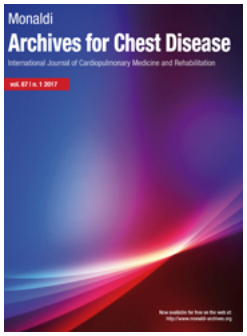

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Exploring the role of Aspergillus galactomannan antigen in assessing the risk factor of acute exacerbations in chronic obstructive pulmonary disease patients: a cross-sectional study

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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 (AECOPD) 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 gold 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 AECOPD.

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

Introduction

Chronic obstructive pulmonary disease (COPD) is currently one of the top three causes of mortality globally, with low-00 and middle-income nations accounting for 90% of all deaths [1,2]. Smoking and indoor biomass exposure pose the greatest risk factors for the developing airflow limitation in developing countries [3]. Also, COPD patients are at risk due to tuberculosis (TB) 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 cause 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 also can 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 acute exacerbations of COPD (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 out at 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 chronic obstructive pulmonary disease (COPD) with acute exacerbation, 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 in COPD patients during acute exacerbation [13]. Symptomatology was assessed using the St. George Respiratory Questionnaire for COPD patients (SGRQ-C). All patient 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 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 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 ELISA 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 PFT 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 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. 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. Thirty-seven 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 AECOPD 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 less than 0.5 were considered as galactomannan-negative. Although the proportion of males were slightly higher in the galactomannan positive group, the difference was not statistically significant ($p = 0.216$). The predominant symptoms were breathlessness followed 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 AECOPD 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 shows 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 the 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 (OR = 1.08, 95% 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 possibly increase serum galactomannan level 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 IgG antibody

In our study, the Receiver Operating Characteristic (ROC) analysis was conducted to compare the effectiveness of serum galactomannan antigen and Serum Aspergillus-specific IgG Antibody in diagnosing pulmonary aspergillosis 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 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 pulmonary aspergillosis.

Discussion

The current study assessed the presence of serum Aspergillus galactomannan antigen in the acute exacerbation of chronic obstructive pulmonary disease (AECOPD) patients requiring hospitalization and examined its associated risk factors. Serum Galactomannan testing was conducted at the time of acute exacerbation as mentioned in methodology. In our study, 24.5% of AECOPD 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 chronic obstructive pulmonary disease (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 versus 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 pulmonary aspergillosis, as outlined by Denning et al. [17]. Among AECOPD 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, six patients received antifungal treatment 200mg bd itraconazole for 6 months as their

CT scans exhibited cavitary lesions with fungal ball and serology positive for Aspergillus specific IgG antibody, treated as per ESCMID guideline for pulmonary aspergillosis.

Effectively managing COPD involves predicting and preventing acute exacerbations of COPD (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 acute exacerbations of COPD (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 risk between patients with increased serum galactomannan antigen and normal serum galactomannan antigen group. Our study did not demonstrate 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 (ICS) 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 pulmonary aspergillosis 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 chronic pulmonary aspergillosis (CPA) and sensitivity and specificity of this test for CPA diagnosis are quite satisfactory [22]. However, the efficacy of galactomannan antigen in diagnosing pulmonary aspergillosis beyond invasive pulmonary aspergillosis (IPA) remains uncertain.

Kono et al. in their study, compared the efficacy of serum and bronchial wash (BW) galactomannan antigen (GA) in diagnosing Pulmonary aspergillosis (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 sensitivity and specificity of 85.7% and 76.3% at a cut-off level of ≥0.5 whereas serum galactomannan antigen showed 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., and he summarized that bronchoalveolar lavage galactomannan antigen would be better for diagnosing chronic pulmonary aspergillosis [24].

The previous studies so far evaluated the precision of serum GM antigen for diagnosing Pulmonary aspergillosis (PA) by comparing it with bronchial washing GM antigen. In our study, we used serum-specific aspergillus IgG antibody as a gold standard test [11,25,26] for diagnosing PA and compared the diagnostic accuracy of serum GM antigen. We found that 15 patients (24.6%) with acute exacerbation of COPD were positive for serum Aspergillus galactomannan antigen taking cut off as ≥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 positive predictive values of 53.30% but they do have negative predictive values of 78.3%. More intensive infection diagnostics and imaging will frequently be required for post tubercular COPD patients to rule out fungal aetiology. 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 severity of COPD.

Our study has some limitations, this is a single centred, descriptive study with modest sample size with absolute precision of 10%. Potential bias exists in the subject selection. Therefore, more cohort studies are required. Second, patients with fungal balls on CT scans, suspected of pulmonary aspergillosis, were not excluded, potentially confounding the serum galactomannan levels and leading to false positives. Third, we only performed serum galactomannan antigen while 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 bronchoalveolar lavage for the predictive markers of AE-COPD, particularly those with a tuberculosis history.

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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)	Serum galactomannan >0.5 (n=15)	p-value
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

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-value
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.

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 (%)
A) Pyogenic isolates	1) Polymicrobial growth (n=5) 2) Monomicrobial growth (n=17)	36.1
B) Fungal isolates	Aspergillus (n=1)	1.6%
C) No organism	n=38	62.2%

Table 4. Receiver operating characteristic analysis evaluating the performance of serum galactomannan antigen and serum aspergillus specific IgG 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.

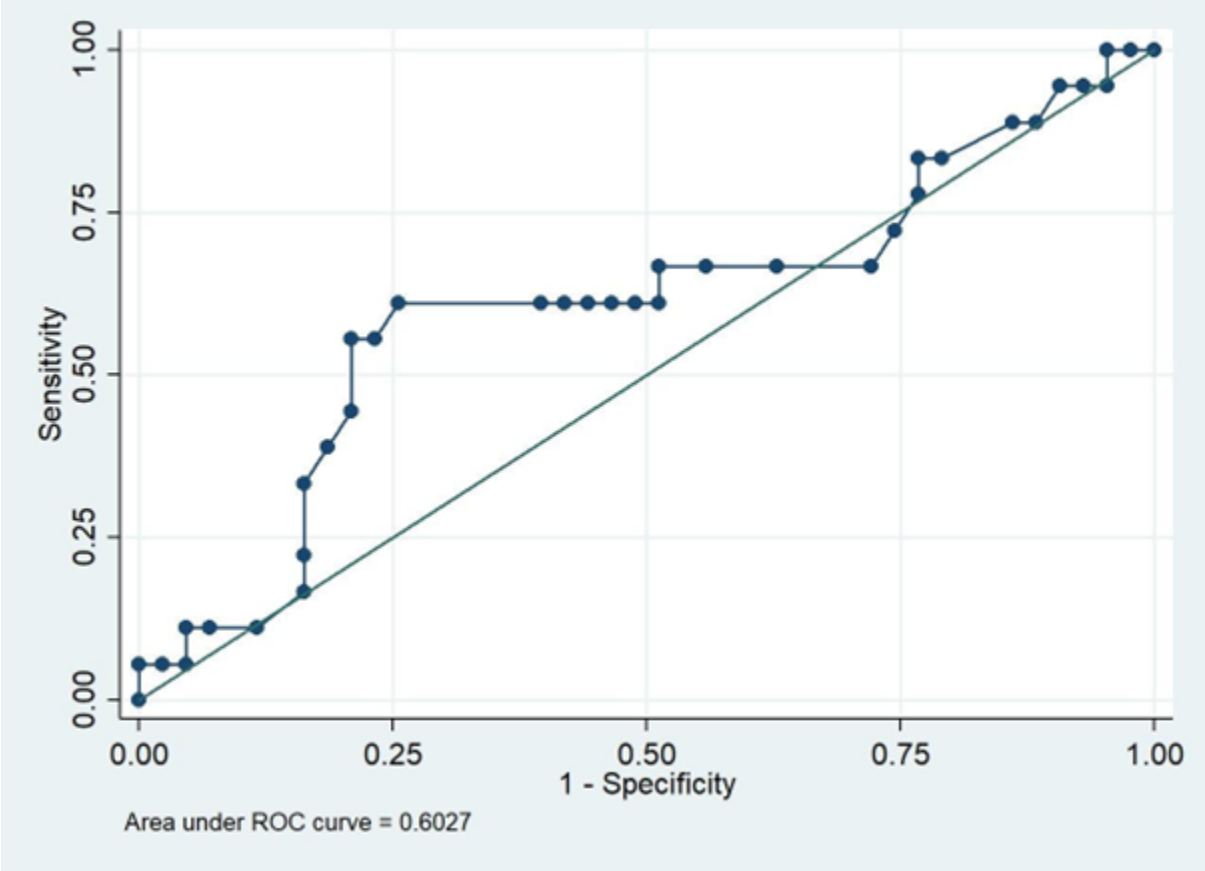

Figure 1. Receiver operating characteristic analysis to evaluate the performance of serum galactomannan antigen and serum aspergillus specific IgG antibody.