Diagnostic value of NGS in bronchoalveolar lavage fluid for pulmonary fungal infection

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Research Article

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Abstract

Metagenomic sequencing (mNGS) has been approved to diagnose lung fungal diseases. However, the test performance of clinical mNGS has not been widely recognized. This study aims to evaluate the value of mNGS in the system of bronchoalveolar lavage fluid through the systematic evaluation of gathered analysis and related research. A total of 1113 patients (265 with proven or probable invasive fungal diseases), included in 6 studies, were analyzed. The pooled sensitivity, specificity, PLR, NLR, and diagnostic odds ratio were 0.89 (95%CI, 0.75–0.96), 0.86 (95%CI, 0.78–0.91), 6.2 (95%CI, 4.0-9.6), 0.12 (95%CI, 0.05–0.32), and 50 (95%CI, 15–163), respectively. The area under the summary receiver operating characteristic curve, with 95% confidence intervals, was 0.93 (95%CI, 0.90–0.95). The accuracy of the metagenomic sequencing (mNGS) is good, has certain clinical characteristics, can explain the results separately, and has the clinical value of early diagnosis of lung fungal infection.

Purpose: This meta-analysis of randomized controlled trials aims to investigate the diagnostic utility and benefits of mNGS in comparison to conventional detection techniques for lung fungal infection in clinical patients.

Patients and methods: A preliminary diagnosis of lung infection based on a patient's medical history, clinical symptoms, and imaging tests is a requirement for inclusion. Using the method of meta-analysis, the sensitivity, specificity, diagnostic odds ratio (OR), positive likelihood ratio (PLR), and negative likelihood ratio (NLR) of BAL-mNGS for identifying lung fungal illness were pooled.

Results: 6 studies involving a total of 1113 patients, 265 of whom had invasive fungal diseases that were proven or likely to have occurred, were examined. The diagnostic odds ratio, PLR, NLR, and diagnostic sensitivity were all pooled, and their respective values were 0.89 (95% CI, 0.75–0.96), 0.86 (95% CI, 0.78–0.91), 6.2 (95% CI, 4.0-9.6), 0.12 (95% CI, 0.05–0.32), and 50 (95% CI, 15–163). With 95% confidence intervals, the area under the summary receiver operating characteristic curve was 0.93 (95%CI, 0.90–0.95).

Conclusion: The clinical value of metagenomic sequencing (mNGS) for the early diagnosis of lung fungal infection is that it is accurate, has specific clinical characteristics, can explain the results separately, and has clinical utility.

Introduction

Lung infections are one of the leading causes of infectious death worldwide. The proportion of fungal infections cannot be ignored. In recent years, the prevalence of pulmonary fungal disease has shown a significant upward trend with the increased use of immunosuppressants. The major sources of human lung fungal infections are opportunistic fungi: Aspergillus, Cryptococcus, Pneumocystis, and endemic fungi. The incidence and importance of fungal infections continue to increase. Prompt diagnosis relies on appropriate laboratory testing in susceptible patients. As one of the types of pulmonary infection, pulmonary fungal disease has been challenging to adopt targeted drug treatment in the early stages of
diagnosis and treatment due to the limited capacity and efficiency of traditional pathogen detection methods in the past, leading to worsening or worsening of symptoms, or even death. The etiology of suspected infection in acutely ill hospitalized patients is often undiagnosed, resulting in delayed or inadequate treatment, prolonged hospital stays, readmissions, and increased mortality and morbidity.

A common problem with traditional testing methods is the limited range of pathogens detected, and clinicians are often confronted with the thorny question of whether traditional tests are negative or whether the acute illness is really caused by an untested infection. For immunocompromised and/or critically ill patients, the rational use of antibiotics is extremely important, and early etiological diagnosis is required. However, traditional cultural methods are time-consuming and have low positive detection rates. For fungal lung infections, traditional detection methods mainly include culture, histopathology, antigen detection, serology, imaging, and molecular diagnosis. Culture methods are generally considered the gold standard for fungal infections, but most fungi are cultured for a long time, and culturing fungal pathogens, especially filamentous fungi, typically requires 1–2 weeks of incubation. Even if the isolation of fungi is successful, identification is sometimes very challenging and requires expertise that may not be routinely available in clinical microbiology laboratories. Staining and microscopy also do not have good sensitivity and specificity. Some even go as far as a month. Because a positive test result can provide clues to the early initiation of antifungal drugs, Therefore, antigen and antibody tests are increasingly used to enable more rapid and specific diagnosis. However, most antigen or antibody detection assays are also nonspecific and insensitive. For immunosuppressed patients and patients with an acute course, the sensitivity of antibody testing may be higher. But the test can be falsely negative. Histopathology is a standardized technique, available in most laboratories, that provides a quick but putative diagnosis. Despite limitations, invasive tests and their sensitivity and specificity largely depend on the pathologist's experience, with a certain rate of misdiagnosis.

With the development of molecular biology, the value of metagenomic sequencing (mNGS) has been gradually recognized in recent years, especially in the detection of rare, atypical, or slow-growing microorganisms. mNGS technology provides a new method for identifying the pathogens of pulmonary fungal infection and has been widely used in clinical practice. Therefore, this paper intends to conduct a meta-analysis on the diagnostic value of mNGS in bronchoalveolar lavage fluid.

Material And Methods

Study selection

Two investigators searched the Embase, PubMed, Web of Science, Cochrane Library databases for pertinent articles published in English on human subjects up to May 2022. The search terms were invasive fungal disease or invasive fungal infection or IFD or IFI or fungal infection or fungal infections or fungal disease or fungal diseases and next generation sequencing or NGS or mNGS or metagenomics or cfDNA or mcfDNA and bronchoalveolar lavage or BAL.
The inclusion criteria for publications were as follows: providing complete data for two-by-two tables. When the same sample group of people was analyzed in several publications, the results were accounted for only once. All of the related articles were scrutinized to judge the eligibility of studies by two reviewers. A third investigator makes the final decision on the disagreements and examines whether any additional studies have been neglected.

**Data extraction**

From each included study, we extracted data regarding the following aspects: characteristics of the patients; types of pulmonary fungal disease included; study quality assessment score; and the criteria value used for the diagnosis of pulmonary fungal disease. The number of true- and false-positive results, as well as true- and false-negative results obtained with the BAL-mNGS assay, were extracted from the patient data. When such data was not provided, we used the specific mathematical formulas to calculate them from the related data on sensitivity, specificity, positive and negative predictive value or contacted the authors directly. The whole process is shown in Figure 1.

**Data analysis**

Researchers conducted a meta-analysis to assess the accuracy of BAL-mNGS by calculating the overall sensitivity, specificity, PLR, NLR, and diagnostic OR and plotting a SROC curve (Figure 3–8). Researchers conducted the two-by-two table analysis because the researchers in the most included studies adopted this mode. Inconsistency (I²) was computed to indicate the percent of the variability caused by unobserved heterogeneity. If I² was larger than 25%, the result was considered significant for heterogeneity. When significant heterogeneity was present, the pooled results (with corresponding 95% CI) were derived by using the DerSimonian-Laird method (random-effects model). The SROC curve, which is shown in Figure 7, represents the relationship between sensitivity and specificity across studies. The area under the curve (AUC) was calculated in order to judge the diagnostic value. The pooled weighted sensitivity, specificity, PLR, NLR, SROC curve, and meta-regression were performed by using Stata version 16. and Review Manager 5.4.

**Results**
Eligible studies and quality assessment

As shown in Figure. 1, we eventually pooled 15 eligible studies, including 15 full texts of the overall 4925 references searched. On the whole, 1113 patients were enrolled, with a mean of 74 patients per study. Data for evaluating the accuracy of BAL-mNGS for the diagnosis of lung fungal disease was extracted from these studies. The small number of lung fungal disease cases in different studies and the variation due to chance mainly led to the wider range of sensitivity. The sensitivity in different studies ranged from 0.40 to 1.00, whereas the specificity ranged from 0.55 to 1.00. The QUADAS questionnaire was used to assess the quality of the selected studies. In conclusion, the quality of most studies was high, as was evident in Figure. 2.

Sensitivity, specificity, PLR, NLR, diagnostic OR and SROC curve

The pooled sensitivity is 0.89 (95%CI, 0.75–0.96), and the I2 value is 69.51 (95%CI, 53.33–85.69). Among the fifteen included studies, the highest sensitivity occurred in five people, including Chen and Wu X. In the study, the values were 1.00(95%CI, 0.74-1.00), 1.00(95%CI, 0.66-1.00), 1.00(95%CI, 0.094-1.00), 1.00(95%CI, 0.48-1.00). The lowest sensitivity occurs in Zhan Y’s study with a value of 0.40 (95%CI, 0.05–0.85). And the pooled specificity is 0.86 (95%CI, 0.78–0.91). The I2 value is 75.61 (95%CI, 63.38–87.83). Among the fifteen included studies, the highest specificity occurs in Peng and Chen J In the studies, the lowest specificity occurs in the Pan T study with a value of 0.50 (95%CI, 0.21–0.79).

The pooled PLR is 6.2 (95%CI, 4.0–9.6) and the I2 value is 60.22 (95%CI, 60.22–87.13). Among the fifteen included studies, the highest positive likelihood ratio is found in Jiang J’s study, whose value is 24.34 (95%CI, 10.73–55.21). The lowest positive likelihood ratio occurs in the Pan T study with a value of 1.50 (95%CI, 0.57–39.95). And the pooled NLR is 0.12 (95%CI, 0.05–0.32). The I2 value is 70.36 (95%CI, 54.74–85.98). Among the fifteen included studies, the highest negative likelihood ratio appears in Lian QY’s study; its value is 0.69 (95%CI, 0.38-1.00). The lowest negative likelihood ratio occurs in Jiang J’s study, with a value of 0.01 (95% CI, 0.01–0.14).

The diagnostic OR was 50.11 (95%CI, 15.44-162.66) and the I2 value was 99.90 (95%CI, 99.89–99.91). Among the fifteen included studies, the highest diagnostic OR was found in Jiang J’s study. The lowest diagnostic OR occurs in the Pan T study. The AUC of the SROC curve was 0.93 (95%CI, 0.90–0.95).

Discussion

The main finding of our meta-analysis is that BAL-mNGS measurement seems to be a good diagnostic assay for pulmonary fungal disease. In our meta-analysis, when comparing patients who had proven pulmonary fungal disease by culture methods with patients who had proven pulmonary fungal disease by BAL-mNG, the AUC was 0.93, while a value between 0.80 and 0.95 has been regarded as a good relationship between the characteristics of studies and the diagnostic OR. Both the sensitivity and the
specificity of BAL-mNGS measurement, 0.88 and 0.86, are greater than 0.7, which seems to have good inspection performance. However, both the PLR and the NLR of BAL-mNGS measurement, 6.20 and 0.12, while the former is less than 10 and the latter is greater than 0.1, mean that there is still a certain gap in the sensitivity and specificity of metagenomic testing compared to traditional testing methods. And the P 0.05 in the Deek’s funnel plot asymmetry test for the diagnosis of lung fungal diseases using BAL-mNGS (Fig. 6). Nevertheless, the above findings should be interpreted in light of the high statistical heterogeneity. As for meta-analysis, it is an important goal to explore the causes of heterogeneity rather than the calculation of summary measures. On one hand, this difference may exist because there are certain differences in the traditional methods of diagnosing pulmonary mycosis among the included studies. On the basis of smear and culture as the main methods, the G test, antigen-antibody detection, PCR technology, pathological activities, and other methods have been included, but not all studies have included all the above detection methods. On the other hand, the higher specificity in this study may be due to some differences in the study population. For example, the study by Peng et al. included the CU-treated population and the study by Lian QY et al. included the lung transplantation crowd. The relatively large proportion of the study population treated in the ICU was associated with a higher rate of Candida colonization of the respiratory tract.

In the present study, BAL-mNGS shows the PLR and the NLR of 6.20 and 0.12, respectively, while there is still a certain gap in the diagnostic value of metagenomic testing compared to traditional testing methods. There are several possible reasons related to the result. Firstly, nucleic acid contamination may occur from specimen collection to specimen processing and may also come from the environment. For example, the sample collection container may become a source of contaminated nucleic acid. Secondly, studies have shown that most of the reagents used for mNGS will also introduce foreign DNA during the sequencing process. This phenomenon is called "kit-ome," which will seriously affect the sample results, and false-positive results may appear in the test report. Thirdly, the diagnostic yield is influenced by the timing of the bronchoscope examination. The false-negative results could be caused by early examination, whereas delayed evaluation may increase the chances of obtaining positive results, so the results have disease progression bias. This issue could be handled only by serial testing in a properly designed prospective study, which is limited during BAL processing as mentioned above. Candida colonization is a major limiting factor that could cause potential false positivity for mNGS diagnosis of pulmonary fungal disease when using BAL. More research is required to determine the best diagnostic cutoff value. On the other hand, some fungi have stiffer cell walls and lower nucleic acid extraction efficiency, resulting in low detection rates and false-negative results.

The limitations of BAL-mNGS have been well discussed and documented in the literature. However, the adverse events arising from the low accuracy have not been sufficiently illustrated. Clearly, a positive BG test cannot indicate a certain fungal pathogen, which places doctors in a dilemma when making decisions. Another much more important concern is that many patients might be missed or misdiagnosed for pulmonary fungal disease if we interpreted the result only with lower sensitivity, leading to delayed treatment while poor specificity causes unnecessary antifungal therapy. In clinical practice, it seems difficult not to use antifungal treatment for a positive test result that may suggest invasive disease
because of the high morbidity and mortality associated with pulmonary fungal disease. Consequently, a
distraction from identifying the actual cause of a patient's disease and excessive use of antifungal
agents would result. The limitations of BAL-MNG have been well discussed and documented in the
pooled literature. Clearly, a positive mMGS test alone doesn't indicate a fungal pathogen that could still
put doctors in a bind to make a decision. Another, more important issue is that if we interpret the results
in terms of only low sensitivity, many patients with pulmonary fungal disease may be missed or
misdiagnosed, resulting in delayed treatment with poor specificity, leading to unnecessary fungal
treatment. A positive test result may seem difficult without the use of antifungal therapy because of the
high morbidity and mortality associated with pulmonary fungal disease. As a result, there will be
distractions from determining the actual cause of the patient's disease and the overuse of antifungal
drugs. In the research reports of Wu X, Li Y, et al., the positive mMGS test of patients under ICU treatment
conditions provided definite help for the clinical diagnosis and treatment of critically ill patients, and the
prognosis of some patients was significantly improved. However, in the study of Liu, Lei Y, et al., they also
pointed out that the false-positive results of mNGS may also lead to the loss of patients' economic,
physical, and mental health.

To our knowledge, this paper is a meta-analysis that summarizes the diagnostic value of BAL-mNGS for
the diagnosis of pulmonary fungal disease. There are still some limitations to our study. The inclusion of
studies with fewer than 10 patients may lead to bias. Additionally, the eligible studies adopt different
diagnostic criteria regarding the accuracy of diagnoses, such as the ESCMID-ECMM-ERS guideline in
2017 and the revised AGIHO/DGHO criteria in 2014, which can cause misclassification bias and lead to
biased results.

**Conclusion**

In conclusion, the current meta-analysis suggests that the accuracy of BAL-mNGS is good. Since the BAL-
mNGS assay is highly specific for lung fungal diseases, the results of that can be interpreted alone and
could be used as a part of a full assessment with clinical features, image findings, and other laboratory
results for the diagnosis of lung fungal diseases. Furthermore, BAL-mNGS detection indicates certain
fungal genuses, leading to the use of targeted treatment in practice. For the desperate need for improved
diagnostic approaches to invasive fungal diseases, we do recommend BAL-mNGS as an independent
screening test for the early detection of lung fungal diseases.

**Declarations**

**Acknowledgments**

**Disclosure**

The author reports no conflicts of interest in this work.

**Ethical Approval**
Competing interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Authors' contributions

Ziyu Kuang and Jing Liu wrote the main manuscript text and Ziyu Kuang prepared figures 1-8. All authors reviewed the manuscript.

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Availability of data and materials

The material and data for this paper can be found in references 15-29.

References


**Figures**

**Figure 1**

Flowchart demonstrating the algorithm for identifying suitable papers for inclusion.
Figure 2

(a). Methodological quality graph and Methodological quality summary.

(b). Methodological quality graph and Methodological quality summary.
Figure 3

Forest plot of the meta-analysis of sensitivity and specificity for the diagnosis of lung fungal diseases using BAL-mNGS.
Figure 4

Forest plot of the meta-analysis of PLR and NLR for the diagnosis of lung fungal diseases using BAL-mNGS.
Figure 5

Forest plot of the meta-analysis of diagnostic score and OR
Figure 6

Deek’funnel plot asymmetry test for the diagnosis of lung fungal diseases
Figure 7

SROC curve for BAL-mNGS.
Figure 8

scattergram of the meta-analysis of PLR and NLR for the diagnosis of lung fungal diseases using BAL-mNGS.