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CASE REPORT

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Rapid and reliable collection of tumor tissue for successful gene panel in a patient with advanced stage lung cancer: A case report

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Abstract

Rapid and reliable identification of targetable driver mutations in patients with advanced stage lung cancer is essential. Adequate amount of tumor tissue biopsies (i.e., genomic biopsies) are required to successfully analyze the gene panel. In the present case, we performed three pleural fluid investigations, including transbronchial biopsy of the primary tumor, transesophageal endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) of lymph node metastasis, and thoracoscopic biopsy of the pleural seeding sites. Among the three investigations, thoracoscopic biopsy alone successfully obtained a sufficient amount of tissue. Thus, it is important to determine the technique and site of biopsy, as multiple biopsies are not only burdensome to the patient, but also lead to significant delays in therapy induction.

KEYWORDS

gene panel, genomic biopsy, lung cancer, rapid on-site evaluation, thoracoscopy

INTRODUCTION

Determination of targetable driver mutation is essential to establish the treatment strategy for patients with advanced lung cancer.[1] Gene panels are required to investigate multiple targetable driver mutations, for which collection of an adequate amount of tumor tissue is mandated. It is often difficult to obtain tumor tissue by surgical biopsy in patients with advanced lung cancer; and in several cases, a gene panel must be successfully performed on small tissue specimens, such as bronchoscopy biopsy specimens.[2] In addition, if multiple sites are available for biopsy, it is necessary to determine the site which would provide adequate amount of tumor tissue sample for a genomic biopsy, in order to perform a successful gene panel analysis. Herein, we present a case of advanced stage lung cancer in which it was difficult to obtain sufficient tissue samples for gene panel analysis, and multiple rebiopsies were required.

CASE REPORT

A 29-year-old woman, with no history of smoking and unremarkable medical history, reported to our hospital with the chief complaint of respiratory distress. The patient was diagnosed with right pleural effusion and a tumor in her right lung (Figure 1a–c). Enhanced brain magnetic resonance imaging (MRI) revealed multiple brain metastases. Her previous physician had performed thoracentesis and examined the pleural fluid, but found no evidence of malignant cells, after which she was referred to our hospital. On her first visit, a second pleural effusion test was performed, but no malignant cells were detected. Bronchoscopy was performed on the fifth day. On transbronchial biopsy (TBB), malignant cells were detected using rapid on-site evaluation (ROSE), and genetic analysis was performed on fresh-frozen specimen using the AmoyDx Pan Lung Cancer PCR panel (Amoy) (Amoy Diagnostics). However, histopathological analysis performed on the eighth day showed insufficient

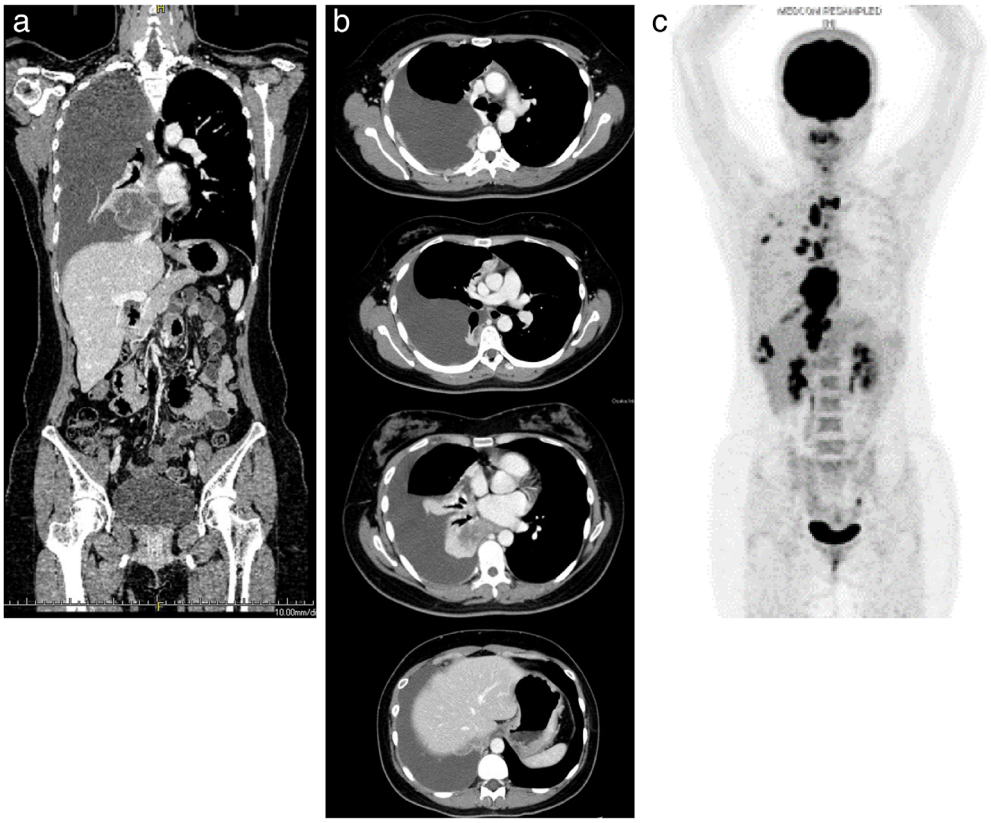

FIGURE 1 (a) Frontal image of enhanced computed tomography (CT) from chest to trunk. (b) Serial transverse images of enhanced CT of chest showing massive right pleural effusion, tumor with low density area in the right lower lobe and no pathological enlargement of the mediastinal lymph nodes. (c) Fluorodeoxyglucose (FDG)-positron emission tomography (PET) image of body showing FDG uptake by multiple lesions.

tissue specimen, and transesophageal endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) was performed on the ninth day. A paraoesophageal lymph node lesion was collected, and a sufficient number of malignant cells were detected using ROSE, but several necrotic components were also found. On the 12th day, histopathological analysis revealed that the EUS-FNA specimen also did not yield a sufficient amount of tissue, and thoracoscopy was performed under local anesthesia on the same day. During thoracoscopy, pleural fluid was submitted for third examination, but no malignant cells were detected. However, sufficient tumor tissue samples were successfully obtained via thoracoscopy. The course of these biopsies is summarized in Figure 2. On the same day, EGFR L858R mutation was detected by Amoy, and treatment with EGFR-tyrosine kinase inhibitor was initiated. Immunohistochemistry was performed on bronchoscopy biopsy specimens, wherein the tissue samples were stained with a specific antibody against EGFR L858R, which confirmed the presence of tumor cells (Figure 3).

DISCUSSION

Two endoscopic biopsies confirmed malignant cells on ROSE, but they did not provide enough tissue to allow gene panel testing. Thoracoscopy eventually yielded a sufficient amount of tumor tissue. We previously reported that even if malignant cells of centrally located tumors were detected on ROSE, it was difficult to obtain sufficient tumor tissue using TBB, thus prompting the use of endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA).[3] In this case, EBUS-TBNA failed to visualize the tumor in the right lower lobe, after which TBB was performed. Contrast-enhanced computed tomography (CT) showed a low-density area within the tumor and lymph node metastasis, suggesting intense necrosis. Specimen obtained by thoracoscopy also showed evidence of necrosis; however, a sufficient number of tumor cells were successfully obtained from the large sample volume. Use of fresh frozen TBB samples led to the identification of *EGFR* L858R mutation; however, we believe that thoracoscopic biopsy is necessary. An archive of sufficient tumor samples enables investigation of a separate gene panel.

Amoy using reverse transcription polymerase chain reaction (RT-PCR) has high sensitivity to detect known sequences.[4] In this case, we used fresh frozen specimen for rapid identification of targetable driver mutations. Based on the results of histopathological analysis, it was likely that the fresh frozen sample did not contain sufficient tumor tissue, but the targetable mutation was successfully detected. When gene panel analysis is performed on a sample which does not contain enough tumor cells, a false negative result is more likely if the mutation is not detected. In this case, biopsy specimen obtained by thoracoscopy was the only suitable specimen for a gene panel. When enhanced CT

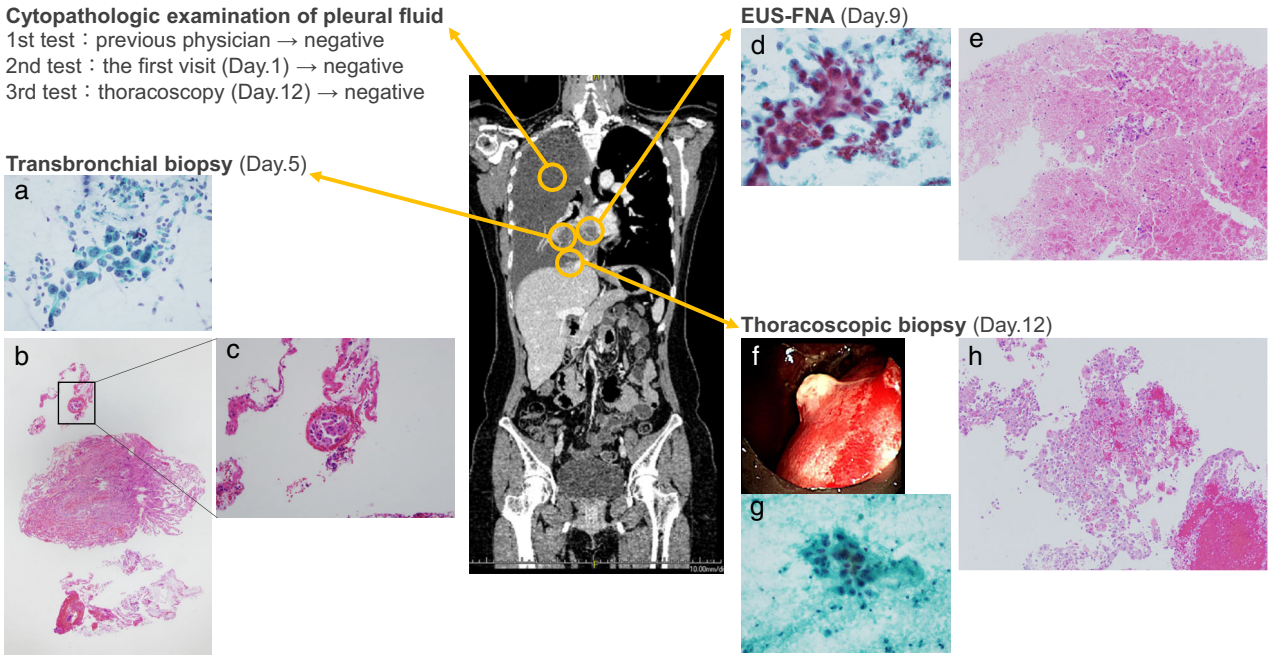

FIGURE 2 List of biopsy sites and dates along the clinical course. Each mentioned biopsy date is in accordance with consideration of the first visit as day 1. Cytopathological examination of the right pleural effusion specimen was performed after collecting samples from the previous physician on the day of visit, the day of first visit at our hospital, and the day of thoracoscopy on day 12, respectively. (a) Transbronchial biopsy (TBB) was performed on day 5, and rapid on-site evaluation (ROSE) showed malignant cells with enlarged atypical nuclei and tracheal epithelial cells (× 400). (b) Hematoxylin and eosin (HE) staining of TBB specimens showed clusters of malignant cells in only one of five collected samples (× 100). (c) An enlarged view is shown. (× 200). (d) ROSE of endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) specimen performed on day 9 showed malignant cells and evidence of necrosis. (e) HE staining showed few clusters of malignant cells in large areas of necrosis. (f) Thoracoscopy performed on day 12 showed numerous white nodules on the pleura including the diaphragm. (g) Stamp cytology of the specimen showed malignant cells and necrosis, and (h) HE staining showed numerous malignant cells.

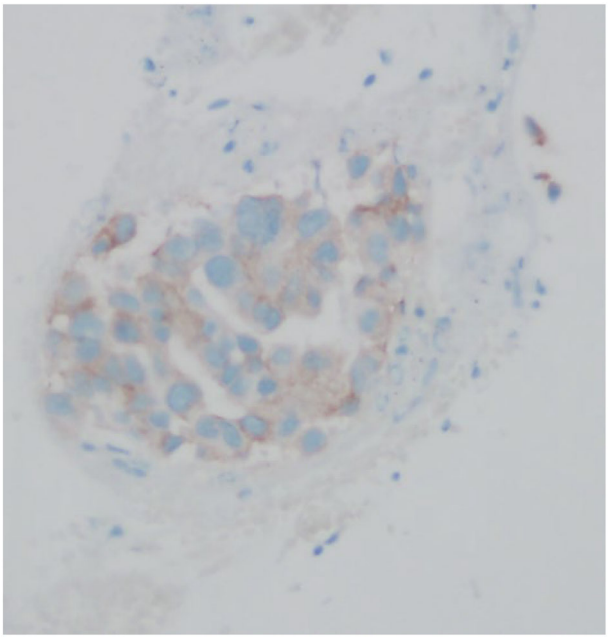

FIGURE 3 Specimens collected with transbronchial biopsy (TBB) were stained with specific antibody against EGFR L858R, and malignant cells were stained (× 400).

findings strongly suggest necrosis at the primary or metastatic site, thoracoscopy should be considered at an earlier stage, rather than endoscopic biopsy.

Among the biopsy methods performed in the present case, for TBB, EBUS-TBNA and fluid samples, which are often used for lung cancer, the success rate of specimen collection for NGS analysis was 87.2% for TBB, 92.9% for EBUS-TBNA and 100% for fluid samples. The success rate of each NGS analysis was 82.8% for TBB, 82.6% for EBUS-TBNA, and 100% for fluid sample when both DNA- and RNA-based NGS were successful.[3,5] Pleural fluid specimens containing malignant cells are ideal for genetic analysis since they can be collected easily and quickly, and the procedure is less invasive for patients.[6] However, obliviousness of the exact content of tumor cells increases the risk of false negatives in NGS analyses. Additionally, since patients with massive pleural effusion require fluid drainage for symptom relief, early consideration of local anesthetic thoracoscopy may be a good strategy to ensure adequate tumor tissue collection for genomic biopsy in these cases.

To conclude, in advanced stage lung cancer cases with multiple sites of biopsy, it is essential to develop a genomic biopsy strategy that aims for rapid and reliable successful gene panel, which would help establish a treatment strategy.

AUTHOR CONTRIBUTIONS

K.K., T.I., Y.K, R.K., S.K, K.Y., K.H., M.T., T.K. and K.N. made substantial contributions to the study conception, data interpretation and writing or critically reviewing the manuscript for important intellectual content. Material preparation, data collection and analysis were performed by K.K. The first draft of the manuscript was written by K.K. All authors read and approved the final manuscript.

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CONFLICT OF INTEREST

Dr Kunimasa reports honoraria for a lecture from AstraZeneca, Chugai Pharma and Novartis, Pfizer Merck and Ono Pharmaceutical, Dr Inoue reports honoraria for a lecture from AstraZeneca, Dr Tamiya reports a grant from Ono Pharmaceutical, Bristol-Myers Squibb and Boehringer Ingelheim and honoraria for a lecture from AstraZeneca, Chugai Pharma, Novartis, Taiho Pharmaceutical, Eli Lilly, Asahi Kasei Pharmaceutical, MSD, Boehringer Ingelheim, Ono Pharmaceutical and Bristol-Myers Squibb, Dr Nishino reports a grant from Boehringer Ingelheim and honoraria for a lecture from Chugai Pharma, AstraZeneca, Boehringer Ingelheim, Eli Lilly, Roche Diagnostics, Novartis, Pfizer Merk and others have no disclosure of conflict.

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