Am J Transl Res 2024;16(9):4885-4893
www.ajtr.org /ISSN:1943-8141/AJTR0157445

Original Article

Diagnostic value and clinical use of metagenomic next-generation sequencing for invasive pulmonary aspergillosis

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Received April 27, 2024; Accepted July 19, 2024; Epub September 15, 2024; Published September 30, 2024

Abstract: Objective: To compare the diagnostic efficacy of metagenomic next generation sequencing (mNGS) with traditional fungal culture, (1,3)-β-D glucan (G) test, and galactomannan (GM) test in diagnosing invasive pulmonary aspergillosis (IPA) and to explore the advantages and disadvantages of mNGS for IPA diagnosis. Methods: A retrospective analysis was conducted on 136 patients admitted to the Department of Respiratory and Critical Care Medicine of Affiliated Hospital of Putian University from March 2018 to March 2020. Among them, there were 66 patients with IPA (IPA group) and 70 without (non-IPA group). Baseline data, inflammatory factors, cytokines, and specimens such as bronchoalveolar lavage fluid (BALF) and blood of these patients were collected. Fungal culture test, G test, GM test and mNGS test were performed. Information included for analysis encompassed patients' host factors, clinical features, chest scanning images, laboratory test results, and treatment outcome. Results: There was no statistical difference in the baseline data or inflammatory factors in patients between the IPA group and the non-IPA group. Further analysis showed that the sensitivity of mNGS in diagnosing IPA was 53.03%, which was higher than that of traditional fungal culture test (27.27%), G test (31.82%), and GM test (34.85%). Notably, when combining fungal culture, G test, GM test, and mNGS, the sensitivity increased to 69.70%, with a specificity of 97.14%. The sensitivity of the combined test was higher than that any of the tests alone for diagnosing IPA. Conclusion: mNGS test offers superior diagnostic performance for IPA in comparison to traditional tests, particularly for testing samples like bronchoalveolar lavage fluid and bronchial secretions. The test result remains valuable even after aspergillus treatment. In addition, the use of mNGS in conjunction with other traditional tests, such as fungal culture test, G test, and GM test, can enhance the diagnostic efficacy for IPA.

Keywords: Invasive pulmonary aspergillosis, fungal culture, traditional test, metagenomic next-generation sequencing

Introduction

Aspergillosis has emerged as the second most common fungus in the 21st century due to the ever-growing use of broad-spectrum antibiotics, corticosteroids and immunosuppressants as well as the continuous development of new treatments such as organ transplantation and cancer chemotherapy [1, 2]. Invasive pulmonary aspergillosis (IPA), as the most critical subtype of aspergillosis, has seen an increasing incidence rate over the years. IPA often presents non-specific clinical symptoms and demonstrates a relatively low positivity rate by traditional microbial culture tests [3, 4]. Therefore, how to realize early confirmation of IPA and initiate prompt treatment remains a hot topicof current research. At present, primary approaches for detecting IPA infections include chest CT scans, fungal culture test, (1,3)-β-D glucan (G) test, galactomannan (GM) test, and antibody testing [5].

https://doi.org/10.62347/LDHU7380

Fungal culture test is a conventional yet the most reliable testing approach, capable of identifying fungal species and their medication sensitivities through morphologic and biochemical analyses, serving as a gold standard for diagnosing aspergillosis infection. However, this method is time-consuming yet has a low detection rate with a positivity less than 5%. The G test for identifying fungal species is limited by the delayed peak concentrations, making early diagnosis challenging. GM test result may be affected by concurrent medication use, such as antibiotics and anti-fungal drugs. Furthermore, chest CT scans, while useful, are not specific to IPA since they are applied for the diagnosis of various diseases.

In this context, metagenomic next-generation sequencing (mNGS) test has been developed. This technique involves high-throughput sequencing of nucleic acids directly from samples, allowing for the comparison with extensive database data to deduce the presence and quantity of all microbial species, thus providing significant value for the diagnosis of critical illnesses and complex cases conditions [6]. Recent studies have demonstrated the potential of mNGS for identifying pathogenic microorganisms and guiding antimicrobial therapies [7]. The aim of this study is to validate further the clinical value of the mNGS test in the diagnosis of IPA.

Materials and Methods

Study design

This is a single-center and retrospective study carried out on 136 patients admitted to the Department of Pulmonary and Critical Care Medicine of Affiliated Hospital of Putian University from March 2018 to March 2020. The study protocol was approved by the ethics committee of Affiliated Hospital of Putian University and complied with the principles of the Helsinki Declaration (revised in 2013). Written informed consents were obtained from all patients prior to the study.

Inclusion criteria: 1) Patients presenting clinical symptoms consistent with invasive pulmonary aspergillosis (IPA) and who had chest CT scans suggestive of IPA; 2) Patients who had undergone at least one bronchoscopy and bronchoalveolar lavage fluid test (bacterial culture test or microscopic examinations); 3) Patients without other concurrent bacterial, viral, or other related infections; 4) Patients presenting typical IPA imaging features. Exclusion criteria: 1) Patients who had undergone radiotherapy/chemotherapy for malignant tumors prior to the study; 2) Patients who were experiencing hemodialysis or peritoneal dialysis; 3) Patients with incomplete clinical data.

IPA diagnostic criteria: Diagnosis of IPA was based on the criteria established by the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group (EORTC/IFICG). A confirmed IPA diagnosis was made if patients showed persistent fever unresponsive to broad-spectrum antibiotics; experienced symptoms such as dyspnea, persistent cough, chest pain, and hemoptysis [8]; had chest CT scanning images displaying crescent signs, halo signs, and satellite lesions; demonstrated the presence of aspergillus hyphae in respiratory secretions or lung tissue, with isolation of Aspergillus from lung tissue or bronchoalveolar lavage fluid (BALF); and showed histopathologic evidence of invasive aspergillus growth, such as hyphae penetrating alveolar walls or tissue necrosis.

Patients who had already been clinically diagnosed with IPA were categorized as the IPA group. Those diagnosed with non-invasive pulmonary aspergillosis were categorized as the non-IPA group. Patients in both groups received appropriate antibiotic treatment.

Specimen collection

Secretion collection: The secretions were collected following instructions specified in the *Chinese Expert Consensus on Pathogen Detection of Bronchoalveolar Lavage Fluid in Pulmonary Infectious Diseases (2017 Edition)* [9]. BALF was obtained through wedging the tip of the bronchoscope into an appropriate bronchial branch, followed by the instillation of 60-120 mL of saline solution. The recovery rate of the lavage fluid ranged from 40% to 60%, and the recovered lavage fluid was distributed into four tubes placed in sterile containers. The secretions of all included patients were collected for bronchoalveolar lavage fluid culture test, G test, GM test, and mNGS test.

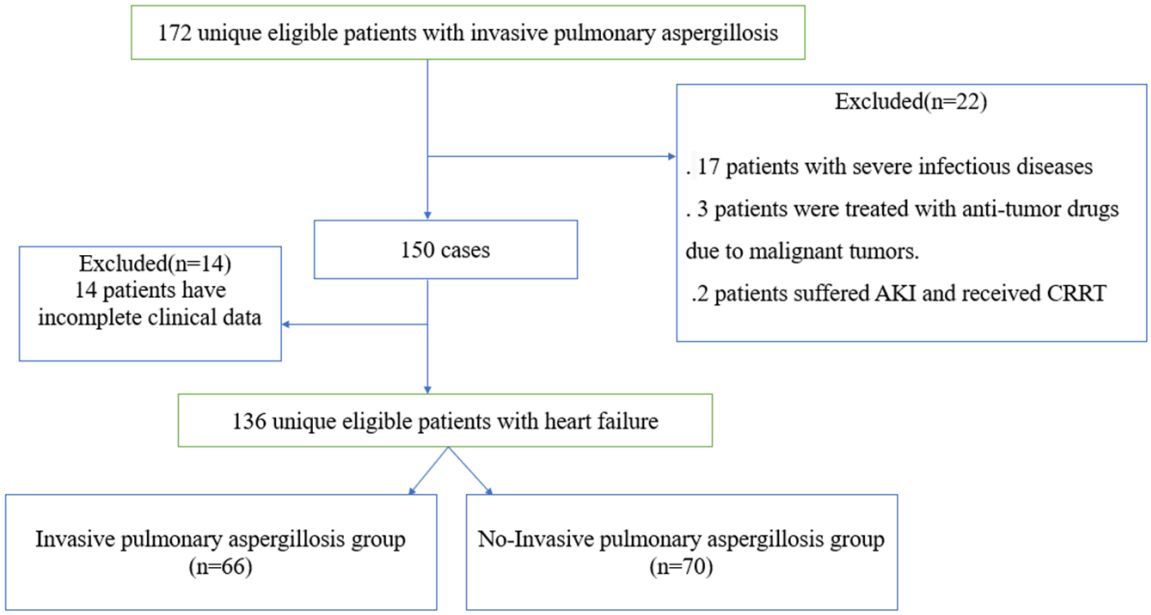

Figure 1. Flowchart of patient enrollment.

Blood sample collection: Samples were collected during fever episodes or prior to the administration of antifungal medications post-admission. Peripheral venous blood (3-5 mL) was drawn from patients, placed into an anticoagulation tube, and centrifuged at 3000 rpm for 3 minutes. Then, the blood samples were transferred into a tube for storage. Blood samples from all included patients were tested using blood culture, G test, GM test, and mNGS test.

Primary outcome measures

The effectiveness and consistency of various testing approaches, including mNGS test, fungal culture test, G test, and GM test, for diagnosing IPA were compared.

Secondary outcome measures

The effects of baseline data, inflammatory factors, and administration of antifungal medications on the results of IPA tests were explored.

Statistical analysis

Data analysis was conducted using SPSS 26.0. Quantitative data conforming to a normal distribution were expressed as mean ± standard deviation (SD) and analyzed using Student t-test. For data not conforming to a normal distribution, non-parametric tests were performed. Counted data were expressed in terms of number and rate, and comparisons between paired samples were conducted using paired chi-square tests. A *p*-value less than 0.05 was considered significant.

Results

Comparison of baseline data between the two groups

A total of 172 patients of having IPA were initially considered for study enrollment. Seventeen patients were excluded for severe infections, 3 patients for undergoing anti-tumor treatments, 2 for continuous renal dialysis treatments, and 14 for incomplete clinical information, resulting in a final cohort of 136 patients. Based on the final diagnostic results, patients were divided into an IPA group (n=66) and a non-IPA group (n=70). **Figure 1** displays the patient screening and grouping procedures. There were no significant differences in gender, age, medical history, or laboratory tests between the two groups, as shown in **Table 1**.

Comparison of blood routine indices between the two groups

No significant differences were found in white blood cell count, absolute neutrophil count, C-reactive protein, or procalcitonin levels in patients between the IPA and non-IPA groups (all P > 0.05, **Table 2**).

Table 1. Comparison of baseline data between the two groups

Variable	IPA group	Non-IPA group	χ^2/t	P
Gender (M/F)	36/30	36/34	0.130	0.723
Age (years)	66.32±5.83	67.47±6.17	1.120	0.272
Medical history			1.490	0.997
None	10	8		
COPD/bronchiectasis/asthma	5/6/7	7/7/6		
Liver, kidney and cardiac insufficiency	8/7/5	7/7/6		
Hypertension/Diabetes/Cerebrovascular Disease	5/7/7	7/9/6		
Antifungal treatment prior to admission (Yes/No)	32/34	31/39	0.241	0.624

Note: COPD: chronic obstructive pulmonary disease; WBC: white blood cell; CRP: C-reactive protein; PCT: procalcitonin.

Table 2. Comparison of blood routine indices between the two groups

Type	IPA group	Non-IPA group	t	P
White blood cell count (*10^9/L)	18.05 (12.73, 20.41)	16.22 (12.34, 20.01)	1.840	0.074
Centriocyte absolute value (*10^9/L)	12.46 (12.32, 16.83)	12.34 (12.21, 15.85)	0.740	0.536
C-reactive protein (mg/L)	113.65±5.42	112.42±4.76	1.830	0.073
Procalcitonin (g/L)	10.25±0.82	10.02±0.88	0.850	0.408

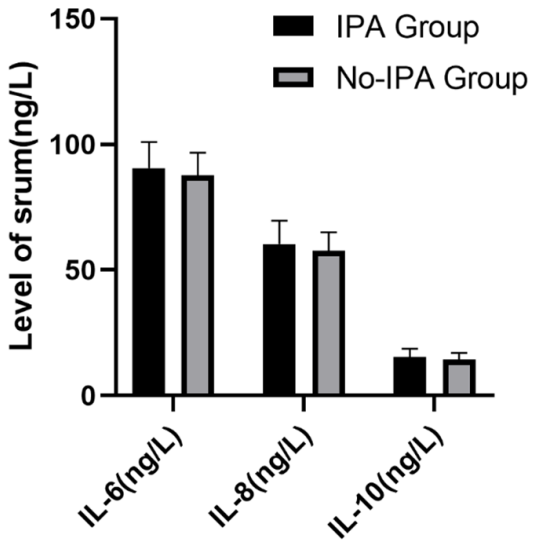

Figure 2. The expression of IL-6, IL-8 and IL-10 in the two groups.

Comparison of inflammatory factors between the two groups

There was no significant difference in cytokine levels of patients between the IPA and non-IPA groups (all P > 0.05, **Figure 2**).

Comparison of the efficacy of different tests for diagnosing IPA

In terms of diagnostic efficacy, mNGS test had a sensitivity of 53.03% for diagnosing IPA, which was significantly higher than that of the traditional fungal culture test (27.27%), G test (31.82%), and GM test (34.85%) (all P < 0.05). The specificity of mNGS test for diagnosing IPA was 88.57%, also significantly higher than that of the traditional fungal culture test (71.43%), G test (74.29%), and GM test (75.71%) (all P < 0.05).

In terms of secretion detection, the sensitivity of mNGS test (69.57%) was higher than that of fungal culture test (36.96%), G test (41.30%), and GM test (45.65%) (P < 0.05). In serological specimen detection, the sensitivity of mNGS test showed no significant difference in comparison to that of the traditional fungal culture test (5.00%), G test (10.00%), and GM test (10.00%) (P > 0.05).

However, the sensitivity for IPA diagnosis reached up to 69.70% with a specificity of 97.14% when the 4 tests were applied jointly. This markedly enhanced the diagnostic efficacy for IPA. See **Table 3** and **Figure 3**.

Influences of anti-aspergillus drugs on patients undergoing various tests

Among the 66 IPA patients, 34 had received antifungal treatment before being transferred to our hospital, and the remaining 32 had not received any antifungal therapies. The sensitivity of fungal culture test, G test, GM test, and

Table 3. Comparison of the diagnostic performance between fungal culture test, G test, GM test, and mNGS test

Variable	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
All specimens				
Fungal culture test	27.27 (18/66)	71.43 (50/70)	47.37 (18/38)	51.02 (50/98)
G test	31.82 (21/66)	74.29 (52/70)	53.85 (21/39)	53.61 (52/97)
GM test	34.85 (23/66)	75.71 (53/70)	57.50 (23/40)	55.21 (53/96)
mNGS test	53.03 (35/66)$^{\#,*,\&}$	88.57 (62/70)$^{\#,*,\&}$	81.40 (35/43)	66.67 (62/93)
Joint test	69.70 (46/66)$^{\#,\&,*,@}$	97.14 (68/70)$^{\#,\&,*,@}$	95.83 (46/48)$^{\#,\&,*,@}$	77.27 (68/88)$^{\#,*,\&}$
Secretions				
Fungal culture test	36.96 (17/46)	60.00 (30/50)	45.95 (17/37)	50.85 (30/59)
G test	41.30 (19/46)	64.00 (32/50)	51.35 (19/37)	54.24 (32/59)
GM test	45.65 (21/46)	66.00 (33/50)	55.26 (21/38)	56.90 (33/58)
mNGS test	69.57 (32/46)$^{\#,\&,*}$	84.00 (42/50)$^{\#,*,\&}$	80.00 (32/40)	75.00 (42/56)
Joint test	89.13 (41/46)$^{\#,\&,*,@}$	96.00 (48/50)$^{\#,\&,*,@}$	95.35 (41/43)$^{\#,*,\&}$	90.57 (48/53)$^{\#,\&,*,@}$
Blood				
Fungal culture test	5.00 (1/20)	75.00 (15/20)	16.67 (1/6)	44.12 (15/34)
G test	10.00 (2/20)	80.00 (16/20)	33.33 (2/6)	47.06 (16/34)
GM test	10.00 (2/20)	85.00 (17/20)	40.00 (2/5)	48.57 (17/35)
mNGS test	15.00 (3/20)	90.00 (18/20)	60.00 (3/5)	51.43 (18/35)
Joint test	25.00 (5/20)	95.00 (19/20)	83.33 (5/6)	55.88 (19/34)

NPV: Negative predictive index; PPV: Positive predictive value. Note: $^{\#}$compared to fungal culture test, $P < 0.05$, $^{\&}$compared to G test, $P < 0.05$, *compared to GM test, $P < 0.05$, $^{@}$compared to mNGS, $P < 0.05$.

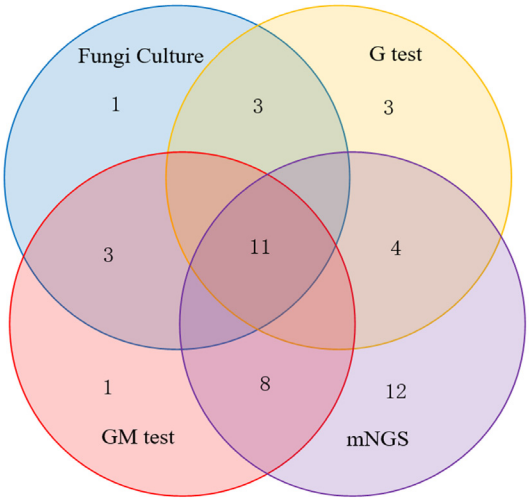

Figure 3. Diagnostic performance of joint tests.

mNGS test in patients without previous antifungal theapies was significantly higher than in those who had received antifungal therapies ($P < 0.05$), with an odd ratio (OR) > 1. Additionally, the sensitivity of mNGS test was significantly higher than that of G test, GM test, and fungal culture test, with significant differences (all $P < 0.05$). See **Table 4**.

Discussion

With the aging population and the widespread use of immunosuppressants and broad-spectrum antibiotics, the risks of aspergillus infection keep increasing over the years, leading to a rising incidence and mortality rate [10, 11]. For patients with invasive pulmonary aspergillosis (IPA), prompt and accurate pathogen identification is of great significance. Imaging of IPA often reveals dynamic changes, starting with "halo signs" and progressing to "crescent signs" as the disease advances [12, 13]. These signs are challenging to discern, often due to the timing of the tests and the interpreting skills of physicians [14]. Particularly for some critically ill patients with complex conditions, undergoing pathogen examinations promptly is of great significance. However, traditional fungal culture test is time-consuming with relatively low sensitivity and specificity, requiring different culture media for various pathogens. Imaging testing is disadvantageous due to its low positivity and poor specificity. G and GM tests are prone to false positivity, which hampers their ability to identify fungal species. The specific IgG antibody test for aspergillus is

Table 4. Comparison of sensitivity between groups with and without treatment (66 cases)

Variable	Sensitivity (non-treatment group)	Sensitivity (treatment group)	OR	P
All specimens	Case (n=32)	Case (n=34)		
Fungal culture test	46.88% (0.29-0.65; 15/32)	8.82% (0.02-0.24; 3/34)	9.12 (2.31-36.01)	< 0.01
G test	50.00% (0.32-0.68; 16/32)	14.71% (0.05-0.31; 5/34)	5.80 (1.79-18.78)	< 0.01
GM test	53.13% (0.35-0.71; 17/32)	17.65% (0.07-0.35; 6/34)	5.29 (1.72-16.25)	< 0.01
mNGS test	75.00% (0.57-0.89; 24/32)#,&	32.35% (0.17-0.51; 11/34)#	6.27 (2.14-18.39)	< 0.01
Joint test	78.13% (0.60-0.91; 25/32)#,&	61.76% (0.44-0.78; 21/34)#,@	2.21 (0.75-6.55)	0.153
Secretion specimen	Case (n=22)	Case (n=24)		
Fungal culture test	63.64% (0.41-0.83; 14/22)	12.50% (0.03-0.32; 3/24)	12.25 (2.76-54.32)	< 0.01
G test	63.64% (0.41-0.83; 14/22)	20.83% (0.07-0.42; 5/24)	6.65 (1.79-24.73)	< 0.01
GM test	72.73% (0.50-0.89; 16/22)	20.83% (0.07-0.42; 5/24)	10.13 (2.60-39.50)	< 0.01
mNGS test	95.45% (0.77-1.00; 21/22)#,*,&	45.83% (0.26-0.67; 11/24)#	24.82 (2.86-215.38)	< 0.01
Joint test	95.45% (0.77-1.00; 21/22)#,*,&	83.33% (0.63-0.95; 20/24)#,@	4.20 (0.43-40.87)	0.349
Blood specimen	Case (n=10)	Case (n=10)		
Fungal culture test	10.00% (0.00-0.45; 1/10)	0.00% (0.00-0.31; 0/10)	3.32 (0.12-91.60)	1.000
G test	20.00% (0.03-0.56; 2/10)	0.00% (0.00-0.31; 0/10)	6.18 (0.26-146.78)	0.483
GM test	10.00% (0.00-0.45; 1/10)	10.00% (0.00-0.45; 1/10)	1.00 (0.05-18.57)	1.000
mNGS test	30.00% (0.07-0.65; 3/10)	0.00% (0.00-0.31; 0/10)	9.80 (0.44-219.25)	0.215
Joint test	40.00% (0.12-0.74; 4/10)	10.00% (0.00-0.45; 1/10)	6.00 (0.53-67.65)	0.304

Note: #Compared to the results of fungal culture test, P < 0.05, &compared to the results of G test, P < 0.05, *compared to the results of GM test, P < 0.05, @compared to the results of mNGS test, P < 0.05.

based on the antigen-antibody reaction that requires known pathogen nucleic acid sequences. Only certain pathogens can be detected using this test, which can be easily influenced by patients' underlying disease, offering limited utility in the early diagnosis of IPA. Additionally, the irregular cellular wall of aspergillus increases the difficulty of detection, complicating the diagnosis [15]. Therefore, there is a pressing need for more sensitive diagnostic methods to ensure early treatment and improve the prognosis for IPA patients.

The metagenomic next-generation sequencing (mNGS) test is a genetic method that detects DNA or RNA sequences to determine the species of pathogens in specimens. In terms of detection accuracy, mNGS test can accurately identify the species of pathogenic bacteria, thus achieving the goal of "precise diagnosis and targeted treatment". mNGS test is capable of identifying the subtypes of pathogenic microorganisms and detecting drug-resistant genes and virulence factors of pathogens, thus enabling a transition from empirical treatment to precision treatment [16, 17]. In this study, mNGS not only detected aspergillus in 23 cases but also identified a range of other specific pathogens, demonstrating its ability to reveal mixed infections. This compensates for the deficiency of traditional fungal culture test, which typically identifies only one bacterium at a time based on clinical experience. Studies have shown that mNGS test is advantageous for diagnosing pathogens in immunocompromised patients, and is able to confirm a large number and high abundance of pathogenic sequences in septic patients, thereby facilitating better outcomes through accurate pathogen detection. The *Expert Consensus on the Application of Metagenomic Analysis and Diagnostic Technology in Acute and Critical Infectious Diseases* recommends the use of mNGS in immunocompromised patients with latent disease. Additionally, the *Expert Consensus on the Application of High-Throughput Sequencing Technology in Pathogen Detection of Infectious Diseases in China* also advocates for the inclusion of mNGS in routine tests for critically ill patients suspected of IPA infection, underscoring the importance of mNGS in diagnosing infectious diseases, especially in both critically ill patients and immunocompromised patients [18, 19].

Studies have shown that the sensitivity of mNGS testing for fungal infection in secretions is 56.1%. In the current study, the sensitivity of

mNGS testing for detecting aspergillus in secretions was 69.57%. Moreover, in secretion specimen detection, the sensitivity of mNGS test was significantly higher than that of fungal culture test, G test, and GM test; however, when the specimen was blood, there was no significant difference in sensitivity among various test groups. The results suggest that the sensitivity of mNGS in secretion samples of patients is significantly higher than that of the serological specimen. This discrepancy is likely due to the early invasion of aspergillus hyphae into lung tissues, leading to their persistent existence in the bronchi and alveoli and resulting in a higher load of aspergillus in the lungs than that in the blood. Therefore, the blood aspergillus DNA load is significantly lower than that of the lung tissue [20, 21].

It has been highlighted that mNGS is strongly recommended for patients who are suspected of respiratory tract infection but fail to be confirmed by traditional laboratory tests within 3 days after the advent of symptoms, and who also have had ineffective antimicrobial therapy. For patients suspected of secondary bloodstream infections, blood samples should be collected from the site of the secondary infection, particularly when samples from the primary infection site are negative or when it is not possible to obtain samples from the primary site for testing. In this study, the consistency of test results was analyzed, and the results suggested that mNGS test, fungal culture test, G test, and GM test all showed "false positive" and "false negative" results, which may be related to different sampling objects. Most of our samples were from respiratory secretions, which may be influenced by the oropharyngeal microbiota. This oversight in the study design needs to be noted and improved in future research.

In this study, the sensitivity, specificity, and PPV of the joint test were significantly better than that of fungal culture test, G test, GM test, or mNGS test alone. We showed that the combination of G test with GM test can improve the specificity and PPV for diagnosing IPA. Similarly, by applying fungal culture test in conjunction with mNGS test, the sensitivity, specificity, and PPV of the test results were better than those of the tests alone. This combined application of the tests is an innovative design of the study. Analysis of the sensitivity, specificity, PPV, and negative predictive value (NPV) results of the separate tests and the combined test within this study indicates that when the results of the G test, GM test, and mNGS test are all negative, the likelihood of a patient having IPA can be confidently ruled out. In contrast, the probability of confirming IPA is extremely high when the results of the G test, GM test, and mNGS test are all positive. Hence, the diagnostic efficacy for confirming IPA can be greatly enhanced through the integrated interpretation of symptoms, vital signs, imaging characteristics, and the results of the combined tests.

Studies have shown that culture media varies greatly in different antifungal therapies, while the results of the mNGS are largely unaffected [22, 23]. In this study, by comparing fungal culture test, G test and GM test to mNGS test for microbiological samples of patients with or without antifungal therapies, it was found that the sensitivity of mNGS test was higher than that of fungal culture test, G test, or GM test both before and after antifungal therapies. Notably, while the sensitivity of all tests decreased following antifungal treatment, the mNGS test maintained a higher sensitivity for secretion specimens compared to the other tests even after such treatment. This analysis demonstrates that the mNGS test offers significant advantages at each stage of diagnosis, showing minimal influence from antifungal therapies.

This study has some limitations: First, this is a single-center study with small sample size, suggesting that the results obtained in the study might not be generalizable. A multi-center study on mNGS test with bigger sample size is necessary in the future. Second, the test results may be less accurate because of the fact that some patients have received treatments before being transferred to our hospital. Third, the employment of different genetic databases and bioinformatic analysis methods by different companies may lead to inconsistent results. Fourth, mNGS test may yield "false positive" or "false negative" results due to variations in the timing and methodology of sample collection. Fifth, the sensitivity of mNGS test to drugs cannot be decided. Sixth, there is currently no universally recognized interpretation standard for mNGS test results. Physicians

need to make comprehensive judgments based on patients' medical history, clinical manifestation and laboratory tests, which are subject to a certain degree of subjectivity. Therefore, it is crucial to adhere strictly to the standards of each operation for collecting specimens, processing samples, extracting nucleic acid, preparing library, and performing mNGS test. Additionally, conducting other tests in parallel with the mNGS test can help form a "rapid diagnostic system" to objectively differentiate background bacteria from true pathogens and analyze the test results in conjunction with clinical facts.

In summary, collecting specimens at appropriate times during the onset of IPA can increase the positivity rate of mNGS test for detecting aspergillus. The mNGS test offers the advantages of non-targeted detection and high accuracy, significantly outperforming fungal culture test, G test, and GM test for diagnosing IPA. Its efficacy in secretion samples is superior to that of blood samples. While antifungal therapy impacts the results of traditional tests to varying degrees, its effect on mNGS test results is minimal. The combination of these four tests can substantially enhance the diagnostic efficacy for IPA.

Acknowledgements

This study was supported by the Natural Science Foundation of Fujian Province (2018-J01196).

Disclosure of conflict of interest

None.

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References

[1] Varga J, Szigeti G, Baranyi N, Kocsubé S, O'Gorman CM and Dyer PS. Aspergillus: sex and recombination. Mycopathologia 2014; 178: 349-62.

[2] Janssens I, Lambrecht BN and Van Braeckel E. Aspergillus and the lung. Semin Respir Crit Care Med 2024; 45: 3-20.

[3] Liu F, Zeng M, Zhou X, Huang F and Song Z. Aspergillus fumigatus escape mechanisms from its harsh survival environments. Appl Microbiol Biotechnol 2024; 108: 53.

[4] Tekin R, Hattapoğlu S and Tekin RC. Aspergillus encephalitis with microabscesses in an immunocompetent patient. Rev Soc Bras Med Trop 2023; 56: e03912023.

[5] Nagashima A, Nagato T, Kobori T, Nagi M and Okochi Y. Uncommon occurrence of pulmonary aspergillosis caused by aspergillus sydowii: a case report. Cureus 2023; 15: e51353.

[6] Liu Z, Sun C, Xiao X, Zhou L, Huang Y, Shi Y, Yin X, Mao Z and Zhang Q. Application of metagenomic next-generation sequencing (mNGS) in diagnosing pneumonia of adults. J Infect Dev Ctries 2023; 17: 1566-1573.

[7] Li X, Feng Y, Li D, Chen L, Shen M, Li H, Li S, Wu X and Lu L. Cerebral abscess infected by *Nocardia gipuzkoensis*. Infect Drug Resist 2023; 16: 7247-7253.

[8] Donnelly JP, Chen SC, Kauffman CA, Steinbach WJ, Baddley JW, Verweij PE, Clancy CJ, Wingard JR, Lockhart SR, Groll AH, Sorrell TC, Bassetti M, Akan H, Alexander BD, Andes D, Azoulay E, Bialek R, Bradsher RW, Bretagne S, Calandra T, Caliendo AM, Castagnola E, Cruciani M, Cuenca-Estrella M, Decker CF, Desai SR, Fisher B, Harrison T, Heussel CP, Jensen HE, Kibbler CC, Kontoyiannis DP, Kullberg BJ, Lagrou K, Lamoth F, Lehrnbecher T, Loeffler J, Lortholary O, Maertens J, Marchetti O, Marr KA, Masur H, Meis JF, Morrisey CO, Nucci M, Ostrosky-Zeichner L, Pagano L, Patterson TF, Perfect JR, Racil Z, Roilides E, Ruhnke M, Prokop CS, Shoham S, Slavin MA, Stevens DA, Thompson GR, Vazquez JA, Viscoli C, Walsh TJ, Warris A, Wheat LJ, White PL, Zaoutis TE and Pappas PG. Revision and update of the consensus definitions of invasive fungal disease from the european organization for research and treatment of cancer and the mycoses study group education and research consortium. Clin Infect Dis 2020; 71: 1367-1376.

[9] Duan H, Li X, Mei A, Li P, Liu Y, Li X, Li W, Wang C and Xie S. The diagnostic value of metagenomic next-generation sequencing in infectious diseases. BMC Infect Dis 2021; 21: 62.

[10] Guo L, Wu X and Wu X. Aspergillus infection in chronic obstructive pulmonary diseases. Clin Respir J 2023; 17: 129-138.

[11] El-Baba F, Gao Y and Soubani AO. Pulmonary aspergillosis: what the generalist needs to know. Am J Med 202; 133: 668-674.

[12] Ledoux MP, Guffroy B, Nivoix Y, Simand C and Herbrecht R. Invasive pulmonary aspergillosis. Semin Respir Crit Care Med 2020; 41: 80-98.

[13] Matthews H, Rohde H, Wichmann D and Kluge S. Invasive pulmonary aspergillosis. Dtsch Med Wochenschr 2019; 144: 1218-1222.

[14] Matthews H, Rohde H, Wichmann D and Kluge S. Invasive pulmonary aspergillosis. Dtsch Med Wochenschr 2019; 144: 1218-1222.
[15] Latgé JP and Chamilos G. Aspergillus fumigatus and Aspergillosis in 2019. Clin Microbiol Rev 2019; 33: e00140-18.
[16] Han D, Li Z, Li R, Tan P, Zhang R and Li J. mNGS in clinical microbiology laboratories: on the road to maturity. Crit Rev Microbiol 2019; 45: 668-685.
[17] Sun L, Zhang S, Yang Z, Yang F, Wang Z, Li H, Li Y and Sun T. Clinical application and influencing factor analysis of metagenomic next-generation sequencing (mNGS) in ICU patients with sepsis. Front Cell Infect Microbiol 2022; 12: 905132.
[18] Zhang B, Gui R, Wang Q, Jiao X, Li Z, Wang J, Han L, Zhou L, Wang H, Wang X, Fan X, Lyu X, Song Y and Zhou J. Comparing the application of mNGS after combined pneumonia in hematologic patients receiving hematopoietic stem cell transplantation and chemotherapy: a retrospective analysis. Front Cell Infect Microbiol 2022; 12: 969126.
[19] Xing XW, Yu SF, Zhang JT, Tan RS, Ma YB, Tian X, Wang RF, Yao GE, Cui F, Gui QP and Yu SY. Metagenomic next-generation sequencing of cerebrospinal fluid for the diagnosis of cerebral aspergillosis. Front Microbiol 2021; 12: 787863.
[20] Bhandari S, Baral MR, Dandwani M, Sandeep F and Hegde A. The ominous aspergillus with cancer of blood vessels: a case of invasive aspergillosis and epithelioid angiosarcoma of the lung. Cureus 2023; 15: e40034.
[21] Schiefermeier-Mach N, Haller T, Geley S and Perkhofer S. Migrating lung monocytes internalize and inhibit growth of aspergillus fumigatus conidia. Pathogens 2020; 9: 983.
[22] Jia H, Liu H, Tu M, Wang Y, Wang X, Li J and Zhang G. Diagnostic efficacy of metagenomic next generation sequencing in bronchoalveolar lavage fluid for proven invasive pulmonary aspergillosis. Front Cell Infect Microbiol 2023; 13: 1223576.
[23] Ao Z, Xu H, Li M, Liu H, Deng M and Liu Y. Clinical characteristics, diagnosis, outcomes and lung microbiome analysis of invasive pulmonary aspergillosis in the community-acquired pneumonia patients. BMJ Open Respir Res 2023; 10: e001358.