To the Editor: Lower respiratory tract infections (LRTIs) are among the deadliest diseases and remain one of the leading causes of death over the last 15 years, especially in low-income countries, and are of great concern to immunosuppressed individuals. Infections located in the peripheral lung field (PLF) are located within the outer-third elliptical regions around the hilum on computed-tomography (CT) scans. They are surrounded by normal lung parenchyma and are unlikely to be visualized and accurately diagnosed by bronchoscopy. Recent studies have demonstrated the value of virtual bronchoscopic navigation (VBN)-assisted bronchoscopy as a diagnostic tool for small peripheral pulmonary lesions. Metagenomics next-generation sequencing (mNGS) is a new sequencing method comparable to first-generation DNA-sequencing technology. Using mNGS, we can perform high-throughput sequencing on clinical samples to analyze microbial communities. mNGS only requires a small amount of DNA directly taken from the sample, in order to screen for pathogens, as well as a bioinformatics tool, which identifies pathogens by linking sequencing reads to an accurate reference genome (or marker) database. The purpose of the present study was to explore the value of VBN combined with mNGS as a potential diagnostic tool in adults hospitalized with LRTIs.

This single-center retrospective study received ethical approval from the Institutional Review Board of the Tianjin Medical University General Hospital (No. IRB2019-133-01), and the requirement for obtaining the informed consent was waived.

We enrolled 136 patients who were referred to the Tianjin Medical University General Hospital between July 2018 and February 2019 with lesions located in the PLF.

Eighty-seven out of 136 patients underwent bronchoscopy with VBN and were categorized into VBN group, and the collected specimens were subjected to regular clinical microbiological assays, as well as tissue and bronchoalveolar lavage fluid (BALF) mNGS testing. Forty-nine out of 136 patients underwent bronchoscopy without VBN, and were categorized into non-VBN group and specimens were collected for mNGS and regular clinical microbiological assays. A total of 136 patients were categorized into two groups, infectious disease (ID) and noninfectious disease (NID) groups, according to the final diagnoses.

All the organisms identified were classified according to the strictly mapped reads and coverage rate of each organism in one sample. For further analysis, a list of the top-ten pathogens with the highest number of strictly mapped reads was obtained. First, bacterial (other than Mycobacterium tuberculosis [MTB]) was considered positive when strictly mapped reads were required to be > 3. Second, for fungi, the number of strictly mapped reads had to be > 3 and exceed the upper limit of the reference range. Third, for mycobacteria, because of the difficulty in DNA extraction and the low probability of contamination, Mycobacterium tuberculosis (MTB) was considered positive when strictly mapped reads were > 1.

Continuous variables with normal distribution were shown as mean ± standard deviation and analyzed using Student's t test. Categorical variables were presented as numbers and percentages and compared using the Pearson Chi-square test or Fisher exact test. The statistical analyses were performed with SPSS version 24.0 (IBM Inc., Chicago, IL, USA). All tests were two-sided, and P values < 0.05 were considered to be statistically significant.
There was no significant difference in gender (44.4% \textit{vs.} 55.6% $\chi^2 = 0.73$, $P = 0.393$) or age (43.1 ± 16.8 years \textit{vs.} 46.0 ± 16.7 years, $t = -1.585$, $P = 0.117$) between the ID and NID group. Forty (44.4%) of 90 patients were male in ID group and 24 (52.2%) of 46 patients were male in the NID group. In the ID group (90/136 [66.2%]), the majority of samples were identified to have pathogens that were bacteria (34/90 [37.8%]), viruses (13/90 [14.4%]), fungi (34/90 [37.8%]), and \textit{Mycobacterium tuberculosis} complex (MTBC) (9/90 [10.0%]). In the NID group, 90 patients were all diagnosed with confirmed pathogen infections using the conventional techniques, including 57 patients diagnosed using culture, 20 patients by pathology, and 13 patients using polymerase chain reaction (PCR).

All the specimens were subjected to conventional clinical culture as well as mNGS. In total, 250 samples underwent mNGS, 133 were from BALF, and 117 were from tissues; 166 samples were identified with pathogen infection. The top-three bacteria diagnosed using mNGS were \textit{Pseudomonas aeruginosa}, \textit{Acinetobacter baumannii}, and \textit{Rothia mucilaginosa}; the reads ranged from 3 to 14,978. The top-three fungi diagnosed using mNGS were \textit{Pneumocystis jirovecii}, \textit{Aspergillus fumigatus}, and \textit{cryptococcus neoformans}; the reads ranged from 3 to 95,738.

The distribution of bacteria obtained by culture was consistent with the results of mNGS, among which \textit{Pseudomonas aeruginosa} and \textit{Acinetobacter baumannii} species were the most common bacteria. The most common fungi obtained by culture were \textit{Aspergillus fumigatus}, and \textit{cryptococcus neoformans}.

In VBN group, the positive predictive value and negative predictive value of BALF mNGS were 90.6% and 63.3%, respectively. The positive predictive value and negative predictive value of tissue mNGSs were 92.1% and 56.7%, respectively. As expected, BALF mNGS and tissue mNGS increased the sensitivity rate in comparison with culture (81.6% \textit{vs.} 31.4%; $\chi^2 = 28.812$, $P < 0.001$; 72.9% \textit{vs.} 31.4%; $\chi^2 = 17.309$, $P < 0.001$), while the specificity differences between mNGS (BALF and tissue) and culture were not significant (79.2% \textit{vs.} 96.2%; $\chi^2 = 3.410$, $P = 0.093$; 85.0% \textit{vs.} 96.2%; $\chi^2 = 1.771$, $P = 0.303$).

The BALF mNGSs combined with VBN increased the sensitivity (81.6% \textit{vs.} 58.6%, $\chi^2 = 5.206$, $P = 0.023$) and specificity (79.2% \textit{vs.} 45.0%, $\chi^2 = 5.503$, $P = 0.019$) in comparison with BALF mNGSs without VBN. The differences in tissue mNGS sensitivity between VBN and non-VBN group were significant (72.9% \textit{vs.} 48.3%, $\chi^2 = 4.743$, $P = 0.029$). The differences in tissue mNGS specificity between VBN group and non-VBN group were not significant (85.0% \textit{vs.} 75.0%, $\chi^2 = 0.625$, $P = 0.429$).

Sixty-four samples were diagnosed with bacterial infections. The sensitivities of BALF mNGS in diagnosing bacterial infection were significantly different between VBN and non-VBN group (95.0% \textit{vs.} 57.1%, $P = 0.012$). However, the specificities of BALF mNGSs between VBN and non-VBN group were not significantly different (96.8% \textit{vs.} 85.7%, $P = 0.093$). The differences in sensitivity and specificity of tissue mNGSs between VBN and non-VBN group were not significant (62.5% \textit{vs.} 50.0%, $P = 0.713$; 98.1% \textit{vs.} 94.3%, $P = 0.562$).

Sixty-four samples were diagnosed with fungal infections. The sensitivities and specificities of BALF mNGSs between VBN and non-VBN group were not significantly different (76.9% \textit{vs.} 62.5%, $P = 0.649$; 94.8% \textit{vs.} 85.4%, $P = 0.157$). The sensitivities and specificities of tissue mNGSs between VBN and non-VBN group were not significantly different (68.2% \textit{vs.} 50.0%, $P = 0.417$; 95.7% \textit{vs.} 92.7%, $P = 0.663$).

In VBN group, 16 samples were identified with virus infections and eight samples were identified with tuberculosis infections. In non-VBN group, eight samples were diagnosed with virus infections and six samples were diagnosed with tuberculosis infections. Due to the relatively small size of the samples, statistical analysis was not performed.

This is a retrospective study to examine the diagnostic value of a VBN system assisting bronchoscopy combined with mNGS. The focus of the present study was on the etiological diagnosis of patients with infections in PLF using mNGS combined with VBN. In this study, there are increased benefits in diagnosis when mNGS is combined with VBN. We found that mNGS combined with VBN was advantageous in several aspects.

First, compared with conventional tissue sample culture, mNGS has significant advantages in the etiological diagnosis of LRTI. Compared with tissue sample culture, the sensitivity of pathogens detection using BALF mNGSs increased from 31.4% to 81.6%, while tissue mNGSs increased from 31.4% to 72.9%. Second, the present study indicated that mNGSs of BALF and tissues combined with VBN can accurately diagnose lung infections. The BALF mNGSs in VBN group increased the sensitivity (81.6% \textit{vs.} 58.6%) and specificity (79.2% \textit{vs.} 45.0%) in comparison with BALF mNGSs in non-VBN group. The tissue mNGS sensitivity of VBN group was significantly higher than that of non-VBN group (72.9% \textit{vs.} 48.3%). Third, mNGS also has high sensitivity and specificity in the diagnosis of bacteria. In our present study, the diagnostic sensitivities of BALF and tissue mNGSs in VBN group were 95.0% and 62.5%, respectively. The specificities of BALF and tissue mNGSs in VBN group were 96.8% and 98.1%, respectively. The sensitivity of BALF mNGS in VBN group increased significantly as compared with non-VBN group.

VBN is a technology that processes CT tomographic data to reconstruct dynamic images. VBN can reconstruct dynamic images of the intracavity to observe the internal surface by computer-simulation-navigation technology. Finally, VBN can be replayed to yield a dynamic reconstructed image, similar to endoscopy, which could move forward, backward, as well as rotate to observe the lesions directly. Recent studies have demonstrated the value of VBN-assisted bronchoscopy using endobronchial ultrasound (EBUS) as a diagnostic tool for small peripheral pulmonary lesions. Moreover, VBN has been recently reported to improve the diagnostic approach of peripheral small lesions. Therefore, we believe that VBN combined
with mNGS can improve the diagnostic efficiency of peripheral infection.

In addition, VBN is a safe method in which the bronchoscope is guided by the real image and the virtual image; no complications of VBN have been reported. Because VBN requires no special apparatus except software, the cost is not generally prohibitive. mNGS combined with VBN may reach the lesion site more accurately, and the specimens can be retained to improve the diagnostic value. However, the sample size of this study is insufficient, so more clinical trials are needed to confirm this finding.

In brief, the present study indicated that mNGS of BALF and tissues combined with VBN could be used to detect pulmonary pathogens in patients. There are increased benefits in sensitivity and specificity when combining mNGS with VBN for infections located in the PFL.

Conflicts of interest

None.

References

1. Baaklini WA, Reinoso MA, Gorin AB, Sharafkaneh A, Manian P. Diagnostic yield of fiberoptic bronchoscopy in evaluating solitary pulmonary nodules. Chest 2000;117:1049–1054. doi: 10.1378/chest.117.4.1049.
2. Langelier C, Zinter MS, Kalantar K, Yanik GA, Christenson S, O'Donovan B, et al. Metagenomic sequencing detects respiratory pathogens in hematopoietic cellular transplant patients. Am J Respir Crit Care Med 2018;197:524–528. doi: 10.1164/rccm.201706-1097LE.
3. Simner PJ, Miller S, Carroll KC. Understanding the promises and hurdles of metagenomic next-generation sequencing as a diagnostic tool for infectious diseases. Clin Infect Dis 2018;66:778–788. doi: 10.1093/cid/cix881.
4. Asano F. Advanced bronchoscopy for the diagnosis of peripheral pulmonary lesions. Respir Investig 2016;54:224–229. doi: 10.1016/j.resinv.2015.11.008.
5. Ishida T, Asano F, Yamazaki K, Shinagawa N, Ozumi S, Moriya H, et al. Virtual bronchoscopic navigation combined with endobronchial ultrasound to diagnose small peripheral pulmonary lesions: a randomised trial. Thorax 2011;66:1072–1077. doi: 10.1136/thx.2010.145490.

How to cite this article: Xu SF, Tian Q, Tian YL, Feng J, Zhao J, Yin XB. Detection of infectious pathogens located in the peripheral lung field by metagenomic next-generation sequencing combined with virtual bronchoscopic navigation. Chin Med J 2021;134:362-364. doi: 10.1097/CM9.0000000000001339