

Original Research Article

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Utility of Galactomannan as Biomarker in Diagnosis of Invasive Aspergillosis: Retrospective Analysis

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ABSTRACT

Invasive Aspergillosis is a systemic infection of *Aspergillus* species which is a filamentous fungus present in the environment causing mild to severe illness. Detection of invasive aspergillosis is recognised by microscopy, conventional culture, biomarkers and by histopathology techniques. Galactomannan antigen detection is an adjunct to early diagnosis of invasive aspergillosis. A retrospective study was done from February 2023 to February 2025 among clinical samples received to microbiology laboratory at a tertiary care. The demographic data of the patients were recorded. The sensitivity and specificity of Galactomannan using FungiXpert Detection Kit on Full-Automatic Chemiluminescence Immunoassay System and its correlation with fungal culture were compared using descriptive statistics. Out of 700 clinical samples, 214 samples were positive for Galactomannan. Among 214 galactomannan positive, 184 BAL, 30 Serum samples were positive. Out of 184 bronchoalveolar lavages, *Aspergillus* species was isolated in 55 samples (29%) and 129 samples showed no growth (70%). *Aspergillus fumigatus* as the most common species isolated in 35 samples (63 %), *Aspergillus flavus* in 12 samples (22%), *Aspergillus terreus* in 4 samples (7.2 %) and *Aspergillus niger* in 4 samples (7.2 %). Age group >70 years showed maximum culture positives 48 (87.2%). In this study, 53 (63.6%) males and 20 (36.3%) females were positive for invasive aspergillosis. Galactomannan is a useful biomarker in the diagnosis of invasive aspergillosis.

Keywords

Invasive aspergillosis, *Aspergillus*, Galactomannan, Bronchoalveolar lavage, FACIS, Filamentous fungus.

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Introduction

Invasive Aspergillosis is a systemic infection caused by inhalation of *Aspergillus* spores, which is a filamentous fungus present in the environment causing mild to severe illness.

Aspergillus species, a protean in its manifestations pose a significant threat to the human population ranging from invasive to disseminated infections leading to high

mortality rates (1). *Aspergillus* causes tissue damage by producing enzymes like proteases, elastases and toxins like gliotoxin.

These compounds facilitate by contributing to the pathogenesis of invasive aspergillosis. It evades the host mechanism by masking the cell wall components and inhibits the cellular functions by forming conidia (spores), hyphae and biofilms which lead to chronic infections and resistance to anti-fungal therapies.

Detection of invasive aspergillosis is by microscopy, conventional culture, biomarkers and by histopathology techniques. Histopathological identification is challenging and difficult (2).

Managing and treating aspergillus has become complex in recent years. Gold standard fungal culture method for identification of the fungal species is fundamental, but is time consuming for the aspergillus growth and difficult when contaminated, accounting for its low sensitivity (3).

Emergence of serological assays, like Galactomannan antigen detection is an effective assay for early diagnosis of invasive aspergillosis (1). Bronchoscopy should be considered in patients for galactomannan antigen detection and PCR testing to rule out invasive aspergillosis (4).

Worsening clinical symptoms to progressive hypoxia and increased pulmonary infiltrates despite aggressive antibiotic therapy raises a suspicion for invasive pulmonary aspergillosis (5) Correlation between the galactomannan and culture positivity aids management of invasive aspergillosis in tertiary care hospitals.

Materials and Methods

Bronchoalveolar lavage (BAL) and Serum samples were collected as per the standard microbiological practices. All the BAL samples were cultured on Sabouraud Dextrose agar (SDA). Culture positives for *Aspergillus* growth were speciated by LPCB mounts and slide cultures microscopically.

Statistical analysis

Descriptive analyses were used to report *Aspergillus* growth, *Aspergillus* speciation, age groups and gender predominance.

Results and Discussion

Out of 700 clinical samples, 214 samples were positive for Galactomannan. Among 214 galactomannan positive, 184 BAL, 30 Serum samples were positive. Out of 184 bronchoalveolar lavages, (Figure 1) *Aspergillus* species was isolated in 55 samples (29%) and 129 samples showed no growth (70%).

(Figure 2) *Aspergillus fumigatus* as the most common species isolated in 35 samples (63 %), *Aspergillus flavus*

in 12 samples (22%), *Aspergillus terreus* in 4 samples (7.2 %) and *Aspergillus niger* in 4 samples (7.2 %).

(Figure 3) Age group >70 years showed maximum culture positives 48 (87.2%).

In this study, 53 (63.6%) males and 20 (36.3%) females were positive for invasive aspergillosis (Figure 4).

Out of 700 clinical samples, 214 samples were positive for Galactomannan. Among 214 galactomannan positive, 184 BAL, 30 Serum samples were positive.

Galactomannan is a polysaccharide component of the *Aspergillus* cell wall that is released into body fluids during active fungal growth (6, 8). Its detection can often precede clinical symptoms and positive cultures, allowing for earlier initiation of antifungal therapy, which is vital for improving survival rates (6, 7, 9). Studies have shown that GM detection can precede definitive IA diagnosis by a median of 17 days (8).

Unlike invasive procedures such as lung biopsy, GM testing can be performed on readily available samples like serum and bronchoalveolar lavage (BAL) fluid, making it a less burdensome and safer option for critically ill and immunocompromised patients (9).

The European Organization for Research and Treatment of Cancer/Mycoses Study Group Education and Research Consortium (EORTC/MSGERC) consensus definitions for invasive fungal diseases include a positive GM assay as a mycological criterion for classifying probable IA, especially in high-risk patient populations (10). This underscores its clinical relevance and acceptance in diagnostic algorithms.

Levels of serum GM levels during antifungal therapy have been correlated with treatment response and patient survival (11, 12). A decline in GM index (GMI) can indicate a favorable outcome, while persistently high or rising levels may suggest treatment failure or progressive disease, guiding clinicians in adjusting therapeutic strategies (13). In HSCT recipients with IA, the serum GMI has been shown to predict mortality (14).

Serial monitoring of GM levels allows for dynamic assessment of fungal burden and disease activity throughout the course of infection and treatment, aiding in therapeutic decisions and duration of antifungal therapy (9, 11).

The *Aspergillus* species was isolated in 55 bronchoalveolar lavage samples (29%). Pulmonary involvement is a hallmark for invasive aspergillosis. A study conducted by Meersseman W *et al.*, in the year 2008, stated that galactomannan for the diagnosis of invasive aspergillosis has high sensitivity and specificity. It also illustrated that galactomannan is particularly beneficial in detecting the early infections and monitoring treatment response.

Compared to serum galactomannan testing, BAL testing has higher sensitivity as it directly samples the respiratory tract where the infection occurs providing accurate results which was clearly inferred from the study (15). Detection of galactomannan in BAL fluid samples of patients at risk of invasive aspergillosis before receiving antifungals, can be used as diagnostic method with reasonable accuracy according to a study conducted by D'Haese J *et al.*, in the year 2012 (16).

In this study, *Aspergillus fumigatus* as the most common species isolated in 35 samples (63 %), *Aspergillus flavus* in 12 samples (22%), *Aspergillus terreus* in 4 samples (7.2 %) and *Aspergillus niger* in 4 samples (7.2 %) which was similar to results from a study conducted by Wattier RL in 2016 (17). In our tertiary care hospital due to the local epidemiological differences, we have reported a predominance in growth of *Aspergillus fumigatus*, "critical pathogen" pathogen" listed under the World

Health Organisation (WHO) fungal priority pathogens, 2022 causing invasive aspergillosis (18) 48 Patients of age group >70 years showed maximum culture positivity of 87.2% when compared to the other age groups of (9%) (50-70 years) and (3.6%) (25 to 50 years), which correlates with the study conducted by Shadrivova *et al.*, in the year 2019.

Age group >70 years showed maximum culture positives 48 (87.2%). In this study, 53 (63.6%) males and 20 (36.3%) females were positive for invasive aspergillosis. This study stated that the most common aspergillus species isolated among the elderly patients was *Aspergillus fumigatus* (19). Another study published in the year 2020, stated that mortality still remains substantial in elderly patients with immunodeficiency.

So, observation of the evolving risks of invasive aspergillosis is needed in any age group without classical risk factors (20). Another study published in the year 2023 stated that lymphopenic complications increase the mortality in the elderly people (21). In this study, 35 (63.6%) males were positive for invasive aspergillosis when compared to 20 (36.3%) females with IPA incidence (M/F: 2.5/1.0) which was similar to large epidemiological studies done by Kim *et al.*, (55.9%) (22), Steinbach *et al.*, (58.9%) (23), Lortholary *et al.*, (62%) (24), Hsiue *et al.*, (58%) (25).

Figure.1 Distribution of *Aspergillus* species in Bronchoalveolar Lavages

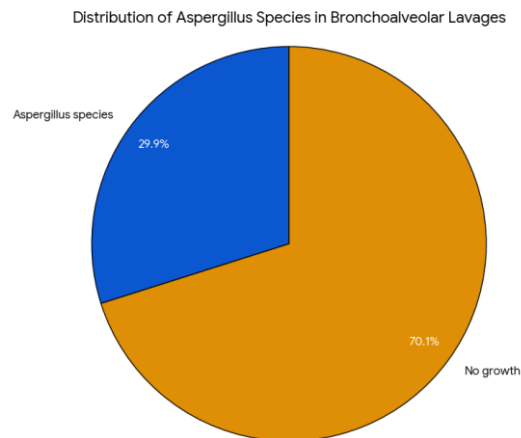


Figure.2 Isolation of *Aspergillus* species in Samples with Percentages

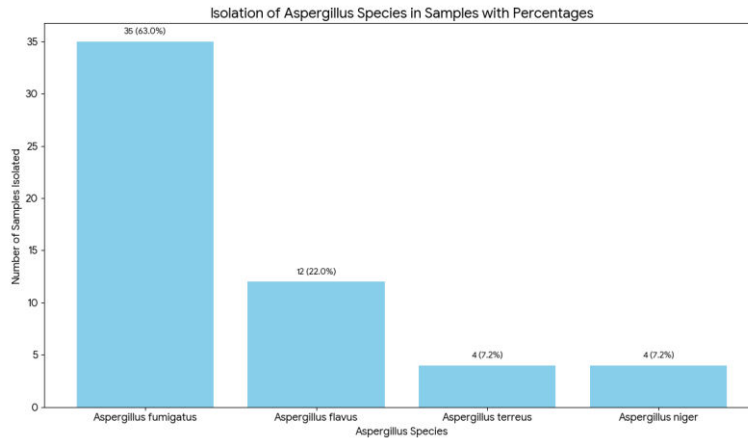


Figure.3 Invasive Aspergillosis Positives by Gender

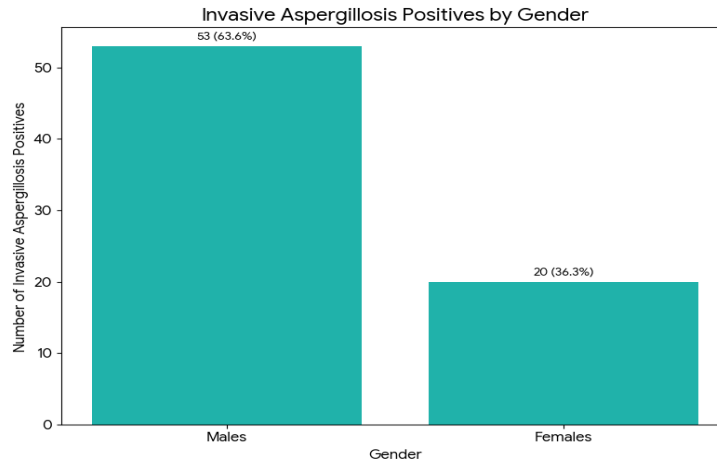
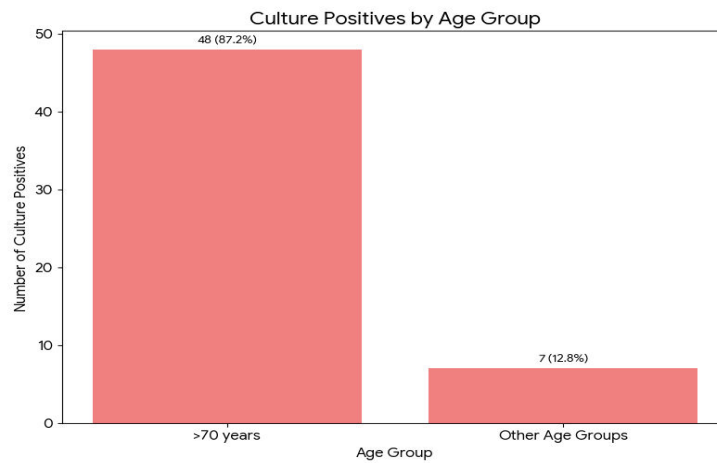


Figure.4 Culture Positive by Age Group



The specific reason for the male gender predominance can be because of the exposure history to the environment and epidemiological features of the underlying disease.

The highest galactomannan value (19.682) correlated with culture and the minimum galactomannan value (0.2084) was culture negative which can probably indicate an early infection or prior antifungal administration in such patients.

Limitations

1. This study is a retrospective study with limited sample size.
2. Beta D Glucan antigen was not included in the study.
3. False positives can occur due to the cross-reactivity with other fungi present in the BAL samples.
4. False Negatives can occur due to the prior or ongoing antifungal prophylaxis which can reduce GM levels,
5. Galactomannan assay do not identify the specific *Aspergillus* species, which may be relevant for guiding tailored antifungal therapy based on susceptibility profiles.

In conclusion, Early diagnosis and prompt therapy can improve clinical outcomes in invasive aspergillosis. Speciation and susceptibility of fungal isolates can add value to diagnostics along with Galactomannan and Beta D Glucan as biomarkers in the diagnosis of invasive aspergillosis.

Author Contributions

Abhishek Velamuri: Investigation, formal analysis, writing—original draft. Krishnaveni: Validation, methodology, writing—reviewing. Rafat Fathima:—Formal analysis, writing—review and editing. K. Navya: Investigation, writing—reviewing. Shiva Priya Eswaran: Resources, investigation writing—reviewing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Consent: Not Applicable

Ethical approval: Verbal ethics committee approval was obtained in view of the nature of the study.

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Guarantor: Dr Abhishek Velamuri holds full responsibility for the work, the conduct of the study.

Declaration of competing interest: Authors declare no competing interest.

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