Diagnosis values of IL-6 and IL-8 levels in serum and bronchoalveolar lavage fluid for invasive pulmonary aspergillosis in chronic obstructive pulmonary disease

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ABSTRACT

Among immunologically normal hosts, patients with chronic obstructive pulmonary disease (COPD) are considered to be at high risk of invasive pulmonary aspergillosis (IPA), and early diagnosis and treatment are the key to improving the prognosis of patients. Here we aimed to evaluate whether interleukin (IL)-6 and IL-8 might be used in the detection and diagnosis of IPA in patients with COPD. We prospectively collected 106 patients with COPD and divided them into non-IPA (n=74), probable/possible IPA (n=26) and proven IPA (n=6). Platelia Aspergillus kit was used to detect galactomannan in bronchoalveolar lavage fluid (BALF), and serum and ELISA kit was used to detect IL-6 and IL-8 levels. Diagnostic efficiency of IL-6, IL-8 and galactomannan in serum and BALF was evaluated by receiver operating characteristic curve. Compared with the non-IPA group, the proven/probable IPA group showed significantly elevated levels of IL-6 and IL-8 in both serum and BALF, which were positively correlated with galactomannan levels. The sensitivity and specificity of IL-6 for diagnosing IPA were 74.32% and 81.25% (cut-off at 92.82 pg/mL, area under the curve (AUC)=0.8366) in serum and 68.92% and 71.88% (cut-off at 229.4 pg/mL, AUC=0.7694) in BALF. The sensitivity and specificity of IL-8 for diagnosing IPA were 83.78% and 81.25% (cut-off at 93.46 pg/mL, AUC=0.8756) in serum and 85.14% and 75.00% (cut-off at 325.4 pg/mL, AUC=0.8252) in BALF. The elevated levels of IL-6 and IL-8 in patients with IPA with COPD could be used as auxiliary indicators to diagnose IPA in addition to galactomannan.

Significance of this study

What is already known about this subject?

- Among immunologically normal hosts, patients with chronic obstructive pulmonary disease (COPD) are considered to be at high risk of invasive pulmonary aspergillosis (IPA).
- IPA is an infectious disease caused by invasion of fungal hyphae to bronchial walls and associated small arteries.
- Inflammatory responses are involved in IPA.

What are the new findings?

- Compared with the non-IPA group, proven/probable IPA group showed significantly elevated levels of IL-6 and IL-8 in both serum and bronchoalveolar lavage fluid (BALF).
- The sensitivity and specificity of IL-8 for diagnosing IPA were 83.78% and 81.25% (cut-off at 93.46 pg/mL, AUC=0.8756) in serum and 85.14% and 75.00% (cut-off at 325.4 pg/mL, AUC=0.8252) in BALF.
- IL-6 and IL-8 levels in BALF are positivelycorrelated with galactomannan in COPD patients with IPA.

How might these results change the focus of research or clinical practice?

- The elevated levels of IL-6 and IL-8 in patients with IPA with COPD could be used as auxiliary indicators to diagnose IPA in addition to galactomannan.

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INTRODUCTION

Invasive pulmonary aspergillosis (IPA) is an infectious disease caused by invasion of fungal hyphae to bronchial walls and associated small arteries. The survey shows that the incidence of IPA is about 2%~26%, and the case fatality rate is about 74%~92%.[1] Due to long-term use of broad-spectrum antibacterial drugs, glucocorticoids and general low immunity of the body, patients with chronic obstructive pulmonary disease (COPD) are at high risk of IPA. According to reports in the literature, more than 10% of patients with COPD have IPA, and the mortality rate of COPD combined with IPA is 72%~95%.[2] In the clinic, for patients with COPD with high suspicion of IPA, a variety of laboratory examination methods should be used as much as possible to assist the diagnosis.[3] More research is still needed to focus on the early diagnosis and treatment of COPD combined with IPA to guide clinical

intervention in a timely manner, thereby reducing mortality and improving prognosis.[4]

Galactomannan is released by germinated spores/conidia and growing hyphae[5] and could be detected in urine, bronchoalveolar lavage fluid (BALF), peripheral blood, pleural fluid or cerebrospinal fluid of patients with IPA.[6] Therefore, evaluating galactomannan levels in BALF has become the standard for diagnosing IPA.[5] In addition, serum galactomannan is also considered to be an outstanding indicator for prognosis prediction and treatment stratification of IPA.[7] Interleukin (IL)-6 is a sensitive indicator for the early diagnosis of acute infection, which has important reference significance for formulating treatment plans and improving prognosis.[8–10] Increased IL-8 is seen in infection, trauma and certain autoimmune diseases.[11] It has also been discovered that IL-8 is the main inflammatory factor in the process of ischemia, reperfusion injury and systemic inflammatory response.[12] Therefore, the detection of IL-8 level could be used for the diagnosis, differential diagnosis and prognosis of inflammatory diseases.[13] Study has shown that IL-6 and IL-8 have clinical value in the diagnosis of IPA in patients with blood diseases.[14] In view of these reports, we plan to study whether IL-6 and IL-8 could be used in the detection and diagnosis of IPA in patients with COPD.

METHODS

Subjects

A total of 106 elderly patients with COPD who were hospitalized from June 2018 to December 2019 were collected. Informed consent was obtained from the research subjects. Exclusion criteria were (1) patients with malignant tumors or organ transplantation, (2) patients with chronic renal insufficiency or diabetes, (3) patients with long-term immunosuppressant, (4) critically ill patients with unstable vital signs, (5) patients with severe heart disease, and (6) patients who could not tolerate bronchoscopy, according to the standards of the European Organization for Research and Treatment of Cancer/Mycoses Research Group.[15] The research subjects are divided into proven, probable or possible IPA or non-IPA.

Histopathological evidence of branching filamentous fungi invasion was used as proven IPA. Probable IPA included (1) finding *Aspergillus* or cytology fungus evidence from lower respiratory tract (LRT) or BALF together with a major criterion (consolidation area with cavity, air-crescent sign and halo sign) or two or more minor signs (new infiltrate, pleural rub and LRT infection); (2) patients with severe COPD who had exacerbation resistant to corticosteroids, pulmonary infiltrates which showed no antibiotic response, and positive culture for *Aspergillus* from the LRT or BALF. Possible IPA was composed of patients with severe COPD treated with corticosteroids, suffered treatment-resistant exacerbation, had infiltrates as shown by chest imaging, and had no positive *Aspergillus* culture. Galactomannan detection was excluded from diagnostic criterion to avoid incorporation bias. IPA group was composed of patients with probable/possible and proven IPA. The non-IPA group was composed of patients with no IPA evidence.

Bronchoscopy and BALF collection

Fiberoptic bronchoscopy was performed; vital signs were monitored, and maintained peripheral oxygenation of ≥90% was maintained. Based on the results of high-resolution CT examination of the chest within the past week, targeted bronchoalveolar lavage (BAL) to areas within the lungs. Within a week of BAL sampling, venous blood was collected for galactomannan assay. Except for galactomannan assay, BALF specimens were also employed for fungal and bacterial culture.

Galactomannan measurement in BALF and serum

The Platelia Aspergillus kit (BioRad Laboratories, Redmond, Washington, USA) was used to detect galactomannan in the serum and BALF.[16] Serum and BALF samples with an optical density of 0.5 are considered positive.

Detection of IL-6 and IL-8 in BALF and serum

ELISA was used to detect the levels of IL-6 and IL-8 in serum and BALF following the standard methods. Human IL-6 ELISA kit (ab178013) and human IL-8 ELISA kit (ab214030) were purchased from Abcam (Shanghai, China).

Statistical processing

Statistical analysis was performed by SPSS V.19.0 software and data were shown as mean±SD The comparison between two groups was applied by Mann-Whitney test. The count data are tested by Fisher's exact probability method or χ^2 test. The diagnostic efficiency of galactomannan, IL-8 and IL-6 detection in BALF and serum is expressed by sensitivity and specificity, and the reliability and best cut-off value were analyzed by using the receiver operating characteristic (ROC) curve. A p value of <0.05 was considered statistically significant.

RESULTS

Clinical data and serum IL-6, IL-8, and galactomannan levels between IPA and non-IPA groups

We prospectively collected 106 patients with COPD admitted to the respiratory department and divided them into proven IPA (n=6), probable/possible IPA (n=26), and non-IPA (n=74), respectively. Proven and probable/possible patients with IPA were classified as the IPA group; patients in whom there was no evidence of IPA were classified as the non-IPA group. Baseline clinical characteristics of patients are shown in table 1. No significant difference was observed between the gender, age, and lung function of the two groups ($p>0.05$). The number of systemic corticosteroids used during hospitalization and mechanical ventilation, admission to ICU were statistically significant ($p<0.05$). We further examined the levels of IL-6, IL-8 and galactomannan in serum of patients with COPD with IPA compared with non-IPA. We found that the serum levels of IL-6 (figure 1A), IL-8 (figure 1B) and galactomannan (figure 1C) were significantly elevated in proven/probable IPA group than in the non-IPA group.

Il-6, IL-8, and galactomannan levels in BALF between IPA and non-IPA groups

A previous study has demonstrated that galactomannan is upregulated in BALF and serum of patients with COPD

Table 1 Demographic and clinical characteristics of the study population

Variable	Study group		P value
	IPA (n=32)	Non-IPA (n=74)	
Age (years)	65.71±13.19	62.85±12.95	0.282
Gender			
Male	22 (68.8)	46 (62.2)	0.659
Female	10 (31.2)	28 (37.8)	
Smoking years	19.33±15.76	17.38±14.75	0.526
Mechanical ventilation	15 (46.9)	14 (18.9)	0.005
Admission to ICU	17 (53.1)	22 (29.7)	0.029
Systemic corticosteroid use			
In the last 3 months	19 (59.4)	39 (52.7)	0.671
During hospitalization	32 (100)	50 (67.6)	<0.001
Lung function			
I	4 (12.5)	15 (20.3)	0.561
II	16 (50.0)	27 (36.5)	
III	10 (31.3)	28 (37.8)	
IV	2 (6.2)	4 (5.4)	

Values were expressed as n (percentage, %) or mean±SD. P values for each group were derived from either unpaired t-test or Mann-Whitney test as appropriate. χ^2 test or Fisher's exact test was used for assessing distribution of observations or phenomena between different groups.
ICU, intensive care unit; IPA, invasive pulmonary aspergillosis.

with IPA. Therefore, we further detected the levels of IL-6, IL-8 and galactomannan in BALF of proven/probable IPA or non-IPA group. Our results revealed that IL-6 (figure 2A), IL-8 (figure 2B) and galactomannan (figure 2C) levels in BALF were significantly elevated in proven/probable IPA group than in non-IPA group.

Il-6 and IL-8 are positively correlated with galactomannan

Since galactomannan has been widely accepted for the auxiliary diagnosis of IPA, here we aimed to evaluate the correlation between galactomannan and IL-6/IL-8 in BALF and serum of patients with COPD with IPA. Our results showed that IL-6 and IL-8 were positively correlated with galactomannan in BALF and serum of patients with COPD with IPA (figure 3A–D).

Diagnostic efficiency of IL-6, IL-8 and galactomannan in serum and BALF

The diagnostic efficiency of galactomannan in BALF and serum is expressed by sensitivity and specificity, and the best cut-off value and reliability of the method were analyzed using the ROC curve. ROC curves for galactomannan, IL-6, and IL-8 are displayed in figure 4. When taking 0.67 as the cut-off value of galactomannan, the sensitivity and specificity of galactomannan detection in serum were 85.14% and 87.50%, respectively; ROC curve analysis revealed that the area under curve (AUC) was 0.9434 (figure 4A). The galactomannan in BALF is respectively 98.65% and 81.25%; the AUC is 0.8750 (cut-off at 1.17, figure 4B).

The sensitivity and specificity of IL-6 detection in serum were 74.32% and 81.25%, respectively (cut-off at 92.82 pg/mL, AUC=0.8366; figure 4C), whereas these were 68.92% and 71.88% in BALF, respectively (cut-off at 229.4 pg/mL, AUC=0.7694; figure 4D). The sensitivity and specificity of IL-8 detection in serum were 83.78% and 81.25%, respectively (cut-off at 93.46 pg/mL, AUC=0.8756; figure 4E), whereas these were 85.14% and 75.00% in BALF, respectively (cut-off at 325.4 pg/mL, AUC=0.8252; figure 4F).

DISCUSSION

Clinically, COPD combined with IPA is easy to be misdiagnosed, which is usually due to its non-specific clinical manifestations and the inability of severely ill patients to undergo invasive examination that could confirm the diagnosis.[17] In addition, laboratory testing methods are still limited; it is difficult to obtain qualified pathogenic bacteria for examination and specimens; and pathogenic microbiology examination is time-consuming, which could easily cause misdiagnosis or missed diagnosis.[18] Therefore, more research is still needed to focus on the early diagnosis and treatment of COPD combined with IPA to guide clinical intervention in a timely manner, to improve prognosis and reduce mortality.[19]

Galactomannan could be released into the blood and other body fluids including BALF when *Aspergillus* invades human tissues,[20] so the detection of galactomannan could indicate *Aspergillus* infection. However, since galactomannan is not released into the blood in the early stage of fungal infection, the sensitivity of early diagnosis of IPA through serum galactomannan detection is not high.[21] Animal and clinical studies at home and abroad have found

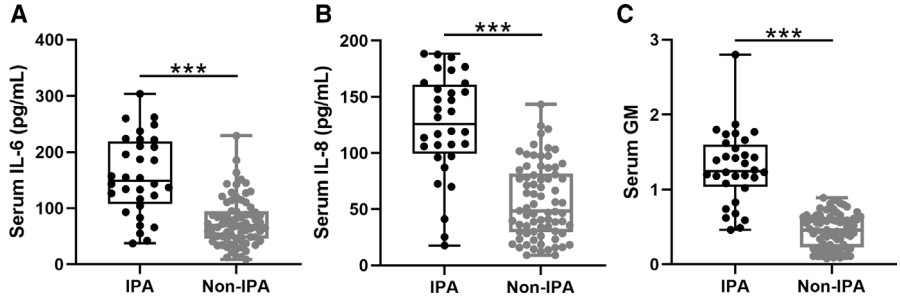

Figure 1 Serum IL-6 (A), IL-8 (B) and galactomannan (C) were elevated in proven/probable IPA group compared with the non-IPA group in patients with chronic obstructive pulmonary disease. Box plots were used to present all individual data. ***P<0.001. IL, interleukin; IPA, invasive pulmonary aspergillosis.

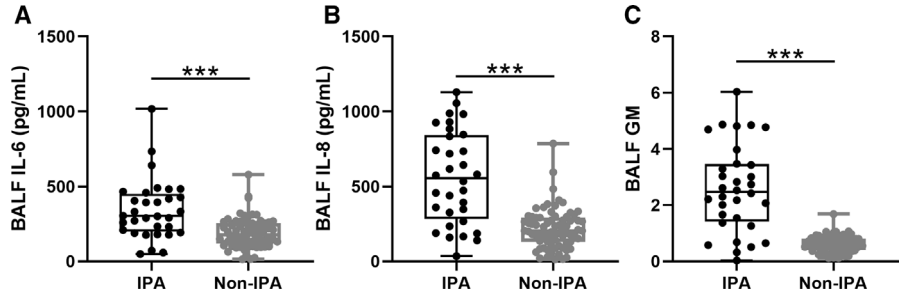

Figure 2 Il-6 (A), IL-8 (B) and GM (C) in BALF were elevated in patients with chronic obstructive pulmonary disease with IPA compared with non-IPA. Box plots were used to present the data with all the data showing. ***P<0.001. BALF, bronchoalveolar lavage fluid; GM, galactomannan; IL, interleukin; IPA, invasive pulmonary aspergillosis.

that galactomannan could be detected earlier in BALF in patients with immunodeficiency such as hematopoietic stem cell transplantation and hematological diseases,[22] suggesting the detection of galactomannan in BALF has higher sensitivity and specificity than in serum and has a higher diagnostic value. In this paper, we found that galactomannan is significantly upregulated in the serum and BALF of patients with COPD with IPA, which is consistent with the previous results. The sensitivity and specificity of galactomannan in serum were 85.14% and 87.50% (cut-off at 0.67, AUC=0.9434), and 98.65% and 81.25% (cut-off at 1.17, AUC=0.8750) in BALF. The value of AUC shows that the accuracy of galactomannan detection in serum is better than that in BALF, whereas the sensitivity of galactomannan detection in BALF is better than that in serum.

Aspergillus infection could lead to elevated levels of several cytokines.[23] Various studies have shown that detecting the difference between the changes in IL levels in IPA combined in patients with COPD and IPA-negative patients with COPD is helpful for the diagnosis and timely treatment of IPA in the early clinical stage.[24] Studies have found that IL-6 and IL-8 levels in the BALF and serum of patients with IPA blood disease are increased.[14] A previous study has demonstrated that IL-6-deficient mice are more susceptible to IPA than wild-type mice, and exposure to exogenous IL-6 restores the antifungal effector activity.[25] IL-8 could be detected in the blood and BALF of patients with IPA,[26 27] and it has been found that patients with IPA showed high serum IL-8 levels (>300 pg/mL).[28] In addition, when combined with BALF PCR analysis, serum IL-8 is also highly specific for IPA.[28] Here, we reported that the BALF and serum levels of IL-8 and IL-6 were significantly elevated in the proven/probable IPA group than in non-IPA group. Furthermore, our results revealed that IL-8 and IL-6 were

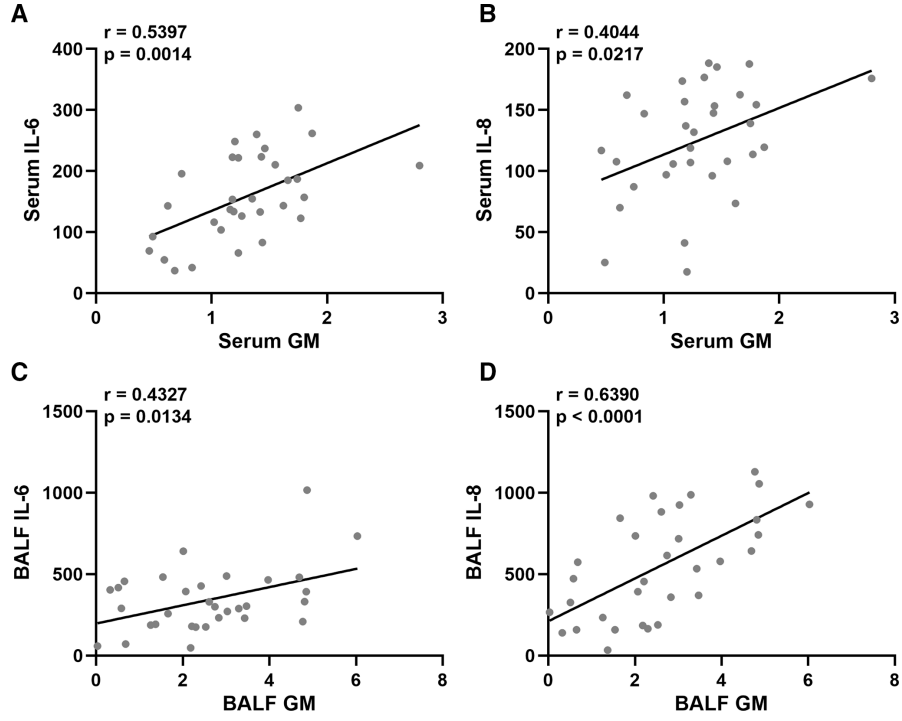

Figure 3 Spearman's correlations between serum GM and IL-6 (A), IL-8 (B), BALF GM and IL-6 (C), IL-8 (D) in proven/probable IPA group in patients with chronic obstructive pulmonary disease. BALF, bronchoalveolar lavage fluid; GM, galactomannan; IL, interleukin; IPA, invasive pulmonary aspergillosis.

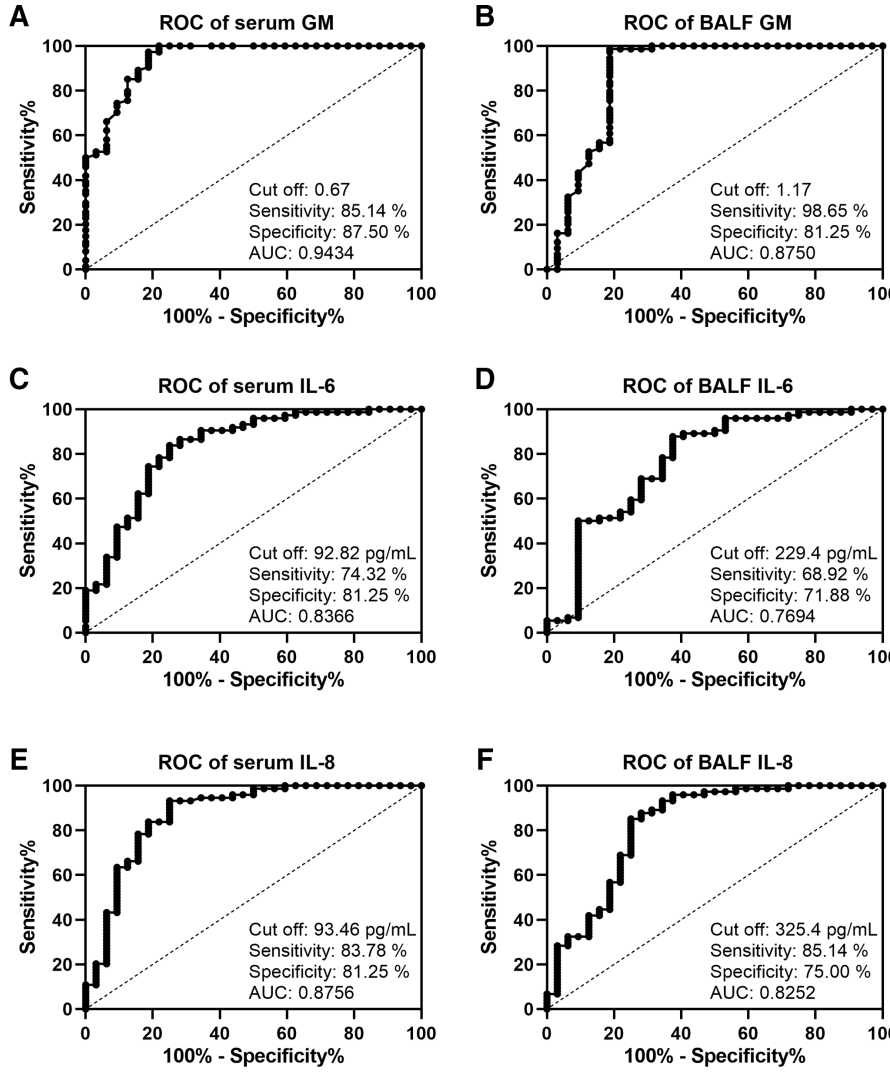

Figure 4 ROC analysis of serum GM (A), BALF GM (B), serum IL-6 (C), BALF IL-6 (D), serum IL-8 (E) and BALF (F) to predict patients with chronic obstructive pulmonary disease with IPA. AUC, area under the curve; BALF, bronchoalveolar lavage fluid; GM, galactomannan; IL, interleukin; IPA, invasive pulmonary aspergillosis; ROC, receiver operating characteristic.

positively correlated with galactomannan in the BALF and serum of patients with COPD with IPA. The value of AUC shows that the accuracy of IL-8 detection in both BALF and serum is better than that of IL-6. In another study, Heldt *et al* analyzed the levels of IL-6 and IL-8 in serum and BALF of hematological patients with IPA.[14] They found that IL-6 and IL-8 were significantly associated with IPA in both serum and BALF, which is consistent with our research. However, they reported that in multivariate conditional logistic regression analysis of serum cytokines, only IL-10 (cut-off 6.75 pg/mL; HR 10.568, 95% CI 1.255 to 89.005; p=0.030) remained a significant predictor of IPA, while IL-6 and IL-8 used for matching were not significant, which is inconsistent with our results. In addition, in multivariate conditional logistic regression analysis of BALF cytokines, only IL-8 (cut-off 710 pg/mL; HR 11.685, 95% CI 1.423 to 95.915; p=0.022) remained significant, while IL-6 as well as covariates used for matching were not significant. It may be due to the inconsistency of the disease and the fact that they only used 10 patients with probable/proven IPA and 20 matched controls without evidence of IPA. Actually, in our study, the number of proven IPA was relatively low when compared with patients without IPA due to resource and time constraints. This may affect the statistical results and need to be taken into consideration.

Notably, galactomannan levels in the serum and BALF performed best in diagnosing IPA. It is unclear based on this study if the IL-6 and IL-8 add any additional diagnostic yield to the galactomannan assay. Although we proposed the limitation of galactomannan level, that is, galactomannan did not increase in the early stage of disease onset, and proposed the role of IL-6 and IL-8 in early diagnosis, this study did not provide stronger evidence to support our view. More research could be carried out in future research, including early detection of IL-6, IL-8 and galactomannan.

CONCLUSION

In conclusion, our results showed that the levels of IL-8 and IL-6 in BALF and serum were significantly increased

in proven/probable IPA group than in non-IPA group and were positively correlated with galactomannan in patients with COPD with IPA. ROC analysis revealed that IL-6 and IL-8 could be used as auxiliary indicators to diagnose IPA in addition to galactomannan.

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