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Research Article
Diagnostic and Preventive Significance of Fungal Detection Combined with (1, 3) β-D Glucan Detection for Pulmonary Fungal Infection

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Abstract

Background and Objective: Pulmonary fungal infections (PFIs) are bronchial or pulmonary diseases caused by fungi. It is necessary to explore an accurate, convenient and rapid diagnostic method for PFIs. This study was conducted to investigate the diagnostic significance of (1,3) β-D-glucan (BDG) detection in PFI. **Materials and Methods:** This is a retrospective study. A total of 139 PFI patients were performed with bronchoalveolar lavage. Alveolar lavage fluid (4 mL) and fasting peripheral venous blood (2 mL) of the patients were prepared to conduct BDG detection using the ELISA method. Following this test, the patient's sputum was collected for fungal culture and the expression level of BDG in alveolar lavage fluid and blood was determined, a diagnostic value of sputum culture and BDG assay results towards PFI was explored. **Results:** As 126 patients were diagnosed with PFI and another 13 patients were diagnosed with bacterial infections after re-examination. Among the diagnosed patients, *Aspergillus* accounted for the highest proportion, 36.51%. For the diagnosed patients, the BDG expression in alveolar lavage fluid and blood specimens was sharply uplifted when compared with the undiagnosed patients ($p<0.001$). Among all pathogenic strains, the BDG expression reached the highest in subjects who were pathogenic to *Cryptococcus* ($p<0.001$). **Conclusion:** The BDG is highly efficient in diagnosing PFI. It can be used as the best clinical diagnosis target for PFI combined with a fungal culture.

Key words: (1,3) β-D glucan detection, alveolar lavage fluid, *Aspergillus*, *Cryptococcus*, pulmonary fungal infection

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Pulmonary fungal infections (PFIs), which include primary and secondary PFIs, are bronchial or pulmonary diseases caused by fungi[1]. With the widespread use of broad-spectrum antimicrobials and immunosuppressants in clinical settings as well as the popularization of indwelling catheters and ventilators, the incidence of PFIs is increasing[2]. According to statistics, there were about 1.2 million new cases of PFIs worldwide in 2016, approximately three times the numbers 5 years ago, with children aged 2-10 years being the most commonly affected[3,4]. At present, the cumulative number of PFIs has exceeded 13 million, which only ranks second to pulmonary mycoplasma infection among all respiratory diseases in children[5]. As the symptom of PFI is nonspecific, it usually coexists with the basic clinical symptoms. Moreover, it is challenging to collect specimens (sputum, airway secretions, alveolar lavage fluid, etc.) and the positive rate of fungal culture is not high, resulting in a low detection rate of PFI[6,7]. This also results in a high rate of missed diagnosis and misdiagnosis of PFIs in clinical settings and most patients cannot be effectively diagnosed in the early stage of the disease, which aggravates the disease with a high probability[8]. Therefore, in clinical settings, continuous efforts have been made to explore an accurate, convenient and rapid diagnostic method for PFIs. As (1,3) β-D glucan (BDG) detection is the current method used for identifying fungal infections. The BDG is the most important component of the fungal cell wall. When invasive fungal infections occur in the body, BDG can be released into the blood and body fluids via cells in large quantities, which can be directly used to determine whether a patient has fungal infections[9,10]. Due to its convenience and rapid examination, BDG detection is the primary choice for the diagnosis of PFIs[11]. However, its specificity has not been validated worldwide.

This study was conducted to investigate the diagnostic significance of BDG detection in PFI, so as to provide reference and guidance for the diagnosis and treatment of PFIs in the future.

MATERIALS AND METHODS

General information: A retrospective analysis was performed on 139 patients who were highly suspected to have a PFI in Qingpu Branch of Zhongshan Hospital, Fudan University from January 2014 to May 2018. The patients ranged in age from 8 to 18 years, with an average age of 8.26±4.35 years. Of these patients, 83 were males and 56 were females. Detailed clinical data was shown in Table 1.

Inclusion and exclusion criteria: The inclusion criteria were as follows: Clinical symptoms that were in accordance with the diagnostic guidelines for PFIs[12], diagnosed as PFIs after medical examination, patients who underwent follow-up BDG test and fungal culture examination or surgery in hospital, those who had a complete medical records and those who were willing to cooperate with the medical staff of hospital. Meanwhile, the exclusion criteria were as follows: Patients who had recently undergone cellulose membrane dialysis or who received blood products, radiochemotherapy, etc., those with cancer, organ failure, other blood diseases, other immune diseases and physical disability, those on prolonged bed rest, pregnant patients and those with mental illness.

Methods: The patients were diagnosed with PFI by a doctor in Qingpu Branch of Zhongshan Hospital, Fudan University and bronchoalveolar lavage was performed. A total of 4 mL of alveolar lavage fluid and 2 mL of fasting peripheral venous blood were collected from the patients for BDG detection using Enzyme-Linked Immunosorbent Assay Method. The kit was purchased from Shanghai Jianglai Biotechnology Co. Ltd., A21349 and the procedure was strictly conducted in accordance with the reagent instructions. Sputum was collected from the patients and inoculated in Sabouraud agar (Shanghai Yihui Biotechnology Co. Ltd., 210986) and CHROMagar (Shanghai Lianmai Bioengineering Co. Ltd., LM70060). The colonies were incubated for 24 hrs. After incubation, the dominant colonies were selected and the pathogens were detected using a microbiological analyzer.

Criterion: The BDG criterion refers to the 2016 BDG application guidelines[13], which were as follows: <0 pg mL^{-1}, negative for BDG and >20 pg mL^{-1}, positive for BDG. The criteria for the classification of potential cases were

Table 1: Clinical data of the patients

Clinical data	n (%)
Gender	
Male	83 (59.71)
Female	56 (40.29)
Incidence	
Primary	84 (60.43)
Secondary	55 (39.57)
Place of residence	
Town	97 (69.78)
Rural	42 (30.22)
Family history	
Yes	69 (49.64)
No	70 (50.36)
Course of disease (d)*	
<7d	24 (17.27)
≥7	115 (82.73)

*Represents the course of the disease, the time from the start of lung discomfort to treatment in the hospital

according to the likelihood of underlying invasive pulmonary aspergillosis: Possible, probable and proven. To diagnose an infection, it is necessary to obtain histopathological evidence or isolate suspected pathogens from normally sterile sites and then classify the cases as probable or possible according to host, clinical and microbiological criteria. The cell wall components in the serum were detected via immunostaining. BDG antigen and galactomannan antigen detections were used to determine the positivity rate.

Observation indicators: The expression level of BDG in the alveolar lavage fluid and blood was detected before and after treatment in diagnosed patients, sputum culture results were assessed. The diagnostic value of BDG on PFIs was identified. That is, the confirmed results were considered as gold standard:

$$\text{Sensitivity}(\%) = \frac{TP}{TP + FN} \times 100$$

$$\text{Specificity}(\%) = \frac{TN}{FP + TN} \times 100$$

$$\text{Diagnostic coincidence rate}(\%) = \frac{TP + TN}{TP + FP + TN + FN} \times 100$$

$$\text{Positive predictive value}(\%) = \frac{TP}{TP + FP} \times 100$$

$$\text{Negative predictive value}(\%) = \frac{TN}{TN + FN} \times 100$$

where, TP refers to true positive, TN to true negative, FP to false positive and FN to false negative.

Statistical analysis: Data were analyzed and processed using the Statistical Package of the Social Sciences software version 24.0 (Shanghai Yuchuang Network Technology Co. Ltd.). The enumeration data, such as the expression level of BDG, were presented as Mean±Standard Deviation (Mean±SD). The comparison between groups was performed by one-way analysis of variance with *post hoc* Bonferroni Test. Data with normal distribution between the two groups were compared using an unpaired t-test and those not conforming to normal distribution were compared using Mann-Whitney U. The counting data, such as diagnostic sensitivity and specificity, were expressed as rate and Chi-square Test was used for comparison between groups. A $p<0.05$ was considered statistically significant.

Ethical consideration: The study was approved by the Ethics Committee of Qingpu Branch of Zhongshan Hospital, Fudan University. All patients provided written informed consent at the initiation of diagnosis, allowing for further clinical research using the clinical records. This research was conducted ethically in accordance with the World Medical Association Declaration of Helsinki.

RESULTS

Diagnostic result: Of the 139 patients, 6 were underwent pathological biopsy examination and another 133 patients underwent sputum culture examination. A total of 126 patients were diagnosed with PFIs and the diagnosed patients accounted for 90.65% of the suspected patients. Another 13 patients were diagnosed with bacterial infection after re-examination. *Aspergillus* was the most common pathological strain, which caused infection in 36.51% of patients (46 cases), followed by *Cryptococcus* (20.63%, 26 cases), *Candida parapsilosis* (14.29%, 18 cases), *Candida tropicalis* (11.90%, 15 cases), *Candida glabrata* (7.14%, 9 cases), *Candida albicans* (5.56%, 7 cases) and other strains (3.97%, 5 cases).

Expression level of BDG: A total of 126 patients diagnosed with PFI were assigned to the fungal group, while 13 patients diagnosed with bacterial infections were assigned to the control group. The two groups exhibited no significant differences in terms of clinical data, such as age, course of disease, gender, morbidity, living environment and family history ($p>0.05$), which validated that the two groups of patients were comparable. The expression level of BDG in the alveolar lavage fluid before treatment was significantly higher in the fungal group than in the control group (275.7±107.8 vs 0.02±0.01 pg mL^{-1}, $p<0.001$) (Fig. 1a). The expression level of BDG in the plasma before treatment was also significantly higher in the fungal group than in the control group (92.4±29.7 vs 6.0±2.1 pg mL^{-1}, $p<0.001$) (Fig. 1b).

The expression level of BDG in the alveolar lavage fluid of all patients was significantly higher before treatment than after treatment (294.2±86.8 vs 51.6±10.8 pg mL^{-1}, $p<0.001$). The expression level of BDG in the plasma of all patients was significantly higher before treatment than after treatment (76.3±18.4 vs 7.6 pg mL^{-1}, $p<0.001$) (Table 2, Fig. 2).

Expression levels of BDG in patients infected with different pathogenic strains: Among the 126 patients with PFI, the expression levels of BDG in the alveolar lavage fluid (Fig. 3a) and blood (Fig. 3b) of patients with *Aspergillus* infection were

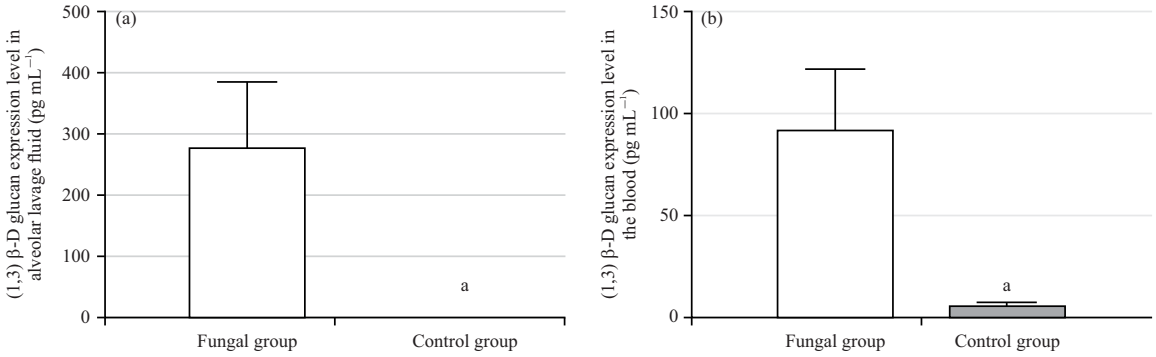

Fig. 1(a-b): Expression levels of (1,3) β-D glucan of the fungal and control groups, (a) Expression levels of (1,3) β-D glucan in the alveolar lavage fluid and (b) Expression levels of (1,3) β-D glucan in the blood

[a]Represents comparison with the fungal group in terms of (1,3) β-D glucan expression level ($p<0.001$)

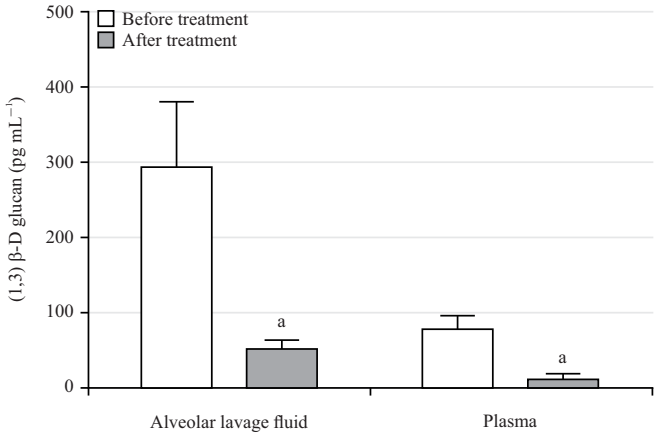

Fig. 2: Results of (1,3) β-D glucan test before and after treatment

[a]Represents a comparison with the (1,3) β-D glucan test results in the pre-treatment alveolar lavage fluid (plasma) ($p<0.001$)

Table 2: Comparison of clinical data

Clinical data	Fungal group (n = 126)	Control group (n = 13)	χ^2 or t	p-value
Age	9.2±2.7	9.2±1.7	0.007	0.995
Course of disease (d)	9.6±5.3	9.5±5.2	0.111	0.912
Gender				
Male	75 (59.52)	8 (61.54)	0.020	0.888
Female	51 (40.48)	5 (38.46)		
Incidence				
Primary	76 (60.32)	9 (69.23)	0.007	0.932
Secondary	50 (39.68)	4 (30.77)		
Place of residence				
Urban	87 (69.05)	10 (76.92)	0.347	0.556
Rural	39 (30.95)	3 (30.95)		
Family history				
Yes	62 (49.21)	7 (53.85)	0.102	0.750
No	64 (50.79)	6 (46.15)		

173.6±26.9 and 96.1±8.7 pg mL^{-1}, respectively, the expression levels of BDG in patients with *Cryptococcus* infection were 405.2±38.8 and 277.7±32.2 pg mL^{-1}, respectively. The expression levels of BDG in patients with *Candida parapsilosis* infection were 197.1±24.4 and 107.7±18.7 pg mL^{-1}, respectively. Meanwhile, in patients with *Candida tropicalis*, the expression levels of BDG were 143.4±16.9 and 93.2±9.3 pg mL^{-1}, respectively. The expression levels of BDG in patients with *Candida glabrata* infection were 115.8±13.0 and 79.6±12.7 pg mL^{-1},

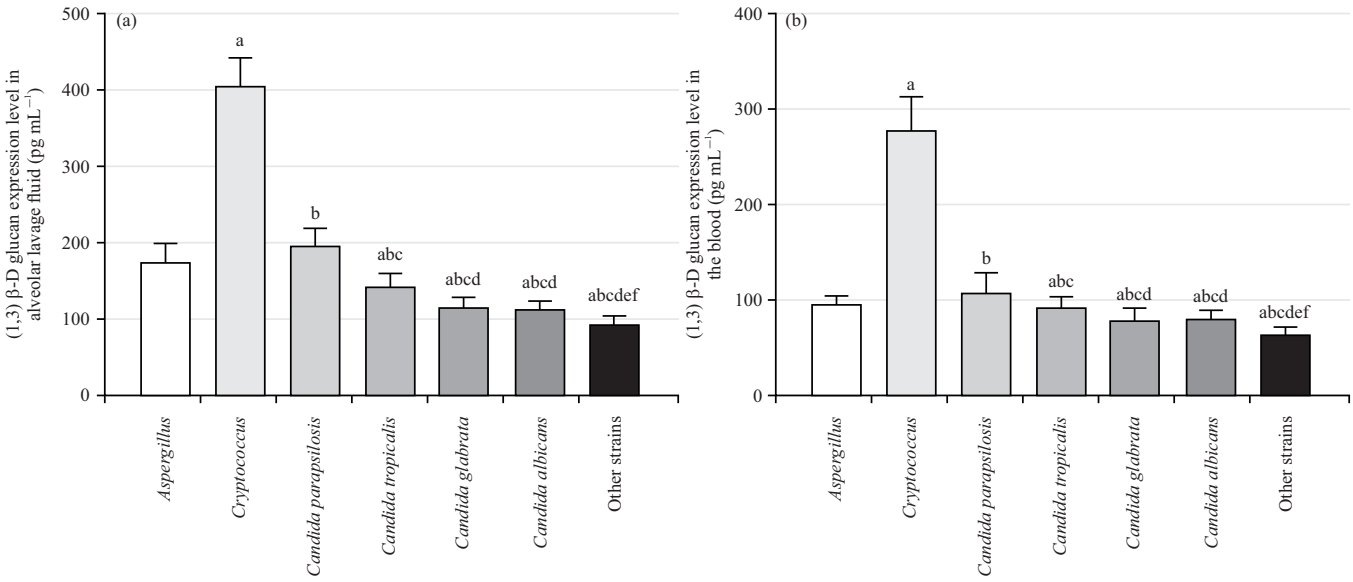

Fig. 3(a-b): Expression levels of (1,3) β-D glucan of different pathogenic bacteria in the fungal group, (a) Expression levels of (1,3) β-D glucan in the alveolar lavage fluid and (b) Expression levels of (1,3) β-D glucan in the peripheral blood
[a]Comparison with *Aspergillus* (p<0.05), [b]Comparison with *Cryptococcus* (p<0.05), [c]Comparison with *Candida parapsilosis* (p<0.05), [d]Comparison with *Candida tropicalis* (p<0.05), [e]Comparison with *Candida glabrata* (p<0.05) and [f]Comparison with *Candida albicans* (p<0.05)

respectively. The expression levels of BDG in patients with *Candida albicans* infection were 114.7±10.6 and 81.1±8.7 pg mL^{-1}, respectively. Moreover, in patients with other strains, the expression levels of BDG were 94.1±10.6 and 64.2 ± 8.3 pg mL^{-1}, respectively. Among all pathogenic strains, the expression level of *Cryptococcus* BDG was the highest (p<0.001), followed by *Candida parapsilosis* (p<0.05). No significant difference was observed in the expression of BDG between *Candida parapsilosis* and *Aspergillus* (p>0.05). Moreover, no significant difference was noted in the expression of BDG between *Candida glabrata* and *Candida albicans* (p>0.05). The expression of BDG was the lowest in patients with other strains infection (p<0.05) (Fig. 2, 3).

Diagnostic efficacy of BDG for PFI: Among the 139 patients, 120 had BDG level >20 pg mL^{-1} as detected using the BDG expression level and a comparison with the gold standard culture results was conducted. The numbers of true-positive and true-negative cases detected by BDG were 118 and 11, respectively and the numbers of false-positive and false-negative cases were 2 and 8, respectively. Therefore, the sensitivity of BDG detection of PFI was 93.65% and the specificity was 84.62%. Moreover, the diagnostic coincidence rate was 92.81% and the positive predictive value was 98.33%. The negative predictive value was 57.89%. Chi-square Test showed that the relative risk of BDG in the diagnosis of PFI was 1.261 and the 95% confidence interval (95% CI) was 0.815~1.951.

DISCUSSION

The clinical manifestations of PFIs are not specific and the commonly used diagnostic methods have certain disadvantages. Therefore, the rapid and accurate diagnosis of PFI has been challenging and is one of the focuses of research. The BDG is a polysaccharide in the fungi other than *Candida parapsilosis*, which is extremely valuable for the reaction of fungal infections[14,15]. However, due to the lack of references for this study worldwide, only few studies have supported the application of BDG detection for PFIs. Therefore, through data analysis, this study is more likely to present the excellent application value of BDG detection for PFIs.

This study showed that PFI was most commonly observed and the number of urban patients was significantly higher than that of rural patients, indicating that PFI was significantly correlated to the living environment. The course of the disease of newly diagnosed patients was extremely long, indicating that the patients should improve their awareness of the disease and physical examination should be carried out at regular intervals for early detection and treatment. After diagnosis, the most common pathogenic bacterium in patients with PFIs was *Aspergillus*, followed by *Cryptococcus* and this result was similar to that of a study by Kamada *et al.*[16]. On distribution status in the study of PFIs, indicating that it should be considered the most important pathogenic fungal group in clinical settings, therefore, effective and targeted treatment measures should be taken as soon as possible.

The expression levels of BDG in the alveolar lavage fluid and blood of the diagnosed and undiagnosed patients were compared and the expression level in diagnosed patients was significantly higher than that of undiagnosed patients. After treatment, the expression level of BDG of patients in the alveolar lavage fluid and plasma was significantly lower than that before treatment, indicating that BDG was significantly correlated to the occurrence and development of PFI. The fungal glucan is widely distributed in fungal cell wall and belongs to the polysaccharide component of the filamentous and yeast fungal cell wall. The glucan content can be reacted using the protein complex formed by the reaction of the fungal glucan-activating enzyme with the corresponding factor in the reagent. BDG was the most sensitive with the most abundant content[17,18]. After fungal infection, the glucan in the fungal cell wall was released in large quantities, which was highly increased in the infected tissue of patients and could penetrate into the blood through the vascular endothelium.

This was also the reason why the expression level of BDG in the alveolar lavage fluid and blood of the fungal group was significantly higher than that of the control group. The expression level of BDG in the alveolar lavage fluid was higher, indicating that the detection results of BDG in the alveolar lavage fluid of patients were more intuitive and sensitive. The expression levels of BDG in patients infected with different pathogenic bacteria were compared, the expression level of BDG was highest in patients infected with *Cryptococcus*, whereas the difference was not significant among other strains, indicating that BDG was the most sensitive to *Cryptococcus*. Its response to other strains was relatively low and the specificity was not significant. *Cryptococcus* is an extremely common pathogen that exits in soil, air, plants, animals, etc. Its vitality is extremely tenacious and all the hyphae have a strong vascular penetrating power. Once *Cryptococcus* infection occurs, it is easy to cause a series of infectious diseases[18]. Therefore, when measuring the expression level of BDG in the alveolar lavage fluid and blood of patients, the patients with *Cryptococcus* infection would release a large amount of BDG due to the massive invasion of fungus, indicating that BDG was more sensitive to invasive fungi and further investigation of its mechanism required deep test analysis.

Compared with the gold standard diagnosis results, the diagnostic sensitivity of BDG for PFI was 93.65% and the specificity was 84.62%. Compared with other diagnostic methods used in clinical settings, BDG is an extremely simple diagnostic method with significantly high sensitivity and can be used as a preferred alternative for the diagnosis of PFI. In clinical settings, after the initial diagnosis of BDG, the patient can be subjected to emergency treatment measures. Afterwards, fungal culture can be carried out to determine the pathogenic bacterium of the patient, thereby implementing an effective treatment plan.

However, pneumocystis pneumonia was not found in this study, which may be due to the fact that spores are uncommon in PFIs. For pneumocystis pneumonia, the diagnostic criteria should be persistent fever >96 hrs, which cannot be cured with antibiotics. At the same time, symptoms and signs of pulmonary infection were observed, including cough, hemoptysis, chest pain and dyspnea, lung vocal or pleural friction sound, all above symptoms did not appear in the subjects included in this study.

The ROC curve analysis required data of glucan levels in undiagnosed and diagnosed patients. The cut-off value (20 pg mL^{-1}) was used as a diagnostic criterion according to the references and the patients were divided into diagnosed and undiagnosed patients. Glucan levels are significantly increased in diagnosed patients with fungal infection. Therefore, the sensitivity of ROC curve analysis is 100%. This result is not rigorous. The sensitivity, specificity and 95% CI of glucan in the diagnosis of fungal infection were obtained by comparison with the gold standard. Therefore, the results of ROC curve analysis were not included in this experiment.

This study had several limitations. The baseline data for participants were inadequate and statistical analysis of large amount of data cannot be performed. Moreover, the number of cases and participants is limited and there might have been different expressions of BDG in varying ethnic groups. Further research about the mechanism of action of BDG on various bacterial groups must be performed. A long-term follow-up survey of the participants of this study will be conducted and constant improvements of the test will be made to obtain the best results.

CONCLUSION

In summary, this study presents the data on the diagnostic significance of BDG detection in PFI and revealed that BDG detection has an extremely high efficiency for the diagnosis of PFI, with a sensitivity of 93.65% and a specificity of 84.62% for PFI. The detection method of BDG combined with fungal culture can be used as the best clinical diagnosis target for the diagnosis of PFIs. The study can provide reference and guidance for the diagnosis and treatment of PFIs in the future.

SIGNIFICANCE STATEMENT

Pulmonary fungal infections (PFIs) are bronchial or pulmonary diseases caused by fungi. In clinical settings, continuous efforts have been made to explore an accurate, convenient and rapid diagnostic method for PFIs. As (1,3) β-D glucan (BDG) detection is the current method used for identifying fungal infections. Due to its convenience and rapid examination, BDG detection is the primary choice for the diagnosis of PFIs. However, its specificity has not been validated worldwide. This study presents the data on the diagnostic significance of BDG detection in PFI and reveals that BDG is highly efficient in diagnosing PFI, which can be used as the best clinical diagnosis target for PFI combined with a fungal culture.

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