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ORIGINAL RESEARCH

Correlation of *Aspergillus fumigatus* Sensitization with Mucus Plugging in COPD

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Background: Both *Aspergillus fumigatus* sensitization and mucus plugs are associated with poor clinical outcomes in COPD. However, little is known about the association between *Aspergillus* hypersensitivity and mucus plugging in patients with COPD.

Methods: We retrospectively enrolled COPD patients who had visited Peking University Third Hospital and received measurement of the *Aspergillus Fumigatus* specific IgE (*Af* sIgE) from Oct 1, 2018 to Sep 30, 2023. The clinical, laboratory, and chest CT features were analyzed, with mucus plugging evaluation using the bronchopulmonary segment-based scoring system. Comparison was performed between COPD patients with and without *Aspergillus* hypersensitivity (AH).

Results: Among the 378 COPD patients with measurement of *Af* sIgE, 29 (7.7%) were classified as having AH (*Af* sIgE>0.35KU/L). By propensity score matching (1:2), 58 patients without AH were included for comparison. Patients with AH had lower FEV1%pred (P=0.008) and FEV1/FVC (%) (P=0.023), and were more likely to have a blood eosinophil count exceeding 300/μL and higher white blood cell and neutrophil counts. The prevalence of luminal plugging on chest CT in subjects with AH was 58.6%, compared to 31.0% in those without AH (P=0.013). Multivariate regression analyses showed that *Af* sIgE more than 0.70 KU/L and blood neutrophil count were associated with mucus plugging.

Conclusion: In patients with COPD, *Aspergillus* sensitization was associated with lower lung function and mucus plugging on chest CT.

Keywords: COPD, *Aspergillus fumigatus*, hypersensitivity, mucus plug

Introduction

Chronic Obstructive Pulmonary Disease (COPD) is currently among the top three leading causes of death globally, posing a significant public health challenge. Fungal sensitization, particularly to *Aspergillus fumigatus*, is increasingly reported in individuals with chronic respiratory diseases, including COPD, asthma, and bronchiectasis, where it is associated with persistent symptoms, increased disease severity, and higher mortality rates.[1–6] However, the role of *Aspergillus* sensitization in the pathogenesis of COPD is largely unknown. Previous studies have shown that exposure to *A. fumigatus* extracts increases mucin production in airway epithelia[7,8], and chronic exposure to *A. fumigatus* spores upregulates the expression of MUC5AC, and induces goblet cell hyperplasia in the airways of asthma rats.[9] However, the relationship between mucus plugging and *Aspergillus* hypersensitivity in COPD remains unclear.

Chronic mucus hypersecretion is a key characteristic of COPD,[10,11] manifesting as cough and sputum production which are associated with greater airflow obstruction, lower oxygen saturation, and worsened quality of life and all-cause mortality. Therefore, studies on risk factors for and mechanisms underlying mucus hypersecretion, a potential treatable trait in COPD, are highly desirable for targeted therapy. A visual chest computed tomography (CT) scoring system was recently developed, demonstrating that high mucus scores on CT were significantly associated with worse lung function in both asthma and COPD.[12,13] Therefore, we used this scoring system to assess mucus plugging and examined its associated features, especially *Aspergillus fumigatus* sensitization in COPD patients. Revealing the association between *Aspergillus s*ensitization and mucus plugging has important clinical implications, as potential interventions, such as environmental isolation or antifungal therapy, may be applied for this subgroup of patients.[14]

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Methods

Patient and Data Collection

Patients visiting the Department of Respiratory and Critical Care Medicine, Peking University Third Hospital and received measurement of blood *Aspergillus fumigatus*-specific IgE (*Af* sIgE) from Oct 1, 2018 to Sep 30, 2023, were retrospectively included for this study. The subject selection and diagnostic process are shown in Figure 1. The study and the exemption from informed consent were approved by the Clinical Research Ethics Committee of Peking University Third Hospital. Patient data confidentiality was maintained in compliance with the Declaration of Helsinki.

Patients were diagnosed with COPD according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria: (1) age over 40 years, (2) consistent symptoms including chronic cough, expectoration, and/or dyspnea, (3) a history of cigarette smoking (≥10 pack-years), and/or exposure to biomass fuel for at least 10 years, and/or occupational exposure to noxious particles and fumes for at least 10 years, and (4) evidence of irreversible obstructive impairment on spirometry, defined by post-bronchodilator FEV1/FVC <70%.

Af sIgE testing was performed within six months before or after the CT examination. Positive *Af* sIgE was defined as a level of more than 0.35 KU/L, indicating *Aspergillus* hypersensitivity (Phadia, Thermo Fisher Scientific, Uppsala, Sweden). Besides, according to whether the *Af* sIgE was greater than 0.7 KU/L, we divided participants with a positive *Af* sIgE into a low-sensitization group and a high-sensitization group.

The clinical data included demographics, smoking history, the number of cigarettes smoked, respiratory symptoms (cough, sputum, hemoptysis, dyspnea), comorbidities, and medication use. The laboratory data included white blood cell count (WBC) and differentials, hemoglobin (HB), serum total IgE, and specific IgE to *Aspergillus fumigatus*. The normal range of total IgE was 0–60 KU/L (Phadia, Thermo Fisher Scientific, Uppsala, Sweden). Lung function was measured post-bronchodilator (after reversibility testing) as forced expiratory volume in the first second in percent predicted values (FEV1% pred) and FEV1/forced vital capacity (FEV1/FVC (%)). Patients avoided active or passive smoking and strenuous exercise for 12 hours before the test, and avoided caffeinated drinks and nitrogen-rich food for 2 hours before the test.

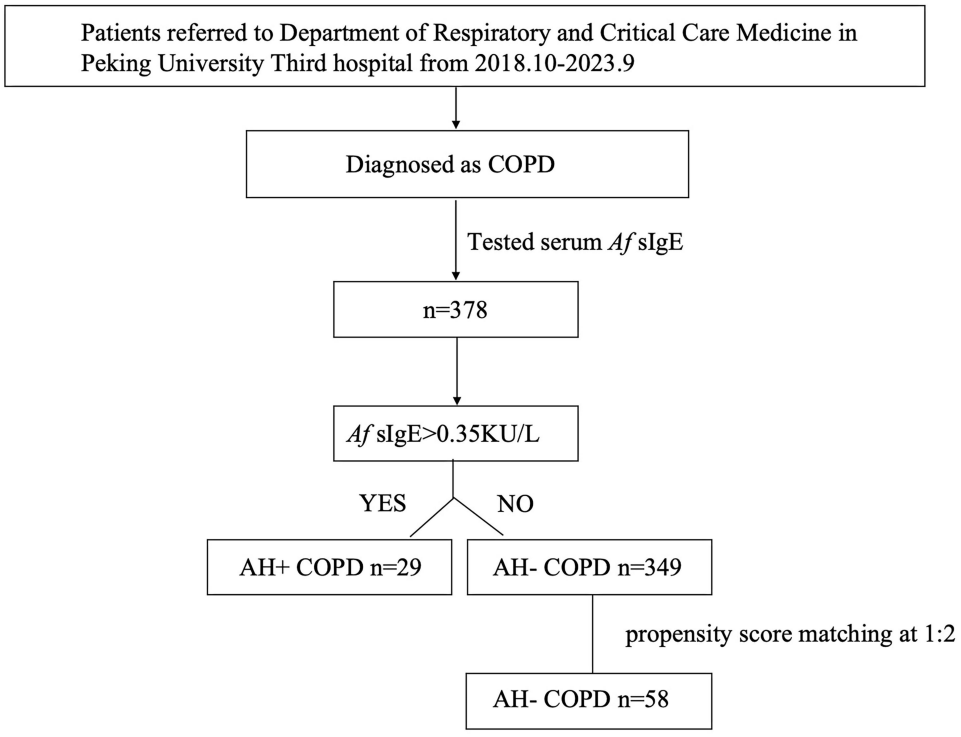

Figure 1 Patient selection process.

Evaluation of Chest HRCT

Mucus Plug Score Evaluation

CT images were scored for mucus plugging using the bronchopulmonary segment-based scoring system by Dunican et al.[13] Mucus plugging was defined as a complete luminal occlusion of the bronchus (Figure 2), irrespective of generation or size. The lung zone within 2 cm of the costal or diaphragmatic pleura was excluded because the airways in that zone are too small to ascertain a complete occlusion by luminal plugs. A total of 18 segmental airways, including ten bronchopulmonary segments in the right lung (3, 2, and 5 segments in the upper, middle, and lower lobes, respectively) and 8 in the left lung (4 segments each in the upper and lower lobes), were evaluated. A luminal plug score was generated for each CT as an aggregate of the number of bronchopulmonary segments with luminal plugging, ranging from 1 to 18. For those without luminal plugging, the score is 0. A standard window width of 1200 hU and a level of 600 hU were used for visual bronchial wall evaluation. Two pulmonary physicians evaluated the CT scans without knowledge of the patient's clinical data. They independently completed the assessments, and differences in readings were resolved through consensus.

Statistical Analysis

All statistical analyses were performed using SPSS 17.0 (Statistics Package for the Social Sciences, SPSS Inc., Chicago, IL, USA). The propensity score matching was performed using SPSS. With *Aspergillus fumigatus* sensitization as the dependent variable and age and gender as independent variables, the propensity scores for the two patient groups were calculated through logistic regression. The nearest-neighbor matching method was applied to match the covariates at a 1:2 ratio, with a caliper value of 0.05. Data were expressed as mean ± standard deviation (SD). Data not normally distributed were expressed as median (interquartile range, IQR). Categorical variables between different groups were analyzed using the chi-square test. Spearman correlations were used for correlation analysis. Comparisons of continuous data between two groups were performed using the independent-samples *T*-test (for normally distributed parameters) and the Mann–Whitney *U*-test (for abnormally distributed parameters). Univariate and multivariable logistic regression analyses were used to assess the relationship of mucus plugging and multiple variables. P-values less than 0.05 were considered statistically significant.

Results

Demographics and Clinical Characteristics of the Patients

During the study period, 378 patients diagnosed with COPD underwent testing for blood *Af* sIgE, of whom 29 (7.7%) had an *Af* sIgE level >0.35KU/L, therefore classified as the *Aspergillus* hypersensitivity (AH) group. We then used propensity score matching at a ratio of 1:2 to recruit controls from the 341 patients without AH. Consequently, 29 patients of the AH+ group and 58 patients from the AH- group were included for the final analysis. Patient demographics and associated clinical characteristics are shown in Table 1.

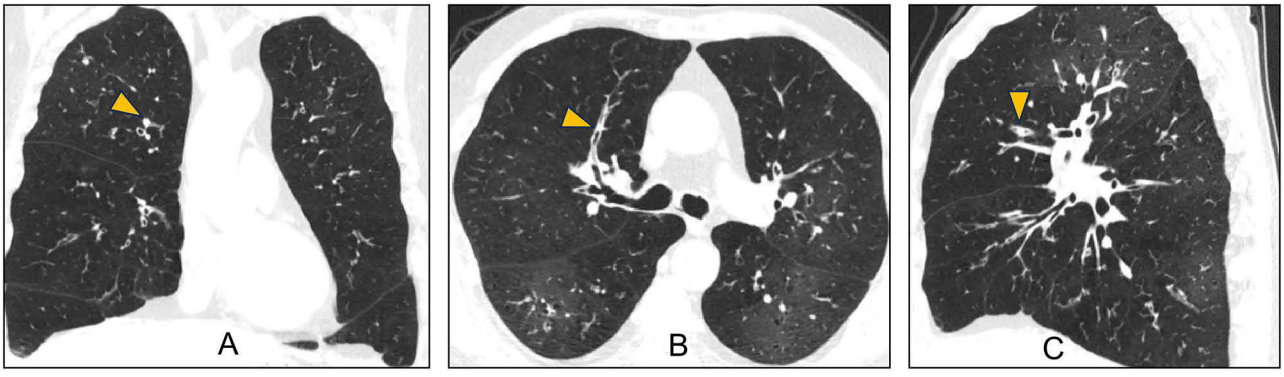

Figure 2 An example of airway mucus plugs (yellow arrowheads) of one participant in this study in the Coronal plane (**A**), the Axial plane (**B**) and Sagittal plane (**C**).

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Table 1 Characteristics of COPD Subjects with Aspergillus Hypersensitivity (AH+) and Without Aspergillus Hypersensitivity (AH-)

	AH(-) (n=58)	AH(+) (n=29)	*p*
Male, n(%)	48 (82.8)	24 (82.8)	1
Age(year)	69 (67, 83)	71 (65, 81.5)	0.81
Ever-smoker, n(%)	46 (80.7)	20 (74.1)	0.489
Af sIgE (KU/L)	0.01 (0, 0.023)	0.85 (0.50, 1.36)	**<0.001**
Total IgE (KU/L)	63.75 (35.5, 137.75)	756 (284.5, 1257)	**<0.001**
Blood WBC (10^9/L)	6.49 (5.43, 7.51)	7.55 (6.40. 8.58)	**0.013**
Blood Neutrophils (10^9/L)	3.97 (3.03, 4.77)	4.86 (4.09, 6.17)	**0.01**
Blood EOS (/uL)	160 (80, 250)	180 (70, 400)	0.304
EOS>300/μL, n(%)	8(13.8)	11(37.9)	**0.01**
FEV1 (%pred)	64.44±22.07	50.43±16.93	**0.008**
FEV1/FVC (%)	58.60 (49.41, 65.71)	51.00 (47.00, 57.87)	**0.023**
History of asthma, n(%)	5(8.6)	4(13.8)	0.455
History of allergic disease[a], n(%)	9(16.1)	6(20.7)	0.596
Mucus plug positive, n(%)	18 (31)	17 (58.6)	**0.013**
Mucus plug score	0 (0, 1.25)	1 (0, 2.5)	0.078
Score stratification, n(%)			**0.007**
Zero (0)	40 (69)	12 (41.4)	
Low (1–2)	5 (8.6)	10 (34.5)	
High (>2)	13 (22.4)	7 (24.1)	
HRCT showing Bronchiectasis	24(41.4)	16 (55.2)	0.224

Notes: Data are presented as mean ± SD or number (%) or median (IQR), unless otherwise indicated; P-values less than 0.05 are bolded to indicate statistical significance. [a]Specific allergic diseases include allergic rhinitis, atopic dermatitis, etc.

Abbreviations: *Af sIgE*: Aspergillus fumigatus specific IgE; Eos: eosinophil; FEV1 (%pred): forced expiratory volume in 1 second percent predicted; FVC: forced vital capacity percent predicted; HRCT: High resolution computed tomography.

As expected, elevated serum total IgE levels were found in the AH+ group (1405 vs 76.75, p<0.01). Subjects in the AH +group exhibited lower FEV1% predicted (50.43 vs 64.44, p=0.008) and FEV1/FVC (%) (51.00 vs 58.6, p=0.023). Additionally, peripheral WBC and neutrophil counts and proportion of blood eosinophils exceeding 300/μL were higher in the AH+ group compared to the AH- group, although there was no difference in the number and percentage of eosinophils between the AH+ and AH- groups. The number of patients with a history of physician-diagnosed asthma or allergic diseases, such as allergic rhinitis and atopic dermatitis, was not significantly different between the two groups.

Mucus Plugging Scores

The prevalence of luminal plugging in the AH+ group was 58.6%, compared to 31.0% in the AH- group, with the difference being statistically significant (p=0.013).

Relationships Between Mucus Plug and Aspergillus Hypersensitivity

Using univariable and multivariable logistic regression models, we found that the blood neutrophil counts and *Af* sIgE >0.7KU/L were independently associated with mucus plugging, whereas ever smoker, FEV1%predicted, and FEV1/FVC ratio were not (Table 2).

Discussion

The relationship between *Aspergillus fumigatus* hypersensitivity and mucus plugging in COPD is poorly understood. Although *Aspergillus* sensitization has been associated with a higher burden of clinical symptoms and decreased lung function in COPD patients,[4,14] to our knowledge, few studies have examined the role of fungal allergy in the pathogenesis of COPD. In this study, we found that COPD patients with *Aspergillus* sensitization were more likely to have mucus

Table 2 Univariate Logistic Regression Analysis of the Associated Factors for Mucus Plugging

Predictor Variables to Mucus Plugging	Univariate Analysis			Multivariable Analysis		
	OR	95% CI	*P*	OR	95% CI	*P*
Ever smoker	2.743	0.816–9.226	0.103			
Blood neutrophils	1.377	1.033–1.835	**0.029**	1.389	1.034–1.865	**0.029**
EOS>300/uL	2.521	0.893–7.115	**0.081**			
FEV1%pred	0.989	0.967–1.011	0.309			
FEV1/FVC (%)	0.997	0.951–1.045	0.903			
Af sIgE >0.7KU/L	3.148	1.248–7.939	**0.015**	4.710	1.397–15.879	**0.012**

Notes: P-values less than 0.05 are bolded to indicate statistical significance.
Abbreviations: OR, odds ratio; CI, confidence interval; Eos: eosinophil count; FEV1 (%pred): forced expiratory volume in 1 second percent predicted; FVC: forced vital capacity percent predicted; *Af* sIgE: *Aspergillus Fumigatus* specific IgE.

plugging on HRCT, in addition to a lower FEV1, as compared to non-sensitized patients. Interestingly, a significant association was found between higher levels of blood *Af* sIgE and mucus plugging, which was also associated with the blood neutrophil count.

Aspergillus sensitization was demonstrated in 7.7% of patients with COPD in our study, whereas previous studies reported a prevalence ranging from 7.9% to 21%.[1,2,4–6] Several methods are available to assess *Aspergillus* sensitization, such as the *Aspergillus* skin test, serum-specific IgE to crude *Aspergillus* antigen extracts (used in our study), and recombinant *A. fumigatus* antigens (rAsp.f),[4] which may account for the discrepancy.

It is notable that previous studies have shown varying results regarding the relationship between *Aspergillus* hypersensitivity and clinical characteristics of chronic respiratory diseases. Most studies demonstrated that fungal sensitization was associated with progressive and persistent symptoms, increased disease severity, and reduced lung function in chronic respiratory diseases[1,4,14–17], though some reported no association between *Aspergillus* sensitization and lung function decline in COPD.[1,5,6] It is proposed that COPD patients with lower lung function are more susceptible to *Aspergillus* sensitization,[2] and sensitization accelerates the decline of lung function.[18]

In this study, we found that *Aspergillus*-sensitized COPD patients showed a higher blood neutrophil counts and were more likely to have a blood eosinophil count of more that 300/μL. The relationship between fungal sensitization and elevated eosinophil counts is expected, as eosinophils can be recruited to the lungs in response to chitin exposure from *Aspergillus fumigatus*, enhancing Th2-mediated immune pathology.[19] Additionally, prior studies have shown that *A. fumigatus* induces neutrophil extracellular traps and eosinophil extracellular traps formation by eosinophils and neutrophils.[20,21]

Recent studies have demonstrated that mucus plugs identified on HRCT scans are frequent in patients with COPD (25%–67%) and are closely associated with clinical measures of the disease, including lung function, quality of life, emphysema on CT, airway wall thickening, and all-cause mortality.[12,22] In this study, we reported mucus plugging in 40.2% of participants with COPD. Importantly, the prevalence of subjects with luminal plugging was higher in the *Aspergillus*-sensitized COPD group than in the non-sensitized COPD group. Multivariate analysis also demonstrated that *Af* sIgE more than 0.7KU/L was independently associated with mucus plugging. Although our study cannot explain the mechanisms underlying the association, possible explanations may be that *A. fumigatus* extracts promote the production of Muc5ac and Muc5b,[7,8,23] which are predominant mucins in normal airway mucus, leading to impaired mucociliary clearance[24,25] and a higher likelihood of mucus plugging. Additionally, evidence suggests that mucus-microbiome shifts occur as COPD progresses,[26] and that COPD patients with very frequent exacerbations and higher mortality are characterized by *Aspergillus* with a concomitant increase in serum-specific IgE levels.[27] It is possible that mucus and allergy in COPD also change over time, which requires further longitudinal studies to confirm. Additionally, compared to Dunican's finding that asthma patients with mucus plugging were more likely to have sputum eosinophilia and systemic eosinophilia,[13] and the sputum neutrophil percentage was higher in smoking patients with a high mucus plug score than in those with a low score,[28] we found that neutrophil count was related to mucus plugging in COPD patients. One

possible mechanism is that human neutrophil elastase mediates mucus hypersecretion via the tumor necrosis factor-α converting enzyme-epidermal growth factor receptor signaling pathway in vivo.[29]

Finally, our study was not without limitations. The sample size of this study was relatively small, and because only cross-sectional assessment was performed, we were unable to ascertain longitudinal changes in both sensitization state and clinical outcomes. Future studies are warranted to elucidate the mechanisms and pathological changes associated with *Aspergillus* sensitization and mucus plugging in COPD.

Conclusion

In summary, COPD patients with *Aspergillus* hypersensitivity exhibited poorer lung function and were more frequently observed to have mucus plugging on lung CT scans. A blood level of *Af* sIgE more than 0.7KU/L was related to mucus plugging, suggesting a potential role of *Aspergillus* sensitization in mucus hypersecretion in a subset of COPD patients.

Abbreviations

COPD, chronic obstructive pulmonary disease; IgE, immunoglobulin E; T-IgE, total IgE; A. Fumigatus, Aspergillus Fumigatus; Af sIgE, Aspergillus Fumigatus specific IgE; AH, Aspergillus hypersensitivity; ABPA, allergic bronchopulmonary aspergillosis; HRCT, high resolution computed tomography; FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity; GOLD, the global Initiative for chronic obstructive lung disease.

Data Sharing Statement

All data generated or analyzed during this study are available from the corresponding author upon reasonable request.

Ethics Approval and Consent to Participate

The study was approved by the Clinical Research Ethics Committees of Peking University Third Hospital (M2023784), and as it was a retrospective study, exemption from informed consent was applied and approved by the Clinical Research Ethics Committees of Peking University Third Hospital.

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Disclosure

The authors declare that they have no conflicts of interest in this work.

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