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ORIGINAL RESEARCH Metagenomic Next-Generation Sequencing for Accurate Diagnosis of Pneumocystis jirovecii Pneumonia: A Comparative Study with Traditional **Methods**

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Background: Metagenomic next-generation sequencing (mNGS) is a high-throughput sequencing technique that identifies a wide array of pathogens directly from clinical specimens. This study evaluates the diagnostic value of mNGS in Pneumocystis jirovecii pneumonia (PJP) and compares its efficacy with traditional detection methods, including Grocott's Methenamine Silver (GMS) staining, serum (1-3)- β -D-Glucan (BDG) testing, and Lactate Dehydrogenase (LDH) testing.

Methods: Seventy-eight patients hospitalized between January 2022 and March 2023 with suspected pulmonary infections were included. Patients were eligible for mNGS if they exhibited symptoms such as fever, cough, dyspnea, or progressive hypoxemia, and met specific clinical criteria for PJP. Specimens obtained included bronchoalveolar lavage fluid, sputum, and peripheral blood. Positive rates and pathogen distributions detected by mNGS and traditional methods were compared.

Results: In the PJP group, 25%, 37.5%, and 9.38% of patients had solid organ tumors, corticosteroid use, and skin diseases, respectively, significantly higher than in the non-PJP group. The sensitivity and specificity of mNGS were both 100%, significantly higher than those of serum BDG (sensitivity 50%, specificity 81.8%) and LDH (sensitivity 9.3%, specificity 91.3%). Significant differences in microbial composition between the PJP and Non-PJP groups were observed. mNGS detected multiple mixed pathogens in 96.88% of PJP cases, with 68.75% exhibiting mixed bacterial and viral infections. Notably, 71% of patients improved following antibacterial treatment based on mNGS results.

Conclusion: mNGS technology shows superior sensitivity and specificity in diagnosing PJP and guides precise treatment for complex pulmonary infections.

Keywords: mNGS, Pneumocystis jirovecii pneumonia, pulmonary infections, dominant pathogens, sensitivity

Introduction

Pneumocystis jirovecii pneumonia (PJP) is an opportunistic, pathogenic fungal infection caused by Pneumocystis *jirovecii* and is the most commonly observed concurrent infection in patients with AIDS.¹ Epidemiological data on PJP in non-HIV Chinese patients indicate that this opportunistic infection is increasingly seen among immunocompromised individuals due to conditions such as hematological malignancies, solid organ transplants, and the use of immunosuppressive therapies.^{2,3} Eighty percent of patients with AIDS experience PJP at a certain stage or at the end of the disease, and 24% of patients with AIDS die directly from PJP.⁴ Due to the wide application of immunosuppressants, the incidence of PJP in patients with non-HIV-infected malignant tumors, hematological diseases, or autoimmune diseases has been increasing.⁵⁻⁷ Patients not infected with AIDS but diagnosed with PJP have a more insidious onset, faster progression, and higher mortality rate than patients with PJP with concurrent HIV infection do.^{8,9}

Because *Pneumocystis jirovecii* cannot be grown in vitro in culture media, the current gold standard for diagnosing PJP is the detection of characteristic cysts or trophoblasts in lower respiratory tract specimens by using staining microscopy. Although traditional hexamine silver staining microscopy can diagnose PJP by detecting cysts or trophozoites, qPCR detection can confirm the diagnosis, and combined with serum (1-3)-β-D-glucan (BDG) levels, it can improve diagnostic accuracy.¹⁰ However, traditional methods can result in missed diagnoses. The symptoms of patients not infected with HIV but diagnosed with PJP were more severe, and the hospitalization rate in the intensive care unit was higher. Therefore, early diagnosis and standardized treatment with sensitive antibiotics are more important.¹¹

Metagenomic next-generation sequencing (mNGS) is a high throughput sequencing technique that does not rely on culture to directly extract nucleic acids from clinical specimens for pathogen detection.¹² Its advantages are high throughput, high sensitivity.¹²⁻¹⁵ Compared with traditional detection, mNGS comprehensively detects thousands of pathogens without bias, including dead pathogens.^{16,17} In 2014, Wilson et al¹² reported the first use of mNGS technology to detect Leptospira in the cerebrospinal fluid of children. Several studies have also explored the potential of mNGS in the diagnosis of PJP. For instance, Lu et al¹⁸ compared mNGS with GMS staining and PCR in non-HIV-infected PJP patients and found that mNGS significantly reduced detection time while maintaining high sensitivity and specificity. Similarly, Li et al^2 conducted a meta-analysis to evaluate the diagnostic accuracy of mNGS for PJP, concluding that mNGS exhibited excellent performance in both immunocompromised and non-HIV patients. Chen et al¹⁹ also reported that mNGS showed satisfactory diagnostic performance in critically ill pediatric patients with PJP and was effective in detecting co-infections. Despite these findings, there is still a lack of largescale, multicenter studies on the application of mNGS in PJP diagnosis. This study aims to explore the diagnostic value of mNGS in PJP and compare its efficacy with traditional detection methods, providing a more comprehensive assessment of its clinical utility. The study's findings advocate for the adoption of mNGS in routine clinical workflows to improve diagnostic accuracy, optimize treatment strategies, and ultimately enhance patient care in complex pulmonary infections.

Methods

Research Design

In this retrospective study, 78 patients hospitalized in the Intensive Care Medicine and Respiratory and Critical Care Department of Meizhou People's Hospital between January 2022 and March 2023 with suspected pulmonary infections were tested for mNGS. Among these, 32 patients were diagnosed with PJP based on clinical judgment, confirmatory tests, and mNGS results. This study was approved by the Ethics Committee of Meizhou People's Hospital (Ethics approval No.: Meishi Lunshen 2021-C-121). Informed consent was obtained from all the participants. This study complies with the principles outlined in the Declaration of Helsinki.

Inclusion criteria for the participants were as follows: (1) fever, cough, dyspnea, and progressive hypoxemia; (2) one of their gomori methenamine silver (GMS) staining or mNGS tests for *Pneumocystis jirovecii* was positive;^{20,21} (3) imaging revealed bilateral interstitial and ground glass opacity in the lungs and alveolar infiltration in the perihilar area; (4) bronchoalveolar lavage fluid (BALF) and peripheral blood (PB) were collected for mNGS; (5) in the immunosuppressive phase,²² that is, within the last 3 months, they had corticosteroid treatment, malignant tumors, rheumatic diseases, solid organ transplantation, or immunosuppressive treatment. The exclusion criteria for the participants were as follows: (1) age<18 years, (2) not undergoing mNGS, and (3) incomplete medical records.

The gold standard for diagnosing PJP was a comprehensive clinical judgement by two senior pulmonary experts. This judgement was based on a combination of clinical symptoms, laboratory test results, chest radiology, microbiological examination, and treatment response. The overall design is presented in Figure 1. All data were subjected to past identification and anonymous processing.

Threshold Criteria for PJP Diagnosis with mNGS.

The diagnosis of PJP with mNGS was based on the detection of *Pneumocystis jirovecii* DNA in clinical samples. The threshold criteria for a positive PJP diagnosis using mNGS were as follows:



Figure I Overview of Research Design.

Notes: One patient in the PJP group simultaneously took two sample types, totaling 33 samples; There were 3 patients in the Non-PJP group who simultaneously took 2 sample types, totaling 49 samples. Traditional Methods: serum (1,3)- β -D-glucan (86.7%) and LDH (55.6%). **Abbreviations**: LDH, Lactate Dehydrogenase; mNGS, Metagenomics next generation sequencing; PJP, pneumocystis jirovecii pneumonia.

- 1. The presence of *Pneumocystis jirovecii* DNA sequences in the sample with a read count threshold exceeding 10 reads per million sequenced reads. This threshold was chosen to distinguish between true infections and potential contaminants or low-level colonization.
- 2. Confirmation of the diagnosis with clinical correlation, including compatible clinical symptoms (fever, cough, dyspnea, and progressive hypoxemia) and radiological findings (bilateral interstitial and ground glass opacity in the lungs and alveolar infiltration in the perihilar area).
- 3. Additional supportive laboratory findings, such as elevated serum BDG levels, were also considered to strengthen the diagnosis of PJP.

Traditional Microbiological Testing

Traditional microbiological testing comprises bacterial and fungal smears and cultures, serum BDG, LDH and Nucleic acid testing. The positive standard for bacterial culture is when the number of bacteria is greater than 10⁴ CFU/mL, and fungal culture must consider clinical characteristics and microbiological evidence.

GMS Staining

The detection of *Pneumocystis jirovecii* cysts or trophozoites in respiratory specimens (eg, BALF, sputum) using GMS staining. A positive result is indicated by the presence of dark brown to black stained cysts against a green background. Specimens were stained following standard laboratory protocols, and slides were examined under a microscope by experienced pathologists.

G Test

The measurement of serum BDG levels, with a threshold of ≥ 80 pg/mL considered positive for fungal infections, including PJP. Blood samples were collected, and serum BDG levels were quantified using a commercially available assay kit. Elevated levels were indicative of a probable fungal infection, supporting the diagnosis of PJP.

Clinical Data Collection

Data on clinical indicators were collected from all patients: namely, patient demographics, underlying diseases, immune deficiency status, laboratory test results, culture results, length of stay, and treatment results.

DNA Extraction and Sequencing

Physicians collected BALF, Sputum and cerebrospinal fluid in accordance with the standard procedures of the Meizhou People's Hospital. EDTA peripheral blood (3–4 mL) was collected from each patient. DNA was extracted using the Full Gold DNA Extraction Kit (TransGen Biotech, china), per the manufacturer's instructions and was used to construct a library. An Agilent 2100 Bioanalyzer was used for quality control of the constructed libraries. Quantification was performed using Qubit 4.0. Finally, an BGISEQ200 from Huada was used for 50PE double-ended sequencing. Finally, the human host sequence was removed from the data, and four microbial genome databases composed of viruses, bacteria, fungi, and parasites were used for classification.

Statistical Analysis

Data were analyzed using SPSS 22.0. Chi-square tests and *t*-tests were used to compare the diagnostic accuracy of mNGS with traditional Methods. We determined the normal distribution of variance by using the *t*-test and the R function Wilcoxon test and by calculating the sensitivity and specificity indicators using the Wilson method. P < 0.05 is considered significant and statistically significant.

Result

Clinical Features

This study included a total of 32 PJP patients and 46 Non-PJP patients. The median age of PJP patients was 64 years old, with 24 males. The most common symptoms of PJP patients included dyspnea (53.13%), fever (28.13%), and cough (62.5%). Compared with the non-PJP group, PJP patients significantly increased the incidence of solid tumors (P = 0.05, 25.0% vs 8.7%), skin diseases (P = 0.034, 9.38% vs 0.0%), and immune suppression (P = 0.013, 65.6% vs 40.0%). More PJP patients have used corticosteroids (P = 0.012, 37.5% vs 13.04%). The lymphocyte ratio of the PJP group was higher than that of the Non-PJP group (P = 0.034, 7.5% vs 5.7%), while C-reactive protein (CRP, P = 0.014, 70.55 vs 132.95 mg/L), procalcitonin (PCT, P < 0.01, 0.21 vs 3.92 ng/mL), and D2 polymers (P = 0.012, 2.04 vs 4.91) were significantly lower than those of the Non-PJP group. The proportion of pleural effusion in the PJP group was 50%. Notably, there were no statistical difference compared to the Non-PJP group. The specific patient characteristics are shown in Table 1.

Evaluation of Diagnostic Effectiveness of mNGS Compared with That of Other Methods

We compared the diagnostic performance of mNGS with serum BDG and LDH. To achieve this objective, we conducted mNGS with BALF and/or PB in all patients; serum BDG staining in 72 patients (28 in the PJP group and 44 in the Non-PJP group); and LDH detection in 78 patients (32 in the PJP group and 46 in the Non-PJP group). The sensitivity of mNGS, serum BDG, and LDH was 100.0% (86.7–100.0), 50.0% (31.1–68.9) and 9.3% (2.4–26.2), respectively; therefore, the sensitivity of mNGS was significantly superior to that of other diagnostic methods. Moreover, the specificity of mNGS was 100.0% (90.8–100.0), significantly higher than that of serum BDG (81.8% (66.8–91.3)). The specificity of LDH reached 91.3% (78.3–97.2), equivalent to the specificity of mNGS. Notably, the PPV and NPV of mNGS also reached 100%, surpassing those of the other detection methods (Table 2). Therefore, mNGS is optimal in sensitivity and specificity for diagnosis.

Differences in Microbial Composition at the Genus Level Between Lower Respiratory Tract Samples from the PJP Group and Non-PJP Group

The microbial composition at the genus level was normalized based on the mNGS Results, and the pathogen profiles of the PJP and Non-PJP group were analyzed at the DNA sequencing and RNA sequencing. The results revealed differences

Characteristics	PJP_Patients (n=32)	Non-PJP_Patients (n=46)	p_value
Male	24(75.00)	27(58.70)	0.137
Age(years)	63.63(28–83) 68.39(26–92)		0.162
Underline conditions			
Use of corticosteroids	12(37.50)	6(13.04)	0.012
Use of immunosuppressive medications	4(12.50)	I (2.17)	0.067
Solid organ transplantation	I (3.12) 0(0)		0.228
Solid organ tumors	8(25.00) 4(8.70)		0.05
Hematologic malignancies	2(6.25)	I (2.17)	0.357
Rheumatic diseases	I (3.12)	I (2.17)	0.794
Connect tissue disease	3(9.38)	2(4.35)	0.373
Skin system diseases	3(9.38)	0(0)	0.034
Other types of immunocompromised disease	21(65.63)	17(40.00)	0.013
Clinical symptoms			
Cough	20(62.50)	29(63.04)	0.665
Fever	9(28.13)	24(51.17)	0.118
Expectoration	(34.38)	21(45.65)	0.237
Dyspnea	17(53.13)	29(63.04)	0.381
Hemoptysis	1(3.13) 2(4.43)		0.782
Chest CT images			
Patchy shadowing	2(6.25)	I (2.17)	0.357
Interstitial patterns	6(18.75)	9(19.65)	0.928
Consolidation	10(31.25)	8(17.39)	0.153
Pleural effusion	16(50.00)	21(45.65)	0.705
Clinical indicators			
Lymphocyte ratio, %	7.5(5.4–13.4)	5.7(3.05–9.5)	0.034
CRP (mg/L)	70.55(39.39–113.14[n = 30])	132.95(63.3275-190.24)	0.014
PCT (ng/mL)	0.21(0.08–0.88)	3.915(0.395–11.58)	<0.01
BNP (pg/mL)	95.8(19.65–208.25[n = 31])	132.4(47.3–431.4)	0.143
Troponin (ng/mL)	0.02(0.01–0.05[n = 31])	0.04(0.01–0.094)	0.153
D2 polymers	2.04(0.765-3.4575[n = 30])	4.91(1.845–9.19)	0.012
ICU intervention			
Vasopressor	I 3(46.43[n = 28])	30(81.08[n = 37])	0.003
Invasive mechanical ventilation	17(53.15)	40(88.89[n = 45])	0
Assistant ventilation	(42.3 [n = 26])	I 3(38.24[n = 34])	0.750
CRRT	5(19.23[n = 26])	7(24.14[n = 29])	0.660
Hospitalization days	14(6–18)	11(8–16.25)	0.693
Mortality	2(6.25)	2(4.35)	0.708

Table I Clinical Characteristics of Patients

Notes: Relative defects of immune function: diabetes, chronic lung disease, liver disease, kidney disease, elderly and infirm.

Abbreviations: PJP, pneumocystis jirovecii pneumonia; CRP, C-reactive protein; PCT, Procalcitonin; BNP, B-type natriuretic peptide; ICU, Intensive Care Unit; CRRT, Continuous renal replacement therapy.

in the pathogen spectra of the two groups. DNA profile analysis revealed that the dominant pathogens in the PJP group were *Mycobacterium tuberculosis complex, Candida, Corynebacterium, Klebsiella*, and *Shigella* (Figure 2a). Commonly observed pathogens in patients without PJP comprised *Acinetobacter, Pseudomonas, Stenotrophomonas*, and *Streptococcus* (Figure 2a). This result is consistent with the advantages of the corresponding microbiota in the RNA sequencing. Notably, in the RNA profile (Figure 2b), the PJP group had more dominant bacterial communities, namely, *Enterobacter, Escherichia, Nocardia*, and *Yersinia*.

Mixed Pathogens in the PJP and Non-PJP Group

Due to immune deficiency, mixed pathogens are commonly observed in patients with PJP. In this study, in the PJP group, 96.88% had more than one pathogen, 3.12% (1/32) were infected with pure PJP, and 68.75% had mixed bacterial and

Methods		PJP Cohort	Non-PJP Cohort	Sensitivity (95% CI)	Specificity (95% Cl)	PPV (95% CI)	NPV (95% CI)
mNGS	+	32	0	100.0%(86.7–100.0)	100.0%(90.8–100.0)	100.0%(86.7–100.0)	100.0%(90.8–100.0)
	-	0	46				
BDG	+	12	8	60.0%(36.4-80.0)	70.6%(56.0-82.6)	44.4%(26.0–64.4)	81.8%(66.8–91.3)
	-	15	36				
LDH	+	3	4	9.3%(2.4–26.2)	91.3%(78.3–97.2)	42.8%(11.8–79.7)	59.2%(46.8–70.5)
	-	29	42				

 Table 2 Evaluation of Diagnostic Efficacy of mNGS Compared with That of Other Methods

Notes: mNGS, metagenomic next-generation sequencing, PJP, pneumocystis jirovecii pneumonia, BDG, (1,3)-b-D-glucan; serum BDG \geq 80 pg/mL was defined as positive. LDH, lactate dehydrogenase, LDH \geq 618 U/L was defined as positive.

Abbreviations: CI, confidence intervals; PPV, positive predict value; NPV, negative predict value.

viral infections (Figure 3a). Betaherpesvirus 5, betaherpesvirus 7, gamma herpesvirus 4, alpha herpesvirus 1, and *Klebsiella pneumoniae* were the most commonly observed pathogens in the PJP group (Figure 3b). In the Non-PJP group, the most commonly observed pathogens were gamma herpesvirus 4, beta herpesvirus 5, beta herpesvirus 6 B, alpha herpesvirus 1, and beta herpesvirus 7 (Figure 3c). The detection rate of beta herpesvirus 6B in the Non-PJP group was significantly higher than that in the PJP group. According to the mNGS results, 71% (10/14) of the patients in the PJP group improved after antibacterial treatment. Notably, Mycobacterium tuberculosis was detected in 13% of the patients in the PJP group.

Pathogen Status in Alveolar Lavage Fluid and Blood Samples

There were 30 BALF samples and three blood samples collected from patients with PJP. One of those patients had BALF and PB samples collected and was undergoing mNGS. The most commonly observed pathogens in the BALF samples





Abbreviations: PJP, pneumocystis jirovecii pneumonia; BALF, bronchoalveolar lavage fluid; PB, peripheral blood.



Figure 3 Pathogen spectrum of the PJP and Non-PJP group. (a) Major types of pathogens with PJP; (b) Co-pathogens in the PJP group; (c) Co-pathogens in Non-PJP group. Abbreviations: BALF, bronchoalveolar lavage fluid; PB, peripheral blood.

were beta herepesviruses 7 and 5; the most commonly observed pathogen in the blood was beta herepesvirus 5 (Figure 4a and b). In all matched BALF and PB samples, the consistency of PJP detected by mNGS reached 100%. There was no statistically significant difference (p = 0.82) between the median *Jirovecii* counts in blood and BALF samples (Figure 4c), which may be due to the small number of blood samples.



Figure 4 Distribution of pathogens in BALF and PB. Histogram of co-pathogens statistics in (a) BALF and (b) PB; (c) Scatter plot of the detection sequence number of *Pneumocystis jirovecii* in BALF and PB. R function Wilcox test was used to test for significant differences.

Adjustment and Effectiveness Evaluation of mNGS Detection for Treatment

We analyzed the antibacterial treatment of the PJP group during hospitalization. 43.8% (14/32) of the patients had their antibacterial treatment regimen adjusted based on their mNGS results, with 71% of those patients (10/14) showing clinical improvement. Furthermore, 24.2% of the PJP group showed a decrease in CRP and PCT indicators, indicating a reduction in inflammatory response and an improvement in infection.

Discussion

PJP is a commonly observed opportunistic pathogen with a high mortality rate in individuals infected with HIV.^{6,23} Patients without HIV but with PJP have a more acute onset and higher mortality rates.^{24,25} Traditional detection methods have low detection rates, long cycles, and low pathogen loads, leading to misdiagnoses, missed diagnoses, and high mortality rates. The primary focus of this study was to evaluate the diagnostic value of mNGS specifically for PJP. Our findings demonstrate that mNGS offers significant advantages in sensitivity and specificity over traditional diagnostic methods, establishing it as a valuable tool for the accurate detection of PJP.

This study compared the detection rates of PJP using mNGS, GMS staining, and qPCR. Our findings indicate that mNGS demonstrated a higher detection rate for PJP compared to traditional methods. The results suggest that mNGS may offer improved diagnostic capabilities for PJP, highlighting its potential utility in clinical settings for the diagnosis of complex pulmonary infections. However, further studies are required to validate these findings and explore the clinical implications of mNGS in the diagnosis and management of PJP. This finding indicates that mNGS contributes to the rapid diagnosis of early PJP. Moreover, mNGS facilitated identifying co-pathogens in mixed lung infections. This finding was consistent with that of Xie et al.²⁶ Moreover, a microbial culture typically reports a dominant bacterium, and mNGS can simultaneously detect more pathogens. Our data showed mixed pathogens in 96.88% of the patients in the PJP group, with 68.75% experiencing mixed viral and bacterial infections. *Mycobacterium tuberculosis* was detected in 13% of the patients in the PJP group, indicating that mixed PJP and tuberculosis infections require further research. Notably, the unbiased broad-spectrum detection by mNGS provides effective guidance for the antibacterial treatment of patients. Moreover, 43.8% of the patients in the PJP group had their antibacterial regimen adjusted based on their mNGS results, decreasing their CRP and PCT indicators and indicating a reduction in inflammatory response and an improvement in infection. Thus, mNGS has an advantage in detecting unknown commonly observed pathogens.

We analyzed the pathogen profiles of the PJP and Non-PJP group at the DNA and RNA levels. Different from the genomic, the transcriptomic identified several additional dominant bacterial groups in the PJP group, namely, *Enterobacter, Escherichia, Nocardia,* and *Yersinia.* Although the four bacterial aforementioned genera were relatively rare at the genetic level, their biological activities in the PJP group were high. *Enterobacter spp.* are conditional pathogens. This bacterium tends to infect individuals with low immunity and can cause bacteremia and purulent diseases. *Nocardia* can also cause brain abscesses and lung infections in immunocompromised patients. This phenomenon consistents with the clinical characteristics of the patients in this study.

We performed mNGS detection in the BALF and PB samples. Three pathogens, *Yersinia, human herpes virus type 5* (*CMV*), and *polycyclic virus type 29*, were detected in PB, and seven pathogens were detected in BALF: *Microeosinophilic Streptomonas, Yersinia, human herpes virus type 4 (EBV), CMV, human herpes virus type 1* (*HSV1*), *human herpes virus type 7*, and *polycyclic virus type 29*. Their detection of *Plasmodium* was consistent. *Plasmodium* was detected in two other blood samples. We found that BALF samples are easier to use to detect pathogens in pneumonia; however, when bronchoscopy is not feasible, blood samples can also be used.

An analysis of PCT and CRP levels, suggests that non-PJP patients exhibit higher incidences of sepsis. This condition may account for the disparity in pathogen detection between the PJP and non-PJP groups. The higher PCT and CRP levels in the non-PJP group indicate a more pronounced inflammatory response, often associated with sepsis, which could lead to a broader detection of pathogens due to the systemic nature of the infection. Given the higher incidence of sepsis in the non-PJP group, it is crucial to consider whether the observed benefits of treatment in both groups are directly related to PJP detection and subsequent management. The specificity of mNGS in identifying *Pneumocystis jirovecii* facilitates targeted treatment, potentially leading to better outcomes for PJP patients. Conversely, the non-PJP group's

response to treatment may be influenced by the management of sepsis and other underlying infections, rather than the direct treatment of PJP.

The main limitations of this study are as follows: the sample size was small, there was a certain degree of bias. Moreover, mNGS cannot directly rule out colonization. Therefore, in further research, we plan to expand the data integration analysis to explore whether the trend of its pathogens is consistent with the current situation.

In summary, mNGS exhibits superior sensitivity and specificity in diagnosing PJP compared to traditional methods. The results highlight the potential of mNGS to improve the diagnostic accuracy for PJP, offering a rapid and comprehensive approach for pathogen detection. However, this study focuses on comparing the detection rates of PJP among these methods, and further research is necessary to explore the broader applications of mNGS in other infections.

Abbreviations

mNGS, Metagenomics next generation sequencing; PJP, pneumocystis jirovecii pneumonia. GMS, Grocott's Methenamine Silver; BDG, (1-3)- β -D-Glucan; LDH, Lactate Dehydrogenase; BALF, bronchoalveolar lavage fluid; PB, peripheral blood; CRP, C-reactive protein; PCT, Procalcitonin; BNP, B-type natriuretic peptide.

Data Sharing Statement

The data used to support the findings of this study are available from the corresponding author upon request.

Ethics Approval and Consent to Participate

This study was approved by the Ethics Committee of Meizhou People's Hospital (Ethics approval No.: Meishi Lunshen 2021-C-121). Informed consent was obtained from all the participants.

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Disclosure

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