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Comparing the diagnostic value of bronchoalveolar lavage fluid galactomannan, serum galactomannanan, and serum 1,3-β-D-glucan in non-neutropenic respiratory disease patients with invasive pulmonary aspergillosis

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Abstract

The incidence of invasive pulmonary aspergillosis (IPA) is increasing higher in non-neutropenic patients. This study aimed to assess the diagnostic performance of bronchoalveolar lavage fluid (BALF). Galactomannan (GM), serum GM, and 1,3-β-D-glucan (BDG) in non-neutropenic respiratory disease patients with IPA.

A total of 333 non-neutropenic patients suspected IPA were recruited from Xiamen University Zhong Shan hospital between January 2016 and February 2019. One, 33, and 92 cases were diagnosed with proven, and possible IPA.

BALF and serum GM were both elevated in the possible IPA group and the probable/proven IPA group ($p < 0.001$). BALF and serum GM showed a fair correlation in the possible IPA group ($r = 0.286$, $p = 0.008$), and moderate correlation in the probable/proven IPA group ($r = 0.466$, $p = 0.005$). When the cutoff value was 0.5, the sensitivity and negative likelihood ratio of BALF GM were superior to serum GM (78.3% vs 47.8%, 96.7% vs 91.6%). The specificity and positive likelihood ratio of BALF GM were slightly weaker than serum GM (91.8% vs 95.4%, 56.7% vs 85.0%). When the cutoff value was 1.0, the sensitivity and negative predictive value of BALF GM were better than serum GM (73.9% vs 26.1%, 94.5% vs 88.8%), and the specificity of were equivalent (99.2%). The optimal cutoff value of BALF GM was 0.6, wherein the sensitivity reached 78.3% and the specificity reached 95.4%. Given the extremely low sensitivity of serum BDG at different cutoff values (≥10 μg/mL = 5.3%, ≥20 μg/mL = 2.1%), it cannot be used as a preferred biomarker.

The diagnostic performance of BALF GM was superior to other biomarkers and the optimal cutoff value was 0.6.

Abbreviations: −LR = negative likelihood ratio, +LR = positive likelihood ratio, BALF = bronchoalveolar lavage fluid, BDG = 1,3-β-D-glucan, COPD = chronic obstructive pulmonary disease, CRP = C-reactive protein, CSF = cerebrospinal fluid, CT = computed tomography, EORTC/MSG = European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy, and Infectious Diseases Mycoses Study Group, GM = galactomannan, ICU = intensive care unit, IPA = invasive pulmonary aspergillosis, NPV = negative predictive value, ODI = optical density index, PCT = procalcitonin, PPV = positive prediction rate, ROC = receiver operating curve.

Keywords: 1,3-β-D-glucan (BDG), bronchoalveolar lavage (BALF), galactomannan (GM), invasive pulmonary aspergillosis (IPA)

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1. Introduction

Invasive pulmonary aspergillosis (IPA) is a type of respiratory infectious disease mainly caused by pulmonary Aspergillus invading the bronchial and vascular walls.[1] IPA usually occurs in neutropenic patients accompanied by immune function damage, including organ transplantation and hematological malignancy; the incidence of IPA is closely related to the mortality of these diseases.[2–4] However, IPA is increasing in non-neutropenic patients with chronic underlying diseases, such as chronic obstructive pulmonary disease (COPD)[5] and diabetes mellitus.[6] Early detection and timely treatment are crucial for non-neutropenic IPA patients. However, the clinical manifestations, including cough, asthma, and dyspnea in non-neutropenic patients are nonspecific.[7] Furthermore, the radiological signs, such as cavity and air-crescent sign are fairly rare in the early phase of IPA.[8] Microbial culture and histopathology are considered as the gold standards of IPA, but they have limited value due to low sensitivity and long turnaround time.[9] Therefore, an efficient and accurate approach to identify IPA in non-neutropenic patients at the preliminary stage is urgently needed.

Galactomannan (GM) is a type of polysaccharide of Aspergillus species. GM widely exists in the cells of Aspergillus and Penicillium. The corresponding commercial ELISA kits have been used to detect GM in the bloodstream at the early stage of infection.[10] According to the Revised Definitions of Invasive Fungal Disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy, and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group, GM detected in bronchoalveolar lavage fluid (BALF), serum, plasma, and cerebrospinal fluid (CSF) is considered as mycological criteria in probable invasive fungal disease. 1,3-β-D-glucan (BDG) is a type of polysaccharide component that universally exists in the cell walls of fungi, including Aspergillus and Candida, but not Mucorales.[11] Rose et al used the negative predictive value (NPV) of BDG to diagnose IPA, which reached 95%.[12] However, these 2 antigens were only measured in IPA patients with neutropenia, and the diagnostic value of these 2 biomarkers in IPA patients with normal immune function remains unclear. Moreover, the cutoff value of BALF GM in diagnosing IPA has not been standardized.

The purpose of this study was to evaluate the diagnostic performance of BALF GM, serum GM, and serum BDG in non-neutropenic patients with IPA, and to define the optimal cutoff value of BALF GM.

2. Materials and methods

2.1. Study patients

Between January 2016 and February 2019, a total of 333 patients with suspected IPA, who received treatment at the pneumology department and intensive care unit (ICU) of Xiamen University Zhong Shan hospital, were enrolled in this study. BALF GM, serum GM, serum BDG, procalcitonin (PCT), and C-reactive protein (CRP) were measured before administering antibiotics. Exclusion criteria[13]: (1) More than 10 days peripheral blood neutrophil count $<0.5\times10^9$/L. (2) Patients with malignant tumors of the blood system. (3) Patients with organ transplant in the last 3 months. The study was approved by the Ethics Committee of Zhong Shan Hospital, Medical College of Xiamen University, and performed in compliance with the Declaration of Helsinki (2008). Written informed consent was obtained from all individual participants included in the study.

2.2. Grouping criteria of IPA

Grouping criteria were according to the (EORTC/MSG) Consensus Group[13] (no reference GM/BDG test). The patients were divided into 4 groups, including the proven IPA group, probable IPA group, possible IPA group, and no IPA (control) group.

Proven IPA group: In blood and sterile specimens, Aspergillus was detected through culture and microscopic analysis.

Probable IPA group: Should satisfy at least 1 host factor: (1) Taking glucocorticoid for at least 3 weeks. (2) Taking immunosuppressants for the past 3 months. (3) Invasive fungal infection history.

At least 1 clinical proof: Computed tomography (CT): (1) Dense, well-circumscribed lesions. (2) Air-crescent sign. (3) Cavity bronchoscopes: ulceration, nodule, pseudomembrane, plaque, or eschar.

At least 1 microbiological proof: (1) detection of mycelium or spores and (2) fungal culture detection in qualified sputum and BALF.

Possible IPA group: Should satisfy at least 1 host factor and clinical features; mycological evidence could be absent.

No IPA group: Confirmation of other pathogens that were effectively treated with non-anti-fungal treatment.

2.3. Sample collection

Serum collection: All participants underwent phlebotomy in the early morning after overnight fasting. For serum GM and BDG tests, blood collection tube with depyrogenation was used. For PCT, EDTA-K3 anticoagulant vacuum tube was used. For CRP, heparin sodium anticoagulant tube was used.

BALF collection: All subjects underwent pulmonary CT imaging examination to identify lung lesion site and extent, followed by bronchoscopy. For BALF collection, 0.9% saline was injected into the lesion site of the lung for lavage and recycled into the bottle of lotion for testing. Sputum sample was collected from the first cough in the morning due to underlying respiratory tract infection (qualification standard: WBC > 25/HP and epithelial cells <10/HP or 10–25/HP).

2.4. Sample test

Serum and BALF GM were tested with an immunoenzymatic sandwich microplate assay (ELISA) from Bio-Rad laboratories. Optical density index (ODI) of serum GM ≥0.5 was considered as a positive specimen as per instructions.

Kinetic-turbidimetric LAL kit and microbial dynamic monitoring system (ELX 808) were used to test serum BDG. The concentration of serum BDG ≥10 μg/ml was identified as a positive specimen as per instructions. The minimum detection limit is 10 μg/ml for this methodology.

Serum PCT was measured by electrochemiluminescence technique using the Cobas 6000 system (Roche Diagnostic, Germany). Serum CRP was measured by AU5800 Biochemical analyzer (Beckman Coulter, USA). Sputum and BALF cultures were measured using Sabouraud fungal medium and identified by spectrum analyzer (Microflex LT/SH).

2.5. Statistical analysis

All statistical analyses were performed by SPSS 24 (IBM Corp, Armonk, NY, USA). Partial drawing was by Graph-pad Prism 6.0 (San Diego, CA, USA). Measurement data, which were tested by the Shapiro–Wilk test, in accordance with normal distribution were expressed by mean and standard deviation, while data not in accordance with normal distribution were expressed by median and interquartile range. Wilcoxon rank-sum test and Kruskal–Wallis *H* test were used to compare the statistical difference between the 2 groups.

Normal distribution correlation analysis was performed using Pearson's correlation test, while non-normal distribution correlation was performed using Spearman's correlation test. A *p*-value of 0.05 was considered statistically significant. Receiver operating curve (ROC) was used to assess the performance of various biomarkers in the diagnosis of IPA. Sensitivity, specificity, positive prediction rate (PPV), NPV, negative likelihood ratio (−LR), and positive likelihood ratio (+LR) were calculated from the ROC curve.

3. Results

3.1. Patient characteristics

A total of 333 patients were enrolled, including 211 males and 122 females. As per the EORTC/MSG criteria, only 1 case was confirmed with IPA by microbiology and histopathology evidence, 33 cases were defined as the probable group, 92 cases served as the possible group, and the remaining 207 patients who had no evidence of IPA were included as the control group. The confirmed patient and the probable group were combined into the probable/proven group for the statistical analysis (Table 1).

Among the definite underlying diseases, the highest percentage was pneumonia as 44.12% (15 out of 34) patients were diagnosed with probable/proven IPA. COPD had the second-highest proportion in the probable/proven IPA group, with 14.71% (5 out of 34). Cardiopathy and hypertension were the top 2 common extrapulmonary diseases among the probable/proven IPA patients, accounting for 32.35% (11 out of 34) and 20.59% (7 out of 34). This tendency was also reflected in the possible IPA group, cardiopathy took up 21.74% (20 out of 92), and hypoproteinemia took up 16.30% (15 out of 92). The highest percentage of extrapulmonary underlying disease was also cardiopathy, as 21.74% (20 out of 92) patients were in the possible group. In prognosis status, the percentage of improved patients in no IPA group attached to 91.79% (190 out of 207) which were higher than the possible IPA group (80.43% 74 out of 92) and probably and proven groups (82.35% 28 out of 34). No patients were completely cured in probably/proven IPA groups.

3.2. Expression of BALF GM, serum GM, serum BDG, PLT, and CRP in each group

The BALF GM level was elevated in the possible and probable/proven groups, with statistically significant differences between

Table 1

Demographic and clinical characteristics of the patients.

	No IPA	Possible IPA	Probable/proven IPA
Gender (total/males)	207/130	92/62	34/19
*Median age	58[37,79]	61[40,82]	60[41,79]
Definite pulmonary disease			
Pneumoconioses	3 (1.45%)	/	/
Lung carcinoma	14 (6.76%)	6 (6.52%)	2 (5.88%)
Pulmonary tuberculosis	19 (9.18%)	5 (5.43)	2 (5.88%)
Pneumonia	95 (45.89%)	44 (47.83%)	15 (44.12%)
Cryptococcosis	3 (1.45%)	1 (1.09%)	/
Bronchiectasis	19 (9.18%)	6 (6.52%)	2 (5.88%)
Autoimmunity	10 (4.83%)	4 (4.35%)	2 (5.88%)
COPD	27 (13.04%)	18 (19.57%)	5 (14.71%)
†Indefinite pulmonary disease			
Pulmonary nodule	5 (2.42%)	/	2 (5.88%)
Pulmonary cavity	5 (2.42%)	/	2 (5.88%)
Lung abscess	7 (3.38%)	8 (8.70%)	2 (5.88%)
Underlying diseases			
Carcinoma	17 (8.21%)	7 (7.61%)	5 (14.71%)
Hypertension	42 (20.29%)	24 (26.09%)	5 (14.71%)
Immune deficiency	10 (4.83%)	4 (4.35%)	1 (2.94%)
Cerebrovascular accident	12 (5.80%)	8 (8.70%)	4 (11.76%)
Hypoproteinemia	22 (10.63%)	15 (16.30%)	7 (20.59%)
Diabetes mellitus	10 (4.83%)	10 (10.87%)	1 (2.94%)
Cardiopathy	19 (9.18%)	20 (21.74%)	11 (32.35%)
No underlying diseases	75 (36.23%)	4 (4.35%)	/
Prognosis status			
Cure	7 (3.38%)	4 (4.35%)	/
Improved	190 (91.79%)	74 (80.43%)	28 (82.35%)
Not cured	7 (3.38%)	5 (5.43%)	4 (11.76%)
Death	3 (1.45%)	9 (9.78%)	2 (5.88%)

COPD = chronic obstructive pulmonary disease, IPA = invasive pulmonary aspergillosis.

*Percentage shows the ratio of pulmonary diseases and underlying diseases in several groups.

†Patients in whom pulmonary diseases could not be identified were classified by radiological proof.

the no IPA group versus the possible IPA group ($p < 0.001$) and the probable/proven IPA group ($p < 0.001$). No statistical difference was observed between the possible IPA group and the probable/proven IPA group ($p = 0.927$). Serum GM was elevated in the possible and probable/proven IPA groups, with the statistical difference between the no IPA group versus the possible IPA group ($p = 0.013$), the no IPA group versus the probable/proven IPA group ($p < 0.001$), and the possible IPA group versus the probable/proven IPA group ($p = 0.035$).

Serum PCT and serum CRP were significantly lower in the no IPA group than in the possible IPA (PCT $p < 0.001$, CRP $p = 0.001$) and probable/proven IPA groups (PCT $p = 0.001$, CRP $p = 0.003$) (Table 2).

3.3. Statistical analysis of the correlation between BALF GM and serum GM

The correlation of BALF GM and serum GM were compared in the no IPA group, possible IPA group, and probable/proven IPA group, respectively. As shown in Figure 1A to C, no correlation was detected between BALF GM and serum GM in the no IPA group ($r = 0.086$, $p = 0.244$), fair correlation was observed in the possible IPA group ($r = 0.286$, $p = 0.008$), and moderate correlation between BALF GM and serum GM was observed in the probable/proven IPA group ($r = 0.466$, $p = 0.005$).

3.4. Diagnostic performance of biomarkers in the no IPA group versus the possible and the no IPA group versus probable/proven IPA groups

Sensitivity, specificity, NPV, PPV, −LR, +LR, and Youden index between the no IPA group versus the possible and probable/proven IPA groups were compared in Table 3. When the cutoff value of GM ODI was ≥0.5, the sensitivity and NPV of BALF GM were higher than serum GM (sensitivity 72.6% vs 22.2%, NPV 85.5% vs 67.4%). The specificity and PPV of serum GM were slightly higher than BALF GM (specificity 96.6% vs 88.8%, PPV 95.5% vs 82.9%). The +LR between BALF GM and serum GM was 6.48 vs 6.52, and the −LR of BALF GM was superior to serum GM (0.31 vs 0.81). When the cutoff value of GM ODI was ≥1.0, the sensitivity, NPV and −LR of BALF GM were superior to serum GM (specificity 47.8% vs 11.1%, NPV 75.5% vs 64.9%, and +LR 17.4 vs 18.5).

The optimal cutoff value of BALF GM was 0.55, the Youden index was 0.628, the sensitivity was 71.8%, and the specificity was 91.6%. The PPV, NPV, +LR, and −LR were 87.5%, 84.5%, 8.54, and 0.30, respectively (Fig. 2A).

When the cutoff value of serum BDG was 10 pg/ml, the sensitivity and specificity were 9.4% and 95.5%, the PPV and NPV were 55.5% and 63.2%, and the Youden index was 0.049. When the cutoff value of serum BDG was 20 pg/ml, the sensitivity decreased to 6.0%, specificity was 97.2%, and the Youden index was 0.032.

3.5. Diagnostic performance of biomarkers in the no IPA group versus the probable/proven IPA group

Diagnostic indicators were also compared between the no IPA group and the probable/proven IPA group (Table 4). When the cutoff value was ≥0.5, serum GM was superior to BALF GM in specificity (95.4% vs 91.8%), PPV (85.0% vs 56.7%), and +LR (10.39 vs 9.54). However, the sensitivity, NPV and −LR of serum GM were inferior to BALF GM (47.8% vs 78.3%, 91.6% vs 96.7%, and 0.55 vs 0.24).

When the cutoff value was ≥1.0, the sensitivity, NPV, and −LR of BALF GM were superior to serum GM (78.3% vs 26.1%, 94.5% vs 88.8%, and 0.26 vs 0.74). The specificity of BALF GM and serum GM were 99.2%. The PPV and −LR of serum GM were better than BALF GM (PPV 97.1% vs 85.0% and +LR 32.62% vs 92.37%).

The optimal cutoff value of BALF GM in the no IPA group versus the probable/proven IPA group was 0.6, the Youden index was 0.736, the sensitivity was 78.3%, and the specificity was 95.4%. The PPV and NPV were 68.0% and 95.8%, respectively (Fig. 2B).

When the cutoff values of serum BDG reached 10 μg/ml and 20 μg/ml, the sensitivity was only 5.3% and 2.1%, the specificity was 95.5%, and the Youden index was 0.048 and 0.016, respectively.

4. Discussion

IPA represents a major threat to non-neutropenic patients with pulmonary diseases. Early diagnosis and treatment are crucial to achieve ideal therapeutic effect, but the initial clinical symptoms are nonspecific. Extensive efforts are being made for improving

Table 2

Expression levels of BALF GM and serum biomarkers in the different groups.

	No IPA	Possible IPA	Probable/proven IPA
BALF GM (ODI)	0.25[0.178 0.368]	0.733[0.426 1.77]	2.66[0.548 4.104]
≥0.5 ODI	26 (12.56%)	64 (69.57%)	26 (75.47%)
≥1.00DI	6 (2.9%)	37 (40.22%)	21 (61.76%)
Serum GM (ODI)	0.163[0.113 0.227]	0.2[0.119 0.34]	0.31[0.185 0.975]
≥0.5 ODI	10 (4.83%)	11 (11.96%)	15 (44%)
≥1.0 ODI	2 (0.96%)	6 (6.52%)	8 (2.35%)
Serum BDG*			
>10 pg/ml	8 (3.86%)	4 (4.34%)	6 (17.64%)
>20 pg/ml	5 (2.41%)	1 (1.08%)	6 (17.64%)
PCT (μg/L)	0.06[0.03 0.12]	0.10[0.04 0.56]	0.12[0.04 1.69]
CRP (mg/L)	20.17[4.63 63.36]	54.45[11.97 144.55]	73.92[40.03,169.72]

BALF = bronchoalveolar lavage fluid, BDG = 1,3-β-D-glucan, CRP = C-reactive protein, GM = galactomannan, IPA = invasive pulmonary aspergillosis, ODI = optical density index, PCT = procalcitonin. The data of ODI, PCT, and CRP in different groups were not in accordance with normal distribution and were expressed by median and interquartile range.

* The methodology of serum BDG used 10 pg/ml as the detection limit of a positive result, so serum BDG was not analyzed as a digital variable.

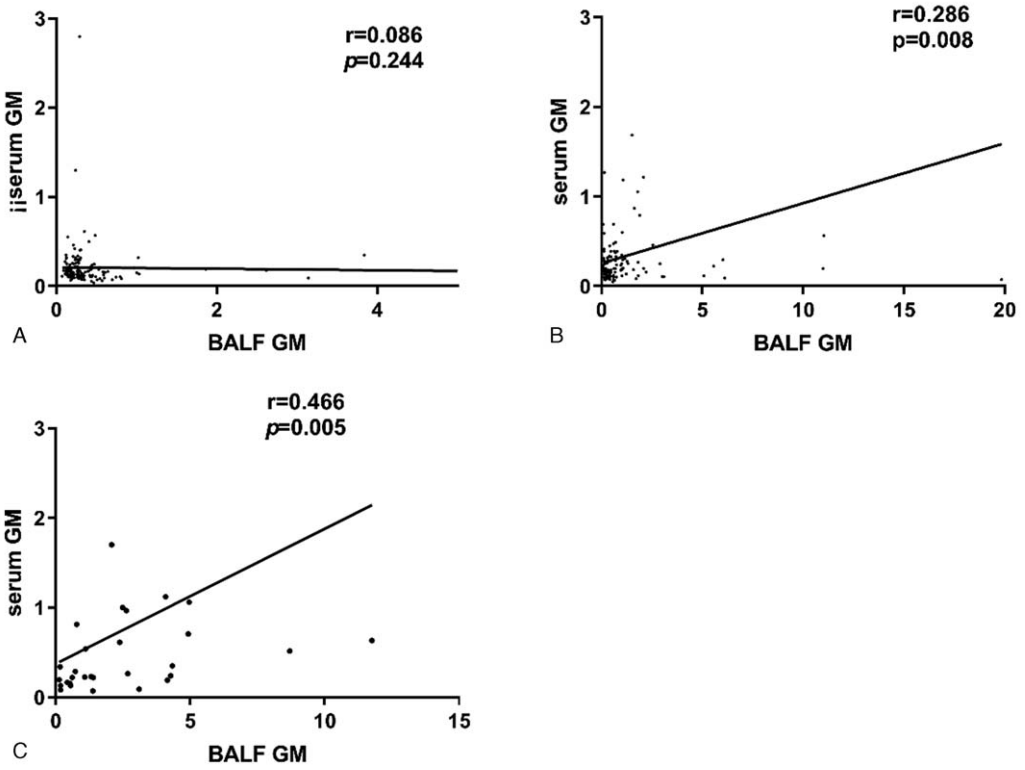

Figure 1. (A) The correlation between serum GM and BALF GM in the no IPA group. (B) The correlation between serum GM and BALF GM in the possible IPA group. (C) The correlation between serum GM and BALF GM in the probable/proven IPA group. BALF = bronchoalveolar lavage fluid, GM = galactomannan, IPA = invasive pulmonary aspergillosis.

the diagnostic efficiency before the appearance of GM and BDG. However, the diagnostic value of these 2 biomarkers was clearly defined only in neutropenic patients. Moreover, the cutoff value to diagnose IPA remains controversial. For non-neutropenic patients with IPA, serum and BALF biomarkers for diagnosing IPA are lacking. To the best of our knowledge, the cutoff value of BALF GM in diagnosing IPA has not been determined.

In this study, statistically significant differences in BALF GM, serum GM, CRP, and PCT were found between the no IPA group and the IPA groups. The latter 2 serum biomarkers are unsuitable for diagnosing IPA because they can be easily confused with other factors such as bacterial infections and inflammation. Fair and moderate correlations of BALF GM and serum GM in the possible and probable/proven IPA groups indicated that these 2 markers might be able to independently assess the diagnostic performance.

In the no IPA group versus the probable/proven IPA group, when the cutoff value of serum GM and BALF GM was ≥0.5, the sensitivity and NPV of BALF GM were significantly higher than serum GM, but the specificity and PPV of BALF GM were slightly lower than serum GM. When the cutoff value was ≥1.0, both the

Table 3

Diagnostic efficiency of biomarkers in possible/probable/proven IPA groups.

Cutoff value	Positive cases	Negative cases	Sensitivity%	Specificity%	PPV%	NPV%	+LR	−LR	Youden index
BALF GM									
≥0.55	90	190	71.8	91.6	87.5	84.5	8.54	0.30	0.634
≥0.5	91	184	72.6	88.8	82.9	85.5	6.48	0.30	0.615
≥1.0	61	201	48.7	97.2	95.5	75.5	17.4	0.52	0.459
Serum GM									
≥0.5	28	200	22.2	96.6	95.5	67.4	6.52	0.80	0.189
≥1.0	14	206	11.1	99.4	99.2	64.9	18.5	0.89	0.106
Serum BDG									
≥10 μg/mL	12	114	9.4	95.5	55.5	63.2	0.95	2.08	0.049
≥20 μg/mL	8	201	6.0	97.2	46.7	62.9	0.93	3.36	0.032

+LR = positive likelihood ration, −LR = negative likelihood ration, BALF = bronchoalveolar lavage fluid, BDG = 1,3-β-D-glucan, GM = galactomannan, IPA = invasive pulmonary aspergillosis, NPV% = negative predictive value, ODI = optical density index, PCT = procalcitonin, PPV% = positive predictive value, Youden index = Sensitivity+ Specificity-1.

The number of possible/probable/proven IPA group was 126 and the number of no IPA group was 207.

The positive case represented the number of cases diagnosed as positive under the corresponding cutoff value.

The negative case represented the number of cases diagnosed as negative under the corresponding value.

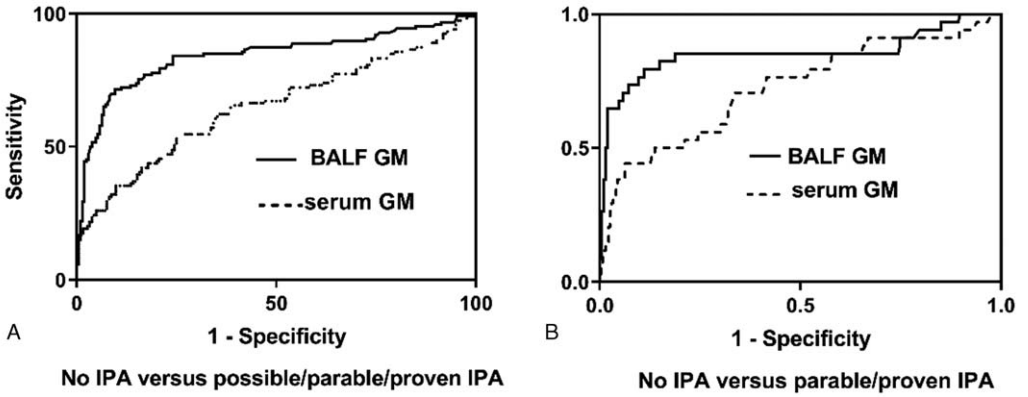

Figure 2. ROC curves of BALF GM and serum GM in the no IPA group versus the possible and probable/proven IPA groups. B shows the ROC curves of BALF GM and serum GM in the no IPA group versus the probable/proven IPA group. BALF = bronchoalveolar lavage fluid, GM = galactomannan, IPA = invasive pulmonary aspergillosis, ROC = receiver operating curve.

sensitivity and NPV of BALF GM declined, while the specificity and PPV were slightly elevated. Moreover, the sensitivity and NPV of BALF GM were superior to serum GM. When the −LR, +LR, and Youden index of BALF GM and serum GM were compared for different cutoff values, BALF GM was superior to serum GM and BDG in diagnosing non-neutropenic IPA patients. Compared with BALF GM, the low sensitivity of serum GM might lead to delayed diagnosis. Given its low sensitivity, serum BDG had a limited reference value in clinical diagnosis. The reason may be that BDG is mainly present in Candida and Aspergillus, but not in Mucorales, which is the main pathogenic fungus in patients with pulmonary diseases.[12]

In this study, the cutoff values of BALF GM set as 0.5 and 1.0 could not maximize the efficiency of sensitivity and specificity. ROC curve analysis showed that when the optimal cutoff value of BALF GM was 0.55, the sensitivity and specificity reached 71.8% and 91.6% by comparing the no IPA group versus the possible and probable/proven IPA groups. When the optimal cutoff value of BALF GM was set at 0.6, the sensitivity and specificity were 78.3% and 95.4%, respectively, by comparing the no IPA group with the probable/proven IPA group. The possible group mixed with no IPA patients may lower the optimal cutoff value, so the cutoff value set at 0.6 would be more reasonable.

Several reports[14–16] indicated that the optimal cutoff value of BALF GM in diagnosing IPA ranged from 0.5 to 1.1. He et al[14] chose 0.8 as the cutoff value of BALF GM, the reason why the cutoff value was higher than other reports may be that the participants in his study were COPD patients whose lung structure and immune system were damaged seriously and Aspergillus could parasite in pulmonary and respiratory tract more easily. Prattes et al[17] chose the appropriate cutoff value as 1.0, perhaps because some of the participants in the study were chosen from ICU, which included neutropenic patients. Kono et al[15] showed that BALF GM had better diagnostic efficacy than serum GM when the cutoff value was set as 0.5, but the number of cases in the study was small and the IPA group included allergic bronchopulmonary aspergillosis and chronic necrotizing pulmonary aspergillosis patients. Nguyen et al[16] chose 1.18 as the optimal cutoff value, which is the highest value of BALF GM reported in diagnosing IPA. Zhou et al[18] chose 0.7 as the diagnostic value of BALF GM for pulmonary aspergillosis, which was closest to the optimal cutoff value that we chose.

Serum GM and serum BDG are universally utilized as diagnostic biomarkers of IPA that are accepted in clinical practice due to their specificity and tolerable sensitivity in neutropenia patients because invasive Aspergillus could transmit

Table 4

Diagnostic efficiency of biomarkers in probable/proven IPA groups.

Cutoff value	Positive cases	Negative cases	Sensitivity%	Specificity%	PPV%	NPV%	+LR	−LR	Youden index
BALF GM									
≥0.6	27	197	78.3	95.4	68.0	95.8	17.02	0.22	0.736
≥0.5	27	190	78.3	91.8	56.7	96.7	9.54	0.23	0.690
≥1.0	25	205	73.9	99.2	85.0	94.5	92.37	0.26	0.731
Serum GM									
≥0.5	16	197	47.8	95.4	85.0	91.6	10.39	0.55	0.432
≥1.0	9	205	26.1	99.2	97.1	88.8	32.62	0.74	0.253
Serum BDG									
≥10 μg/ml	2	206	5.3	99.5	42.8	87.6	3.42	0.96	0.048
≥20 μg/ml	1	206	2.1	99.5	54.5	87.8	1.36	0.99	0.016

+LR = positive likelihood ration, −LR = negative likelihood ration, BALF = bronchoalveolar lavage fluid, BDG = 1,3-β-D-glucan, GM = galactomannan, IPA = invasive pulmonary aspergillosis, NPV% = negative predictive value, ODI = optical density index, PCT = procalcitonin, PPV% = positive predictive value, Youden index = Sensitivity+ Specificity-1.

The number of probable/proven IPA group was 34 and the number of no IPA group was 207.

The positive case represented the number of cases diagnosed as positive under the corresponding cutoff value.

The negative case represented the number of cases diagnosed as negative under the corresponding value.

to various organs of the body through blood circulation and the antigen is easily detected. However, patients with non-granulocyte deficiency have a normal immune function so their circulatory system can resist the invasion and the antigen can be eliminated, and hence serum GM often has low sensitivity.[19] BALF GM is more sensitive than serum GM in diagnosing fungal colonization of the lungs. Detection of colonization site of Aspergillus in lungs and respiratory tract could easily provide reliable evidence.

This study had several limitations. First, the number of probable and proven IPA cases was small due to the stringent inclusion criteria. Another limitation was the lack of a reliable definition of possible and probable IPA in non-neutropenic patients, which requires a revision of the definition used for patients with neutropenia. Lastly, the total volume, frequency, and recovery of saline in BALF are difficult to standardize when performing bronchoscopy and lavage, which might lead to inconsistent concentrations of BALF GM.

In summary, BALF GM is more valuable than serum GM and serum BDG for diagnosing IPA in non-neutropenic patients with underlying respiratory diseases. The ODI of BALF GM set to 0.6 showed optimal diagnostic efficiency. Serum BDG could complement BALF GM in improving the specificity of diagnosing non-neutropenic patients with IPA.

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