Original Article

Galactomannan detection in bronchoalveolar lavage fluid corrected by urea dilution for the diagnosis of invasive pulmonary aspergillosis among nonneutropenic patients

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Background: To investigate the diagnostic performance of galactomannan (GM) detection in bronchoalveolar lavage fluid (BALF) corrected by urea dilution and modification of the AspICU clinical algorithm.

Methods: GM detection in serum and BALF samples was performed in nonneutropenic patients on the day of clinically suspected invasive pulmonary aspergillosis (IPA) between January 2016 and June 2018, and urea was measured in the plasma and BALF. The BALF GM concentration was corrected by urea dilution, and receiver operating characteristic (ROC) curves were generated to determine the optimal cut-off value.

Results: A total of 184 patients who were suspected of IPA, were enrolled in this prospective study together with 30 patients with lung cancer as a control group. Seventy-eight patients were diagnosed with IPA, including 37 who were verified by pathology. The urea plasma-to-urea BALF ratio in the IPA group [4.18 (IQR, 3.52–4.91)] was greater than that in the non-IPA group [3.42 (IQR, 3.12–3.76), P<0.001]. The ROC curve showed that defining the cut-off value as 2.94 optical density index (ODI) for the corrected BALF GM resulted in a sensitivity and specificity of 85.91% and 94.07%, respectively, and was more accurate than the use of the uncorrected values (P<0.05).

Conclusions: The corrected BALF GM was valuable for diagnosing IPA in nonneutropenic patients. The modified AspICU clinical algorithm based on this measurement represents a reliable diagnostic instrument in clinical settings.

Keywords: Invasive pulmonary aspergillosis; galactomannan (GM); bronchoalveolar lavage fluid (BALF); urea; intensive care unit (ICU)

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Introduction

Invasive pulmonary aspergillosis (IPA) is a serious filamentous fungal respiratory infection that causes high mortality and morbidity. Previously, IPA was considered to appear mostly in patients with neutropenia, such as haematopoietic stem cell transplantation (HSCT) patients. Currently, a rising incidence has been observed in nonneutropenic hosts, most of whom are admitted to an intensive care unit (ICU) (1).

Accurately diagnosing of IPA in ICU patients is quite challenging, because the symptoms and signs are nonspecific, subtle and unable to be distinguished from those of bacterial pneumonia or even non-infectious diseases (2). Conventional culture methods are considered to have significant importance for identifying *Aspergillus* spp., although positive results are obtained for only approximately 50% of patients with IPA (3). The AspICU clinical algorithm (listed in Supplementary file 1) has been proposed to discriminate IPA in ICU patients due to its relatively high diagnostic utility (4). However, this clinical algorithm includes at least one positive *Aspergillus* culture in a respiratory tract specimen as an inclusion criterion, which leads to misdiagnosis or missed diagnosis of IPA patients with negative culture results.

Galactomannan (GM) detection in bronchoalveolar lavage fluid (BALF) is recommended in the practice guidelines for IPA diagnosis due to its higher sensitivity and specificity than its detection in serum. However, the optimal cut-off point for GM in BALF samples for the diagnosis of IPA has not been unified due to the use of varying dilutions. An ODI of 1.0 was recommended by the Infectious Diseases Society of America (IDSA) for clinical testing, whereas an ODI of 0.5 to 1.0 was applied in the new aspergillosis disease guideline of the ESCMID-ECMM-ERS (5,6). Urea is a diffusible substance that can easily be detected in all body compartments, including capillaries and the alveolar space (7), and the urea concentrations in both compartments are maintained at a constant ratio (8). The ratio of the plasma to BALF urea concentration was previously applied as an index of the epithelial lining fluid (ELF) dilution, and the real concentrations of antibiotics in the ELF were corrected by urea dilution in many pharmacokinetics studies (9,10).

These theories led to the hypothesis that urea dilution could be used as an index to correct the real GM concentration in the BALF. Therefore, the present study aimed to investigate the optimal cut-off point of a corrected BALF GM value and to determine whether the modified AspICU clinical algorithm had a better diagnostic performance when based upon the corrected concentration.

Methods

Research briefs

This multi-centre, prospective, observational study was performed in the Department of Critical Care Medicine in three teaching hospitals in Shanghai that accounted for a total of 155 ICU hospital beds. The annual volume of patients admitted to these three ICUs was approximately 3,200. The three participating units were Renji Hospital, Ruijin Hospital (both affiliated with Shanghai Jiao Tong University School of Medicine) and Minhang District Central Hospital (affiliated with Fudan University School of Medicine). This study was approved by the Ethics Committee of Shanghai Jiao Tong University (No. 2016-Clinical-Res-083), and informed written consent was obtained from either the patients or their next of kin.

Study population

Patients admitted to the ICU with suspected IPA (possible diagnosis) were enrolled in our study from January 2016 to June 2018. Patients were included if they met the following criteria: (I) peripheral blood neutrophil count $>0.5\times10^9$/L; (II) BALF and serum GM detection tests were performed; (III) other necessary differential diagnostic tests, such as chest computed tomography (CT) and lower respiratory tract specimen culture, were performed; (IV) urea was tested in both plasma and BALF samples; and (V) age between 18 and 80 years. Patients were excluded from the study if the following criteria were met: (I) they received antifungal treatment prior to the GM test; (II) the medical history was incomplete; (III) the final diagnosis was unclear; and (IV) they were moribund and not expected to survive for 48 h due to irreversible primary diseases. Thirty lung cancer patients without any infectious diseases were enrolled in our study as the control group.

Diagnostic criteria for IPA

According to the standard criteria (11), the cases were classified into three categories: proven cases, probable cases and possible cases. A biopsy was needed for the proven cases, and histopathological evidence must be acquired.

Microbiological evidence was demanded for the probable cases, and a host factor and a radiological criterion were also required. Microbiological proof was not demanded for the possible cases, although host factors and radiological criteria were needed. The colonization group was defined as patients with a positive *Aspergillus* culture from the sputum or BALF whose clinical symptoms were relieved without antifungal therapy.

BALF procedure and sample collection

A bronchoalveolar lavage (BAL) was performed, and samples (serum, plasma and BALF) were collected for measurement on the day when IPA was suspected. The guideline of the British Thoracic Society (BTS) was followed during the procedure (12). The segment selected for the BAL was guided by a recent chest CT scan, and the right middle lobe was selected when diffuse infiltrates were present. A 50 mL saline solution was instilled two times and aspirated gently. BALF was collected from the suction channel and stored in a sterile tube for microbiological culture, microscopic examination and further analysis, including GM and urea measurement.

GM and urea detection

Urea was detected in both of the plasma and BALF based on the following reaction principle: urea is hydrolysed by urease to generate ammonium carbonate, and then 2-ketoglutarate reacts with ammonium to produce L-glutamate in the presence of GLDH and NADH. In this reaction, 2 mol of NADH are oxidized to NAD^+ when 1 mol of urea is hydrolysed. Thus, a decrease in the NADH concentration reflects the urea concentration, which is measured by spectrophotometric analysis.

The urea nitrogen assay was carried out on the Hitachi 7600 automatic biochemical analyser according to the manufacturer's handbook. GM antigen was measured in both the sera and BALF with a double-sandwich enzyme-linked immunosorbent assay (ELISA) using the Platelia *Aspergillus* enzyme immunoassay (Bio-Rad, USA).

Each 300 μL BALF sample was used to determine the GM and urea levels. Positive and negative controls were included in each assay. The corrected GM concentration in the BALF sample was calculated as:

(Corrected BALF GM) = (BALF GM) × (Urea plasma)/(Urea BALF)

Data collection and clinical assessment

The demographic information and clinical characteristics of each patient enrolled in our study were obtained from the hospital's electronic medical records, which included age, gender, comorbidities, body mass index (BMI), the Acute Physiology and Chronic Health Evaluation II (APACHE II) score, and the Sequential Organ Failure Assessment (SOFA) score. The chest CT scan, BALF culture and histological evidence were also recorded, as were the GM and urea concentrations.

Statistical analysis

Data from the study were analysed with SPSS 22.0 (IBM for Windows). Categorical variables were compared with the Chi-square test, and the results were presented as percentages (n). Continuous variables were reported as the means when the data were normally disturbed or as medians with 25 to 75 interquartile ranges for skewed data. Differences among means were analysed with the Kruskal-Wallis test to compare three or more groups.

According to the guidelines of IDSA and ESCMID, the proven and probable cases of IPA should be treated early to improve the prognosis while the possible cases could remain under observation (5,6). Therefore, the proven and probable cases of IPA were defined as the gold standard in our study and the receiver operating characteristic curve (ROC) analysis was performed to determine the optimal cut-off value. A two-sided $P<0.05$ was considered statistically significant. The ROC was generated using MedCalc 15.0, and the other figures were drawn with GraphPad Prism 7.0.

Results

Patient characteristics

GM was detected in BALF and serum samples from 225 patients with suspected IPA during the study period, 41 (18.2%) of whom were excluded due to an unclear final diagnosis, moribund state and other reasons. Finally, 184 patients were enrolled in our study. Among them, 78 patients (42.4%) were diagnosed with IPA, and the other 106 patients were not diagnosed. IPA was proven in 37 patients from a lung biopsy, and 28 patients were diagnosed with *Aspergillus* colonization in the non-IPA group (*Figure 1*).

The clinical characteristics of the IPA and non-IPA groups are listed in *Table 1*. The age range, gender, BMI,

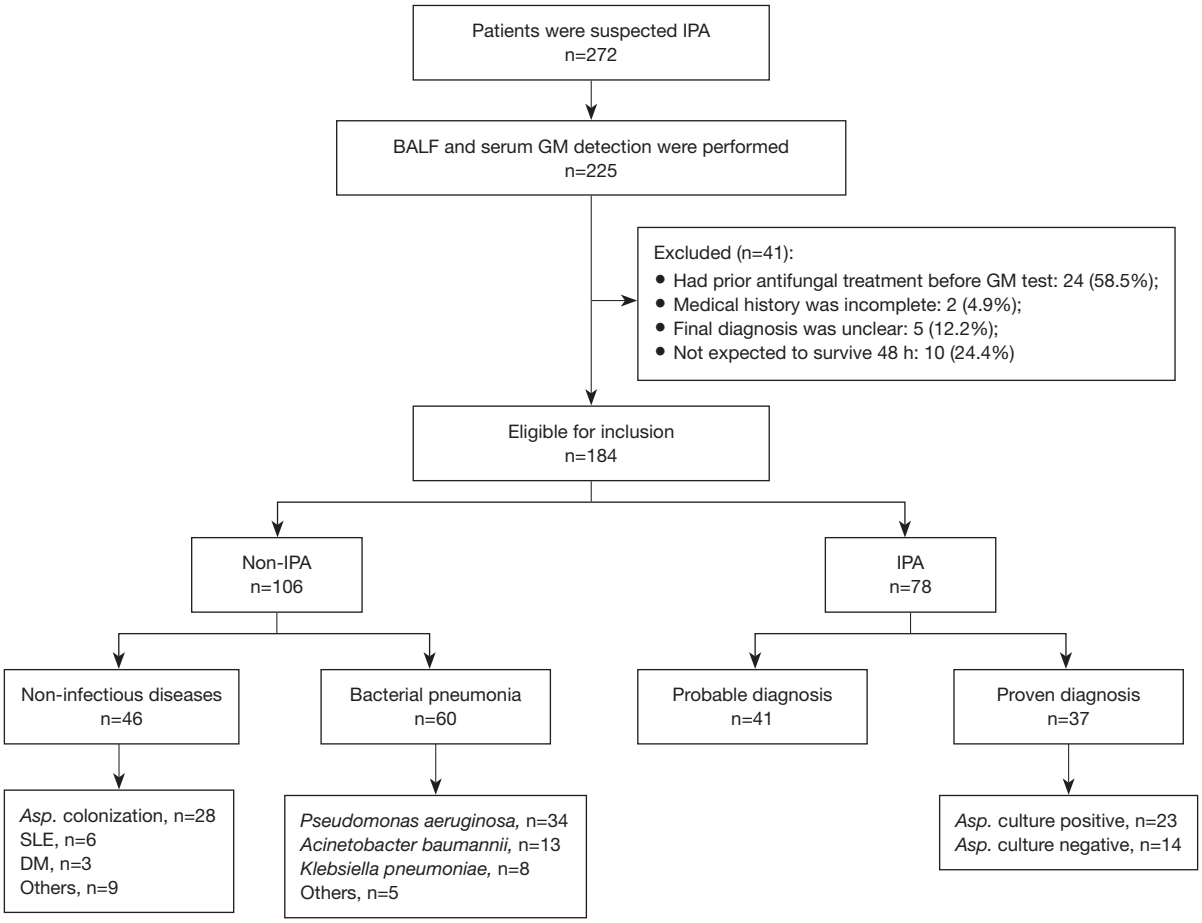

Figure 1 Flow chart of the study. IPA, invasive pulmonary aspergillosis; BALF, bronchoalveolar lavage fluid; GM, galactomannan; SLE, systemic lupus erythematosus; DM, dermatomyositis; *Asp.*, *Aspergillus*.

and Charlson Comorbidity Index, APACHE II and SOFA scores did not significantly differ between the two groups (P>0.05). The proportions of patients with diabetes mellitus, interstitial lung disease, solid organ transplantation and systemic lupus erythematosus were also similar between the two groups (P>0.05), whereas more patients with chronic obstructive pulmonary disease (COPD) were detected in the IPA group (39.7% *vs.* 21.7%, P<0.01). The value of PO_2/FiO_2 in the IPA group [126 (IQR, 77–215)] was much lower than that in the non-IPA group [158 (IQR, 98–262)] (P<0.05). A higher percentage of patients who received immunosuppressive treatment (26.9% *vs.* 10.4%) and corticosteroid therapy (35.9% *vs.* 12.3%) was found in the IPA group than in the non-IPA group (P<0.05). The 30-day mortality rate was significantly higher in the IPA group and was almost 4-fold that in the non-IPA group (24.4% *vs.* 6.6%, P=0.001).

GM and (1→3)-β-D-glucan detection

The value of GM in serum samples was not found to be significantly different between the IPA proven group [1.02 ODI (IQR, 0.54–1.38)] and the IPA probable diagnosis group [1.04 ODI (IQR, 0.65–1.38)] (P>0.05). The serum GM concentration was significantly higher in the IPA cohort than in all of the non-IPA groups (P<0.001), (*Figure 2A*). The median uncorrected BALF GM value in the IPA proven cohort was 1.04 ODI (IQR, 0.86–1.45), which was higher than the values in all non-IPA groups (P<0.001) (*Figure 2B*).

As a conventional biomarker for IPA discrimination,

Table 1 Demographics and clinical characteristics of IPA and Non-IPA groups

Characteristics	IPA (n=78)	Non-IPA (n=106)	P value
Age, median [IQR] (years)	58 [36–71]	56 [38–70]	0.872[a]
Gender (male), n (%)	31 (39.7)	37 (34.9)	0.502[b]
BMI, mean ± SD (kg/m^2)	22.7±3.2	23.5±4.1	0.154[c]
Comorbidity, n (%)			
Diabetes mellitus	12 (15.4)	18 (16.9)	0.084[b]
Chronic obstructive pulmonary disease	31 (39.7)	23 (21.7)	0.008[b]
Interstitial lung disease	8 (10.3)	9 (8.5)	0.683[b]
Solid organ transplantation	8 (10.3)	7 (6.6)	0.371[b]
Systemic lupus erythematosus	16 (20.5)	22 (20.8)	0.968[b]
Charlson Comorbidity Index score, mean ± SD	3.1±1.1	3.2±1.3	0.583[c]
APACHE II score, median [IQR]	19 [12–25]	18 [11–23]	0.773[a]
SOFA score, mean ± SD	4.3±1.4	4.5±1.7	0.397[c]
PO_2/FiO_2, median [IQR]	126 [77–215]	158 [94–262]	0.025[a]
Immunosuppressive therapy, n (%)	21 (26.9)	11 (10.4)	0.003[b]
Corticosteroid therapy, n (%)	28 (35.9)	13 (12.3)	0.001[b]
30-day mortality, n (%)	19 (24.4)	7 (6.6)	0.001[b]

[a], Mann-Whitney U test; [b], Chi-square test; [c], Student's *t*-test. IPA, invasive pulmonary aspergillosis; BMI, body mass index; APACHE II, acute physiology and chronic health evaluation II; SOFA, sequential organ failure assessment; IQR, interquartile range; SD, standard deviation.

(1→3)-β-D-glucan (BDG) was also measured in all 184 included patients. The BDG values were not significantly different among the lung cancer control group [40.51 pg/mL (IQR, 27.33–74.25 pg/mL)], bacterial pneumonia group [42.51 pg/mL (IQR, 23.07–122.56 pg/mL)] and *Aspergillus* colonization group [52.01 pg/mL (IQR, 32.57–1.48.2)] (P>0.05). The BDG levels in the IPA group were higher than those in the non-IPA group (P<0.001) (*Figure 2C*).

GM and BDG were also detected in thirty serum samples from health volunteers. It indicated that the concentrations of GM [0.21 ODI (IQR, 0.15–0.23)] and BDG [41.29 pg/mL (IQR, 25.39–70.18 pg/mL)] were lower than those in IPA groups respectively (P<0.001).

Corrected BALF GM value and urea dilution

Figure 3A displays the urea plasma-to-urea BALF ratios. The median value of this ratio in the IPA group [4.18 (IQR, 3.52–4.91)] was greater than that in the non-IPA group [3.42 (IQR, 3.12–3.76)] (P<0.001). The corrected BALF GM values were not significantly different between the IPA proven diagnosis group and the IPA probable diagnosis group. However, the values in these two groups were approximately two times higher than that in the non-IPA group (P<0.001) (*Figure 3B*, *Table 2*).

The GM values in the 37 patients with a lung biopsy (IPA proven) were also compared (*Figure 3C*). The corrected BALF GM values [4.28 ODI (IQR, 3.30–6.12)] were considerably higher than the uncorrected values [1.04 ODI (IQR, 0.78–1.57)] (P<0.001). No difference was found between the GM values in the serum samples [1.03 ODI (IQR, 0.64–1.35)] and the uncorrected BALF samples (P>0.05).

Correlation of GM among different samples

A scatter plot was applied to determine whether correlations existed between the GM values in the serum and BALF samples. No significant correlation was identified between GM in the uncorrected BALF and serum samples (R^2

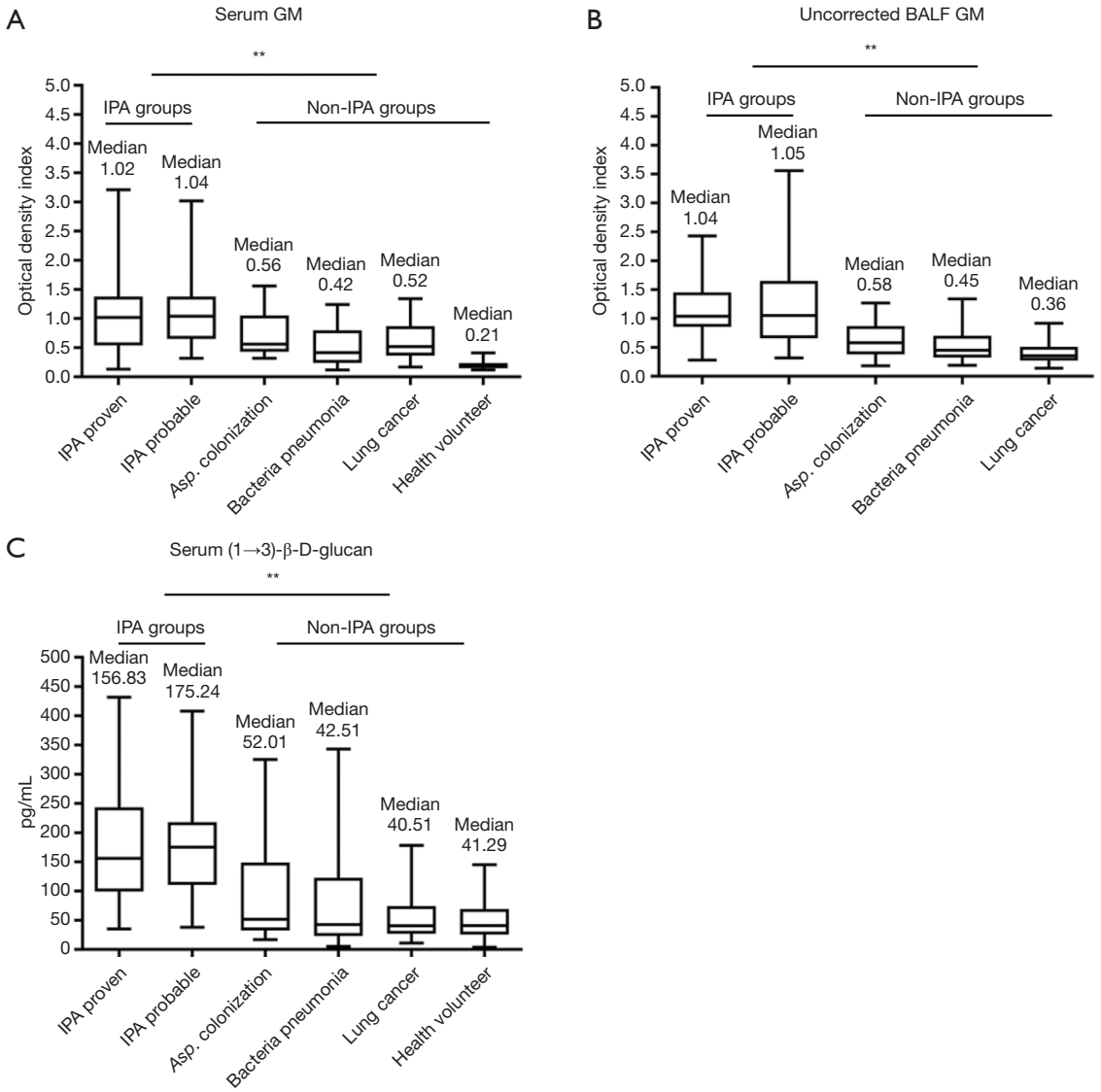

Figure 2 Box plot comparing GM with (1→3)-β-D-glucan among different groups. (A) Comparison of serum GM values in 6 different groups; (B) comparison of uncorrected GM values in 5 different groups; (C) comparison of serum (1→3)-β-D-glucan values in 6 different groups. **, P<0.001. BALF, bronchoalveolar lavage fluid; GM, galactomannan; IPA, invasive pulmonary aspergillosis.

=0.302, P>0.05). However, when the GM values in the BALF sample were corrected by urea dilution, a significant correlation was detected (R^2 =0.642, Y =1.89X +2.486, P<0.001) (*Figure 4*).

Diagnostic efficiency of GM for diagnosing IPA at different cut-off values

Because no consensus exists on the optimal cut-off value of GM in serum and BALF samples in the practice guidelines, meta-analysis or randomized controlled trials (5,6,13-15), the diagnostic efficiency of GM for IPA at different cut-off values was verified for our 184 enrolled patients. Lower accuracy (61.7%) was found in serum samples when the optimal cut-off value was defined as ≥0.5 ODI and ≥1.0 ODI (71.4%). In the uncorrected BALF samples, when the cut-off value was defined as a GM concentration ≥0.8 ODI, the accuracy was as high as 81.6%, with a better positive predictive value (PPV) (78%) and negative predictive value (NPV) (84%) than those of the other cut-off values (*Table 3*).

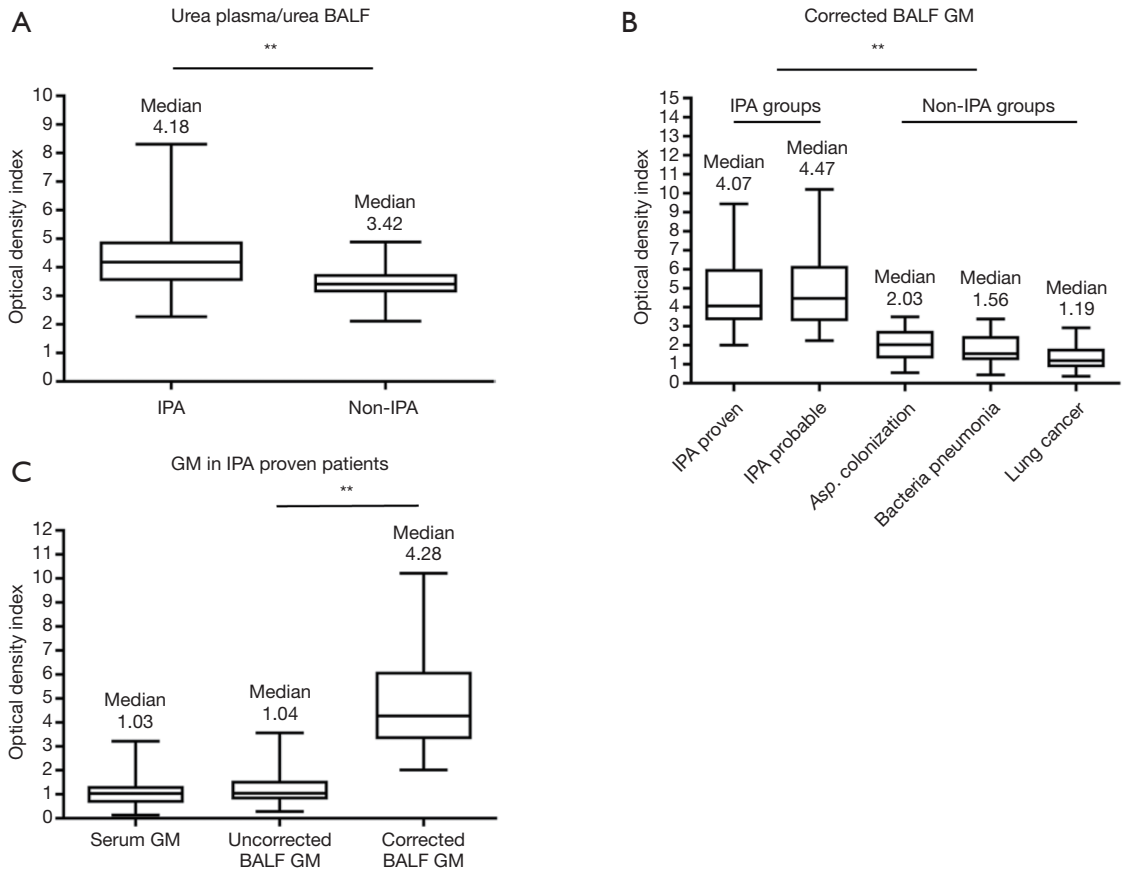

Figure 3 Box plot comparing the urea dilution with GM among different groups. (A) Urea dilution between the IPA and non-IPA groups; (B) corrected GM values in 5 different groups; (C) the GM value in the IPA-proven patients. **, P<0.001. BALF, bronchoalveolar lavage fluid; GM, galactomannan; IPA, invasive pulmonary aspergillosis.

Table 2 Corrected BALF GM median values and interquartile range between different groups

Groups	p25	Median	p75	Min	Max	P value
IPA diagnosed (n=78)	3.31	4.28	6.12	2.01	10.21	<0.001[a]
IPA proven diagnosis (n=37)	3.32	4.07	6.03	2.01	9.45	<0.001[a]
IPA probable diagnosis (n=41)	3.27	4.47	6.18	2.25	10.21	<0.001[a]
Asp. colonization (n=28)	1.31	2.03	2.76	0.56	3.49	<0.05[b]
Bacteria pneumonia (n=60)	1.22	1.55	2.48	0.44	3.39	>0.05[c]
Lung cancer (n=30)	0.84	1.19	1.82	0.36	2.92	>0.05

[a], compare with three non-IPA groups; [b], compare with the other non-IPA groups; [c], compare with lung cancer group. p25, 25th percentile; p75, 75th percentile; IPA, invasive pulmonary aspergillosis; BALF, bronchoalveolar lavage fluid; GM, galactomannan.

Diagnostic efficiency of different variables for diagnosing IPA

The ROC curves are shown in *Figure 5*. A cut-off value of 2.94 ODI for the corrected BALF GM resulted in a sensitivity of 85.91% and specificity of 94.07%, which corresponded to an area under the curve (AUC) of 0.961. A cut-off value of 0.86 ODI for the uncorrected BALF GM led to a low sensitivity of 74.36% and specificity of 88.14%.

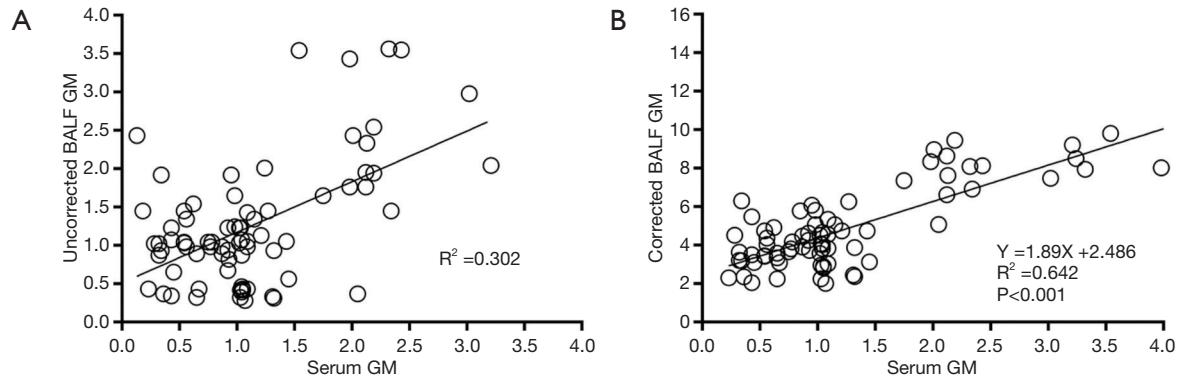

Figure 4 Correlations of GM in different samples among IPA patients. (A) Correlation of the serum GM and uncorrected BALF GM values; (B) correlation of the serum GM and corrected BALF GM values. BALF, bronchoalveolar lavage fluid; GM, galactomannan; IPA, invasive pulmonary aspergillosis.

Table 3 Diagnostic efficiency of galactomannan for diagnosing IPA at different cutoff values

Variables	TP	FP	TN	FN	Se (%)	Sp (%)	PPV (%)	NPV (%)	Acc (%)
Serum GM ≥0.5	65	62	56	13	83.3	47.5	51	81	61.7
Serum GM ≥1.0	43	21	97	35	55.1	82.2	67	73	71.4
Uncorrected BALF GM ≥0.5	62	47	71	16	79.5	60.2	57	82	67.9
Uncorrected BALF GM ≥0.8	59	17	101	19	75.6	85.6	78	84	81.6
Uncorrected BALF GM ≥1.0	45	6	112	33	57.7	94.9	88	77	80.1
Uncorrected BALF GM ≥1.5	20	0	118	58	25.6	100	100	67	70.4
Uncorrected BALF GM ≥2.0	11	0	118	67	14.1	100	100	64	65.8
Uncorrected BALF GM ≥3.0	4	0	118	74	5.1	100	100	61	62.2

TP, number of true positives; FP, number of false positives; FN, number of false negatives; TN, number of true negatives; Se, sensitivity; Sp, specificity; PPV, positive predictive value; NPV, negative predictive value; Acc, rate of accuracy of the risk score model; IPA, invasive pulmonary aspergillosis; BALF, bronchoalveolar lavage fluid.

The AUC was 0.832, which was lower than that of the corrected BALF GM value (P<0.05). The optimal cut-off value for serum GM was 0.87 ODI, which led to an AUC of 0.746 and both lower sensitivity (64.11%) and specificity (80.51%) (*Table 4*).

Diagnostic efficiency of the modified AspICU clinical algorithm

Thirty-seven patients diagnosed with IPA by lung biopsy were enrolled in our study. The *Aspergillus* culture results from sputum or BALF samples were negative in 14 (37.8%) patients, who would be misdiagnosed by application of the AspICU clinical algorithm. According to the optimal cut-off value for the corrected BALF GM (2.94 ODI), one criterion in the AspICU clinical algorithm ("*Aspergillus*-positive lower respiratory tract specimen culture") was changed to "*Aspergillus*-positive lower respiratory tract specimen culture or a corrected BALF GM value ≥2.94 ODI". High sensitivity and specificity (both 100%) were revealed with a satisfactory AUC (1.00), which was verified in the 37 patients with a proven IPA diagnosis. For the 106 non-IPA patients in our study (including 28 patients with *Aspergillus* colonization), the NPV of modified AspICU clinical algorithm was 87.4% while the rate of accuracy was 86.3% which were still satisfactory.

Discussion

Accurately diagnosing IPA among ICU patients is challenging, although molecular and conventional approaches have been improved over the past two decades. Recently, BALF GM detection has been strongly recommended in the practice guidelines of the IDSA due to its high sensitivity, especially for neutropenic patients (5). However, the optimal cut-off value is unidentified due to differences in BALF dilutions, patient selection and BAL procedures (16). Moreover, evidence for application of BALF GM in nonneutropenic patients is limited. The ratio of the urea plasma-to-urea BALF concentrations was previously applied as an index of ELF dilution. Thus, we hypothesized that the BALF GM value corrected by urea dilution might serve as an adjunct for diagnosis of IPA among nonneutropenic patients.

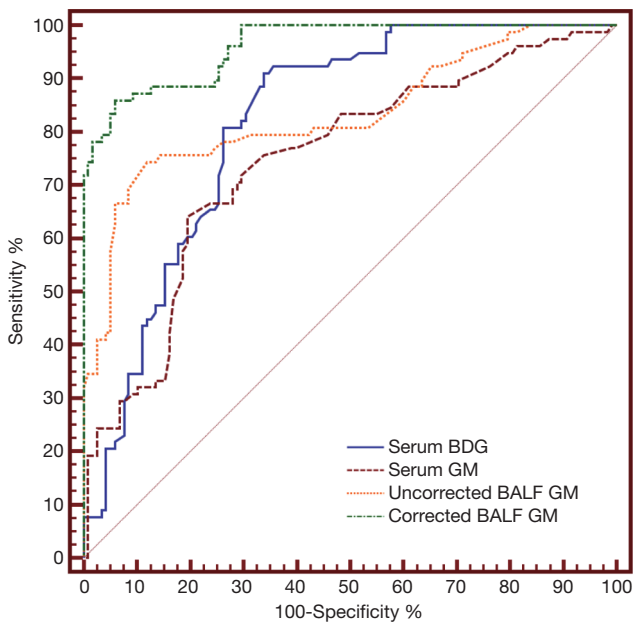

Figure 5 Receiver operating characteristic curves for the serum BDG, serum GM, uncorrected BALF GM and corrected BALF GM for the diagnosis of IPA. BALF, bronchoalveolar lavage fluid; BDG, (1→3)-β-D-glucan; GM, galactomannan.

As indicated in our study, the proportion of patients with COPD was higher in the IPA group than in the non-IPA group (39.7% *vs.* 21.7%, P<0.01). Possible explanations could be alterations of the alveolar structure, decreased mucociliary clearance, mucosal lesions and an impaired immunologic response, which might lead to frequent hospital stays, invasive procedures, such as intubation, and a long course of antibiotics treatment (17). The other two risk factors were immunosuppressive use (26.9% *vs.* 10.4%, P<0.01) and corticosteroid therapy (35.9% *vs.* 12.3%, P<0.01) due to the accompanying humoral and cellular immunity functional disorders (18). Not unexpectedly, a dismal prognosis of IPA was also found in our study, which led to a long-term search for better therapeutic strategies.

Generally, the optimal cut-off value for GM in sera is set at 0.5 ODI, but many factors can lead to a false-positive or false-negative result (1). As shown in our study, the serum GM value in the lung cancer group was 0.52 ODI (IQR, 0.36–0.87), which could be interpreted as albumin treatment in cancer patients. We also found that the serum GM value was greater than 0.5 ODI in 26 (43.3%) patients with bacterial pneumonia due to the prescription of piperacillin-tazobactam for *Pseudomonas aeruginosa* treatment. Thus, the PPV was only 51% and the NPV was 81% when the cut-off value was defined as 0.5 ODI for serum GM in our study.

Although GM in BALF samples was reported to have better sensitivity than that in sera (19), the optimal cut-off value varied significantly. We measured the diagnostic efficiency of different cut-off values of uncorrected BALF GM (0.5, 0.8 1.0, 1.5, 2.0 and 3.0) reported by previous clinical trials. An uncorrected BALF GM value of 0.8 ODI might be the best choice with 81.6% diagnostic accuracy,

Table 4 Diagnostic efficiency of different variables for diagnosing IPA

Variables	Cut-off	Sensitivity	Specificity	AUC	95% CI	Youden Index
Serum BDG	68.23	91.03	66.12	0.818	0.757–0.871	0.571
Serum GM	0.87	64.11	80.51	0.746	0.671–0.805	0.446
Uncorrected BALF GM	0.86	74.36	88.14	0.832	0.773–0.882	0.625
Corrected BALF GM	2.94	85.91	94.07	0.961	0.924–0.983	0.801

AUC, area under the curve; BALF, bronchoalveolar lavage fluid; BDG, (1→3)-β-D-glucan; GM, galactomannan; IPA, invasive pulmonary aspergillosis.

although the sensitivity (75.6%) and PPV (78%) were still unsatisfactory.

To overcome the problem, BALF was corrected by urea dilution in our study. The urea plasma-to-urea BALF ratios in the IPA group was higher than that in the non-IPA group. The value of PO_2/FiO_2 in the IPA group was much lower than that in the non-IPA group which indicated that more patients were found under oxygen deficit circumstances in the IPA group and resulted in poor tolerance to BAL. More coughing and shortness of breath occurred during the procedure, and less urea was obtained from the BALF samples in the IPA group.

IPA is primarily caused by inhalation of fungal spores, and BALF samples are taken from the site of infection. Thus, the GM concentration in the BALF sample can be reasonably expected to be higher than that in the serum (7,20,21). The BALF GM level was corrected by urea dilution in our study, and we found that the corrected BALF GM values were more than quadruple the uncorrected values in the IPA-proven patients.

Non-corrected BALF GM values could be altered due to the different volumes obtained by BAL, and that the urea dilution method could standardise these figures. The ROC curve analysis verified that a 2.94 ODI for the corrected BALF GM concentration was an optimal cut-off value, with sensitivity as high as 85.91% and 94.07% specificity. The AUC was 0.961, which was a satisfactory index. Compared to that of the uncorrected BALF GM values, the corrected values showed much higher sensitivity and specificity, which might decrease the rate of misdiagnosis or missed diagnosis.

A lack of correlation between the BALF and serum GM values was revealed among the IPA patients in our study. This lack might be interpreted as meaning that the BALF dilution was diverse and uncontrollable, which concurred with the studies of Duettmann *et al.* and Jackson *et al.* (21,22). Therefore, we attempted to minimize the confounding factors and corrected the BALF GM value by urea dilution, which showed a statistically significant correlation between the corrected BALF GM value and the serum GM value (R^2 =0.642, P<0.001).

The isolation rate of *Aspergillus* spp. was only 50% in the respiratory samples from the IPA patients, which was far from satisfactory (3). In addition, positive culture results always raise a clinical dilemma, because ICU doctors must discriminate colonization from real infection. Moreover, a negative culture result cannot rule out infectious diseases. Based on the theories proposed above and the optimal corrected BALF GM value (2.94 ODI), one criterion in the AspICU clinical algorithm ("*Aspergillus*-positive lower respiratory tract specimen culture") was changed to "*Aspergillus*-positive lower respiratory tract specimen culture or a corrected BALF GM value ≥2.94 ODI". The modified algorithm was verified using the 37 patients with an IPA diagnosis based on histopathological evidence, and high sensitivity and specificity (both 100%) were revealed with a satisfactory AUC (1.00). These findings indicated that the modified AspICU clinical algorithm might be applied as a reliable diagnostic instrument in clinical settings in the future.

To the best of our knowledge, this study is the first prospective study to correct the BALF GM value by urea dilution and modify the AspICU clinical algorithm accordingly. However, some limitations still exist. First, the number of patients who underwent biopsy was limited (n=37). Although histopathological evidence was recommended as a gold standard in the IDSA guideline (5), biopsy was uncommon in ICU admission patients because of the high incidence of complications, such as bleeding or pneumothorax. Second, we were unable to recruit health volunteers as a control group because BAL in these samples was disapproved by the Ethics Committee. Only blood samples from health volunteers were obtained from our study to determine the baseline value of BDG and GM. Lastly, some cases of false-positive BALF GM were unavoidably enrolled in our study, which led to unnecessary antifungal treatment. The incidence was reported to be as high as 20% (23,24). Further studies investigating new ideal marker verification and methods to discriminate false-positive results are needed in the future.

In summary, we demonstrated the potential of corrected BALF GM values by urea dilution as a biomarker for the diagnosis of IPA with high accuracy. The modified AspICU clinical algorithm based on the corrected BALF GM value should become a reliable diagnostic instrument in clinical settings.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

Ethical Statement: This study was approved by the Ethics Committee of Shanghai Jiao Tong University (No. 2016-Clinical-Res-083), and informed written consent was obtained from either the patients or their next of kin.

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Supplementary

Supplementary file 1

AspICU criteria

Putative invasive pulmonary aspergillosis (all four criteria must be met)

(I) *Aspergillus*-positive lower respiratory tract specimen culture;

(II) Compatible signs and symptoms (one of the following):

- Fever refractory to at least 3 days of appropriate antibiotic therapy;
- Recrudescent fever after a period of defervescence of at least 48 h while still on antibiotics and without other apparent cause;
- Pleuritic chest pain;
- Pleuritic rub;
- Dyspnoea;
- Haemoptysis;
- Worsening respiratory insufficiency despite appropriate antibiotic therapy and ventilator support.

(III) Abnormal medical imaging by portable chest X-ray or CT scan of the lungs;

(IV) Either IVa or IVb:

(IVa) Host risk factors (one of the following conditions);

- Neutropenia (absolute neutrophil count 500/mm^3) preceding or at the time of ICU admission;
- Underlying haematological or oncological malignancy treated with cytotoxic agents;
- Glucocorticoid treatment (prednisone equivalent 20 mg/day);
- Congenital or acquired immunodeficiency.

(IVb) Semi-quantitative *Aspergillus*-positive culture of BAL fluid without bacterial growth together with a positive cytological smear showing branching hyphae.