Application Value of BALF mNGS for Screening Patients With *Mycobacterium Tuberculosis* Infection in General Hospitals

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Abstract

Background

To evaluate the diagnostic value of metagenomic next-generation sequencing (mNGS) in patients with *Mycobacterium tuberculosis* infection.

Methods

Patients hospitalized for pneumonia in the Department of Pulmonary and Critical Care Medicine of Wuhan Central Hospital from January to June 2021 were enrolled in the study. Patients were examined by mNGS and divided into two groups according to the *M. tuberculosis* infection. We compared the clinical indicators of infected patients of both groups. All patients in the *M. tuberculosis* infection group underwent bronchoalveolar lavage (BAL). Different detection methods were used to detect pathogenic bacteria.

Results

A total of 111 patients agreed to undergo mNGS. Of these, 15 were eventually enrolled in the study group. We found that patients with decreased white blood cells had a higher risk of infection with *M. tuberculosis* than those infected with other pathogens (OR=0.807, 95%CI: 0.672–0.969, P=0.022). We also found that mNGS had a higher positive rate of *M. tuberculosis* infection detection than traditional detection methods. The positive detection rates of TB-DNA-PCR, Xpert MTB, and T-SPOT. TB were 69.23%, 83.33%, 77.77%, respectively.

Conclusions

BALF mNGS assay could quickly and effectively detect *M. tuberculosis* complex in patients with pulmonary infection in a general hospital setting.

Background

Tuberculosis (TB) is a pulmonary infectious disease caused by *Mycobacterium tuberculosis* that seriously affects human health and is one of the most critical public health problems of the world(1, 2). According to the report of the World Health Organization, 10.4 million new TB patients were diagnosed globally in 2016. The number of TB patients in China accounts for about 14.3% of the world, and the incidence rate in some areas is increasing yearly. Approximately 1.5 million deaths worldwide have been attributed to TB alone(3).The early onset of TB has no typical clinical symptoms and signs, and is not easy to detect. However, if not treated in a timely manner, it will cause severe damage to the respiratory system, and even endanger patients' life in severe cases. Early diagnosis and treatment is key to the prevention and control of TB epidemic(4).

The typical clinical manifestations of pulmonary TB include cough, hemoptysis, and other symptoms, such as low fever, anemic, et al, and lung computed tomography (CT) usually shows tree bud sign and cavity formation(5). However, *M. tuberculosis* infection is more often found in hospitalized pneumonia patients in the respiratory department of general hospitals rather than non-tuberculosis specialized hospitals, although they do not have the above typical pulmonary TB manifestations and imaging findings.

Therefore, it is very challenging but important to screen out patients infected with *M. tuberculosis* from general pulmonary infection as soon as possible.

Real-time fluorescent quantitative nucleic acid amplification (Xpert Mtb/RIF), T cell SPOT test (T-SPOT.TB), acid-fast staining smear, TB-DNA-polymerase chain reaction (PCR) and *M. tuberculosis* culture are commonly used detection methods of TB(6). Xpert Mtb/RIF is an automatic, quantitative, and semi-nested real-time fluorescence PCR detection technique, which is used as the initial test for TB to increase case detection directly from sputum(7). However, the Xpert's sensitivity for TB detection is inadequate when only few bacilli are present in a clinical specimen, which limits the usefulness of Xpert in patients with sputum smear-negative(8). T-spot can be used for rapid TB diagnosis with high sensitivity. However, studies have reported that the sensitivity of T-SPOT is very high, resulting in a high false-positive rate. Acid-fast staining smear is a traditional TB detection method with high specificity but low sensitivity, and can easily cause missed detection. *Mycobacterium tuberculosis* culture is the gold standard for diagnosis of TB, but the time required is too long, which is not conducive to clinical diagnosis and treatment.

With the wide application of bronchoscopy in the examination of pulmonary diseases, metagenomic next-generation sequencing (mNGS) assay using bronchoscopic alveolar lavage fluid (BALF) is gradually recommended for clinical diagnosis of *M. tuberculosis* complex. mNGS assay is a high-throughput sequencing technology, which is characterized by fast detection speed, high accuracy, low cost, wide coverage, and huge output. The mNGS assay works by running all nucleic acids in a sample that may contain mixed populations of microorganisms, and assigning these to their reference genomes to identify which microbes are present and in what proportions. The examination technology combining bacteriology and molecular biology is one of the important advances in medicine, and is considered a convenient and efficient technology to study the epidemiology of infectious diseases, which is expected to change the understanding of clinical microbiology.

At present, there are relatively few studies using mNGS assay of BALF to screen for *M. tuberculosis* complex infection in hospitalized pneumonia patients in the respiratory department of general hospitals, and it is unknown whether its diagnostic ability is superior to that of conventional test methods. Therefore, we designed this study to investigate whether mNGS assay can effectively screen patients infected with *M. tuberculosis* complex from hospitalized patients with pulmonary infection in general hospitals. The application value of the above different detection methods was compared to explore their clinical diagnostic value of *M. tuberculosis* complex in patients hospitalized with pneumonia.

Methods

Study Design and Participants

This was a cross-sectional study. The study population included all patients hospitalized in the Department of Pulmonary and Critical Care Medicine of Wuhan Central Hospital due to pulmonary infection from January to June 2021. The Wuhan Central Hospital is a tertiary teaching hospital that admits a large number of patients with pulmonary infection every year. Depending on the severity of illness and the will of patients, mNGS assay of some patients was tested by Vision Medicals. Vision Medicals is a company founded in 2008 that specializes in mNGS. Based on extensive metagenomics data, the company has published 15 high-quality articles and applied for nearly 30 invention patents. Moreover, Vision Medicals was the only company to receive a full score in the external quality assessment conducted by the National Center for Clinical Laboratories in 2020. Therefore, the company is a reliable inspection agency, and the data it provides are credible.

The inclusion criteria of patients were as follows: positive respiratory symptoms, lung CT showing signs of infection, complete basic laboratory tests, BALF sent for mNGS assay, and *M. tuberculosis* complex was detected in the mNGS results. The exclusion criteria were as follows: *M. tuberculosis* complex was not found in the mNGS results. In such cases, the BALF mNGS of the same patients was sent repeatedly. Patients with mNGS of non-BALF sample were submitted for examination. Patients with missing data were also excluded. The patient selection process is depicted in a flowchart in Fig. 1.

This study was approved by the Ethics Committee of Wuhan Central Hospital. As it was a retrospective study and did not require protection of patients' privacy, we were exempted from obtaining informed consent.

Data collection

We reviewed electronic medical records (EMRs), from which we obtained chronic comorbidities, demographic data, laboratory examination results, and chest CT images. From the nursing records, we can obtain the patient's vital signs at the time of admission. Data on counts or levels of white blood cells (WBCs), hemoglobin (Hb), lymphocytes (L), neutrophils (N), alanine transaminase (ALT), aspartate aminotransferase (AST), serum albumin (ALB), serum creatinine (sCr), blood urea nitrogen (BUN), serum procalcitonin (PCT), and C-reactive protein (CRP) were included. The results of T-spot TB test, TB-DNA-PCR, and X-pert MTB assay were also obtained. From the medical records, we could learn the patient's outcome, complain, and course of the disease. All data were checked by another researcher to ascertain their accuracy.

The mNGS of BALF

Owing to the high cost of mNGS testing and the inability of our hospital to carry out this examination, all BALF samples for mNGS were sent to Vision Medicals for testing with the consent of patients and their

families. BALF was obtained via a bronchoscope by a skilled physician; the samples were then stored and transported at 4–8°C after collection, and the NGS results were usually available within 48 h. We included only DNA sequence mNGS results.

Statistical analysis

Continuous variables were used for the mean and standard deviation (SD) and compared using *t*-tests if they were normally distributed; if not, they were described using the median and interquartile range (IQR) values. The Mann–Whitney U test was used for comparisons. Categorical variables were expressed as count (%) and compared by chi-squared test or Fisher's exact test. Logistic regression analysis was used to assess the risk factors of *M. tuberculosis* infection. A two-sided α value < 0.05 was considered statistically significant. We used SPSS software (version 23.0) for statistical analysis.

Results

Basic Characteristics

A total of 111 patients hospitalized for pneumonia in the Department of Pulmonary and Critical Care Medicine of Wuhan Central Hospital from January to June 2021 agreed to undergo mNGS assay, and 117 results were obtained. According to the inclusion and exclusion criteria, 15 subjects were finally enrolled in the study.

The mean age of the participants was 66 years (11 male and 4 female). Eight participants were treated in the respiratory intensive care unit and seven were treated in the general ward. Four (26.67%) patients had underlying pulmonary diseases, including two cases of chronic obstructive pulmonary disease and one case each of pulmonary fibrosis and bronchiectasis. Four of the participants had a history of type 2 diabetes. In addition, three of the participants had high blood pressure and two had malignant tumors (Table 1).

Table 1
Comparison of clinical features between patients with and without *M. tuberculosis* infection

	Patients with <i>M. tuberculosis</i> infection (n = 15)	Patients without <i>M. tuberculosis</i> infection (n = 71)	P
Male (%)	11 (73.33%)	48 (67.60%)	0.767
Age (years)	65.93 (12.91)	64.06 (15.98)	0.671
Previous lung disease (%)	4 (26.67%)	21 (29.58%)	1.0
Diabetes mellitus (%)	4 (26.67%)	14 (19.72%)	0.507
Hypertension (%)	3 (20%)	24 (33.8%)	0.371
Malignant tumor (%)	1 (6.67%)	9 (12.68%)	0.484
Smoking (%)	3 (20%)	32 (45.07%)	0.088
Drinking (%)	1 (6.67%)	10 (14.08%)	0.680
Non-survivors (%)	3 (20%)	14 (19.72%)	1.0
WBC (10 ⁹)	7.76 (2.58)	11.49 (5.92)	0.019
Hb (g/L)	117.13 (21.04)	123.27 (19.67)	0.281
N (10 ⁹)	6.02 (4.77,7.87)	8.80 (5.71,11.82)	0.000
L (10 ⁹)	0.97(0.78)	1.16(0.73)	0.385
CRP(mg/dL)	7.25(7.47)	7.51(9.94)	0.927
PCT(ng/ml)	1.95(2.88)	5.70(17.61)	0.415
Cr(umol/L)	68.53(25.19)	77.28(56.19)	0.558
BUN(mmol/L)	6.49(2.04)	6.66(4.75)	0.892
ALT(U/L)	15.50 (10.30,33.50)	16.70 (11.00,27.20)	0.475
AST(U/L)	26.10 (17.50,58.50)	22.00 (15.00,30.00)	0.338
ALB(g/L)	30.00 (24.40,39.40)	36.20 (31.20,41.00)	0.128
WBC: White blood cell, Hb: Hemoglobin, L: Lymphocytes; N: Neutrophils; ALT: Alanine Transaminase, AST: Aspartate aminotransferase, ALB: Serum albumin, Cr: Serum creatinine, BUN: Blood urea nitrogen, PCT: Serum procalcitonin, CRP: C-reactive protein.			

To further understand the clinical characteristics of patients infected with *M. tuberculosis* complex, we compared the clinical indicators of patients infected with and without *M. tuberculosis*. According to the

analysis, levels of white blood cells in patients infected with *M. tuberculosis* were significantly lower than those infected with other pathogens ($P = 0.019$). Other factors such as age, sex, hypertension, diabetes, liver function, and kidney function showed no significant difference between the two groups (Table 1). To adjust for other confounding factors, we conducted two logistic regression analyses to further verify the relationship between levels of white blood cells and the risk of *M. tuberculosis* infection. We found that patients with decreased white blood cells had a higher risk of infection with *M. tuberculosis* than those infected with other pathogens ($OR = 0.807$, $95\%CI = 0.672-0.969$, $P = 0.022$).

Clinical Characteristics of Patients with M. Tuberculosis

The chief complaints of patients at admission were varied. Fever, cough, dyspnea, chest pain, fatigue, and sore throat were the common complaints of these patients, among which cough accounted for the highest proportion (33%) (Fig. 2). Before admission, the average course of disease was about 13 days, the shortest was 1 day, and the longest was 90 days.

CT imaging features of the lung were also analyzed. The lung CT data of one patient was lost, so the lung CT characteristics of only 14 patients are discussed. The distribution of lung lesions was shown in Fig. 3. We found that the lower lobe of the right lung was the most common lesion site (29%), followed by the middle lobe of the right lung (24%). Diffuse lesions in both lungs were not rare (19%). We further scrutinized the imaging features of pulmonary lesions. Figure 4 shows that lung consolidation was the most common imaging signs of *M. tuberculosis*. Tree bud signs, interstitial lesions, and cavities were also common imaging manifestations of pulmonary disease caused by *M. tuberculosis* infection.

Results of Etiological Examination

All 15 included patients underwent BAL. Different detection methods are used to detect pathogenic bacteria, and the positive rate was diverse (Fig. 5). The BALF mNGS reports of 15 patients revealed *M. tuberculosis* complex. The sequence number of *M. tuberculosis* complex detected was at least 1 and at most 328080. Fourteen patients also underwent acid-fast staining using Ziehl–Neelsen (Z–N) stain for sputum smear microscopy, but the results were negative. TB-DNA-PCR arrays was performed on 13 of the enrolled patients using 12 BALF specimens and one lung tissue obtained by ultrasound bronchoscopy. The results of TB-DNA-PCR arrays were positive in nine patients and negative in four, and the positive rate was 69.23% compared with the result of BALF mNGS. Xpert MTB was detected in six patients, one of whom was negative and the rest positive, with a positive rate of 83.33%. In addition, nine patients were tested for the T-SPOT.TB from blood samples, of which seven were positive and two were negative, with a positive rate of 77.77%.

In BALF mNGS results of some included patients, other pathogens such as bacteria and fungi besides *M. tuberculosis* were also found. *Lactobacillus salivarius* and *L. gernerii*—normal bacterial flora in the human body—were identified as non-pathogenic bacteria based on the clinical conditions presented. According to the results of BALF mNGS, eight and seven patients were found with and without bacterial infection, respectively. The mixed pathogenic bacteria were *Haemophilus parahaemolyticus*, *H. parainfluenzae*,

Escherichia coli, *Streptococcus* spp., *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Moraxella catarrhalis*, and *Legionella pneumophila*.

Fungal infection mainly by *Candida albicans*, was found in the BALF mNGS results of six patients. *Aspergillus* and *Pneumocystis yersinia* were also detected.

Outcome of patients with *M. tuberculosis*

As *M. tuberculosis* infection is a national statutory infectious disease, it needs to be treated in designated specialist hospitals, so it is our duty to refer the patient into a specialist hospital after diagnosis. Of the 15 patients in this study who were diagnosed with *M. tuberculosis* infection, 12 were transferred to a specialist hospital, while the remaining three were not transferred, because they were critically ill and died before or shortly after the diagnosis. Of these three patients, two received endotracheal intubation and invasive ventilator-assisted ventilation, and one received nasal high-flow oxygen therapy.

By analysis, the ratio of neutrophils to lymphocytes in the dead patients was significantly higher than that in the survivors (9.34 vs. 28.43, $P = 0.002$). Furthermore, the ratio of neutrophil to lymphocyte was positively correlated with the sequence number of *M. tuberculosis* ($P = 0.025$). In addition, all three patients who died were found to be co-infected with other bacterial infections.

Discussion

This is a retrospective study carried out in the department of respiratory medicine of general hospital. Throughout the study, we found that the mNGS assay could quickly and effectively screen patients with *M. tuberculosis* infection from patients with pulmonary infections in a general hospital setting. In addition, we also found that not all patients with *M. tuberculosis* infection had typical clinical and pulmonary imaging findings, which made it difficult to screen patients early for *M. tuberculosis* infection.

Patients in our study had varying degrees of severity at admission. Nearly half of the patients required treatment in the respiratory intensive care unit because of their condition, while the other half were treated in the general ward. The chief complaints of patients at admission varied, and the duration of onset was also different. Not all patients had chronic cough, hemoptysis, night sweats, low fever, and other typical clinical manifestations(3). In addition, lung CT analysis showed that the most common sites of lung infection in the included patients were right lower lobe and right middle lobe. The most common pulmonary CT imaging feature was pulmonary consolidation, so it was difficult to provide any evidence of etiology and potential *M. tuberculosis* infection(9). Therefore, it was very challenging for respiratory specialists to quickly and accurately determine whether a patient was infected with *M. tuberculosis* based on experience alone, although we found that decreased white blood cells can increase the risk of infection with *M. tuberculosis*; hence, finding an appropriate test is an urgent task.

mNGS is an encouraging, culture-independent approach for identifying potentially pathogenic microorganisms(10), which could detect a range of pathogens such as bacteria, fungi, viruses, and *M. tuberculosis*, rather than just a specific pathogen(11). We collected data on 111 patients who underwent

BALF mNGS assay, and searched for *M. tuberculosis* complex in BALF in 15 of them, with a positive rate of 15.31%. Another study, also utilizing mNGS assay, detected *M. tuberculosis* complex in BALF in only 3 of 132 patients(12). This discrepancy in results is likely due to geography. The sequence number of *M. tuberculosis* detected was at least 1. As it is difficult for *M. tuberculosis* to break the cell wall during nucleic acid extraction, even if the detected sequence number of *M. tuberculosis* is low, it should be considered as a pathogenic microorganism(13, 14). Langelier et al. considered that the mNGS results can be judged as positive as long as the sequence number of *M. tuberculosis* complex detected was ≥ 1 (15).

Besides BALF mNGS, some patients also underwent other assays to detect *M. tuberculosis*, such as acid-fast staining of sputum smear microscopy, TB-DNA-PCR, T-SPOT.TB, and Xpert MTB. It is well known that acid-fast staining of sputum smears has low sensitivity, because it requires high bacillary content (5000–10,000 CFU/mL) in the sputum and multiple sputum samples. In our study, acid-fast staining results of sputum smears were negative, and the reason may be that we do not inspect sputum specimens repeatedly. TB-DNA-PCR assays were tested in 13 of 15 patients, with a positive rate of 69.23%, which is similar to that of Muhammad Idrees' study (70%); however, in other samples such as blood, urine, and cerebrospinal fluid, the positive rate of PCR detection was significantly lower than 70%(16). Xpert MTB testing was carried out in six patients, and the positive rate was 83.33%, which was higher than 70% for smear-negative samples in another study(17) and maybe it's because the specimen by tested was BALF, so as to improve the positive rate of detection. Nine patients were tested using T-SPOT.TB from blood samples, of which seven and two were positive and negative, respectively, with a positive rate of 77.77%, which was slightly different from those of other studies with a positive rate of 61.44%(18). The difference may be due to different prespecified cut-off points. The above results suggested that T-SPOT.TB is of great significance in eliminating *M. tuberculosis* infection.

In our study, mNGS assay also detected other pathogens including bacteria and fungi, in the BALF of 53.33% patients with *M. tuberculosis* infection. Through the mNGS assay, Ye et al. also ascertained that some patients, especially immunosuppressed ones, were often infected with a mixture of multiple pathogens(12). Moreover, all of the patients who died were infected with *M. tuberculosis* combined with other bacteria or fungi. Thus, mNGS can also detect pathogens other than *M. tuberculosis* such as bacteria or fungi, which other tests do not. Being able to identify pathogens can be encouraging for clinicians superficially, but remains a challenge. When an mNGS assay simultaneously shows *M. tuberculosis* and other pathogenic bacteria, the actual causative pathogenic bacteria should be judged by clinicians based on the symptoms and imaging manifestations of patients.

Although mNGS assay can effectively detected *M. tuberculosis* complex, mNGS also has certain limitations. According to the working principle of mNGS assay, it was difficult to distinguish whether *M. tuberculosis* is active or dormant. At present, TB is widely perceived as a bacterial infection with a long-term pathogen persistence in a latent or an active form(19, 20) that can remain in the host for a long time because it has the ability to remain dormant; thereby not being cleared by the host's immune cells(20, 21). Dormancy is the state when bacteria cease to divide yet do not die and the host is regarded as having latent TB infection (LTBI). According to epidemiological data, approximately 1.6–1.9 billion people

worldwide suffer from LTBI(22).Therefore, determining whether *M. tuberculosis* is a pathogenic bacterium is a problem that the mNGS assay cannot solve at present. In addition, the cost of mNGS is still high in China, and it cannot be carried out routinely in general hospitals.

Our study has some limitations. First, our study was a retrospective study, and a prospective study is needed to evaluate the ability of mNGS assay to screen *M. tuberculosis* infection in general hospitals. Second, due to the high cost of mNGS, fewer patients underwent mNGS testing, so the study enrolled a small number of patients. Subsequently, we will continue to collect more patient data to further confirm our findings.

Conclusion

BALF mNGS assay is a rapid and effective method for screening *M. tuberculosis* infection in a general hospital. Its sensitivity is higher than that of other conventional detection methods, and it can also detect other pathogens, which has important guiding value for clinicians and is of great significance to curb the spread of TB. Further large-scale studies are needed to evaluate the application value of BALF mNGS in the diagnosis of *M. tuberculosis* infection in general hospitals.

Declarations

Funding

No any funding for this study.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Wuhan Central Hospital. As it was a retrospective study and did not require protection of patients' privacy, we were exempted from obtaining informed consent.

Availability of data and materials

All data generated or analysed during this study are included in this published article.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author contributions

Y.Z., X.Y.L. and Y.F.W. had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: S.L.Y. Acquisition, analysis, or interpretation of data: Y.F.W., Y.Z. and X.C. Drafting of the manuscript: Y.F.W., Y.Z., X.C. Critical revision of the manuscript for important intellectual content: all authors. Statistical analysis: Y.F.W, X.C. Administrative, technical, or material support: S.L.Y. Study supervision: Y.F.W. and S.L.Y.

References

1. Arman A, Artin V, Aram Y, Abdul Latif AK, Vishwanath V. Review of the Effectiveness of Various Adjuvant Therapies in Treating Mycobacterium tuberculosis. *Infectious disease reports*. 2021;13(3):821-34.
2. Gautam K, Negi S, Saini V. Targeting endogenous gaseous signaling molecules as novel host-directed therapies against tuberculosis infection. *Free Radical Research*. 2021(7):1-16.
3. Organization WH. Global tuberculosis report 2018. 2018.
4. Ye E, Schluger NW. Advances in the diagnosis and treatment of latent tuberculosis infection. *Current Opinion in Infectious Diseases*. 2020;33(2).
5. Khan AU, Khanduri S, Tarin Z, Abbas SZ, Jain S. Dual-Energy Computed Tomography Lung in patients of Pulmonary Tuberculosis. *Journal of Clinical Imaging Science*. 2020;10:39.
6. Bin A, Joohae K, Chul-Gyu Y, Whan KY, Koo HS, Jae-Joon Y. Changes in Diagnostic Methods for Pulmonary Tuberculosis between 2005 and 2013. *Tuberculosis and respiratory diseases*. 2020;78(3):227-31.
7. Aninagyei E, Ayivor-Djanie R, Attah J, Dakorah MP, Acheampong DO. Molecular detection of Mycobacterium tuberculosis in blood stained sputum samples using GeneXpert PCR assay. *Diagnostic Microbiology and Infectious Disease*. 2021(2):115363.
8. Theron G, Peter J, Dowdy D, Langley I, Squire SB, Dheda K. Do high rates of empirical treatment undermine the potential effect of new diagnostic tests for tuberculosis in high-burden settings? *Lancet Infectious Diseases*. 2014;14(6):527-32.
9. Pletz MW, Blasi F, Chalmers JD, Cruz C, Aliberti S. International perspective on the new 2019 ATS/IDSA CAP guideline – a critical appraisal by a global expert panel. *Chest*. 2020;158(5).
10. Ramachandran PS, Wilson MR. Metagenomics for neurological infections – expanding our imagination. *Nature Reviews Neurology*. 2020;16(10):1-10.
11. Chiu CY, Miller SA. Clinical metagenomics. *Nature Reviews Genetics*. 2019.
12. Zhan Y, Xu T, He F, Guan WJ, Li Z, Li S, et al. Clinical Evaluation of a Metagenomics-Based Assay for Pneumonia Management. *Front Microbiol*. 2021 Sep 16;12:751073.
13. Clsa B, Peng HC, Pjta B, Mmc C, Zjya B, Mywa B, et al. Clinical metagenomic sequencing for diagnosis of pulmonary tuberculosis - ScienceDirect. *Journal of Infection*. 2020;81(4):567-74.
14. Schlaberg R, Chiu C, Miller S, Procop GW, Weinstock G. Validation of Metagenomic Next-Generation Sequencing Tests for Universal Pathogen Detection. *Archives of Pathology & Laboratory Medicine*.

2017:arpa.2016-0539-RA.

15. Langelier C, Zinter MS, Kalantar K, Yanik GA, Derisi JL. Metagenomic Sequencing Detects Respiratory Pathogens in Hematopoietic Cellular Transplant Patients. *Am J Respir Crit Care Med*. 2018;197(4).
16. Amin I, Idrees M, Awan Z, Shahid M, Hussain A. PCR could be a method of choice for identification of both pulmonary and extra-pulmonary tuberculosis. *Bmc Research Notes*. 2011;4.
17. Catharina, C., Boehme, Pamela, Nabeta, Doris, et al. Rapid Molecular Detection of Tuberculosis and Rifampin Resistance. *New England Journal of Medicine*. 2010.
18. Zhong H, Wu H, Yu Z, Zhang Q, Huang Q. Clinical evaluation of the T-SPOT.TB test for detection of tuberculosis infection in northeastern Guangdong Province, China. *The Journal of international medical research*. 2020;48(5):030006052092353.
19. Stewart GR, Robertson BD, Young DB. Tuberculosis: A problem with persistence. *Nature Reviews Microbiology*. 2003;1(2):97-105.
20. Esmail H, Barry CE, Young DB, Wilkinson RJ. The ongoing challenge of latent tuberculosis. *Philosophical Transactions of The Royal Society B Biological Sciences*. 2014;369(1645):20130437.
21. Chao MC, Rubin EJ. Letting sleeping dos lie: does dormancy play a role in tuberculosis?. [Review]. *Annual Review of Microbiology*. 2013;64(1):293-311.
22. Houben R, Dodd P. The Global Burden of Latent Tuberculosis Infection: A Re-estimation Using Mathematical Modelling. *Plos Medicine*. 2016;13(10):e1002152.

Figures

Figure 1

Screening flow chart of patients included in the study.

Figure 2

Chief complaints of patients with mycobacterium tuberculosis on admission

Figure 3

Distribution of lung lesions of patients with mycobacterium tuberculosis

Figure 4

Imaging features of pulmonary lesions of patients with mycobacterium tuberculosis

Figure 5

Positive rate of different detection methods