Case Report
Peripheral pulmonary nodule diagnosed as mycobacterium chelonae using electromagnetic navigation bronchoscopy combined with next generation sequencing: a case report

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Abstract: We report the case of a 29-year-old female with a 1.1 cm × 1.1 cm solitary nodule adjacent to the pleura in the upper lobe of the right lung that was diagnosed as Mycobacterium chelonae using electromagnetic navigation bronchoscopy combined with next generation sequencing. This diagnostics technology shows great promise in identifying peripheral pulmonary nodules, especially infectious lesions.

Keywords: Peripheral pulmonary nodules, electromagnetic navigation bronchoscope, next generation sequencing, mycobacterium chelonae

Introduction

With the application of high-resolution computed tomography (HRCT), pulmonary nodules are detected frequently; however, peripheral pulmonary nodules, especially those smaller than 2 cm, are difficult to diagnose [1, 2]. Benign lesions, such as infectious lesions, account for a significant proportion of peripheral nodules, but a thoracotomy for segment removal is costly. In particular, clinical manifestations and imaging are not specific and conventional culture techniques are difficult for detecting non-tuberculosis mycobacterium. Next generation sequencing (NGS) is a convenient and efficient technique for the diagnosis of infectious diseases [3]. Tissue sampling is required to provide microbiological material for characterization and sensitivity analysis. Conventional bronchoscopy or ultrasonic bronchoscopy does not reach the lesion site, so tissue samples are unobtainable. Computed tomography (CT)-guided percutaneous puncture biopsy (PCNB) has pneumothorax complications. Ultrasound-guided PCNB requires that nodules are adjacent to the chest wall, and the smaller the

diameter of the nodules, the higher risk of pneumothorax, hemothorax, and other complications. Electromagnetic navigation bronchoscopy (ENB) biopsy can accurately reach the peripheral lesion site with few complications. In this case, we performed ENB biopsy combined with NGS, which enabled the patient to obtain a definitive diagnosis and avoided the pain of surgical resection.

Case presentation

A 29-year-old female presented with a nodular lesion in the upper lobe of the right lung. The patient was free from any respiratory, neurological, or endocrine paraneoplastic symptoms. She had no smoking or radioactive substance exposure history. No history of recurrent pulmonary infections, including tuberculosis or family history of cancer, was present. Physical examinations and laboratory tests were unremarkable. The tumor biomarkers CEA, FRT, NSE, CA125, CYFRA21-1, CA50, and SCC were negative. Only the T-SPOT test was positive. CT with contrast of the chest (**Figure 1**) showed a 1.1-cm × 1.1-cm well-defined solitary nodule

Figure 1. Computed tomography (CT) with contrast of the chest. A 1.1-cm × 1.1-cm well-defined, solitary, uniformly dense nodule adjacent to the pleura in the upper lobe of the right lung.

adjacent to the pleura in the upper lobe of the right lung. The lesion was homogenous in density suggesting a benign lesion. The patient rejected 1-month follow-up or an immediate lobectomy but agreed to and underwent an ENB.

A bronchoscopist in our institute performed the ENB in this case report using Super Dimension Bronchus System Version 7.0 (SDBS, Herzliya, Israel) for navigation. The biopsy was performed using 16-mm biopsy forceps. The patient's chest CT (ideal 0.625 slice thickness) was uploaded into the specialized planning software that created a three-dimensional map to find a pathway to the lesion (**Figure 2**). The patient lies on an electromagnetic board. The bronchoscope with the extended working channel and locatable guide is introduced in the airway. When the patient's airway is matched to the virtual bronchial tree, the bronchoscope is advanced along the planned pathway illustrated by the virtual bronchoscopy. Once at the nodule, the extended working channel is locked into position, and the biospy was carried out (**Figure 3**). ENB successfully procured a sample and hematoxylin-eosin staining confirmed it was a small amount of lung tissue with hemorrhage (**Figure 4**). The bronchoalveolar lavage fluid (BALF) was tested using loop-mediated amplification (LMAP), traditional isolation, and cultivation. The results showed no presence of pathogen (**Tables 1** and **2**). Therefore, NGS was performed on the specimens using Segene Science and Technology Ltd. The NGS results showed that Mycobacterium chelonae was detected in both BALF and biopsy tissue (**Tables 3** and **4**). The patient rechecks the chest CT after 6 months and there is no obvious change on the nodule of upper lobe of the right lung (**Figure 6**).

Discussion

Peripheral pulmonary nodules are increasingly detected via CT screening, especially HRCT. Apart from malignant lesions, infectious lesions are also a common cause of peripheral pulmonary nodules, such as fungus and non-tuberculous mycobacterium (NTM). CT imaging of NTM lung disease presents with various characterizations, including nodular lesions. However, the identification of a peripheral pulmonary nodule is more difficult if a small nodule is detected. Bronchoscopy is widely used for the diagnosis and treatment of diseases of the proximal bronchial tree. The nodules located in the peripheral third of the lung are not normally accessible by conventional bronchoscopy. Therefore, for peripheral nodules of sizes under 2 cm, the diagnostic rate is as low as 14% [4].

PCNB is a common diagnostic method for peripheral lung lesions. PCNB is divided into core/cutting needle biopsy (CNB) and fine-needle aspiration (FNA). The most common complications of CNB and FNA are pneumothorax, accounting for 25.3% and 18.8% of complications with 5.6% and 4.3% of patients needing intervention, respectively [5]. The incidence of pulmonary hemorrhage after CNB and FNA is 18% and 6.4%, among which the incidence of hemoptysis is 4.1 and 1.7%, respectively [6]. Small lesions and cross-lung parenchyma are predictors of higher complication rates. Yamamoto reported higher efficacy and safety of ultrasound-guided percutaneous needle biopsy for lesions adjacent to the chest wall compared with PCNB because of its higher diagnostic rate, longer lesion-pleura contact arc length, and reduced complications [7]. However, other studies confirm that diagnostic accuracy generally decreases with the decrease
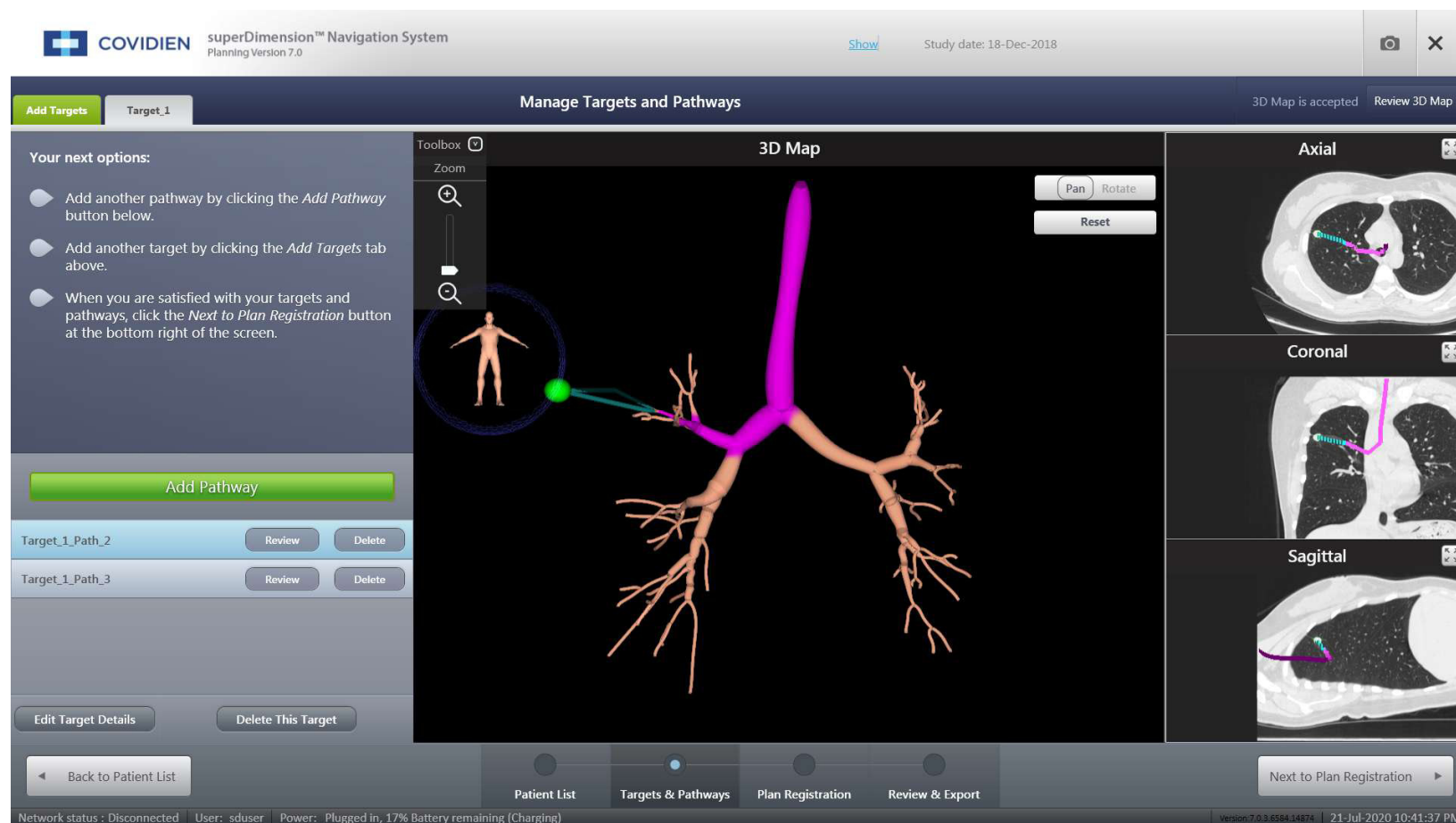
Figure 2. Electromagnetic navigation bronchoscopy planning. A pathway was created to locate the right upper lobe nodule. The operator selects the target, shown here by the green circle. The software then plans the quickest pathway to the lesion (pink line and blue line) automatically.
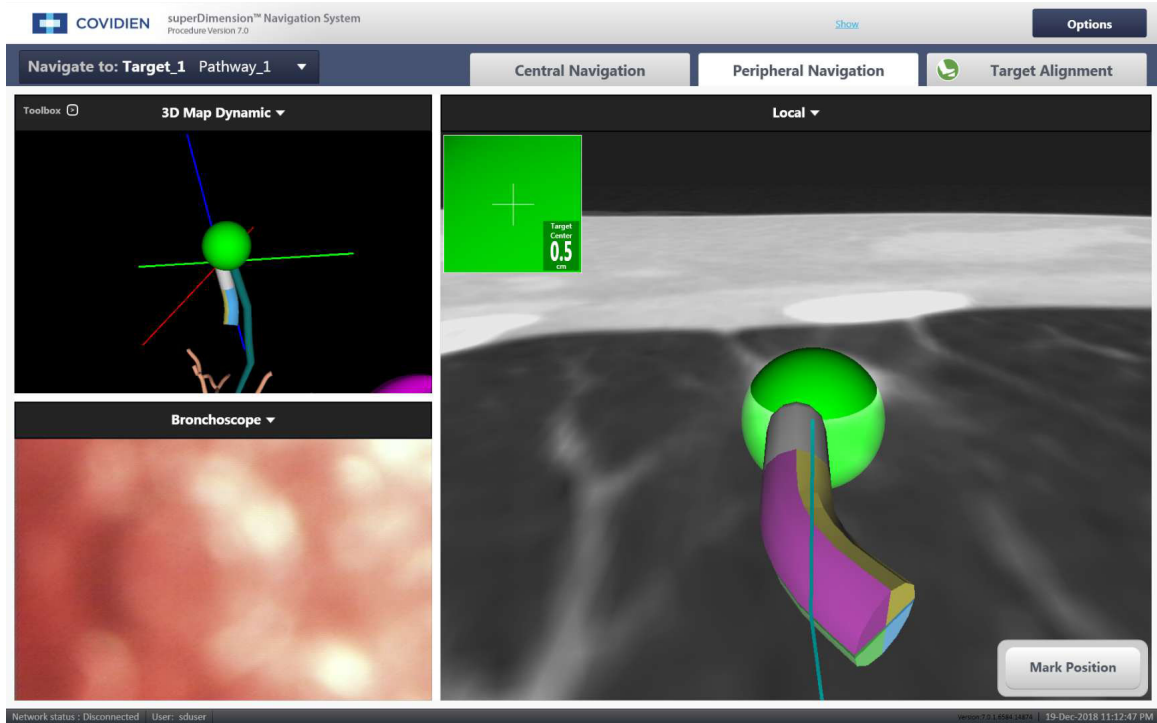
Figure 3. Electromagnetic navigation bronchoscopy. Electromagnetic navigation bronchoscopy guide. The broncho-scope is in a wedged position and cannot be advanced further (left lower image). The dynamic computed tomography (CT) reconstruction allows virtual visualization of the pathway and the target to enable navigation despite lack of vision.
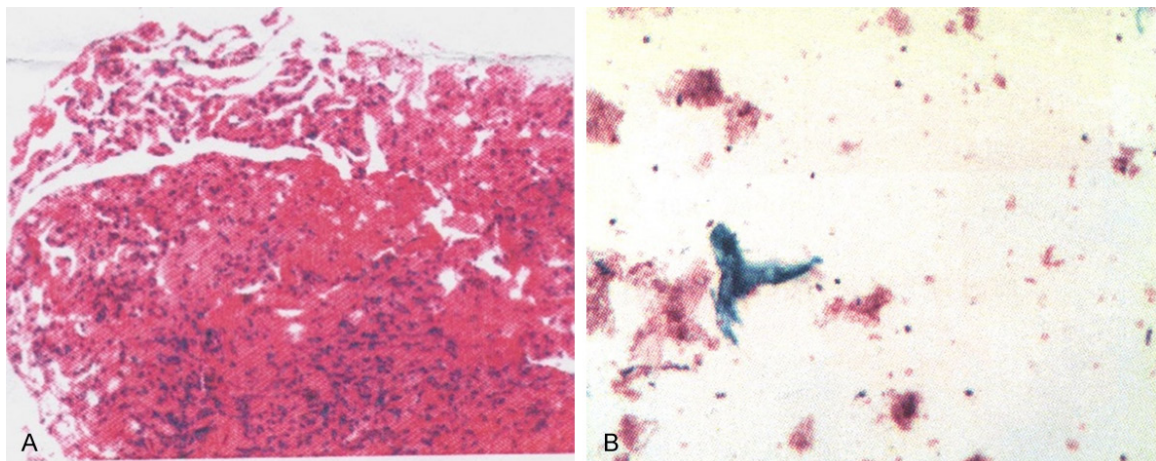
Figure 4. Hematoxylin-eosin staining. A: Biopsy of the upper lobe of the right lung: a small amount of lung tissue with hemorrhage. B: Bronchoscopy of the upper and anterior lobe of the right lung: neither tumor cells nor pathogens.

in lesion size, especially for an isolated peripheral pulmonary nodule [2, 8]. To distinguish benign from malignant nodules, we also performed a clinical prediction of pulmonary nodules using a mathematical prediction model (Dexin Medical Imaging Technology Co. Ltd.). The result showed that the lung imaging reporting and data system (Lung-RADS) classifi-

cation is 4A and suggested a biopsy (**Figure 5**). However, the patient refused an immediate lobectomy or a thoracoscopic biopsy.

ENB, first used in the United States in 2005, has become a reliable and effective endobronchial intervention technique over the past 10 years [9]. It is used for the diagnosis of difficult

Table 1. Loop-mediated amplification report of 13 respiratory pathogens in bronchoalveolar lavage fluid

No.	Name	Test results
1	*Streptococcus pneumoniae*	negative
2	*Staphylococcus aureus*	negative
3	*Methicillin-resistant staphylococcus*	negative
4	*Escherichia coli*	negative
5	*Klebsiella pneumoniae*	negative
6	*Pseudomonas aeruginosa*	negative
7	*Acinetobacter baumannii*	negative
8	*Stenotrophomonas maltophilia*	negative
9	*Haemophilus influenzae*	negative
10	*Legionella*	negative
11	*Mycobacterium tuberculosis complex*	negative
12	*Mycoplasma pneumoniae*	negative
13	*Chlamydia pneumoniae*	negative

Table 2. The loop-mediated amplification report of 17 mycobacteria in bronchoalveolar lavage fluid

No.	Name	Values
1	*Mycobacterium tuberculosis complex*	34
2	*Mycobacterium intracellular*	18
3	*Mycobacterium avium*	30
4	*Mycobacterium gordonae*	10
5	*Mycobacterium kansasii*	32
6	*Mycobacterium fortuitum*	8
7	*Mycobacterium scrofulaceum*	35
8	*Mycobacterium gilvum*	8
9	*Mycobacterium terrae*	25
10	*Mycobacterium chelonae/Mycobacterium abscessus*	6
11	*Mycobacterium phlei*	20
12	*Mycobacterium nonchromogenicum*	7
13	*Mycobacterium marinum/Mycobacterium ulcerans*	21
14	*Mycobacterium aurum*	7
15	*Mycobacterium szulgai/Mycobacterium malmoense*	18
16	*Mycobacterium xenopi*	5
17	*Mycobacterium smegmatis*	17

Text result: no mycobacterium

lesions near the lung periphery (such as lung tumors, pulmonary tuberculosis, and interstitial pulmonary diseases) and for the diagnosis of lymph node enlargement (such as lymph node metastasis, lymph node tuberculosis, and sarcoidosis). ENB can be extended for the treatment of peripheral lung lesions or mediastinal lesions (such as tumor interventional therapy or tuberculosis interventional therapy) and can guide the site determination for minimally inva-sive thoracic surgery [10-12]. The combined use of ENB with a peripheral ultrasound system can improve the diagnostic rate. Numerous studies have shown that ENB has distinct advantages over traditional operation methods. Ashraf reviewed the diagnostic rates and complications of ENB in peripheral pulmonary nodules and found it has a similar diagnosis rate to that of percutaneous pulmonary puncture, but a lower pneumothorax incidence [13]. Memoli assessed 39 studies with 3004 patients with pulmonary lung nodules and found that the pooled diagnostic yield of ENB was 70% [14]. Gex included 15 trials with 971 patients with peripheral lung nodules and the overall diagnostic accuracy was 73.9% [4]. Zhang looked at 17 studies consisting of 1106 patients with peripheral lung nodules and found a sensitivity of 82% and specificity of 100% with the diagnostic yield ranging from 60% to 94% [15].

In this case report, the patient had no respiratory symptoms or extrapulmonary symptoms. Only a 1.1-cm × 1.1-cm peripheral nodule was found using CT, which was adjacent to the pleura in the upper lobe of the right lung. Initially the conventional bronchoscopy showed a negative result. We used electromagnetic navigation through a laryngeal mask airway, with the patient under anesthesia. Transbronchial biopsies and BALF samples were collected. Hematoxylin-eosin staining confirmed that the nodule was a small amount of lung tissue with hemorrhage. The BALF was tested using LMAP, traditional isolation, and cultivation. The results showed that there was no pathogen present. Based on the imaging findings and serological examination results of the patient (the tumor biomarkers were negative and the T-SPOT test was positive), we concluded that this lesion was likely benign and a result of mycobacterial infection. However, the results from traditional culture and LMAP were negative. Therefore, we

Table 3. Next generation sequencing report of the microorganisms in bronchoalveolar lavage fluid

Type	Genus	Species	Total reads percent	Total reads
B:G+	Streptococcus	Retarded streptococcus	56.06%	37
B:G+	Streptococcus	Streptococcus pneumoniae	42.42%	28
B:G+	Mycobacterium	Mycobacterium sp.	1.52%	1

B:G+: gram-positive bacteria; Total reads percent: the ratio of the microbial fragment number in comparison to the total sequence fragment number of the same microorganism type; Total reads: the total number of fragments of the microbial sequences in comparison.

Table 4. Next generation sequencing report of the microorganisms in biopsy samples

Type	Genus	Species	Total reads percent	Total reads
B:G+	Mycobacterium	Mycobacterium sp.	77.50%	31
B:G+	Mycobacterium	Mycobacterium chelonae	22.50%	9

B:G+: gram-positive bacteria; Total reads percent: the ratio of the microbial fragment number in comparison to the total sequence fragment number of the same microorganism type; Total reads: the total number of fragments of the microbial sequences in comparison.
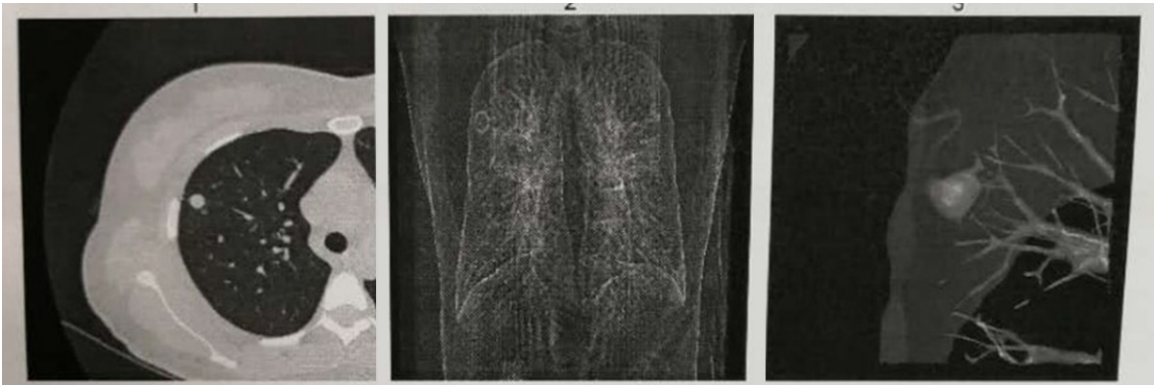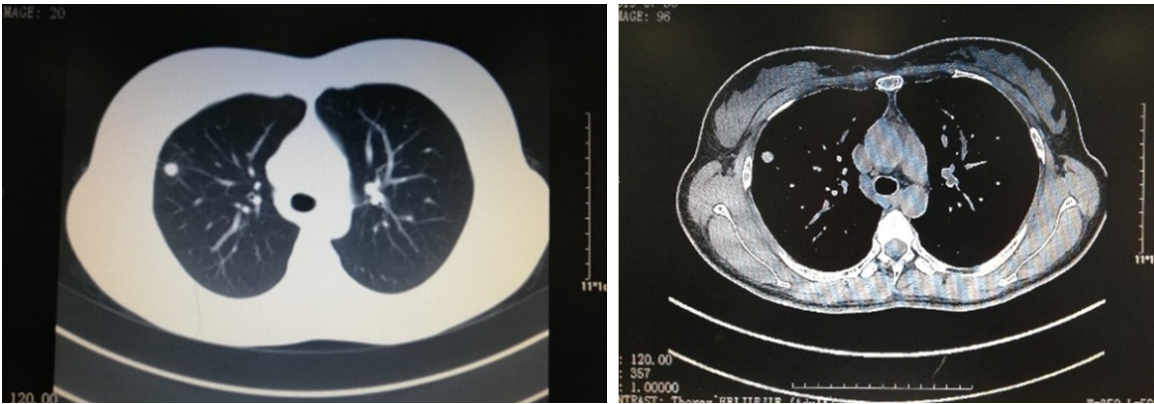

Figure 5. Pulmonary nodules (tumor) analysis. The Lung-RADS classification is 4A, suggesting a need for a biopsy.

Figure 6. Computed tomography (CT) with contrast of the chest after 6 months: Six months later, the patient re-checks the chest CT, and there is no obvious change on the node of upper lobe of the right lung.

looked for a new method to identify the biopsied lesion.

NGS technology provides a new method to detect microbial pathogens for the accurate diagnosis of clinical infection. NGS is characterized by fast detection speed, high accuracy, low cost, wide coverage, and huge output [16]. NGS is used in some clinical microbiology laboratories, and is often used in the investiga-

tion of infectious diseases outbreaks in hospitals, identification of unknown pathogens, virulence analysis, and the study of drug-resistant genomes [17, 18]. As a new molecular diagnostic method with high clinical value, it quickly and accurately detects pathogens in the blood stream, respiratory tract, central nervous system, abscesses, focal tissue, and other specimens [19-22]. In order to make a definitive diagnosis, NGS was performed on the biopsies and BALF samples. The NGS results showed that Mycobacterium chelonae (an NTM) was detected in both BALF and biopsy tissues. NTM can invade the lung, lymph node, skin, soft tissue, etc. Patients with NTM often present without distinctive respiratory symptoms. Sputum acid-fast staining in both TB and NTB patients are positive, and it is not possible to differentiate mycobacterium tuberculosis and non-mycobacterium tuberculosis using sputum acid-fast staining. The conventional microbiological methods are time-consuming and produce a low yield. With the rapid development of molecular biology technology, TB diagnosis and research methods have also made significant progress. One such method is NGS technology, which has become an important method to determine if and what type of mycobacterium infection is present.

Through a combination of ENB and NGS, the patient acquired an accurate diagnosis and prompt medical treatment and avoided the cost of surgery.

Conclusions

This case is the first to use ENB in combination with NGS to diagnose peripheral pulmonary nodules caused by Mycobacterium chelonae infection. Through this diagnostic technology, the peripheral pulmonary nodules of the patient were clearly diagnosed. In addition, the pain of surgery was avoided and the length and cost of hospitalization were reduced. Thus, this method provides a new way for the accurate diagnosis of peripheral pulmonary nodules.

Disclosure of conflict of interest

None.

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