Case Reports in Oncology

Case Rep Oncol 2024;17:865–873
DOI: 10.1159/000540356
Received: June 28, 2024
Accepted: July 9, 2024
Published online: August 5, 2024

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Case Report

Successful *EGFR* Mutation Detection in Cytological Specimens of Lung Cancer with Challenging Biopsies by Integrating Virtual Bronchoscopy Navigation and Endobronchial Ultrasound Guidance with Highly Sensitive Next-Generation Sequencing: A Case Report

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Keywords
Bronchoscopy · Case report · Lung cancer · Next-generation sequencing

Abstract
Introduction: This case report presents the successful detection of an *EGFR* exon 19 deletion using virtual bronchoscopic navigation (VBN) and endobronchial ultrasound with guide sheath (EBUS-GS) brushing, integrated with highly sensitive next-generation sequencing (NGS), even in challenging biopsy scenarios. The growing prevalence of driver gene alterations in non-small cell lung cancer necessitates effective bronchoscopic technology and reliable multiplex gene NGS panels. However, data regarding the optimal bronchoscopic techniques when using highly sensitive NGS panels are limited. Herein, we report a case utilizing VBN-guided EBUS-GS brushing as an exploratory approach to address this challenge. ***Case Presentation:*** A 71-year-old man was evaluated for a band-like lesion near the left pleura during spinal cord infarction. Transbronchial specimens were obtained from lesions invisible on conventional chest radiography and X-ray fluoroscopy using VBN and EBUS-GS brushing. Cytological brushing

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specimens revealed lung adenocarcinoma, and highly sensitive NGS identified an *EGFR* exon 19 deletion. He was diagnosed with stage IB disease and underwent radical radiotherapy owing to his fragile condition. If recurrence occurs, the patient will be treated with an EGFR inhibitor. ***Conclusion:*** VBN-guided EBUS-GS brushing, a minimally invasive approach, combined with highly sensitive NGS has the potential to provide accurate molecular diagnoses to more patients with lung cancer, thereby offering opportunities for personalized treatment. Our findings warrant further investigation to determine optimal bronchoscopic technologies for obtaining tumor specimens.

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Introduction

The accurate detection of driver gene alterations in non-small cell lung cancer is crucial for personalized treatment. Conventional multiplex gene panels based on next-generation sequencing (NGS) or real-time polymerase chain reaction have been developed to simultaneously examine these alterations [1, 2]. Unfortunately, these panels require a substantial number of cancer cells and a high tumor cell percentage of at least 20–30% within tissue specimens [2]. The need for substantial amounts of high-quality samples reduces the feasibility of conventional multiplex gene panels for some patients [3, 4]. The Lung Cancer Compact Panel™ (Compact Panel; DNA Chip Research, Tokyo, Japan), a high-sensitivity NGS panel, was authorized in Japan as the first panel for small tissue and cytology samples. Notably, this panel can analyze specimens containing as few as 5% cancer cells [5, 6].

The identification of driver gene alterations necessitates accurate gene analysis tests and effective sampling modalities, such as bronchoscopic technologies. However, limited data exist regarding the optimal bronchoscopic technologies when using highly sensitive NGS. This case report aimed to explore clues to address this gap. Herein, we present a case of lung adenocarcinoma in which virtual bronchoscopic navigation (VBN) and endobronchial ultrasound with guide sheath (EBUS-GS), followed by brush cytology and the application of a compact panel, successfully identified an *EGFR* mutation, despite technically challenging biopsies. The authors completed the CARE Checklist for this case report, which is attached as online supplementary material (for all online suppl. material, see https://doi.org/10.1159/000540356).

Case Report

A 71-year-old male, ex-smoker was evaluated for a band-like lesion in the left upper lung lobe (Fig. 1). He had smoked 20 cigarettes per day for 51 years and had no family history of malignancies. A month earlier, he developed acute muscle weakness in both legs, resulting in a diagnosis of thoracic spinal cord infarction due to anterior spinal artery syndrome. Antiplatelet therapy with clopidogrel was initiated. The patient eventually required a wheelchair to carry out daily activities and developed bladder-rectal dysfunction. During systemic evaluation, incidental chest computed tomography (CT) revealed a 37 × 7 mm band-like lesion extending along the left pleural fissure (Fig. 2a, b); however, conventional chest radiography failed to detect the lesion. Due to the patient's fragile condition and the lesion's radiological appearance, suggestive of a possible inflammatory lesion, chest CT was performed every several months.

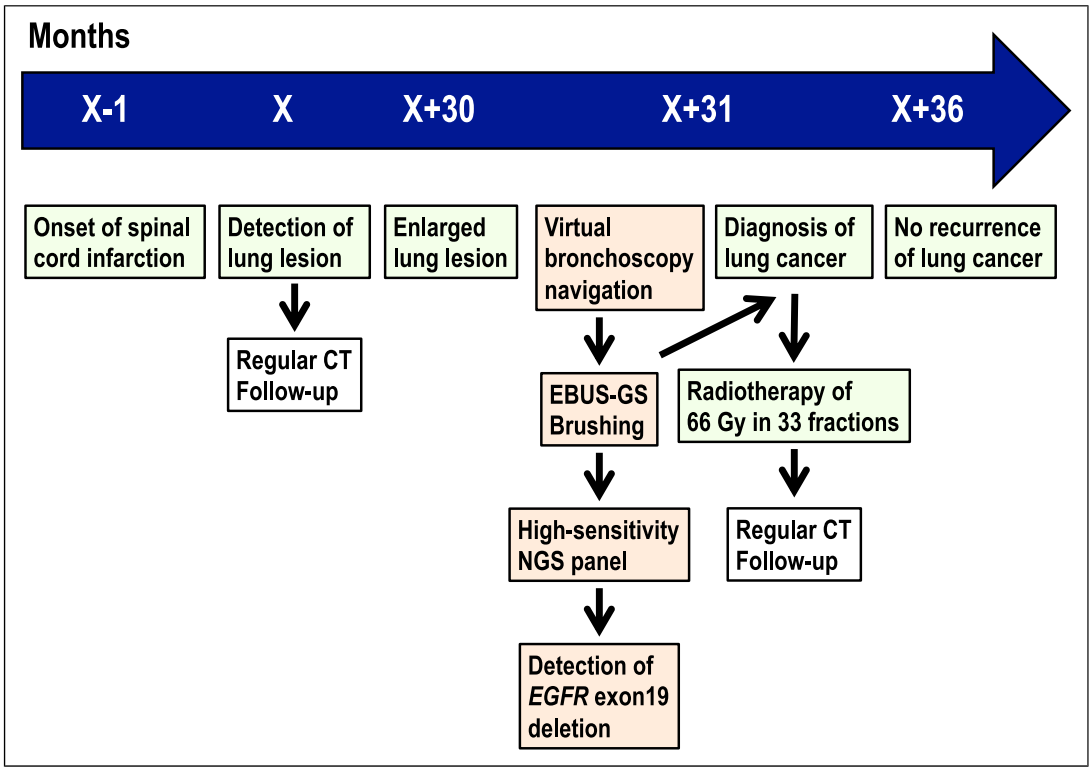

Fig. 1. Timeline of the patient with lung adenocarcinoma. CT, computed tomography; EBUS-GS, endobronchial ultrasound with a guide sheath; NGS, next-generation sequencing.

Two and a half years later, the band-like lesion had enlarged from 37 × 7 mm to 38 × 10 mm (Fig. 2c, d) but remained invisible on conventional chest radiography (Fig. 2e). Physical examination and laboratory tests for diagnosing any malignancies yielded unremarkable results, including blood cell counts and serum levels of liver and renal function, C-reactive protein, carcinoembryonic antigen, cytokeratin 19 fragmentation, and pro-gastrin releasing peptide. Additionally, other blood laboratory tests regarding infectious diseases also produced results within the reference ranges. These tests included interferon-γ release assay for *Mycobacterium tuberculosis*, anti-glycopeptidolipid core IgA antibody for *Mycobacterium avium complex*, β-glucan for fungal diseases, and Cryptococcus antigen. Positron emission tomography revealed the accumulation of ^{18}F-fluorodeoxyglucose in the pulmonary lesion, suggesting a potential malignancy (Fig. 2f). Using the SYNAPSE VINCENT system (Fujifilm, Tokyo, Japan), a virtual bronchoscopy image of the target lesion was reconstructed, identifying a small B^{1+2} ciiα bronchus that reached the inferior region of the lesion rather than through the B^{3}aiα (Fig. 3a, b, 4a). Subsequently, EBUS-GS was employed to explore the lesion and reduce the risk of bleeding and pneumothorax associated with antiplatelet medication and near-pleural lesions. Bronchoscopy was performed using a bronchoscope (BF-1T260; Olympus, Tokyo, Japan), guide sheath (SG-201C, Olympus), bronchial ultrasonic probe (UM-S20-20R, Olympus), cytology brushes (BC-202D-2010, Olympus), and normal-diameter biopsy forceps (FB-231D, Olympus). Although X-ray fluoroscopy failed to detect the pulmonary lesion, the ultrasound probe successfully identified the lesion through the B^{1+2} ciiα (Fig. 4b, c). A guide sheath was then placed within the lesion (Fig. 4d), allowing two brushings and four biopsies without massive bleeding or pneumothorax. The brush was rinsed in a tube containing a sterile saline solution. This solution was divided into two samples: one for

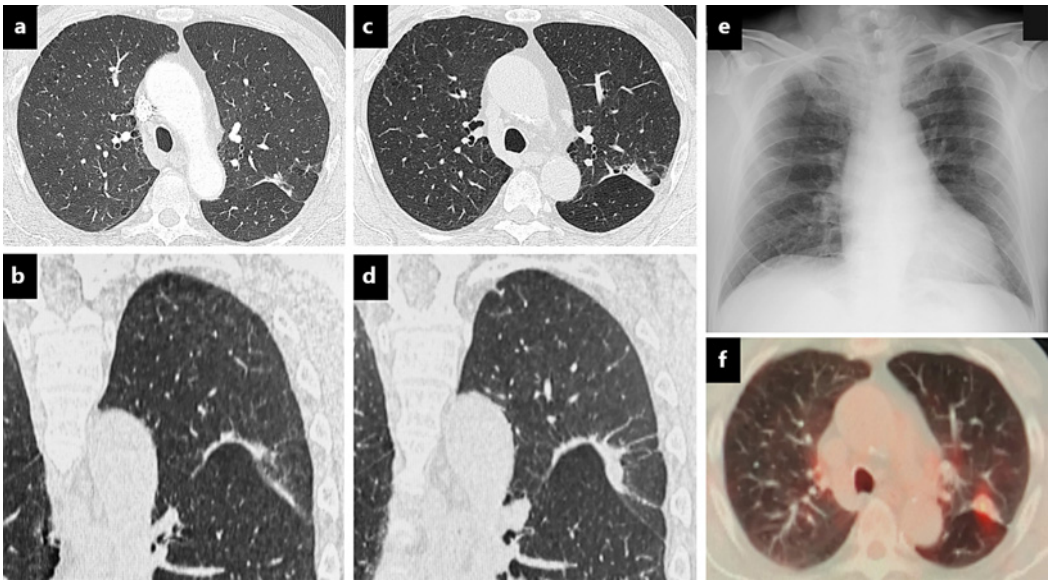

Fig. 2. Chest radiological images of the patient with lung adenocarcinoma. A band-like lesion extends along the left major pleural fissure in the horizontal view (**a**) and in the frontal view (**b**). Two and a half years after the initial presentation, the lesion is enlarged as shown in the horizontal view (**c**) and frontal view (**d**). **e** This lesion remains invisible on conventional chest radiography. **f** The lesion shows the accumulation of ^{18}F-fluorodeoxyglucose on positron emission tomography.

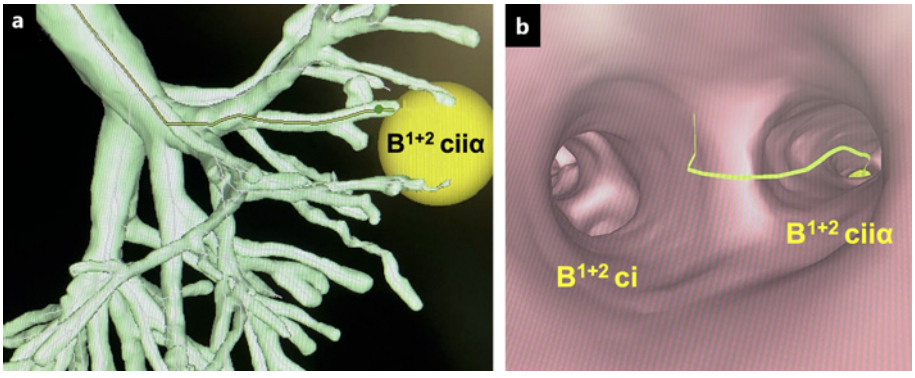

Fig. 3. Virtual bronchoscopic images of the patient with lung adenocarcinoma. The tracking line (yellow line) is drawn to the target lesion (yellow circle). **a** The oblique view of the bronchial tree is reconstructed from the data obtained from the thin-slice CT, indicating the route to the lesion through the left B^{1+2}ciiα. **b** The endoscopic view is also created, showing the orifice of the B^{1+2}ciiα.

cytological diagnosis and the other for the GM tube (GeneMetrics, Osaka, Japan) containing an ammonium sulfate-based nucleic acid stabilizer. The GM tube was then stored at 4°C.

Histological examination revealed that thyroid transcription factor (TTF)-1-positive adenocarcinoma cells were scattered in highly fibrotic tissues (Fig. 5a, b). Macrodissection proved challenging and yielded a tumor cell percentage of <5% despite the use of normal-diameter biopsy forceps. By contrast, the cytological specimens from the brushing wash fluid contained a high percentage of cancer cells, exceeding >30% (Fig. 5c). Consequently, we analyzed the cytological specimens using a compact panel, which successfully extracted

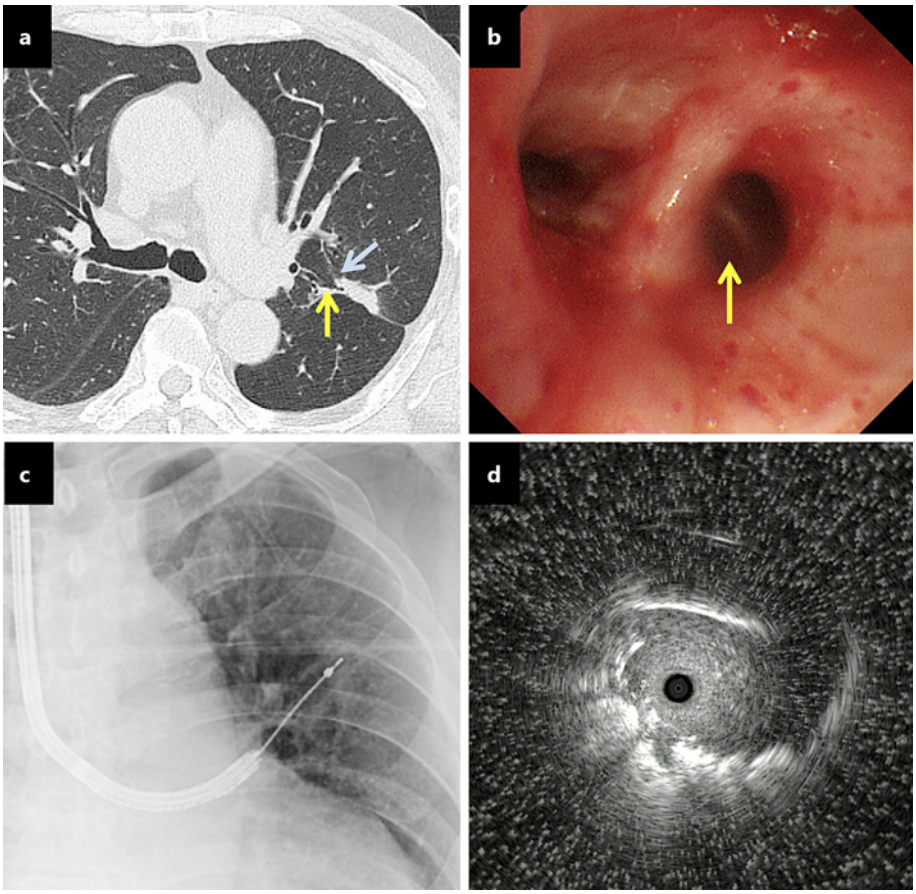

Fig. 4. The patient with lung adenocarcinoma. **a** Chest CT shows that the left B^{1+2}ciiα leads to the inferior region of the lesion (yellow arrow), while the left B^{3}aiα is located near the lesion (blue arrow). **b** Bronchoscopy indicates the orifice of the left B^{1+2}ciiα (yellow arrow). **c** X-ray fluoroscopy shows an endobronchial ultrasound probe with a guide sheath through the left B^{1+2} ciiα. **d** The endobronchial ultrasound probe and a guide sheath are placed precisely within the lesion.

1,040 ng of deoxyribonucleic acid (DNA) and 1,031 ng of ribonucleic acid. The analysis identified an *EGFR* exon 19 deletion (c.2236_2250del GAATTAAGAGAAGCA; p.E746_A750del) with a read depth of 56,591 and an allele fraction of 36.2%. No other driver alterations (*ERBB2*, *KRAS*, *BRAF*, *ALK*, *ROS1*, *RET*, or *MET*) were detected by the panel.

The patient was diagnosed with stage IB (cT2aN0M0) lung adenocarcinoma carrying *EGFR* exon 19 deletions. After discussing the treatment options with the patient with a fragile condition, we decided to proceed with radical radiotherapy. The patient received 66 Gy of radiation in 33 fractions to the primary lung lesion, including the hilar and mediastinal lymph nodes. One month after the initiation of radiotherapy, the patient complained of grade 2 radiation esophagitis (Common Terminology Criteria for Adverse Events version 5.0), which was briefly alleviated by treatment with an oral sodium alginate solution. The patient completed radiotherapy as scheduled. The lesion showed no significant change, and no recurrence or radiation pneumonitis was observed 5 months after the initiation of radiotherapy. In the case of lung cancer recurrence, an EGFR inhibitor will be administered.

The patient was disappointed upon receiving a diagnosis of lung cancer following a spinal cord infarction but was relieved that the lung cancer had not metastasized. He hoped to

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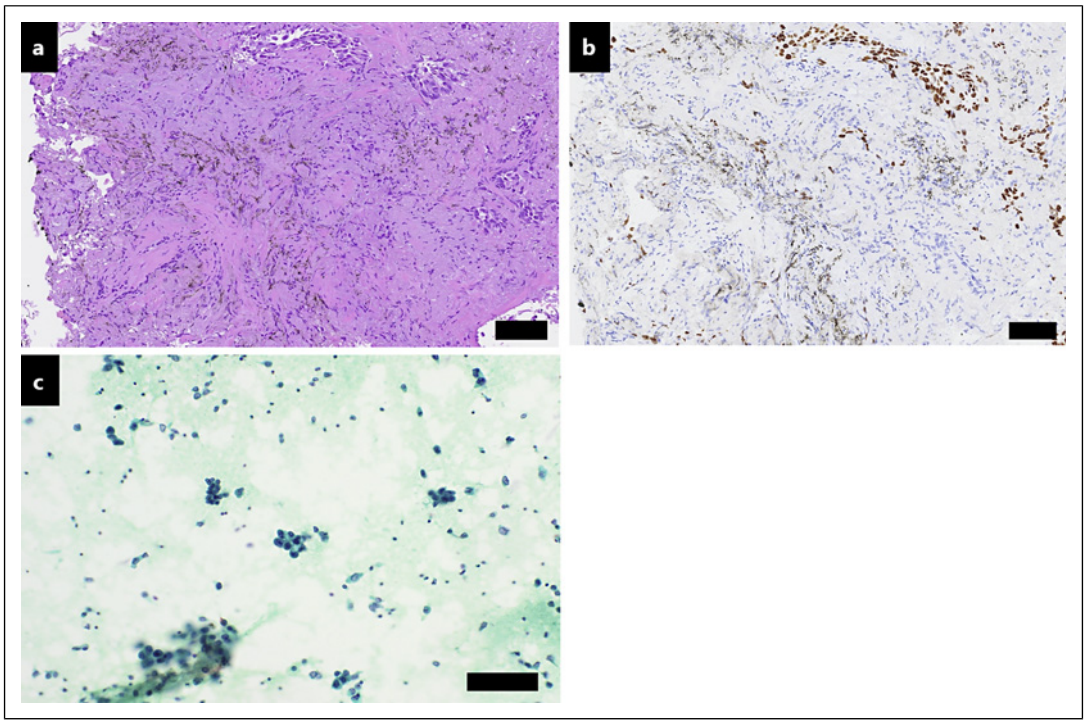

Fig. 5. Photomicrographs of the specimens obtained from the patient with lung adenocarcinoma. Scale bars indicate 100 μm. **a** A small number of cancer cells are scattered in the highly fibrotic tissue (hematoxylin and eosin stain). **b** The immunohistological examination shows that the scattered cancer cells are positive for TTF-1. **c** Many cancer cells with deformed nuclei, consistent with adenocarcinoma, are found in the transbronchial brushing wash fluid (Papanicolaou stain).

undertake a multiplex gene panel to ensure optimal treatment with molecular-targeted therapy in the future if required. He continued undergoing rehabilitation to improve his leg paralysis and lung cancer treatment.

Discussion

This case report demonstrates the successful use of VBN and subsequent EBUS-GS brushing with a compact panel to identify driver gene alterations in a patient with lung cancer despite challenging biopsy conditions. A previous case report on lung adenocarcinoma described a similar situation but with notable differences, involving challenging biopsies [7]. In contrast to our case, this previous case report did not involve the use of VBN, included *KRAS* mutation-positive cytological specimens with no cancer cells and had low DNA yield and allele frequency. Our study achieved a higher DNA yield and allele frequency, likely due to precise navigation and thorough sample collection. Thus, the successful detection of driver gene alterations highlights the importance of effective bronchoscopic methods and precise gene analysis tests.

First, VBN and subsequent EBUS-GS brushing improved the diagnostic efficacy for lung cancer. A previous randomized study demonstrated that combining VBN with EBUS-GS resulted in a higher diagnostic efficacy for peripheral lesions compared with EBUS-GS alone (80.4 vs. 67.0%) [8]. Another prospective cohort study found that EBUS-GS brush cytology often outperformed EBUS-GS biopsied tissues in terms of DNA yield [6]. The authors of this cohort study assumed that the lower DNA yield in tissue specimens resulted from nucleic acid

deterioration and fragmentation during formalin fixation [6]. Utilizing VBN and subsequent EBUS-GS brushing can help in obtaining sufficient specimens for molecular diagnosis.

Second, the compact panel demonstrates higher sensitivity to driver gene alterations compared with conventional multiplex gene panels. This panel uses the amplicon amplification method and increases the read depth of eight druggable gene alterations to detect variants with an allele fraction of 1% in samples containing ≥5% tumor cells [5]. By contrast, conventional multiplex gene panels can only detect variants with an allele fraction of 1–6% in samples containing at least 20–30% tumor cells [4]. A retrospective cohort study found that only 52 (64.2%) of 81 patients undergoing transbronchial biopsies yielded complete results from a conventional multiplex gene NGS panel [3], underscoring the challenges of employing multiple gene NGS panels universally.

This study has several limitations. First, advanced bronchoscopic technologies are expensive and specialized, particularly for some community hospitals [9]. The present case report did not evaluate the cost-effectiveness of VBN-guided EBUS-GS brushing, compared with conventional bronchoscopy and CT-guided biopsy, in terms of equipment costs and the number of patients eligible for this procedure due to the accessibility of peripheral lesions. Second, EBUS-GS brushing requires technical expertise, and the results may vary depending on the skill of the pulmonologists [10]. Despite this expertise, mobile cone beam CT guidance can enhance the performance of EBUS-GS biopsy and brushing [11]. Third, this case did not undergo rapid on-site evaluation (ROSE) on the cytological specimens obtained by EBUS-GS brushing due to a shortage of cytologists trained in ROSE procedures. A randomized controlled trial demonstrated that the combination of ROSE with EBUS brushing improved the diagnostic yield of peripheral lung nodules by 24% [12]. Incorporating ROSE into VBN-guided EBUS-GS brushing can be beneficial to ensure the collection of adequate tumor specimens. Finally, the compact panel cannot identify pathogenic mutations, except for druggable gene alterations, which may affect the efficacy of molecular-targeted therapies [13]. Hence, further studies are warranted to advance bronchoscopic technologies and develop multiplex gene NGS panels with broader coverage of oncogenes.

Conclusion

This case report demonstrates that VBN-guided EBUS-GS brushing with a compact panel can accurately detect mutations, even in technically challenging biopsies. These findings suggest that emerging minimally invasive bronchoscopic techniques may enable a broader range of patients with lung cancer to receive accurate molecular diagnoses and potentially benefit from personalized treatment. This encourages further investigation of bronchoscopic technologies for obtaining tumor specimens.

Acknowledgment

The authors would like to thank Editage for providing excellent English language editing assistance.

Statement of Ethics

Ethical approval was not required for this case report in accordance with the policy of the Institutional Ethical Committee of Sasebo City General Hospital. Written informed consent was obtained from the patient for the publication of his medical case and any accompanying images.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Funding Sources

This study was not supported by any sponsor or funder.

Author Contributions

All authors have approved the final version of the manuscript for publication. H.S.: conceptualization. Y.U. and H.S.: writing – original draft. Y.U., H.S., H.H., R.O., and M.I: resources. H.S., H.T., and S.T: writing – review and editing. T.T., N.S., Y.F., and H.M: supervision.

Data Availability Statement

All data generated or analyzed during this study are included in this article and its online supplementary material. Further inquiries can be directed to the corresponding author.

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