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ORIGINAL ARTICLE

Evaluation of galactomannan assay in diagnosing invasive aspergillosis

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ABSTRACT

Background and Objectives: In most laboratories, diagnosis of invasive aspergillosis (IA) depends on culture and histopathological examination of the involved tissues. Galactomannan (GM) is a polysaccharide antigen detectable in body fluids and represents an ideal assay for diagnosing invasive pulmonary aspergillosis. The main objectives of this study were to estimate the proportion of patients with proven, probable, and possible aspergillosis and to compare different diagnostic methods.

Materials and Methods: Samples for culture, microscopy, and GM assay were taken from a total of 60 patients. The enrolled patients were divided into proven, probable, and possible aspergillosis groups.

Results: Among the 60 patients in the study, there were 9 (15%) cases of probable aspergillosis. galactomannan assay was positive in 19 patients. Galactomannan assay had a higher sensitivity (66.7%) when compared to other fungal assays for diagnosing probable aspergillosis.

Conclusion: Diagnosing invasive aspergillosis is extremely challenging, and biomarkers such as galactomannan assay are important for early diagnosis. Raising the cutoff to >1 can eliminate false-positive results associated with the GM assay.

Keywords: Galactomannan; Aspergillus; Assay; Culture; Microscopy

INTRODUCTION

The frequency of invasive fungal infections (IFI) has risen exponentially in recent years (1, 2). This rise is mainly due to increasing number of immunocompromised patients. These infections are associated with high morbidity and mortality in 60-90% of cases, while studies have shown prevalence of invasive fungal infections in haematological malignancies to be ranging from 24-31% (3-11). The vast majority of IFI occur in patients with hematological malignancies (HM), particularly in patients with acute myeloid leukemia (AML) and those who have undergone allogeneic hematopoietic stem cell transplantation (allo-HSCT) (12-14). *Aspergillus* species is a saprophytic fungus found ubiquitously in the environment and is the cause of infections such as allergic aspergillosis, invasive pulmonary aspergillosis, chronic necrotizing pulmonary aspergillosis, and CNS aspergillosis. Invasive aspergillosis (IA) is a serious disease seen in immunocompromised patients, especially those with hematological malignancy, acute leukemia, solid tumors, solid organ and hematopoietic stem cell transplant recipients, and those with defective neutrophil function (15-17). Infection is caused by inhalation of fungal spores, mainly *Aspergillus fumigatus*, but can also be caused by other species such as *Aspergillus flavus* and *Aspergillus niger.*

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In most laboratories, the diagnosis of IA depends on culture and histopathological examination of the involved tissues (4). Microscopy and culture of sputum and bronchoalveolar lavage (BAL) samples are not highly sensitive methods, and accurate diagnosis is not always possible. Another frequently encountered problem is that in critically ill patients obtaining a tissue biopsy may not be feasible due to thrombocytopenia and other coagulation defects (18). Diagnosis is mainly dependent on a combination of clinical scenarios, such as the setting of high-risk neutropenia, persistent fever despite broad-spectrum antibiotics, along with radiologic abnormalities and clinical experience. Unfortunately, the clinical symptoms and radiological signs of IA are usually non-specific and appear later in the disease, making early diagnosis extremely challenging for the treating physician (19). There is an urgent need to explore non-culture-based tests for the diagnosis of IA, such as enzyme-linked immunosorbent assay (ELISA) for circulating serum galactomannan (GM), assay for circulating 1-3-β-D-glucan (BDG), and polymerase chain reaction (PCR) for fungal DNA in the blood. The PCR assays are not routinely available, while the BDG assay is not specific for aspergillosis (20).

Galactomannan (GM) is a polysaccharide antigen found primarily in the cell walls of *Aspergillus* species. It is a heat-stable component of the fungal cell wall that is released during tissue invasion by fungal hyphae. It can be detected in body fluids, and it is an ideal assay for the diagnosis of invasive pulmonary aspergillosis (IPA) because it appears in the blood during the early stages, even before clinical or radiological signs manifest (21). The sensitivity and specificity of ELISA for Galactomannan assay may vary considerably according to the patient population and cut off level used (22). The galactomannan assay is included in the microbiological criterion for probable invasive pulmonary aspergillosis according to the European Organization for the Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) (23).

The main objectives of the study were:

1. To estimate the percentage of people with proven, probable and possible aspergillosis as per the 2019 revised guidelines given by the European Organization for Research and Treatment of Cancer and the Mycoses Study Group (EORTC/MSG).

2. To compare the percentage of probable IA detected using GM assay with Microscopy.

3. To validate GM ELISA against proven IA infection based on either Culture or Biopsy

MATERIALS AND METHODS

This study was conducted by the Department of Microbiology, Believers Church Medical College, Thiruvalla, over a period of 3 years from September 2019 to September 2022, after obtaining clearance from the Institutional Ethics Committee. Serum and bronchoalveolar lavage (BAL) specimens were collected from 60 patients admitted to Believers Church Medical College Hospital who were at high risk of invasive aspergillosis, as determined by the treating team. Details of any risk factors for acquiring IFI, such as neutropenia and prolonged antibiotic intake, were recorded. Investigations included fungal microscopy (KOH mount), culture, and histopathology. Culture was inoculated onto SDA (Sabouraud Dextrose Agar, HiMedia, India), with or without antibiotics, and incubated at 25°C and 37°C for 6 weeks. The demographic details, radiological findings, and clinical course were then recorded for all enrolled patients. The enrolled patients were divided into proven, probable, and possible aspergillosis according to the guidelines recommended by the European Organization for the Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) (23). Even though the EORTC/MSG guidelines are recommended for the diagnosis of invasive aspergillosis in neutropenic/transplant patient groups, in this study, they were extrapolated to include other populations.

The GM assay was carried out using the Platelia *Aspergillus* enzyme immunoassay test kit (Bio-Rad Laboratories, France). The steps of a routine ELISA were followed, except for the initial heating of 300 µL of serum at 120°C after the addition of 100 µL of serum treatment solution. Optical density of the wells was read at 450 nm (reference filter 620 nm) in an ELISA reader (BioRad, India). Cutoff values were calculated as per manufacturer's instructions and the samples that qualified the validity criteria and had an optical density index of >0.5 were labelled positive.

Inclusion criteria. All patients who were clinically suspected of having invasive aspergillosis were included in the study.

Definitions. Proven aspergillosis- a patient with histopathological evidence of IPA; Probable aspergillosis-simultaneous presence of at least one host factor,

one clinical feature and the mycological evidence of *Aspergillus* in the LRT; Possible aspergillosis- a host factor and clinical features are present, without mycological positive tests (23).

Statistical analysis. Descriptive statistics will be presented as proportions (%). Proportions will be compared using the z-test. Validation of the galactomannan assay will be performed using sensitivity, specificity, positive and negative predictive values, and their respective 95% confidence intervals.

RESULTS

Among the 60 patients in the study, there were 9 (15%) cases of probable aspergillosis, no cases of proven aspergillosis, and the remaining 51 (85%) cases fell into the category of possible aspergillosis. The mean galactomannan (GM) antigen levels were as follows: probable aspergillosis, GM value; possible aspergillosis, GM value. Details of risk factors, comorbid conditions, and their association with probable and possible aspergillosis are shown in Table 1. The galactomannan assay was positive in 19 patients (optical density index >0.5). Bivariate analysis showed that gender, fever, neutropenia, bronchiectasis, prolonged antibiotic use, and steroid use had no relationship with the diagnosis of IPA ($p > 0.05$) (Table 1). Dyslipidemia had a statistically significant association with the diagnosis of probable aspergillosis ($p = 0.002$).

The sensitivity, specificity, PPV, and NPV of various fungal assays in diagnosing probable aspergillosis are summarized in Table 2. The galactomannan assay had a higher sensitivity (66.7%) when compared to other fungal assays for diagnosing probable aspergillosis. There were only four samples that were culture-positive for *Aspergillus* species (3 *Aspergillus fumigatus* and 1 *Aspergillus flavus*). Among the culture-positive samples, only two were galactomannan-positive. There were only two samples in which microscopy was positive for fungal elements and showed narrow septate hyphae. Both samples that were positive by microscopy also had positive GM values.

DISCUSSION

The diagnosis of invasive pulmonary aspergillosis is extremely challenging due to the need for invasive sampling, nonspecific radiological findings, and the limitations associated with culture and microscopy. If not diagnosed early, IPA can be fatal. The GM assay has been extremely useful in diagnosing invasive aspergillosis, as GM is released into the blood and other body fluids during the early stages of *Aspergillus* invasion and can be detected for 1 to 8 weeks (24). EORTC/MSG diagnostic criteria are ideally recommended for immunocompromised patients (23). In this study, this criterion was extrapolated to include all patients with suspected aspergillosis from whom samples were sent for GM testing. According to the criteria, mycological evidence is required for diagnosing possible aspergillosis, and one of the fungal assays mentioned is the GM assay. Galactomannan positivity from a single serum, plasma, or BAL fluid sample is required, with an optical density index of ≥1. Other fungal assays include microscopy, culture, and *Aspergillus* PCR (23).

In our study, there was a male preponderance among patients with both probable (88.89%) and possible aspergillosis (68.63%). A similar finding was reported in studies by Malhotra et al. and Sun et al. (24, 25). In our study, only 15% of cases met the criteria for probable aspergillosis, while the remaining 85% were classified as possible aspergillosis. This is discordant with studies by Malhotra et al. and Toienemercier et al., who reported probable aspergillosis rates of 70% and 68%, respectively (24, 26). Our study did not find any proven case of invasive aspergillosis as per the EORTC/MSG diagnostic criteria.

According to the EORTC/MSG diagnostic criteria, galactomannan positivity in a single serum, plasma, or BAL fluid sample with an optical density index of ≥1 is required for diagnosing probable aspergillosis. In this study, the galactomannan assay at a cutoff of ≥1 had a sensitivity of 66.7% for diagnosing probable aspergillosis, which was superior to both microscopy and culture. A study conducted in Iran reported a sensitivity of 77% at a cutoff of ≥1 for diagnosing aspergillosis (27). A study conducted in India by Mohindra et al. reported a sensitivity of 95.8% at a cutoff of ≥1 for diagnosing probable aspergillosis (28). Another study conducted in India reported a lower sensitivity of only 33.3% at a cutoff of ≥1 for diagnosing probable aspergillosis (29). In our study, culture and microscopy had a sensitivity of only 44.4% and 22.2%, respectively, in diagnosing probable aspergillosis. This was concordant with the study conducted

Table 1. Distribution of baseline characteristics.

	Total	Probable	Possible	P Value
Gender				
Male	43 (71.7%)	8 (88.89)	35 (68.63)	0.2
Female	17 (28.3%)	1 (11.11)	16 (31.37)	
Risk Factors				
Fever	38 (63.3%)	5 (55.6%)	33 (64.7%)	0.2
Neutropenia	9 (15.0%)	2 (22.2%)	7 (13.7%)	0.3
Bronchiectasis	23 (38.3%)	4 (44.4%)	19 (37.3%)	0.3
Ground Glass Opacities	17 (28.3%)	4 (44.4%)	13 (25.5%)	0.2
Prolonged Antibiotics Use	43 (71.7%)	4 (44.4%)	39 (76.5%)	0.1
Prolonged Steroid Use	17 (28.3%)	2 (22.2%)	15 (29.4%)	0.3
ICU Stay	33 (55.0%)	4 (44.4%)	29 (56.9%)	0.2
Central Line	30 (50.0%)	3 (33.3%)	27 (52.9%)	0.2
Comorbid Conditions				
Diabetes Mellitus	37 (61.7%)	7 (77.8%)	30 (58.8%)	0.2
Hypertension	30 (50.0%)	5 (55.6%)	25 (49.0%)	0.3
Dyslipidemia	13 (21.7%)	6 (66.7%)	7 (13.7%)	0.002
Asthma	12 (20.0%)	1 (11.1%)	11 (21.6%)	0.3
Chronic Artery Disease	19 (31.7%)	3 (33.3%)	16 (31.4%)	0.3
Chronic Kidney Disease	8 (13.3%)	2 (22.2%)	6 (11.8%)	0.3

Table 2. Fungal assays in diagnosing probable aspergillosis

Fungal assay	Sensitivity	Specificity	PPV	NPV
GM ≥1	66.7 (6/9)	100 (54/54)	100 (6/6)	94.7 (54/57)
Microscopy positive for Fungal Elements	22.2 (2/9)	100 (58/58)	100 (2/2)	88.9 (56/63)
Culture Positive for Aspergillus Species	44.4 (4/9)	100 (56/56)	100 (4/4)	91.8 (56/61)

GM- Galactomannan

by Malhotra et al., who reported that culture and microscopy were positive in only 18.1% of cases (24).

Among the 19 (31.7%) patients who were positive for the galactomannan assay (optical density index >0.5), ten patients had galactomannan values between 0.5 and 1. Of these ten patients, only one was classified as having probable aspergillosis, based on a positive microscopy finding. The remaining patients were classified as having possible aspergillosis due to the absence of mycological evidence, according to the EORTC/MSG diagnostic criteria. Among the nine patients classified as having possible aspergillosis with galactomannan values between 0.5 and 1, 88.9% were on antibiotics at the time of sample collection for the GM assay. These patients were on meropenem, piperacillin-tazobactam, or amoxicillin-clavulanate. False-positive results in the galactomannan assay have been reported with drugs such as carbapenems, piperacillin-tazobactam, amoxicillin-clavulanate, and cefepime by Boonsarngsuk et al. (30). Ghosh et al. reported that raising the assay cutoff to >1 and collecting the sample immediately before antibiotic administration can reduce false positives (29). However, collecting samples before antibiotic administration was not possible in our study.

In conclusion, diagnosing invasive aspergillosis is extremely challenging, and biomarkers such as the galactomannan assay are important for early diagnosis. In our study, there were 9 (15%) cases of probable aspergillosis and no cases of proven aspergillosis. The galactomannan assay was positive in 19 patients. Dyslipidemia had a statistically significant association with the diagnosis of probable aspergillosis ($p = 0.002$). The galactomannan assay had a higher

sensitivity (66.7%) when compared to other fungal assays for diagnosing probable aspergillosis. Raising the cutoff to >1 can eliminate false-positive results associated with the GM assay.

This study had a few limitations. Serial monitoring of galactomannan could not be performed due to the high cost of the assay. Additionally, the EORTC/MSG diagnostic criteria were extrapolated for this study, and samples could not be collected before antibiotic administration.

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