The role of Pentraxin3 in plasma and bronchoalveolar lavage fluid in COPD patients with invasive pulmonary aspergillosis

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Abstract

BACKGROUND

The use of galactomannan testing in plasma and bronchoalveolar lavage fluid (BALF) has improved diagnosis of invasive pulmonary aspergillosis (IPA) in COPD patients; However, the high false positive rate leads to overdiagnosis. This study aimed to investigate the diagnostic value of PTX3 in COPD patients with invasive pulmonary aspergillosis.

METHODS

A total of 165 patients initially suspected of COPD with invasive pulmonary aspergillosis were included in the study. Among these, 35 cases were proven or probable to be invasive pulmonary aspergillosis (35 plasma samples and 28 BALF samples). The remaining 130 cases were non-aspergillosis controls (130 plasma samples and 83 BALF samples). PTX3 levels and GM were measured by enzyme-linked immunosorbent assay.

Results

Median plasma and BALF PTX3 level was significantly higher in COPD patients with invasive pulmonary aspergillosis compared with non-aspergillosis patients (3.74 [2.57–5.61] ng/ml vs 1.29 [0.62–2.88] ng/ml, $P < 0.001$; 3.88 [2.28–8.29] ng/ml vs 1.58 [0.85–2.13] ng/ml, $P < 0.001$). When the plasma GM/PTX3 and BALF GM/PTX3 assays were used for patients included in the study, the sensitivity/specificity values were 60%/77.1%/78.6%/89.3%, 73.8%/69.2%/80.7%/77.1%, respectively. Thus, The sensitivity of PTX3 in plasma and BALF was higher than that of GM. However, There was no significant difference in the specificity of PTX3 and GM between the IPA group and non-aspergillosis group. When PTX3 and GM were both positive in plasma or BALF, the specificity for the diagnosis of pulmonary aspergillosis can reach more than 90%.

Conclusions

BALF and plasma PTX3 measurements were significantly higher among patients with IPA. The sensitivity of PTX3 was superior to GM in the diagnosis of IPA in COPD patients. The combination of GM and PTX3 is beneficial to the diagnosis of IPA in COPD patients.

Introduction

Invasive pulmonary aspergillosis is a serious opportunistic infection disease by *Aspergillus*. In recent years, the number of patients with invasive pulmonary aspergillosis in COPD has gradually increased. Current research suggests that the occurrence of pulmonary *Aspergillus* infection in patients with COPD

is related to the long-term use of glucocorticoids and antibiotics. COPD patients with pulmonary invasive aspergillosis often have atypical symptoms and imaging[1]. Therefore, the early diagnosis of these patients is difficult and the fatality rate is high[2]. The IDSA guideline recommend plasma and bronchoalveolar lavage (BALF) galactomannan (GM) testing for diagnosing pulmonary invasive aspergillosis[3]. However, Some studies believe that the diagnostic value of the GM test in patients with Non-neutropenic (include COPD) Invasive Pulmonary Aspergillosis is limited[4]. Therefore, it is necessary to find better biomarkers to help diagnose invasive pulmonary aspergillosis.

Pentraxin 3 (PTX3), a member of the family of long pentraxins, is produced by dendritic cells, epithelial cells, endothelial cells, and macrophages at the sites of inflammation[5]. A study have shown that PTX3 knockout mice are more susceptible to *Aspergillus*, because of reduced PTX3 production[6]. Recent report has described a link between PTX3 polymorphisms and susceptibility to aspergillosis in patients undergoing hematopoietic stem-cell transplantation (HSCT) and lung transplant recipient[7]. Our previous research found that the COPD patients with pulmonary aspergillosis is also closely related to PTX3 polymorphism. plasma PTX3 levels significantly increased in patients with COPD with invasive pulmonary aspergillosis compared with patients with COPD alone[8]. But we have not evaluated the diagnostic value of PTX3 for invasive pulmonary aspergillosis. It is well known that bronchoalveolar lavage fluid is more directly to reflect lung inflammation than blood in lung infections. This provides a new potentially biomarker for clinical diagnosis of invasive pulmonary aspergillosis. Thus, In this study, we evaluated the diagnostic value of BLAF and plasma PTX3 in COPD patients with invasive pulmonary aspergillosis.

Patients And Methods

The medical records of 165 COPD patients, who were suspected of invasive pulmonary aspergillosis in Changzhou First People's Hospital from September 2017 to November 2020, were retrospectively analyzed. The Institutional Review Board of Changzhou first people's Hospital approved this study (No.2019-020).

All patients had a previous diagnosis of COPD. According to their lung function, they are classified into GOLD 1, 2, 3, and 4, respectively. All the enrolled patients had clinical symptoms such as fever, cough, sputum, dyspnea or hemoptysis. Furthermore, the computed tomography (CT) scan showed consolidation with or without a halo sign, pulmonary nodules or cavitary lesions. Patients who satisfied the definition were included in this study. Among them, 35 cases were finally diagnosed with invasive pulmonary aspergillosis with COPD according to the Infectious Diseases Society of America guideline criteria (3 proven; 33 probable), proven patients were diagnosed by histopathology (*Aspergillus* hyphae were found in Bronchoscopy biopsy specimens). Among IPA probable patients, 23 patients had positive *Aspergillus* cultures from qualified BLAF or sputum specimens, 10 patients had 2 consecutive positive serum or BLAF galactomannan detection results. 130 COPD patients without IPA were enrolled as controls. Patients who previously underwent hematopoietic stem-cell or solid-organ transplant or who were diagnosed with neutropenia were excluded from the study.

Collection and Measurement of BALF and plasma samples

Peripheral blood from each subject was collected before initiating treatment. The bronchoscopy procedures were performed by a bronchoscopist with more than 3 years of bronchoscopy experience. CT was used to locate the segment or subsegmental bronchus of the lesion. This area was rinsed twice with 50ml saline. BALF was collected in a sterile tube and sent for laboratory.

Plasma and BLAF PTX3 levels were measured using an enzyme-linked immunosorbent assay kit according to the manufacturer’s protocol (DPTX30, Quantikine Human Pentraxin 3 Immunoassay, R&D, Abingdon, UK). Both Plasma and BLAF specimens were performed using a double-sandwich ELISA according to the manufacturer’s instructions for the Platelia Aspergillus kit (Bio-Rad Laboratories, CA, USA).

Statistical analysis

Continuous variables were expressed as mean ± standard deviation or medians and interquartile ranges, according to distribution. Categorical variables were expressed as proportions. For counting data and categorical variables, we used the χ^2 or Fisher exact tests. For quantitative variables, we used the Student’s t-test or Mann-Whitney test, depending on whether or not data distribution was normal. All data were analyzed by the SPSS 19.0 software (Chicago, IL). A p value <0.05 was taken to indicate statistical significance. Receiver operating characteristic curve (ROC curve) analysis was used to determine the optimal cutoff value.

Results

Patient characteristics

A total of 165 patients received both the GM and PTX3 tests in plasma(35 cases were diagnosed with invasive pulmonary aspergillosis with COPD; 130 cases were non-aspergillosis controls). Among these people, 111 patients received bronchoscopy and had the BALF GM/PTX3 tested. No significant difference was observed in terms of sex, age, smoking history and Pulmonary function between the case and control groups (Table1). More patients in the IPA group used corticosteroids and the difference was statistically significant(P<0.05).

Table 1. Demographic Characteristics of the Study Population

Variables	COPD (n=130)	invasive pulmonary aspergillosis (n=35)	P value
Male	95	26	0.87
Age,y	66.4±9.00	65.57±8.90	0.63
History of smoking	113	30	0.85
Steroid treatment*	84	32	0.002
Pulmonary function			
GOLD 1	23	4	0.71
GOLD 2	34	8	
GOLD 3	51	17	
GOLD 4	22	6	

* Steroid treatment >3 months.

PTX3 levels in plasma and BALF

The median [IQR] plasma PTX3 levels in IPA (3.74[2.57-5.61]ng/ml) were significantly higher than that in COPD group (1.29[0.62–2.88]ng/ml,P<0.001). Similarly, the level of PTX3 in the BLAF of the IPA group was significantly higher than that of the COPD group (3.88[2.28-8.29]ng/ml vs 1.58[0.85-2.13]ng/ml,P<0.001).

Diagnostic efficiency among PTX3 and GM

As shown in the receiver operating characteristic (ROC) curve, the plasma GM optimal cutoff value was 0.55, at which value the sensitivity and specificity of the test were 60% and 73.8%, respectively (AUC = 0.704). According to the ROC curve, the plasma PTX3 threshold for diagnosing aspergillosis was 2.57ng/ml, At this point, the sensitivity and specificity were 77.1% and 69.2% (AUC = 0.751). The sensitivity of plasma PTX3 is significantly increased compared with the plasma GM(P=0.04).however, The difference between the rates of specificity of the plasma PTX3 and GM was not statistically significant.(P=0.69)

In the detection of BLAF, ROC curve analysis showed that the BALF GM Optimal threshold was 0.8, while the sensitivity and specificity was 78.6% and 80.7% (AUC = 0.813). In BLAF PTX3, the sensitivity and specificity was 89.3% and 77.1% for the diagnosis of IPA (AUC = 0.889) , when the ptx3 value was 2.16ng/ml . Similarly, the sensitivity of PTX3 is higher than that of GM in the detection of BLAF, but there was no statistical difference(P=0.37).

The diagnostic values of the combined GM and PTX3

Double positivity of the plasma GM and PTX3 tests for the diagnosis of IPA in COPD patients, was associated with 60% sensitivity and the specificity increased to 93.8% (AUC = 0.804). In BLAF sample, the double positive strategy for the GM and PTX3 tests in IPA showed 75% sensitivity and 94% specificity (AUC = 0.920).(Figure1, Table2)

Table 2. The diagnostic values of the combined GM and PTX3

	Plasma GM(+)/PTX3(+)	BLAF GM(+)/PTX3(+)
Sensitivity	60%	75%
Specificity	93.8%	94.0%
Positive predictive value	72.4%	80.8%
Negative predictive value	89.7%	91.8%

Discussion

PTX3 is a soluble pattern recognition receptor that can be immediately released into the extracellular space in response to inflammation soluble pattern recognition receptor[9]. A number of studies have shown that PTX3 plays a key role in innate immunity to aspergillus infections[10, 11]. A total of 165 consecutive inpatients with COPD were included in this study. Among them, 35 cases were finally diagnosed with IPA with COPD, whereas 130 cases were determined to have COPD without pulmonary aspergillosis. More patients had a history of corticosteroid use in IPA group, which was consistent with previous studies[4].

PTX3 is an acute phase protein that can be detected within a few hours of inflammation[12]. In our study, we found plasma PTX3 levels in IPA subjects were greater than for the COPD without pulmonary aspergillosis groups. A previous report indicated that plasma PTX3 levels significantly increased after infection with fungi, but c-reactive protein levels did not increased[13]. In another study, The plasma PTX3 for aspergillosis was significantly higher than for non-aspergillosis group in non-neutropenic patients[14]. Our previous research results are also consistent with the above conclusions[8]. ROC curve analysis showed that plasma GM and PTX3 thresholds were 0.55 and 2.57ng/ml. at those point, The sensitivity of plasma PTX3 were significantly higher than for plasma GM, and the difference is statistically significant. However, There is no statistical difference between the specificity of plasma GM and PTX3.A previous study described the sensitivity of plasma PTX3 in non-neutropenic patients were greater than for BALF GM, but differences were not statistically significant[14].

In previous studies, alveolar lavage fluid was considered to be a better indicator of lung inflammation than plasma[15]. At present, the BLAF GM test is the best biomarker for the diagnosis of pulmonary aspergillosis. In our study, According to the ROC curve show the BALF GM Optimal threshold was 0.8 ,the sensitivity and specificity were 78.6% and 80.7%. Other studies in which ROC curve analysis was applied have reported optimal BALF GM cutoff values ranging from 0.8 to 1.25 in COPD patients with pulmonary

aspergillosis[16–18].The difference in BLAF GM values was due to differences in study population selection, such as critically ill with COPD or COPD patients are only part of the study population.

In a study of hematopoietic stem cell transplant patients[7], they found higher levels of PTX3 in BLAF, but not in plasma, from patients with invasive aspergillosis than in patients without invasive aspergillosis. In Kabbani's study[19], PTX3 measurements in BAL samples were significantly higher among patients with invasive aspergillosis in lung transplant recipients. The above-mentioned studies mainly focused on the transplant population, but our study selected COPD patients. We also found the BLAF PTX3 levels in the IPA group were significantly higher than those in the controls. According to the ROC curve show the BALF PTX3 Optimal threshold was 2.16ng/ml, the sensitivity and specificity were 89.3% and 77.1%. the sensitivity of BALF PTX3 were greater than for BALF GM, though differences were not significant. These findings suggest that PTX3 is superior to GM in the diagnosis of COPD patients with pulmonary aspergillosis in both plasma and BLAF samples.

Then, We evaluated the diagnostic value of GM combined with PTX3. our study found that 72.4% of patients with both plasma GM and PTX3 positive had aspergillosis in COPD patients. However, Patients positive for BALF GM and PTX3 in the meantime were more than 80% likely to have aspergillosis. When PTX3 and GM were both positive in plasma or BLAF, the specificity for the diagnosis of pulmonary aspergillosis can reach more than 90%. These findings suggest that GM combined with PTX3 can increase the specificity of the diagnosis of COPD with pulmonary aspergillosis.

Conclusions

In conclusion, The sensitivity of PTX3 was superior to GM in the diagnosis of IPA in COPD patients. Double positive for GM and PTX3 in plasma or BLAF is helpful for diagnosis of IPA in COPD patients. our findings have diagnostic implications and need to be validated in a larger cohort.

Declarations

Ethics approval and consent to participate The study was approved by the Institutional Review Board of Changzhou first people's Hospital (No.2019-020).

Consent for publication Not applicable.

Availability of data and materials The datasets generated and/or analysed during the current study are not publicly available due other manuscripts will be published from this data, but are available from the corresponding author on reasonable request.

Competing interests: The authors declare no competing interests.

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Authors' contributions: Qian He and Chunlai Feng designed the study. Qian He and Ming Zhang collected and analyzed the data. Qian He and Chunlai Feng wrote the paper. All authors read and approved the final manuscript.

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Figures

Figure 1

A:ROC curve for plasma GM/PTX3, The area of plasma GM/PTX3/Double(+) under the ROC curve was 0.704/0.751/0.804. B. ROC curve for BLAF GM/PTX3. The area of BLAF GM/PTX3/Double(+) under the ROC curve was 0.813/0.889/0.920.