R, 4; left paratracheal #4L, 3; left main bronchus #4L, 1; left hilar #11L, 1; and posterior carina #7, 1) were aspirated.[9–11] The mean LN short-axis diameter was 12.2 ± 0.4 mm and the mean number of needle passes was 3.2 ± 0.6.

TBNA provided positive results of malignancy in 27 patients with lung adenocarcinoma. A true negative result was confirmed in six patients. The sensitivity and accuracy were 65.9% and 70.2%, respectively. Among them, 17 patients (63.0%) had cancer cells on the cellblock eligible for *EGFR* mutation testing. Bronchial biopsy including TBLB ($n = 6$), neck LN FNA ($n = 1$), and brushing ($n = 1$) provided higher tumor cell fractions for analysis in eight patients. TBNA was the exclusive method used in nine patients (19.1%) (Figs 2–3a). For patients with an inadequate TBNA cellblock, bronchial biopsy including TBLB ($n = 5$), neck LN FNA ($n = 3$), CT-guided TTNB ($n = 1$), and brushing ($n = 1$) were used for analysis. *EGFR* mutations were detected in 16 patients (59.3%): 11 had an in-frame deletion of exon 19, and 5 had a point mutation (L858R) of exon 21. During follow-up, the response rate to EGFR-TKIs was 75% (12/16) and the disease control rate was 87.5% (14/16).

In the 14 patients with a false negative TBNA result, video-assisted thoracoscopic surgery LN biopsy ($n = 4$), CT-guided TTNB ($n = 4$), bronchial biopsy ($n = 3$), neck LN FNA ($n = 2$), or brushing ($n = 1$) provided a salvage specimen for *EGFR* mutation testing. *EGFR* mutation was detected in eight patients (57.1%): three had an in-frame deletion of exon 19, three had a point mutation (L858R) of exon 21, and two had an exon 20 in-frame insertion. The latter is associated with decreased EGFR-TKI sensitivity. The response rate to EGFR-TKIs was 50% (3/6) and the disease control rate was 83.3% (5/6).

After modifications to the specimen processing method in June 2016, there was a significantly higher rate of positive cellblocks eligible for *EGFR* mutation testing (9/10, 90% after vs. 8/17, 47.1% before; $P = 0.042$) (Fig 3b,c).

Discussion

Conventional TBNA is a useful method for hilar-mediastinal diagnosis and staging.[11,16] It is a less invasive procedure for patients than mediastinoscopy or mediastinotomy. EBUS-TBNA has been introduced using real-time imaging to confirm that the needle is placed within the LN, and has resulted in a higher diagnostic yield.[17–19] Cumulative studies have also shown the adequacy of EBUS-TBNA for molecular analysis.[20–22] When compared to the fixed penetration angle of EBUS-TBNA, the flexibility of the conventional TBNA needle allows for a more versatile approach to target the LN, particularly peripherally. The expense of setting up an EBUS-TBNA system and specialized single-use needles have limited its widespread use, with the majority performed in tertiary centers. However, EBUS-TBNA could assist trainees to become more confident to perform conventional TBNA and shorten the learning curve. Complementary use of conventional TBNA and EBUS-TBNA is more cost-effective for hilar-mediastinal staging in an era of constrained medical costs.

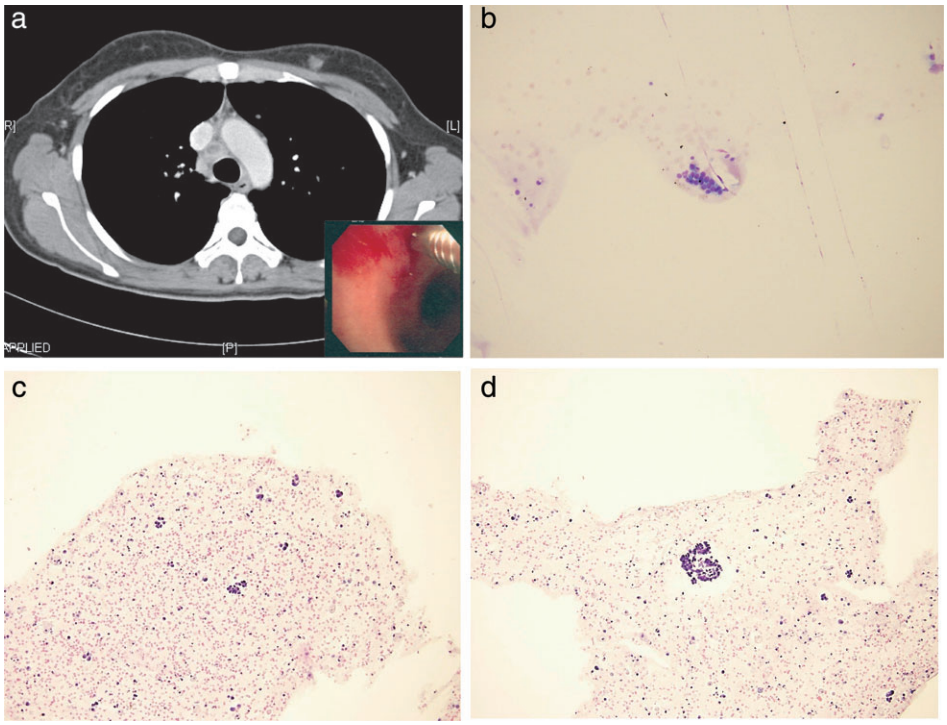

Figure 2 A 37-year-old female patient with right upper lobe lung adenocarcinoma. Analysis of a cellblock obtained via conventional transbronchial needle aspiration of the right paratracheal #4R lymph node (**a**) was negative for *EGFR* mutation (**b-d**) (hematoxylin and eosin, original × 100).

In this study, we sought to analyze the adequacy of specimens obtained by conventional TBNA for detecting *EGFR* mutations. TBNA provided positive results of malignancy in 27 out of 41 lung adenocarcinoma patients (sensitivity, 65.9%), which is decisive for the choice of treatment modalities (i.e. surgical versus non-surgical). Seventeen of

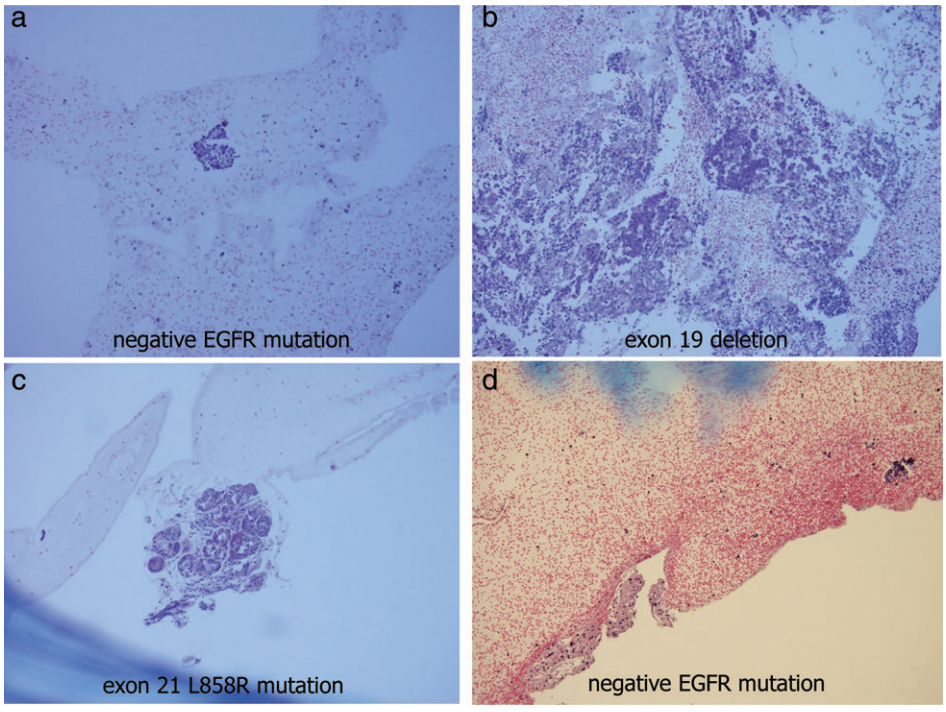

Figure 3 Conventional transbronchial needle aspiration using different needles and specimen processing methods for *EGFR* mutation testing in lung adenocarcinoma. The cellblock obtained using a 21 G NA-2C-1 needle (Olympus Optical, Tokyo, Japan) from the same patient in Figure 2. (**a**) The cellblocks obtained using a 21 G needle from the right paratracheal #4R LN with all needle pass rinses processed for the cellblock (except the first for onsite examination) in (**b**) a 45-year-old male patient and a (**c**) 71-year-old male patient with lung adenocarcinoma demonstrated exon 19 deletion and exon 21 L858R mutation, respectively. (**d**) The cellblock obtained using a 19 G eXcelon needle (Boston Scientific, Boston, MA) from the right paratracheal #4R LN in a 72-year-old patient with left upper lobe lung adenocarcinoma revealed a negative *EGFR* mutation result (hematoxylin and eosin, original × 100).

the 27 patients (63.0%) had cancer cells on their cellblock eligible for *EGFR* mutation testing. Although the introduction of radial EBUS has improved the diagnosis of peripheral pulmonary lesions by TBLB or brushing and provides a higher tumor cell fraction for *EGFR* mutation analysis, TBNA was the exclusive method used in approximately one-fifth of the patients.[23] The needle passes required to attain a cytology diagnosis and the molecular testing was comparable to the optimal numbers reported for EBUS-TBNA.[24–26] The plasma-thrombin method provided an adequate cellblock, even for brushing specimens (Fig 4). Garcia-Olive *et al.* reported that no additional tumor cells were available for genetic analysis after examination for a cytology diagnosis in 10 out of 36 (27.8%) patients undergoing EBUS-TBNA.[27] Modification of our specimen processing method with needle passes exclusively for molecular testing has yielded a significantly higher rate of positive cellblocks eligible for *EGFR* mutation analysis.

Technological advances have increased the resectability of N2 disease. Patients with bulky extranodal multi-station N2 disease are directly triaged to concurrent chemoradiotherapy. Both of these developments have led to a reduced number of conventional TBNAs being performed in recent years. The introduction of PET-CT staging detects smaller ^{18}F-fluorodeoxyglucose-avid LNs but has increased the technical difficulty of conventional TBNA and requires more needle passes than previously (conventional TBNA: accuracy 76.7%, mean LN short-axis diameter 14.4 $\pm$ 3.0 mm, and mean needle passes 2.4 $\pm$ 0.6 for 440 LNs in 275 patients in the historical control between September 1999 and March 2013; all $P < 0.001$).[5] However, conventional TBNA is still advantageous as a one-step diagnosis and for the staging of lung adenocarcinoma under topical anesthesia and conscious sedation.

The TaqMan PCR and the amplification-refractory mutation system PCR used in this study can easily detect mutations in exons 18–21 of the *EGFR* gene, which is predictive of the therapeutic response to EGFR-TKIs. Simplicity and sensitivity allow their widespread use; however, efforts to reduce the cost and turnaround time continue.[28–30]

The utility of biopsy, pleural fluid, and serum for *EGFR* mutation analysis is limited by the contamination of noncancerous cells in samples.[31–34] A large number of wild-type genes may reduce the yield. Cellblock preparation obtained by conventional TBNA comprises mainly tumor cells and a small amount of lymphocytes, with less bronchial epithelial cells, which reduces the problem of contamination, similar to that of EBUS-TBNA.[20]

Less than 30% of lung adenocarcinoma patients are eligible for surgical resection at the time of diagnosis. The development of molecular analysis on smaller nonsurgical samples is decisive for subsequent treatment, targeted therapy, or chemotherapy. However, a study by Sholl *et al.* reported that 26% of the biopsies and 35% of the cytology specimens in the sample did not undergo molecular testing because there were an insufficient number of tumor cells.[35] The results of our study show that *EGFR* mutation status

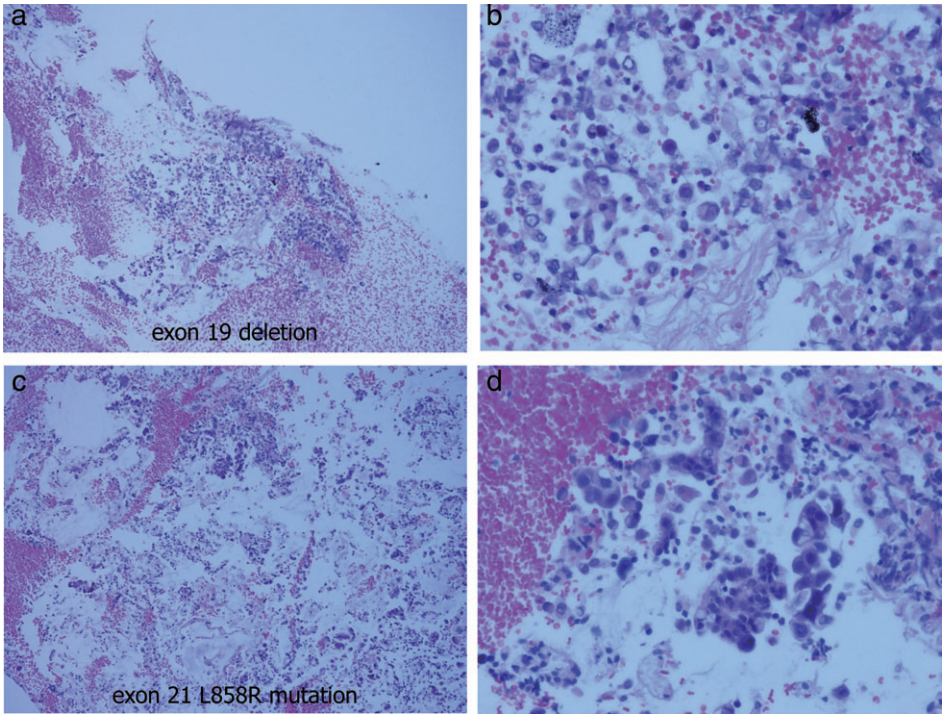

Figure 4 (**a,b**) Analysis of a cellblock obtained via brushing revealed an exon 19 deletion in a 63-year-old male patient with left lower lobe lung adenocarcinoma whose transbronchial needle aspiration cellblock was positive for malignancy but inadequate for analysis (hematoxylin and eosin, original × 100 and 400). (**c,d**) Analysis of a cellblock obtained via brushing revealed an exon 21 L858R mutation in a 71-year-old male patient with right upper lobe lung adenocarcinoma (hematoxylin and eosin, original × 100 and 400).

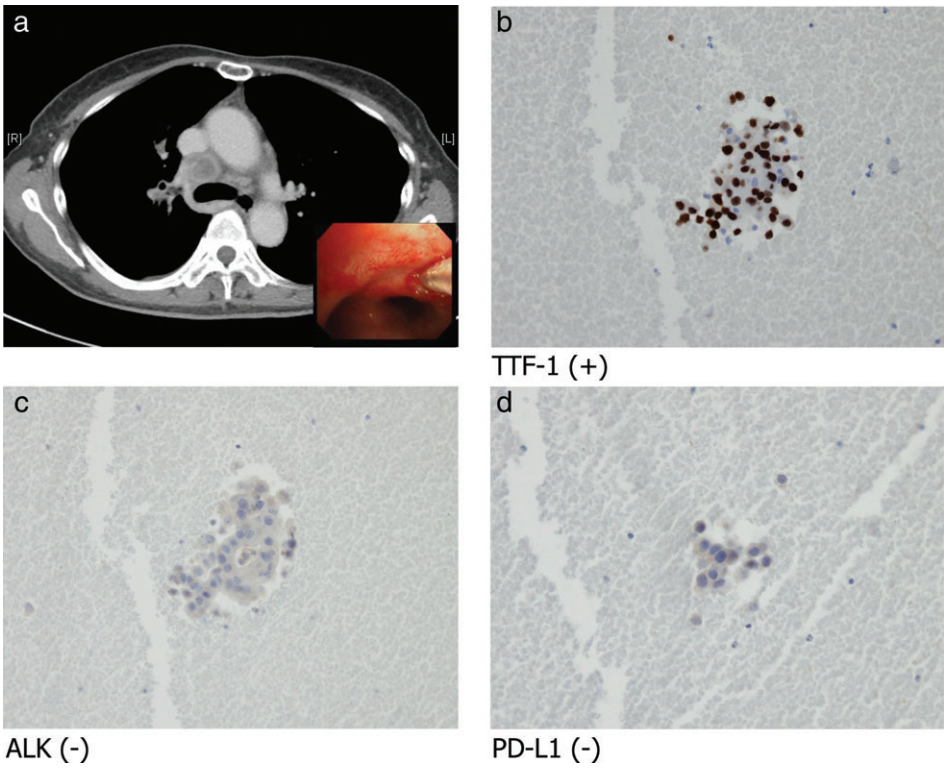

Figure 5 A 65-year-old female patient with right lower lobe lung adenocarcinoma. A cell-block obtained via conventional transbronchial needle aspiration of the right main bronchus #4R lymph node (**a**) showed strong positive nuclear staining of TTF-1 (**b**) (Leica, clone SPT24) and a negative EGFR mutation result; subsequent testing for ALK (**c**) (Roche, clone D5F3) and PD-L1 (**d**) (Roche, clone SP263) showed no cytoplasmic staining in cancer cells (original × 200).

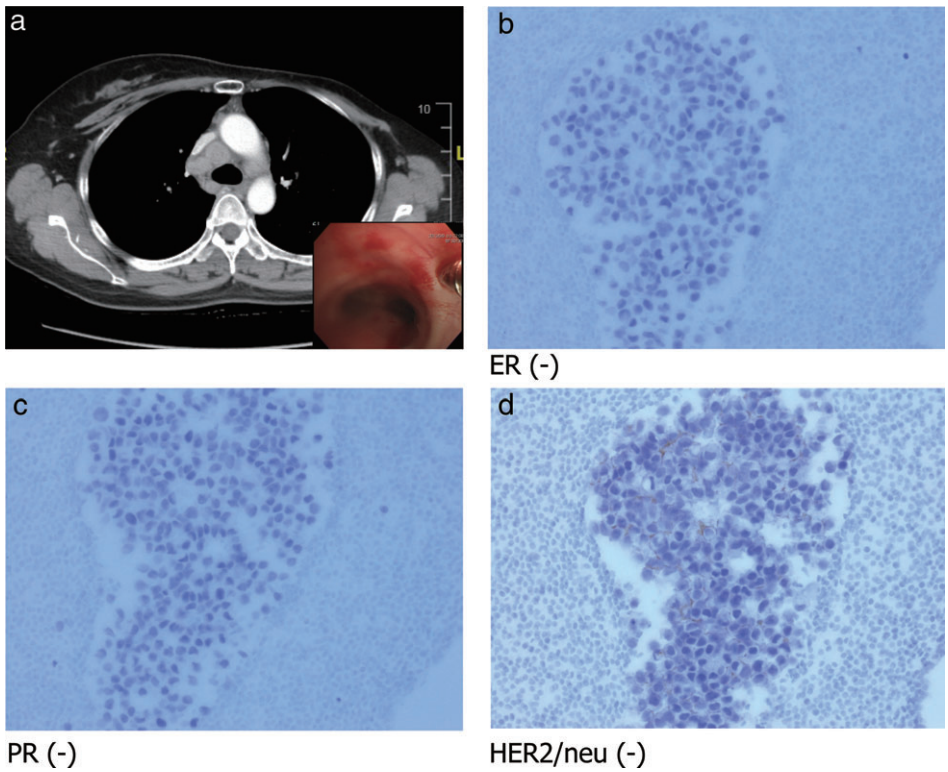

Figure 6 (**a**) A 46-year-old female breast cancer patient was diagnosed with mediastinal recurrence by conventional transbronchial needle aspiration of the right main bronchus #4R lymph node. The cellblock illustrated triple negative for (**b**) ER, (**c**) PR, (**d**) HER2/neu, contrast with ER 3+, PR 2+ and no overexpression of HER2/neu in the primary tumor (original × 400).

can be analyzed from samples obtained by conventional TBNA, and that patients with clinically important mutations benefit from targeted molecular therapies. In TBNA cellblocks positive for cancer cells without the *EGFR* mutation, the adequacy of immunohistochemistry of cellblock samples for determining *ALK* rearrangements and PD-L1 status will be evaluated in future research (Fig 5),[36,37] whereas we have demonstrated the utility of conventional TBNA for identifying ER, PR, and HER2/neu status for breast cancer mediastinal recurrence (Fig 6).

A limitation of this study is that we did not consider the mean tumor cell fraction of corresponding reference samples in the same patient. Our study sample was small, therefore more patient recruitment and follow-up will be helpful to confirm the benefits achieved after our specimen processing method was modified. In addition to EBUS-TBNA real-time guidance to improve the diagnostic yield of smaller LNs, how to obtain more cellular specimens, the significance of a larger bore needle (Fig 3d), and how to handle specimens are inevitable challenges in this era of precision medicine, especially when faced with requirements of re-biopsy and competition from liquid biopsy.[38]

In conclusion, this study shows that conventional TBNA is promising for identifying *EGFR* mutations in lung adenocarcinoma and these findings have implications for the challenges of doing more with less in the future.

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Disclosure

No authors report any conflict of interest.

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