

RESEARCH ARTICLE

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Galactomannan Antigen Assay for the Diagnosis of Invasive Aspergillosis: A Study from a Tertiary Care Hospital of Central India

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Abstract

Invasive Aspergillosis (IA) is a severe and fatal infection, especially in immunocompromised patients. Galactomannan is a polysaccharide antigen present in the cell wall of *Aspergillus* species, which is secreted into the blood and other body fluids during hyphal growth. Therefore, detecting galactomannan antigen is very useful in diagnosing IA, along with clinical features and radiological findings. The study period was one year (January 2022 to December 2022). The data was collected retrospectively from the medical records and case sheets of all clinically suspected invasive aspergillosis patients. Galactomannan antigen assay was performed using an FDA-approved Platelia *Aspergillus* EIA test kit, and results were interpreted according to the manufacturer's instructions (Cut off > 0.5). A total of 236 clinically suspected Invasive Aspergillosis cases were enrolled in the study. Galactomannan positivity was predominantly seen in patients aged 40 – 60 years, with male preponderance. Of 236 patients, 14.40% were immunocompromised, and 85.59% were immunocompetent. According to EORTC/MSG definitions, we got one proven IA case, 21 probable cases (8.89%), and nine (3.81%) possible cases. In immunocompetent individuals also, we observed 48.72% (115/236) galactomannan positivity, especially in old Pulmonary Tuberculosis (PTB) patients. Galactomannan positivity was higher in Broncho alveolar lavage (BAL) samples (n=70, 85.36%) than in serum samples (n=77, 46.67%). We found culture positivity of 14.06%, with *Aspergillus fumigatus* being the commonest isolate, followed by *Aspergillus flavus*. There is increased positivity of galactomannan in BAL samples compared to serum; hence BAL is a better specimen for diagnosis of Invasive Pulmonary Aspergillosis (IPA).

Keywords: BAL, Galactomannan, Invasive Aspergillosis

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Citation: Keche A, Patil G, Ganga RT, Sahu D. Galactomannan Antigen Assay for Diagnosis of Invasive Aspergillosis: A Study from a Tertiary Care Hospital of Central India. J Pure Appl Microbiol. Published online 28 March 2024. doi: 10.22207/JPAM.18.2.01

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INTRODUCTION

Aspergillus is a ubiquitous saprophytic fungus causing a variety of clinical syndromes. The spectrum ranges from invasive aspergillosis (IA), chronic pulmonary aspergillosis (CPA), and aspergilloma to various allergic manifestations. The interaction between the pathogen and host immunity determines the spectrum of clinical presentation. IA is seen in patients with immune dysfunction, like solid organ and hematopoietic stem cell transplant recipients, and patients on cancer chemotherapy and immunosuppressants.¹

In recent years, the prevalence of IA has also increased in immunocompetent hosts with previously damaged lungs, like chronic obstructive pulmonary disease (COPD), old pulmonary tuberculosis, and bronchiectasis.² Early diagnosis of IA is difficult since the radiological and clinical features are often nonspecific and occur late in the course of the disease.³

Galactomannan (GM) is a cell wall polysaccharide antigen released by *Aspergillus* hyphae during growth.⁴ It is released during growth and invasion. GM can be detected in blood and other body fluids since it is water soluble and released even in the early stages of *Aspergillus* invasion.⁵ Recently, GM antigen detection has been included in the diagnostic criteria of IA by EORTC/MSG guidelines 2020.⁶

This study was conducted to determine the utility of galactomannan antigen in the diagnosis of IA.

MATERIALS AND METHODS

This study was conducted from January 2022 to December 2022 at the Microbiology department of the tertiary care teaching hospital of Central India. Data was collected retrospectively from the medical records of the patients. All clinically suspected patients of invasive aspergillosis were included in the study. Patients already on mold-active antifungals at the time of specimen collection were excluded from the study.

The demographic, clinical, and radiological details were recorded for all enrolled patients. Also, the results of mycological investigations like culture and galactomannan antigen tests were recorded. A total of 236 clinically suspected

Invasive Aspergillosis cases were enrolled in the study. Of this, 54.66% were males (n=129), and 45.34% were females (n=107). Patients range from neonates (10 days) to older people (83 years). Fungal culture of BAL specimens were done on Sabouraud dextrose agar and incubated at 25°C and 37°C for four weeks. Fungal culture was requested for 128 patients. Galactomannan antigen testing was requested for 165 serum and 82 BAL samples (for 11 patients, both BAL and serum samples were tested). Galactomannan antigen assay was performed using an FDA-approved Platelia *Aspergillus* EIA (Bio-Rad, Vienna, Austria) test kit, and results were interpreted according to the manufacturer's instructions (Cut off > 0.5).

All the enrolled patients were categorized into proven IA, probable IA, and possible IA as per definitions of EORTC/MSG.⁶

Statistical analysis

All data collected were entered and analyzed using Microsoft Excel. Data were expressed as percentages. Sensitivity and specificity were calculated using OpenEpi software.

RESULTS

Galactomannan positivity was predominantly seen in patients from 41-60 years, followed by 21-40 years with male preponderance (Male-61% vs female-39%).

We have analyzed specificity and sensitivity only for serum samples in immunocompromised patients since they were the only patients we could categorize as having proved, probable, or suspected IA according to EORTC/MSG definitions. In our study, sensitivity for serum galactomannan was 75%, and specificity was 100% against diagnosis by EORTC/MSG guidelines. We calculated only positivity for BAL galactomannan since we received very few BAL samples from immunocompromised hosts. We got 85% galactomannan positivity in BAL samples. Of 236 patients, 14.40% were immunocompromised, and 85.59% were immunocompetent. According to EORTC/MSG definitions, one patient had IA, 21 probable IA (8.89%), and nine possible IA (3.81%) (Figure 1). The proven IA patient had undergone surgery for renal calculi. Immunocompromised

patients in the study consisted of the majority (29.41%) of hematological malignancy, followed by corticosteroids use (25%), neutropenia (8.82%) and solid organ transplant (5.88%). We got some immunocompromised conditions not included in EORTC/MSG criteria, like solid organ malignancy (11.76%) and acquired immunodeficiency syndrome (8.82%) (Figure 2).

Among immunocompetent individuals, 48.72 % (115/236) and 36.44 % (86/236) patients were galactomannan positive and negative, respectively. The common risk factors in this group

were old Pulmonary Tuberculosis (PTB), post-COVID, diabetes mellitus (DM), chronic kidney disease (CKD), and admission to the intensive care unit (ICU) (Figure 3). We observed that all these risk factors were more associated with galactomannan positivity.

Radiological abnormalities on the CT chest were analyzed only for galactomannan-positive patients as shown in Table 1. Galactomannan antigen positivity was relatively more in BAL specimen (85.36%) as compared to serum specimen (46.67%) (Table 2). Fungal culture

Table 1. Radiological abnormalities on CT chest of galactomannan positive patients

Radiological Abnormality	BAL Galactomannan positives (n = 71)	Serum Galactomannan positives (n = 66)	Total *(n = 137)
Well Circumscribed lesions with or without a halo sign	05	-	5 (3.64%)
Air crescent sign	03	02	5 (3.64%)
Consolidation	09	07	16 (11.67%)
Cavity	22	05	27 (19.7 %)
Cystic lesions	05	04	9 (6.56%)
Opacities	06	09	15 (10.94%)
CT not available / not done	21	39	60 (43.79%)

* Note: Radiological abnormalities were analyzed only in 137 galactomannan antigen positive patients

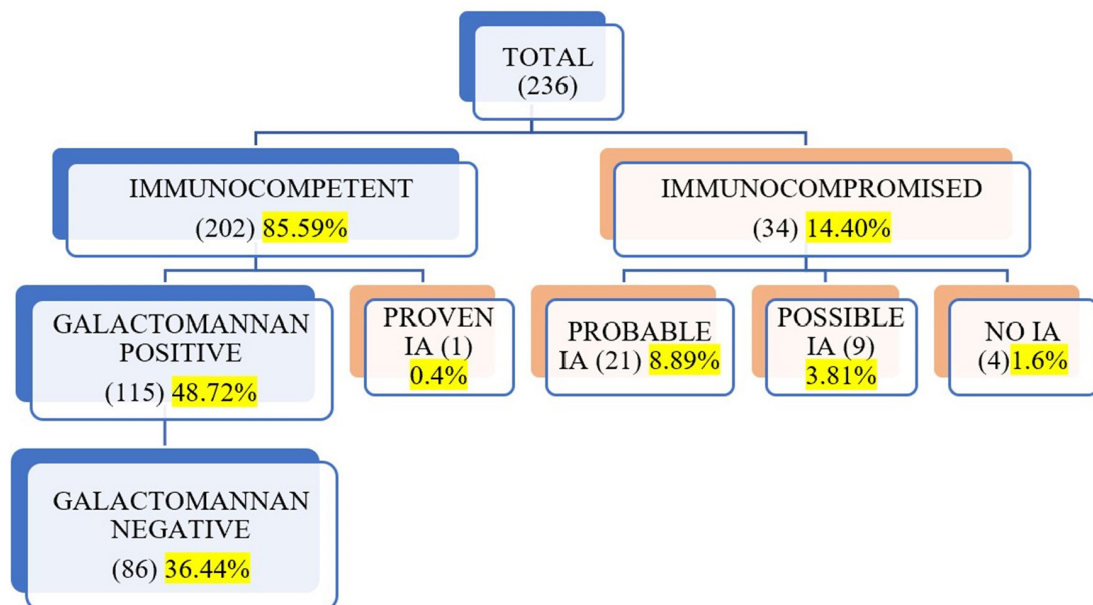


Figure 1. Categorization of patients according to EORTC/MSG definition

was requested for only 128 patients. We found culture positivity of 14.06%, with *Aspergillus fumigatus* being the commonest isolate, followed by *Aspergillus flavus* (Figure 4). A comparison of galactomannan antigen and culture positivity is shown in Table 3.

DISCUSSION

Invasive aspergillosis is a life-threatening infection in the immunocompromised as well as immunocompetent patients. Its diagnosis is challenging because of nonspecific clinical and

radiological features and also due limitations of fungal culture. Galactomannan Antigen detection, a relatively rapid and noninvasive test, plays a vital role in diagnosis at an early stage of infection. Recently WHO released a priority list for fungal pathogens in which *Aspergillus fumigatus* is included in a critical priority group.⁷

Male preponderance was noted in the current study, similar to Malhotra *et al.*,⁸ and Khanna *et al.*⁹ Males who participate in outdoor activities more frequently are more likely to contract a fungus. There were 3.22%, 67.74%, and 29.03% of proven, probable, and possible IA

Table 2. Comparison of galactomannan positivity in serum and BAL samples

Specimen	Galactomannan antigen tests results	
	Positive (%)	Negative (%)
Serum (n=165)	77 (46.67)	88 (53.54)
BAL (n=82)	70 (85.36)	12 (14.63)

Table 3. Comparison of culture results and galactomannan positivity

Culture	Galactomannan Ag Test		Total
	Positive	Negative	
Positive	15 (11.71%)	3 (2.34%)	18
Negative	76 (59.37%)	34 (26.56%)	110
Total	91	37	128

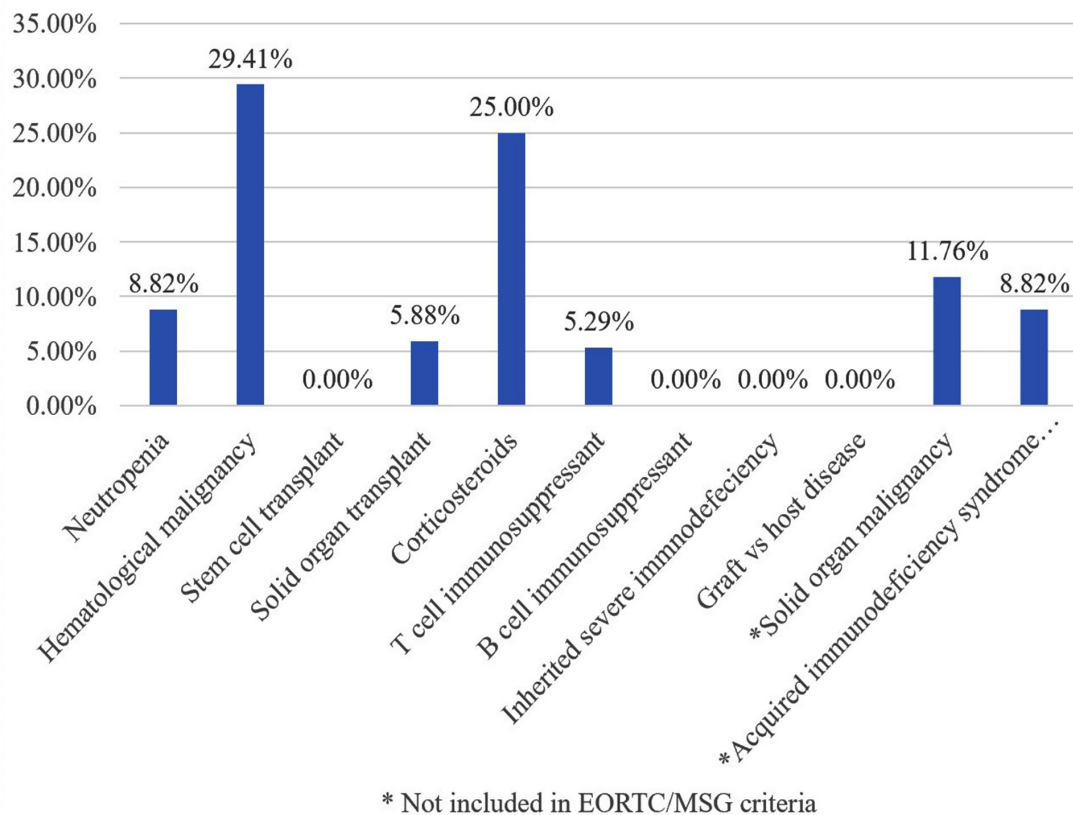
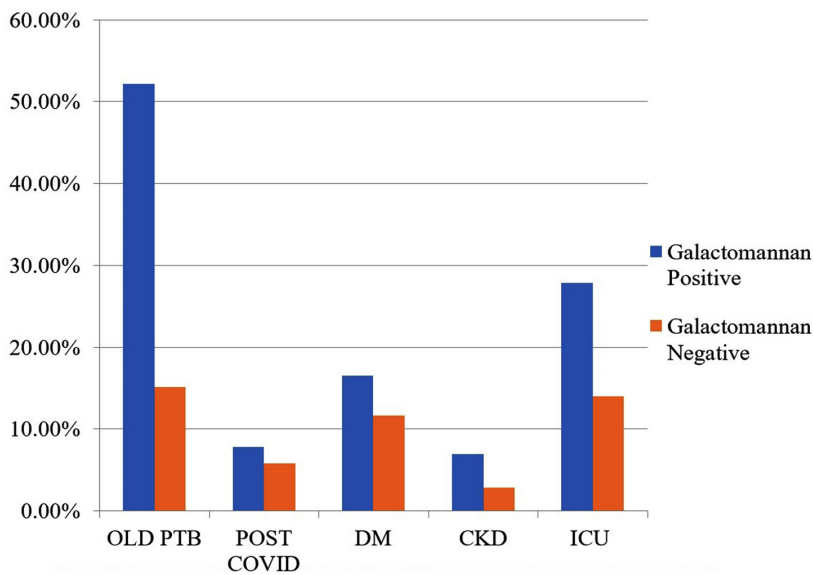


Figure 2. Host factors according to EORTC/MSG Criteria in immunocompromised patients

cases, respectively. Mohindra *et al.* found a similar prevalence (Proven IA - 1.8%, Probable IA - 69.37%, and Possible IA - 2.83%).¹⁰ Khanna *et al.* observed a relatively higher percentage of proven IA cases (14.8%) and a lower prevalence of probable IA cases (48.2%).⁹

Amongst the immuno-compromised host, the maximum number of patients were of hematological malignancy (29.41%), followed by

corticosteroids use (25%), neutropenia (8.82%) and solid organ transplant (5.88%). There were a few immunocompromised conditions not included in EORTC/MSG criteria, like solid organ malignancy (11.76%) and acquired immunodeficiency syndrome (8.82%). In a study by Singh *et al.*, most patients had hematological malignancy (60.93%), followed by solid organ transplant (26.56%).¹¹ The common risk factors observed by Vazquez JA *et*



Note: PTB-Pulmonary tuberculosis; COVID-Coronavirus (SARS CoV-2) disease; DM-Diabetes mellitus; CKD-chronic kidney disease; ICU-Intensive care unit

Figure 3. Comparison of risk factors in galactomannan positive and negative immunocompetent patients

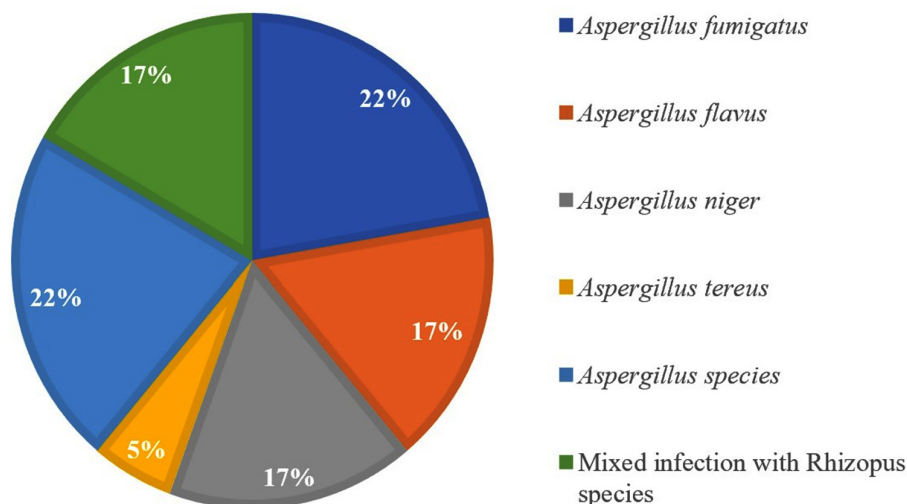


Figure 4. Culture results of various *Aspergillus* species

*al.*¹² were the use of immunosuppressants (39%), solid organ transplant (33%), malignancy (22%), etc. A study from Pune reported 48.75% cases of hematological malignancy, followed by solid organ tumors (12.5%) and organ transplant recipients (2.5%).¹³ All these patients were neutropenic and immunocompromised due to chemotherapy and immunosuppressive drugs, hence more prone to Invasive Aspergillosis.

In our study, the most common radiological abnormality was cavitation (19.70%), followed by consolidation (11.67%), nodules (3.64%), and air crescent sign (3.64%). S. Raveendran *et al.* reported nodules in 38.7%, consolidation in 19.4%, and crescent sign followed by cavitations in 29% of IA cases.¹⁴ In the study by Tong *et al.*, the radiological abnormalities commonly seen were consolidation (60.7%), nodules (50.4%), cavity (39.3%), opacity (17.1%), and the halo sign (12.0%).¹⁵ These radiological findings are nonspecific; galactomannan plays a crucial role in diagnosing IA. In the medical records of 43.79% of patients, either the chest CT scans were not performed or reports were not accessible. In 43.79% of patients, chest CT chest was either not done or reports were unavailable in medical records. Of these, 29.41% of patients were children with hematological malignancy without a CT scan.

Among immunocompetent patients, BAL and serum galactomannan positivity was 85.52% and 42.75%, respectively. Similarly, Zhou W *et al.* observed that among nonneutropenic patients, the sensitivity of galactomannan in BAL and serum samples was 75.68% and 37.84%, respectively.¹⁶ Among old PTB and post-COVID cases, galactomannan positivity was 52.17% and 55.56%, respectively. Yusuf *et al.* reported galactomannan positivity in BAL samples between 10% and 28% and in serum samples between 0.9% to 6.7% from post-COVID patients.¹⁷ In research by Zhou W *et al.*, out of 37 IA cases (diagnosed by EORTC/MSG), nine (24.32%) had pulmonary tuberculosis.¹⁶ In a study by Sivasankari *et al.*, among 80 PTB patients, 8 (10%) were IA cases and were diagnosed by culture and PCR.¹⁸ Invasive Aspergillosis cases are increasing in immunocompetent patients like old PTB and post-COVID patients due to previous lung damage. Any recent change in symptoms or radiological findings should not be ignored in such patients and can be

supplemented with a galactomannan antigen test to diagnose IA.

We have analyzed specificity and sensitivity only in immunocompromised patients since they were the only patients we could categorize as having proved, probable, or suspected IA according to EORTC/MSG definitions. In our study, sensitivity for serum galactomannan was 75%, and specificity was 100% against diagnosis by EORTC/MSG guidelines. Singh *et al.*¹¹ observed relatively high sensitivity and specificity (both 90%), and Zhou W *et al.*¹⁶ observed relatively low sensitivity (37.8%) and specificity (87.1%). We calculated only positivity for BAL galactomannan since we received very few BAL samples from immunocompromised hosts. We got 85% galactomannan positivity in BAL samples, similar to that of Singh *et al.* (90%).¹¹

Galactomannan can be detected in serum, BAL & CSF in invasive aspergillosis. It is also successfully detected in sputum, endotracheal aspirate, plasma, urine, and fluid from abscesses. Nuh *et al.*¹⁹ performed a galactomannan antigen test on sputum samples, demonstrating its utility in diagnosing chronic pulmonary aspergillosis. Sputum GM showed sensitivity and specificity of 70% and 71%, respectively, compared to clinical diagnosis. When sputum GM was tested against *Aspergillus* culture/IgG, a sensitivity of 77% and specificity of 78% were discovered. Hence sputum can also be a suitable sample for GM testing in critically ill patients unable to tolerate bronchoscopy procedures.

Culture positivity (14.06%) observed in the present study is similar to that of Mohindra *et al.* (17.22%)¹⁰ and Kaur *et al.* (19.4%).²⁰ However, Roohani *et al.* (31.11%)²¹ and Khanna *et al.* (59.25%)⁹ observed relatively more culture positivity. Our 2.34% patients were culture positive and galactomannan negative. This is probably due to colonization. Also, our 59.37% patients were culture negative and galactomannan positive. Sensitivity of fungal culture in diagnosing IA depends on the population tested and the prevalence of infection in that area, and thus it is higher among immunocompromised patients and in endemic areas.²² The most common isolated species in the present study was *A. fumigatus*, followed by *A. flavus*, similar to the findings of Roohani *et al.*²¹ However, many other researchers

got *A. flavus* most common pathogen.^{9,10,20} A fungal culture is less sensitive and takes time, and there are also chances of contamination during processing. Here galactomannan has a significant role along with chest CT for diagnosing IA.

In the present study, there were 15 patients (10.94%) with galactomannan antigen positive, but they were not immunocompromised and had no radiological abnormality. These patients may be considered false positives. Of these 15 patients, nine were on piperacillin-tazobactam, and five were on amoxicillin-clavulanic acid. Previous researchers also found galactomannan false positivity with beta-lactam antibiotics like piperacillin-tazobactam and amoxicillin-clavulanic acid.²³⁻²⁵ Hence, physicians should cautiously interpret galactomannan results in such patients.

CONCLUSION

BAL samples are better for diagnosis of Invasive Pulmonary Aspergillosis. Galactomannan positivity was seen in both immunocompromised and immunocompetent individuals. PTB is the most common risk factor for GM positivity in immunocompetent patients. Hence, PTB may be considered for diagnosing IPA in TB-endemic regions. More prospective studies are required to evaluate the performance of the galactomannan antigen test for diagnosing IPA in immunocompetent hosts, especially in PTB patients. GM detection can prove an excellent diagnostic test for IPA, but a panel of tests should be implemented in patients suspected of pulmonary aspergillosis.

ACKNOWLEDGMENTS

None.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

None.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

Not applicable.

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