Exploring the role of Aspergillus galactomannan antigen in assessing the risk factor of acute exacerbations in chronic obstructive pulmonary disease patients: a cross-sectional study

Jayabharathi Palanivel,[1] Madhusmita Mohanty Mohapatra,[1] Manju Rajaram,[1] Debasis Gochhait,[2] Sunitha Vellathussery Chakkalakkoombil,[3] Rakesh Singh[4]

[1]Department of Pulmonary Medicine, Jawaharlal Institute of Postgraduate Medical Education and Research, Puducherry; [2]Department of Pathology, Jawaharlal Institute of Postgraduate Medical Education and Research, Puducherry; [3]Department of Radiology, Jawaharlal Institute of Postgraduate Medical Education and Research, Puducherry; [4]Department of Microbiology, Jawaharlal Institute of Postgraduate Medical Education and Research, Puducherry, India

Correspondence: Madhusmita Mohanty Mohapatra, Department of Pulmonary Medicine, Jawaharlal Institute of Postgraduate Medical Education and Research, Puducherry, India.
E-mail: drmadhusmita1@gmail.com

Key words: Aspergillus, serum galactomannan, chronic obstructive pulmonary disease, pulmonary Aspergillosis.

Contributions: MMM, JP, MR, contributed to the conception and design of the study, acquisition of data, or analysis and interpretation of data; MMM, MR, JP, RS, SNC, DG were involved in drafting the article or revising it critically for important intellectual content. All authors provided final approval of the version to be submitted.

Conflict of interest: the authors declare no potential conflict of interest.

Ethics approval and consent to participate: the study protocol received approval from the Institutional Ethical Committee for observational studies DHR REG.NO.EC/NEW/INST/2020/331issued by Jawaharlal Institute of Postgraduate Medical Education & Research (JIPMER) (Protocol no. JIP/IEC/2021/077 dated 25/06/2021).

Informed consent: written informed consent was obtained for data collection. The manuscript does not contain any individual person's data in any form.

Availability of data and materials: all data underlying the findings are fully accessible.

Funding: JIPMER intramural fund.

Received: 28 April 2024.
Accepted: 29 July 2024.
Early view: 12 September 2024.

Monaldi Archives for Chest Disease 2025; 95:3041
doi: 10.4081/monaldi.2024.3041

Abstract

Chronic obstructive pulmonary disease (COPD) is characterized by permanent airflow obstruction due to abnormalities of the airways and alveoli. This study investigated the potential role of Aspergillus species in acute exacerbations of COPD (AE-COPD) and evaluated the diagnostic utility of serum Aspergillus galactomannan antigen. This cross-sectional study, carried out at the Jawaharlal Institute of Postgraduate Medical Education and Research from January 2021 to June 2022, involved COPD patients aged ≥40 years. Serum galactomannan and serum Aspergillus-specific antibodies were analyzed, along with the collection of demographic details, symptoms, and comorbidities. Statistical analyses, including univariate analysis and receiver operating characteristic (ROC) curve analysis, were performed. Among the 61 recruited COPD patients, 24.5% showed serum galactomannan positivity. Significant associations were found between galactomannan positivity, hemoptysis, and previous tuberculosis. ROC analysis revealed modest diagnostic accuracy (area under the ROC=0.6027) with a sensitivity of 44.4% and a specificity of 83.7% at a cut-off of 0.5. Univariate analysis did not show any potential links between diabetes, hypertension, previous exacerbations, and severe Global Initiative for Chronic Obstructive Lung Disease stages with a risk of exacerbation. Serum galactomannan antigen showed limited sensitivity, and its routine testing may not be justified for predicting exacerbation risk. Further studies are warranted to validate these findings and explore other diagnostic methods using bronchoalveolar lavage galactomannan antigen in AE-COPD.

Introduction

Chronic obstructive pulmonary disease (COPD) is currently one of the top three causes of mortality globally, with low- and middle-income nations accounting for 90% of all deaths [1,2]. Smoking and indoor biomass exposure pose the greatest risk factors for the development of airflow limitation in developing countries [3]. Also, COPD patients are at risk due to tuberculosis infection, which leaves behind residual effects as sequelae, thereby causing tuberculosis-associated obstructive pulmonary airway disease [4]. It is widely acknowledged that many COPD exacerbations go unreported and untreated, despite often being shorter in duration [5]. Physicians need to understand what triggers symptoms and causes exacerbations in COPD patients to provide better

care; meanwhile, patients themselves should be educated on seeking timely medical attention [6,7].

While respiratory viral and bacterial infections are the main causes of COPD exacerbations, the role of fungal infections in exacerbating these events is not well understood. Additionally, environmental factors like extreme heat and air pollution can also play a significant role in causing or worsening exacerbations. Filamentous fungi, particularly Aspergillus species, may be present in the sputum samples of COPD patients during moderate or severe exacerbations, but their clinical significance remains unknown [8-10]. These findings indicate a potential involvement of Aspergillus species in the progression and prognosis of COPD. Nevertheless, there is a lack of research examining the correlation between Aspergillus species and acute exacerbations of COPD (AE-COPD).

The diagnosis of Aspergillus disease in clinical settings is often challenging. However, the measurement of Aspergillus galactomannan antigen in serum samples offers a practical diagnostic approach and has been widely employed [11]. Our study focused on determining if serum levels of Aspergillus galactomannan antigen can function as an indicator for evaluating the risk of AE-COPD. Additionally, the study aimed to assess the sensitivity and specificity of serum galactomannan in comparison to serum Aspergillus-specific antibodies in diagnosing pulmonary aspergillosis.

Materials and Methods

This descriptive cross-sectional study was conducted at the Jawaharlal Institute of Postgraduate Medical Education & Research (JIPMER) from January 2021 to June 2022. The study focused on patients aged 40 years or older diagnosed with AE-COPD, as defined by the Global Initiative for Chronic Obstructive Lung Disease (GOLD) [12]. Patients with pre-existing diseases such as asthma, bronchiectasis, interstitial lung disease, lung cancer, chronic kidney disease, and risk factors for invasive aspergillosis, such as those with immunosuppressed status like HIV infection, hematological malignancies, and long-term use of oral steroids or immunomodulators for more than 3 months, were excluded from the study.

Following ethical approval and obtaining written informed consent from participants, comprehensive demographic data, including smoking history, exposure to biomass fuel, past exacerbation history, and comorbidities, were documented. The methodology for patient recruitment and demographic data collection was adapted from our previously published study on the prevalence of chronic pulmonary aspergillosis (CPA) in COPD patients during acute exacerbation [13]. Symptomatology was assessed using the St. George Respiratory Questionnaire for COPD patients. All patients were subjected to sputum investigations, including gram staining, pyogenic culture, KOH staining, fungal culture, cytology, and acid-fast staining.

Venous blood samples were obtained within 2 hours or before any treatment administration in the emergency department for further investigations, including complete blood count with absolute eosinophil count, renal function test, liver function test, serum galactomannan, and Aspergillus immunoglobulin G (IgG) serology. The Bio-Rad Platelia Aspergillus Ag kit, a single-stage immunoenzymatic sandwich microplate assay employing the rat monoclonal antibody EBA-2, was utilized for the detection of serum galactomannan. Test outcomes were presented as index values, with a cut-off of 0.5 or higher considered positive. Aspergillus fumigatus IgG antibodies were assessed through a qualitative immunoenzymatic method utilizing the enzyme-linked immunosorbent assay technique. According to the manufacturer's recommendations, antibody concentrations below 5 AU/mL were categorized as negative, concentrations ranging between 5 and 10 AU/mL were considered intermediate, and concentrations equal to or exceeding 10 AU/mL were classified as positive.

For eligible patients, baseline chest X-rays (posterior-anterior view) and high-resolution computed tomography (HRCT) scans of the thorax during full inspiration were performed using a SIEMEN 6 slice CT scanner located in the Department of Radiodiagnosis at JIPMER. HRCT findings were evaluated by a single radiologist without blinding. Spirometry was conducted 6 weeks post-discharge, after patients had stabilized and were no longer in respiratory distress, using the Jaeger master screen pulmonary function test machine. Spirometry results were interpreted according to American Thoracic Society guidelines, with COPD severity categorized as per GOLD criteria [12].

Statistical analysis

The data were collected and organized in an Excel spreadsheet, and SPSS v19 (IBM, Armonk, NY, USA) was employed for the analysis. Categorical variables were presented as numbers and percentages, while continuous variables were described using means with standard deviations or medians with interquartile ranges, depending on the normality of their distribution, as assessed by the Shapiro-Wilk test. Associations between categorical variables and serum galactomannan were examined using either the chi-square test or Fisher's exact test. To identify risk factors for AE-COPD, univariate analysis was performed. Receiver operating characteristic (ROC) curve analysis was performed to determine the sensitivity and specificity of serum galactomannan. The significance level of $p<0.05$ was adopted, indicating statistical significance in the analyses conducted.

Results

During the study period from December 2020 to June 2022, a total of 98 patients underwent screening. A total of 37 patients were ineligible for the study for various reasons: 12 were unable to undergo spirometry during follow-up visits, 10 had concurrent asthma, 7 exhibited bronchiectasis on HRCT chest scans, and 8 were lost to follow-up. Consequently, the study recruited 61 patients within the same timeframe. A summary of the demographic characteristics and clinical symptoms of these participants is provided.

An overview of the baseline characteristics of AE-COPD patients is provided in Table 1, stratified by their serum galactomannan status. The patients with serum galactomannan antigen levels above 0.5 were considered as galactomannan-positive, and those with levels below 0.5 were considered as galactomannan-negative. Although the proportion of males was slightly higher in the galactomannan-positive group, the difference was not statistically significant ($p=0.216$). The predominant symptoms were breathlessness, followed by cough and expectoration. Notably, the presence of hemoptysis was significantly associated with serum galactomannan positivity (7% *vs*. 50%, $p<0.01$), indicating its potential as a marker for identifying AE-COPD patients with fungal infection. Additional factors such as biomass fuel exposure and underlying conditions like diabetes mellitus, systemic hypertension, and previous tuberculosis were also evaluated. Of these,

patients with previous tuberculosis show a significant difference between the two subgroups (28.26% *vs*. 24.59%, p=0.026), suggesting a potential association with serum galactomannan positivity. These patients had a temporal progression to COPD as a sequela of pulmonary tuberculosis.

Univariate analysis to assess risk association based on serum galactomannan antigen positivity

The results of univariate analysis to evaluate the risk with various factors in COPD are presented in Table 2. The analysis indicates that there was no significant difference in factors such as diabetes mellitus, hypertension, or recent use of inhalational steroids between the two groups. However, there seemed to be a trend towards an association between smoking and serum galactomannan positivity, although statistical significance was not achieved [odds ratio (OR)=1.08, 95% confidence interval (CI) 0.29-4.02, p=0.905]. Smoking can lead to chronic respiratory inflammation and epithelial damage, which might create an environment conducive to fungal colonization, which could increase serum galactomannan levels in smokers. Similarly, there was an association between serum galactomannan positivity and GOLD stage IV (OR=1.50, 95% CI 0.35-6.40, p=0.584), although it did not reach statistical significance.

The microbiological composition of sputum samples obtained from a subgroup of patients was assessed, revealing growth in 23 patients (37%). Among these patients, 22 (36.1%) exhibited growth in pyogenic culture, whereas fungal culture yielded positive results in one patient, indicating the presence of Aspergillus species (Table 3). The prevalent pathogens identified predominantly belonged to the Gram-negative group, encompassing *Pseudomonas aeruginosa* (31.8%), *Acinetobacter baumannii* (22.7%), *Enterobacteriaceae* (18.2%), *Klebsiella pneumoniae* (18.2%), and others (9%).

Interpretation of receiver operating characteristic curve analysis for serum galactomannan antigen and serum Aspergillus-specific immunoglobulin G antibody

In our study, the ROC analysis was conducted to compare the effectiveness of serum galactomannan antigen and serum

Table1. Baseline characteristics among serum galactomannan positive and negative in acute exacerbation of chronic obstructive pulmonary disease patients.

	Total	Serum galactomannan <0.5 (n=46) n (%)	Serum galactomannan >0.5 (n=15) n (%)	p
Demographics				
Male gender	51	40 (86.95)	11 (73.3)	0.216
Female gender	10	6 (13.04)	4 (26.67)	
Smoker	44	33 (71.74)	11 (73.3)	0.905
Biomass fuel exposure	30	23 (50)	7 (46.67)	0.823
Symptoms				
Cough	50	38 (82.61)	12 (80.0)	0.819
Expectoration	45	34 (73.91)	11 (73.33)	0.965
Chest pain	8	5 (10.8)	3 (20)	0.363
Hemoptysis	10	3 (6.5)	7 (46.6)	<0.01
Comorbidities				
Diabetes mellitus	29	25 (54.35)	4 (26.67)	0.062
Systemic hypertension	25	20 (43.48)	5 (33.3)	0.48
Previous tuberculosis	22	13 (28.26)	9 (60)	0.026
Stable period treatment				
Use of inhalational steroids	34	26 (56.52)	8 (53.3)	0.83
Previous admission history				
COPD exacerbation in previous year≥ 1	31	24 (52.17)	7 (46.67)	0.711

COPD, chronic obstructive pulmonary disease.

Table 2. Univariate analysis to assess risk association based on serum galactomannan antigen positivity in acute exacerbation of chronic obstructive pulmonary disease patients.

Variables	Serum galactomannan >0.5	Serum galactomannan <0.5	OR	CI	p
Smoker	11	33	1.08	0.29-4.02	0.905
Diabetes mellitus	4	25	0.30	0.08-1.10	0.070
Hypertension	5	20	0.65	0.19-2.20	0.489
GOLD stage III	4	20	0.60	0.13-2.85	0.521
GOLD stage IV	7	14	1.50	0.35-6.40	0.584
Use of inhalational steroids in last 3 months	8	26	0.884	0.27-2.83	0.829
Previous history of exacerbations in last 1 year	7	24	0.80	0.25-2.58	0.711

GOLD, global initiative for obstructive lung disease; OR, odds ratio; CI, confidence interval.

Aspergillus-specific IgG antibody in diagnosing pulmonary aspergillosis (PA) infection. The ROC analysis in Figure 1 shows the area under the ROC curve (AUC), which was 0.6027, suggesting modest diagnostic accuracy. With a cut-off value of 0.5, the sensitivity was 44.4%, and the specificity was 83.7% (Table 4). The sensitivity of serum galactomannan in our study was less than 50%, which is relatively low. However, the specificity was 83.7%, suggesting it might be useful for ruling out non-infected patients (true negatives). The positive predictive value (PPV) at this cut-off was 53.30% and the negative predictive value (NPV) was 78.30% (Table 4). Despite this, due to the low sensitivity, serum galactomannan cannot be recommended as a standalone diagnostic tool. The test might be more useful in a targeted patient population with a high suspicion of PA.

Discussion

The current study assessed the presence of serum Aspergillus galactomannan antigen in the AE-COPD patients requiring hospitalization and examined its associated risk factors. Serum galactomannan testing was conducted at the time of acute exacerbation, as mentioned in the methodology. In our study, 24.5% of AE-COPD patients showed a positivity for serum Aspergillus galactomannan antigen. Some patients also exhibited positivity for serum Aspergillus-IgG around 29.5% indicating prior sensitization or an immune response to Aspergillus. The significance of a positive Aspergillus galactomannan antigen in COPD remains uncertain; however, it may signify conditions such as moderate colonization or latent/inapparent infection rather than the mere presence of antigens in the bloodstream, independent of nonspecific elevations or false-positive results.

In the study conducted by Yashimoro *et al.* [14], the presence of serum Aspergillus galactomannan antigen was assessed in stable COPD patients, revealing that 40% of the participants tested positive for the galactomannan antigen with a value exceeding 0.5. Reports of false positives have also been documented. Hamaki *et al.* noted instances of false positives of serum galactomannan in patients with chronic graft *vs.* host disease post-bone marrow transplantation [15]. Recent progress in microbiome research has unveiled a wide variety of microbial species in the airways, previously undetected by conventional methods like mycotic cultures and antigen tests. Notably, Aspergillus species consistently emerge in the lower respiratory tract [16].

In our study, out of 61 patients, twenty-two were post-tuberculosis treatment. Positive serum galactomannan antigen was significantly associated with hemoptysis (Table 1).

Hemoptysis is recognized as a key symptom, prompting suspicion of various forms of PA, as outlined by Denning *et al*. [17]. Among AE-COPD patients with a previous history of pulmonary tuberculosis and hemoptysis presentation, HRCT thorax was assessed for the presence of cavities with fungal balls, pericavitatory fibrosis, or thickening. The role of *Aspergillus* spp. colonization in contributing to the heightened frequency and severity of exacerbations, or acting as a marker of more severe disease, remains unclear. In our study, 6 patients received antifungal treatment, 200 mg bd itraconazole for 6 months, as their computed

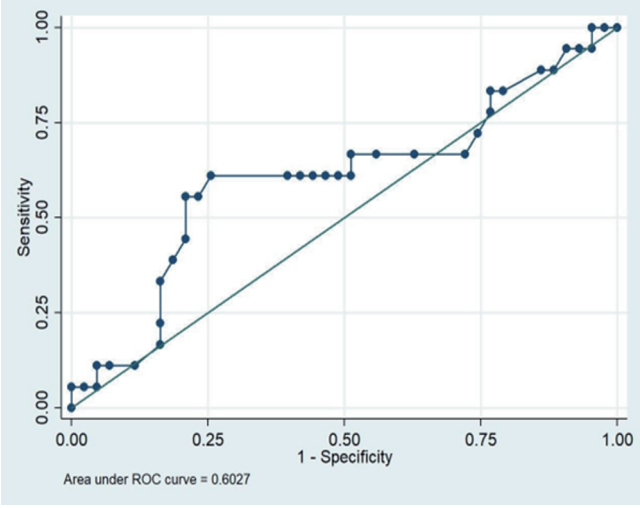

Figure 1. Receiver operating characteristic (ROC) analysis to evaluate the performance of serum galactomannan antigen and serum Aspergillus-specific immunoglobulin G antibody.

Table 3. Microbiological pattern of sputum sample isolated from patients with acute exacerbation of chronic obstructive pulmonary disease (n=23). Modified from: Palanivel *et al.* (2024).

Sputum	Number of study subjects	Percentage (%)
Pyogenic isolates	1) Polymicrobial growth (n=5) 2) Monomicrobial growth (n=17)	36.1
Fungal isolates	Aspergillus (n=1)	1.6
No organism	n=38	62.2

Table 4. Receiver operating characteristic analysis evaluating the performance of serum galactomannan antigen and serum Aspergillus-specific immunoglobulin G antibody with cut-off values, sensitivity, and specificity.

Variable	Area	Cut-off value	PPV	NPV
Serum galactomannan	0.6027	0.5	53.30%	78.30%
Cut-off value	Sensitivity	Specificity	Likelihood ratio +	Likelihood ratio -
0.4	61.11%	51.6%	1.2513	0.7601
0.5	44.4%	83.7%	2.0476	0.7963
0.6	22.22%	83.72%	1.3651	0.929

PPV, positive prediction value; NPV, negative prediction value.

tomography scans exhibited cavitary lesions with a fungal ball and serology positive for Aspergillus-specific IgG antibody, treated as per the European Society of Clinical Microbiology and Infectious Diseases guideline for PA.

Effectively managing COPD involves predicting and preventing AE-COPD. AE-COPD not only directly contributes to mortality but also indirectly affects the quality of life, worsens pulmonary function, and exacerbates symptoms [18]. Various risk factors for AE-COPD have been proposed, with a history of prior exacerbation emerging as the single most reliable predictor [18]. Indeed, the GOLD guidelines recommend considering a combination of factors, including history of exacerbation, history of hospitalization for exacerbation, and symptoms, to evaluate exacerbation risk.

Filamentous fungi, notably Aspergillus species, can be detected in sputum samples from patients experiencing moderate or severe exacerbations [8,9]. In our study, sputum cultures yielded positive growth in 22 individuals (36.1%). Aspergillus species were identified in the sputum of only one patient (1.63%) (Table 3). The low incidence of Aspergillus isolation may be attributed to the quality of sputum samples collected for culture, which could have been suboptimal, as well as the inherent difficulty in cultivating fungi in culture. Inhaled Aspergillus conidia bind to the airway surface through galactomannans, subsequently triggering the activation of the innate immune response. This activation can lead to persistent inflammation, potentially contributing to an increased incidence of AE-COPD in individuals with a high level of Aspergillus galactomannan antigen. In the study done by Yoshimura *et al*. [14], they proposed that serum Aspergillus galactomannan antigen can be used to determine the risk of exacerbation in patients with COPD. In our study, we performed univariate analysis to ascertain the risk between patients with increased serum galactomannan antigen and the normal serum galactomannan antigen group. Our study did not demonstrate a significant association between serum Aspergillus galactomannan antigen and AE-COPD with severe GOLD stages (Table 2).

Additionally, Aspergillus sensitization serves as an indicator of an elevated risk of exacerbations [19]. Notably, the use of high-dose inhaled corticosteroids and oral corticosteroids has been linked to Aspergillus colonization [8,20]. Sensitization to Aspergillus is associated with impaired lung function, although there are no reported associations with overall survival [9]. Smoking can lead to chronic respiratory inflammation and epithelial damage, creating an environment conducive to fungal colonization. The association between aspergillosis and smoking has been reported in case studies involving immunocompetent patients [21]. Although our study did not find a statistically significant difference, further studies with larger sample sizes could help elucidate the potential link between smoking and increased serum galactomannan levels.

Receiver operating characteristic curve analysis to estimate the diagnostic accuracy of serum galactomannan antigen

Diagnosing PA poses challenges due to the limited sensitivity of conventional methods like culture and cytology for Aspergillus detection. In an effort to enhance sensitivity, the exploration of galactomannan antigen detection has been undertaken. The detection of serum-specific Aspergillus IgG antibodies is indeed a significant diagnostic tool for aspergillosis, particularly CPA, and the sensitivity and specificity of this test for CPA diagnosis are quite satisfactory [22]. However, the efficacy of galactomannan antigen in diagnosing PA beyond invasive PA remains uncertain.

Kono *et al.*, in their study, compared the efficacy of serum and bronchial wash (BW) galactomannan antigen in diagnosing PA and found that BW galactomannan antigen was a better diagnostic tool for PA than serum galactomannan antigen. The AUC for serum galactomannan antigen was 0.41, and for BW galactomannan antigen was 0.89. BW galactomannan antigen had a sensitivity and specificity of 85.7% and 76.3% at a cut-off level of ≥0.5, whereas serum galactomannan antigen showed a sensitivity of only 14.7% [23]. Comparing serum galactomannan testing with bronchoalveolar lavage (BAL) fluid, the BAL fluid revealed a more acceptable sensitivity of 77.2% and specificity of 77% with a cut-off of 0.4, as mentioned by Izumikawa *et al*., who summarized that BAL galactomannan antigen would be better for diagnosing CPA [24].

The previous studies so far evaluated the precision of serum GM antigen for diagnosing PA by comparing it with BW GM antigen. In our study, we used serum-specific Aspergillus IgG antibody as a gold standard test for diagnosing PA and compared the diagnostic accuracy of serum GM antigen [11,25,26]. We found that 15 patients (24.6%) with AE-COPD were positive for serum Aspergillus galactomannan antigen, taking a cut-off of ≥0.5. The AUC analysis obtained for serum galactomannan antigen was 0.6027 (30%) (Figure 1) with 95% CI, with the sensitivity and specificity of 44.4% and 83.7% respectively, for the cut-off value of ≥0.5. The specificity did not alter by increasing the cut-off (Table 4).

Although serum assays for galactomannan Aspergillus antigen are not as satisfactory, with a PPV of 53.30% but they do have an NPV of 78.3%. More intensive infection diagnostics and imaging will frequently be required for post-tubercular COPD patients to rule out fungal etiology. The best results were observed for Aspergillus antibody detection in serum, Aspergillus PCR, and culture testing of respiratory samples. Hence, we recommend that further studies should be directed to explore Aspergillus association in post-tubercular COPD patients and to evaluate the utility of BAL galactomannan in predicting the severity of COPD.

Our study has some limitations; this is a single-center, descriptive study with a modest sample size, with an absolute precision of 10%. Potential bias exists in the subject selection. Therefore, more cohort studies are required. Second, patients with fungal balls on computed tomography scans, suspected of PA, were not excluded, potentially confounding the serum galactomannan levels and leading to false positives. Third, we only performed serum galactomannan antigen, whereas Aspergillus galactomannan antigen in BAL is more sensitive [27]. Fourth, the study did not individually establish whether fungal sensitization, bacterial infection, or inadequate medication use were distinct causes for exacerbations. This presents a limitation in the analysis as it does not offer a separate assessment of each potential contributing factor.

Conclusions

The significant association between serum galactomannan positivity and hemoptysis, as well as a history of tuberculosis, highlights the need for careful clinical evaluation and the need for targeted diagnostic approaches. Given the complexity and multifactorial nature of COPD exacerbations, our findings suggest that routine serum galactomannan testing may not be justified for predicting risk factors for exacerbation. This indicates the necessity for more extensive cohort studies to validate these findings and

other factors in COPD exacerbations to enhance management strategies. It would be worthwhile to assess potential values of Aspergillus galactomannan antigen levels in BAL for the predictive markers of AE-COPD, particularly those with a tuberculosis history.

References

1. Halpin DMG, Celli BR, Criner GJ, et al. The GOLD Summit on chronic obstructive pulmonary disease in low- and middle-income countries. Int J Tuberc Lung 2019;23:1131-41.
2. Meghji J, Mortimer K, Agusti A, et al. Improving lung health in low-income and middle-income countries: from challenges to solutions. Lancet 2021;397:928-40.
3. Vestbo J, Edwards LD, Scanlon PD, et al. Changes in forced expiratory volume in 1 second over time in COPD. N Engl J Med 2011;365:1184-92.
4. Jain NK. Chronic obstructive pulmonary disease and tuberculosis. Lung India 2017;34:468-9.
5. Vijayasaratha K, Stockley RA. Reported and unreported exacerbations of COPD: analysis by diary cards. Chest 2008;133: 34-41.
6. Wilkinson TMA, Donaldson GC, Hurst JR, et al. Early therapy improves outcomes of exacerbations of chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2004;169: 1298-303.
7. White AJ, Gompertz S, Stockley RA. Chronic obstructive pulmonary disease. 6: the aetiology of exacerbations of chronic obstructive pulmonary disease. Thorax 2003;58:73-80.
8. Bafadhel M, Mckenna S, Agbetile J, et al. Aspergillus fumigatus during stable state and exacerbations of COPD. Eur Respir J 2014;43:64-71.
9. Huerta A, Soler N, Esperatti M, et al. Importance of Aspergillus spp. isolation in acute exacerbations of severe COPD: prevalence, factors and follow-up: the FUNGI-COPD study. Respir Res 2014;15:17.
10. Hammond EE, McDonald CS, Vestbo J, Denning DW. The global impact of Aspergillus infection on COPD. BMC Pulm Med 2020;20:241.
11. Mennink-Kersten MA, Donnelly JP, Verweij PE. Detection of circulating galactomannan for the diagnosis and management of invasive aspergillosis. Lancet Infect Dis 2004;4:349-57.
12. Celli BR, Fabbri LM, Aaron SD, et al. An updated definition and severity classification of chronic obstructive pulmonary disease exacerbations: the Rome proposal. Am J Respir Crit Care Med 2021;204:1251-8.
13. Palanivel J, Mohapatra MM, Rajaram M, et al. Prevalence and risk factors for chronic pulmonary aspergillosis in chronic obstructive pulmonary disease patients with acute exacerbations. Monaldi Arch Chest Dis 2024. doi: 10.4081/monaldi.2024.2927.
14. Yoshimura K, Suzuki Y, Inoue Y, et al. Utility of serum Aspergillus-galactomannan antigen to evaluate the risk of severe acute exacerbation in chronic obstructive pulmonary disease. PLoS One 2018;13:e0198479.
15. Hamaki T, Kami M, Kanda Y, et al. False-positive results of Aspergillus enzyme-linked immunosorbent assay in a patient with chronic graft-versus-host disease after allogeneic bone marrow transplantation. Bone Marrow Transplant 2001;28: 633-4.
16. Nguyen LDN, Viscogliosi E, Delhaes L. The lung mycobiome: an emerging field of the human respiratory microbiome. Front Microbiol 2015;6:89.
17. Denning DW, Cadranel J, Beigelman-Aubry C, et al. Chronic pulmonary aspergillosis: rationale and clinical guidelines for diagnosis and management. Eur Respir J 2016;47:45-68.
18. Bongomin F, Gago S, Oladele RO, Denning DW. Global and multi-national prevalence of fungal diseases-estimate precision. J Fungi 2017;3:57.
19. Tiew PY, Ko FWS, Pang SL, et al. Environmental fungal sensitisation associates with poorer clinical outcomes in COPD. Eur Respir J 2020;56:2000418.
20. Barberan J, Sanz F, Hernandez JL, et al. Clinical features of invasive pulmonary aspergillosis vs. colonization in COPD patients distributed by gold stage. J Infect 2012;65:447-52.
21. Pourbaix A, Lafont Rapnouil B, Guéry R, et al. Smoking as a risk factor of invasive fungal disease: systematic review and meta-analysis. Clin Infect Dis 2020;71:1106-19.
22. Mei ZX, Han JF, Yu HW, et al. Detection of serum Aspergillus-specific IgM and IgG antibody levels for the diagnosis of chronic pulmonary aspergillosis developed in patients with tuberculosis. Eur J Clin Microbiol Infect Dis 2023;42: 1081-9.
23. Kono Y, Tsushima K, Yamaguchi K, et al. The utility of galactomannan antigen in the bronchial washing and serum for diagnosing pulmonary aspergillosis. Respir Med 2013;107: 1094-100.
24. Izumikawa K, Yamamoto Y, Mihara T, et al. Bronchoalveolar lavage galactomannan for the diagnosis of chronic pulmonary aspergillosis. Med Mycol 2012;50:811-7.
25. Yu Q, He J, Xing B, et al. Potential value of serum Aspergillus IgG antibody detection in the diagnosis of invasive and chronic pulmonary aspergillosis in non-agranulocytic patients. BMC Pulm Med 2020;20:89.
26. Baxter CG, Denning DW, Jones AM, et al. Performance of two Aspergillus IgG EIA assays compared with the precipitin test in chronic and allergic aspergillosis. Clin Microbiol Infect 2013;19:E197-204.
27. Park SY, Lee SO, Choi SH, et al. Serum and bronchoalveolar lavage fluid galactomannan assays in patients with pulmonary aspergilloma. Clin Infect Dis 2011;52:e149-52.