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Original Research Article

Comparative Analysis between Aspergillus-PCR and Galactomannan ELISA for the Detection of Invasive Aspergillosis

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Abstract: In the realm of medical mycology, Invasive aspergillosis (IA) is a severe fungal disease primarily affecting individuals with weakened immunity. Because conventional diagnostics like fungal cultures are often slow and lack sensitivity, this study focuses on evaluating two advanced testing methods- *Aspergillus*-specific PCR and galactomannan ELISA (GM-ELISA)- to identify which delivers more reliable and timely results across sample types and patient profiles. The research was conducted over 13 months in a hospital setting, analyzing bronchoalveolar lavage fluid (BALF) and serum samples from patients suspected of having IA. GM-ELISA targeted the presence of fungal antigens, while PCR was used for genetic detection and species differentiation. Thorough statistical methods were applied to assess the performance and correlations between both techniques. Patients aged 61–70 years, predominantly male (60%), were most affected. GM-ELISA showed more positives than PCR, though overlap between methods was limited. Antigen levels were higher in BALF than serum, and PCR cycle thresholds inversely correlated with GM-ELISA values, indicating higher fungal loads lead to quicker detection. *Aspergillus fumigatus* showed the highest antigen burden, while *Aspergillus flavus* had lower nucleic acid levels. No significant link was found with haematological markers or conditions like diabetes and renal issues.Radiological findings often matched IA pulmonary changes, and Voriconazole was the most common treatment. In conclusion, GM-ELISA proved to be a sensitive tool, particularly for respiratory samples, while PCR contributed valuable specificity and early-stage detection. When used together, they offer a more complete diagnostic picture, enhancing early identification and treatment strategies for IA.

Keywords: Galactomannan ELISA, *Aspergillus*-PCR, Invasive Aspergillosis, *Aspergillus* sp., BAL fluid, Serum.

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INTRODUCTION

Invasive aspergillosis (IA) constitutes a formidable and frequently fatal mycosis mainly affecting immunocompromised individuals, with *Aspergillus fumigatus* as the leading pathogen [11]. Though initially linked to neutropenic and transplant patients, its incidence has broadened to include those on corticosteroids and with congenital immunodeficiencies [11, 12]. A comprehensive ICU study by Chakrabarti *et al*., revealed *A. flavus* (47%) and *A. fumigatus* (39.4%) as the most recurring isolates, especially among patients with unconventional risk factors [2]. Given its high mortality, early and accurate diagnosis is crucial, which remains paramount in initiating appropriate antifungal therapy and mitigating adverse clinical outcomes [14].

Conventional methods- microscopic examination, culture, and histopathology- suffer from suboptimal sensitivity, extended turnaround times, and the requirement for invasive procedures [10,14], prompting reliance on non-culture diagnostics like galactomannan enzyme-linked immunosorbent assay (GM-ELISA) and *Aspergillus*-specific polymerase chain reaction (PCR) [9, 10].

Galactomannan, a polysaccharide constituent of the *Aspergillus* cell wall, is released during hyphal growth [18] and can be detected in serum or bronchoalveolar lavage fluid (BALF) via GM-ELISA [21]. The assay is widely recognized in international diagnostic guidelines for its strong sensitivity and specificity [1, 8], though its accuracy may be affected by antifungal therapy, immune status, and cross-reactivity with certain pathogens or drugs like β-lactam antibiotics [6].

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Conversely, PCR identifies fungal DNA across specimen types and often precedes antigenemia [19]. El-Baba *et al.,* reported serum PCR sensitivity and specificity at 80.5% and 78.5%, respectively, which improved dramatically in BALF (90.2% sensitivity, 96.4% specificity) [6]. Moreover, PCR facilitates prompt diagnosis and identification of azole resistance [9].

BALF is preferred for GM and PCR testing due to its close proximity to pulmonary infections. Pooled meta-analytical data indicates that BALF PCR over 85–90% sensitivity and >95% specificity in high-risk cases [8]. GM testing also shows strong accuracy and may be more sensitive in CPA [21]. GM levels in BALF are strong indicators of IA, particularly with an Optical Density index (ODI) of 0.5-1.0 [4]. An ODI ≥ 1.0 is now the accepted criteria per European Organization for Research and Treatment of Cancer and Mycoses Study Group Education and Research Consortium (EORTC/MSGERC) [5]. False positives can occur due to asymptomatic colonization or other fungal infections [7].

PCR and GM-ELISA, though promising, have inherent drawbacks—PCR is highly sensitive but lacks standardization and may produce false positives [10, 16], while GM-ELISA cannot identify species and may yield false-negative results in certain cases [1, 6]. To overcome these issues, combining both methods has shown improved sensitivity and specificity, boosting diagnostic accuracy [3, 19]. Machado et al.'s 2023 review stressed the need for comparative biomarker studies [14], and ongoing research continues to evaluate the performance of these assays across varied clinical settings [10,16].

Accordingly, the present study seeks to conduct a comparative evaluation of *Aspergillus*-PCR and GM-ELISA in the diagnosis of invasive aspergillosis. The findings are anticipated to inform the refinement of diagnostic protocols and facilitate timely, precise therapeutic interventions in patients with suspected IA.

MATERIALS AND METHODS

This retrospective cross-sectional investigation, conducted from May 2024 to June 2025, at Peerless Hospital and B. K. Roy Research Centre, Kolkata, India; investigated patients suspected of Invasive Aspergillosis in Medicine and Respiratory wards. Samples including BAL fluid and serum were meticulously collected under aseptic conditions - through fiberoptic bronchoscopy and venipuncture respectively – and preserved at 2–8 °C before prompt laboratory processing. Diagnostic testing was then performed using Galactomannan ELISA (GM-EIA) and *Aspergillus*-specific PCR.

Galactomannan ELISA

The Bio-Rad Platelia *Aspergillus* GM-EIA assay, widely used in IA diagnostics, operates on a microplate format with EB-A2 monoclonal antibodies targeting galactomannan's galactofuranosyl components. These antibodies, capture the antigen and detect it via peroxidase conjugation. A chromogenic substrate (TMB) generates a colour change, read spectrophotometrically, indicating antigen concentration. These antibodies, validated by Stynen *et al.,* (1992) [17], demonstrate high specificity and affinity for *Aspergillus fumigatus* galactomannan.

***Aspergillus*-PCR**

DNA extraction was standardized using the QIAamp Mini Kit, targeting BAL, serum, plasma samples etc. BAL fluid was treated with Dithiothreitol and Proteinase K, lysed with AL buffer, vortexed, incubated, and processed through ethanol precipitation. The sample was loaded onto spin columns with internal control, centrifuged and sequentially washed with AW1 and AW2 buffers followed by high-speed centrifugation. The DNA was then eluted with AE Buffer. The purified DNA was stored at -20 °C for PCR.

Molecular analysis was then carried out using the AsperGenius® 2.0 Species Multiplex realtime PCR kit. Each 25 µL reaction included a master mix and 5 µL of extracted DNA, and amplification was conducted on the CFX96™ Real-Time System. The thermal protocol involved initial denaturation and enzyme activation at 95°C followed by 45 cycles of denaturation and annealing/extension at 60°C. Real-time fluorescence monitoring allowed simultaneous detection of *Aspergillus* species and resistance markers.

Statistics and Graphs

The comparison of means and Pearson's correlation coefficient was performed using MedCalcSoftware Ltd., Version 23.2.8. The standard deviation was calculated using Calculator.net. Graphical representations were created in Microsoft Excel and all the data presented are based on original experimental observations.

RESULTS

1. Demographics

A total of 61 patients tested positive for *Aspergillus* using the PCR method, while 234 patients were found positive by GM ELISA. Among these, 26 patients showed concurrent positivity in both PCR and GM ELISA tests. The patients' ages ranged from 1 month to 90 years, with the highest proportion falling in the 61–70 age group (25.4%). Males comprised 60% (n=177) and females comprised 40% (n=118) of the total cohort (Figure 1).

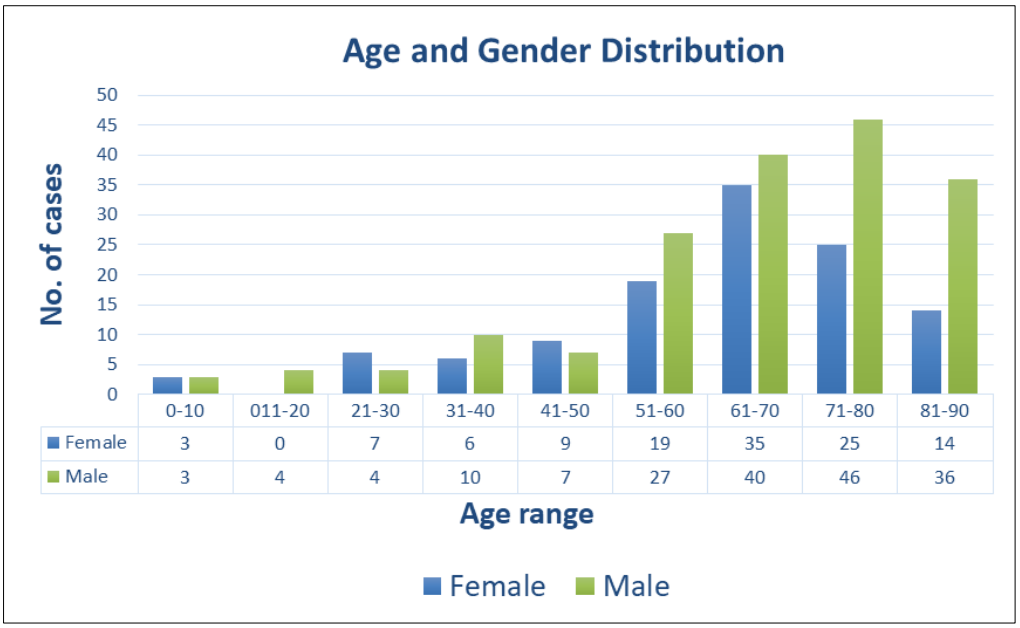

Figure 1: Age and Gender distribution of total positive patients. Clustered bar chart showing the number of *Aspergillus* positive cases across age groups, stratified by gender.

2. PCR CT Value and GM Index Analysis

GM ELISA-Positive Patients

In GM-positive cases, a total of 104 BAL fluid samples, 129 serum samples, and 1 endotracheal tube (E.T.) suction sample were included in the analysis. Mean GM index for BAL fluid samples (n = 104)was 1.39 ± 0.79, while for serum samples (n =129), it was 1.08 ± 0.63. The single E.T. suction sample showed no aggregated statistics due to its solitary nature. The standard error (SE) for BAL and serum samples was 0.07 and 0.05, respectively. A t-test comparison between BAL fluid and serum yielded a mean difference of 0.310, with a t-value of 3.340 and a p-value of 0.001 (Table 1).

Table 1: The table shows higher GM index in BAL fluid (1.39 ± 0.79) than serum (1.08 ± 0.63), with a significant difference (p = 0.001)

Sample	Mean GM Index	Standard Deviation	Standard Error	P value	Difference	t value
BAL Fluid	1.39	0.79	0.07	0.001	0.310	3.34
Serum	1.08	0.63	0.05			

PCR-Positive Patients

Among the specimens analyzed in PCR-positive patients, BAL fluid samples (n = 47) had a mean Ct value of 31.90 ± 2.81, with a SE of 0.41. In comparison, serum samples (n = 9) showed a mean Ct value of 32.84 ± 1.97, with an SE of 0.65. The mean difference in Ct values between BAL fluid and serum was 0.66, with a p-value of 0.46 and a t-value of 0.73 (Table 2).

Table 2: The table shows similar mean Ct values for BAL (31.90± 2.81) and serum (32.84 ± 1.97) samples, with no significant difference (p = 0.46).

Sample	Mean GM Index	Standard Deviation	Standard Error	P value	Difference	t value
BAL Fluid	31.90	2.81	0.41	0.46	0.66	0.73
Serum	32.84	1.97	0.65			

Patients Who Tested Positive by Both PCR and GM ELISA Methods

Out of the 26 patients who tested positive for *Aspergillus* by both PCR and GM ELISA, 22 had available BAL fluid samples. A correlation analysis was conducted to examine the relationship between the two, in these 22 samples. Figure 2 displays a scatter plot illustrating the correlation between Ct values and GM index, with the Pearson correlation coefficient (r) calculated as 0.573 (Figure 2).

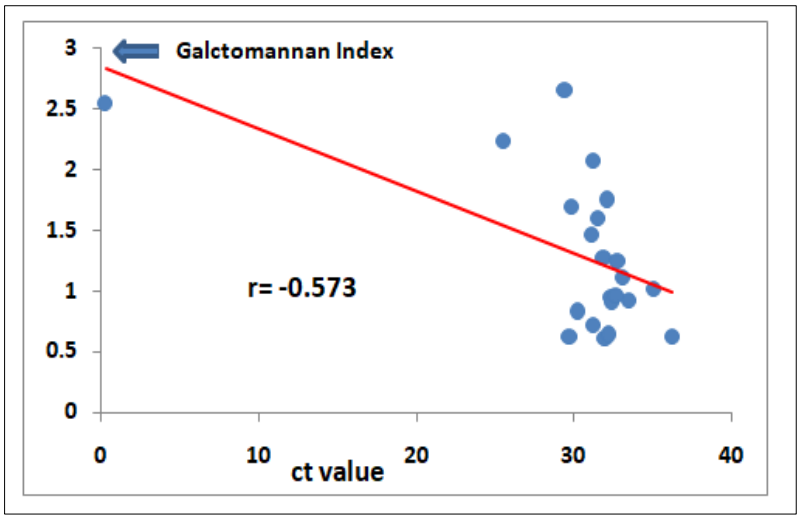

Figure 2: A significantly high negative correlation between Galactomannan Index and PCR Ct values of BAL fluid samples.

3. Species

The mean Ct value was highest in *A. flavus* (34.42 ± 2.33 ± 1.16) and lowest in *A. fumigatus* (31.63 ± 3.06 ± 2.17) whereas the GM index was highest in *A. fumigatus* (1.72 ± 0.82 ± 0.47), followed by other *Aspergillus* species (1.21 ± 0.57 ± 0.12) and *A. flavus* (1.06 ± 0.37 ± 0.18). A statistically significant difference was observed in Ct values between *A. flavus* and other *Aspergillus* species (p=0.02). In contrast, all other comparisons showed non-significant p-values (p>0.05) for both Ct values and GM indices (Table 3).

Table 3: The table shows the highest Ct value in *A. flavus* and the highest GM index in *A.fumigatus*,with a significant Ct difference between *A.flavus* and other species (p=0.02); other differences were not significant (p > 0.05).

Species	PCR Ct value (Mean±SD±SEM)	GM Index (Mean±SD±SEM)	p-value (between Ct values)	p-value (betweenGM Index)
A.flavus	34.42±2.33±1.16	1.06±0.37±0.18	Between*A.flavus*and*A. fumigatus*:0.22	Between*A.flavus*and*A. fumigatus*:0.20
A. fumigatus	31.63±3.06±2.17	1.72±0.82±0.47	Between*A.fumigatus*and other species : 0.96	Between*A.fumigatus*and other species: 0.18
Other *Aspergillus* species	31.68±1.98±0.43	1.21±0.57±0.12	Between*A.flavus*andother species:0.02	Between*A.flavus*andother species:0.62

4. Co-Morbidities

Patients with Diabetes Mellitus exhibited higher mean fungal load and GM Index levels (1.42±0.66±0.17) compared to other comorbid groups. However, none of these differences were statistically significant (p>0.05), although the GM Index comparison between Diabetes Mellitus and CKD showed a trend toward significance (p=0.08) (Table 4).

Table 4: The table indicates higher GM Index and fungal load in diabetic patients, with a near-significant difference in GM Index between DM and CKD(p=0.08)

Co-morbidities	PCR Ct value (Mean±SD±SEM)	GM Index (Mean±SD±SEM)	p-value(between Ct values)	p-value (between GM Index)
Diabetes Mellitus (DM)	31.43±2.01±0.53	1.42±0.66±0.17	Between DM & CKD: 0.11	Between DM & CKD: 0.08
Chronic Kidney Disease (CKD)	32.83±2.08±0.65	0.98±0.45±0.14	Between CKD & HTN: 0.32	Between CKD & HTN: 0.43
Hypertension (HTN)	32.03±1.80±0.48	1.18±0.70±0.18	Between DM & HTN: 0.41	Between DM & HTN: 0.35

5. Correlation between C - reactive protein (CRP) and GM Index

The correlation coefficient (R = 0.1529) between CRP and GM Index indicates a weak positive relationship, and the P-value of 0.1369 confirms that this association is not statistically significant (Figure 3).

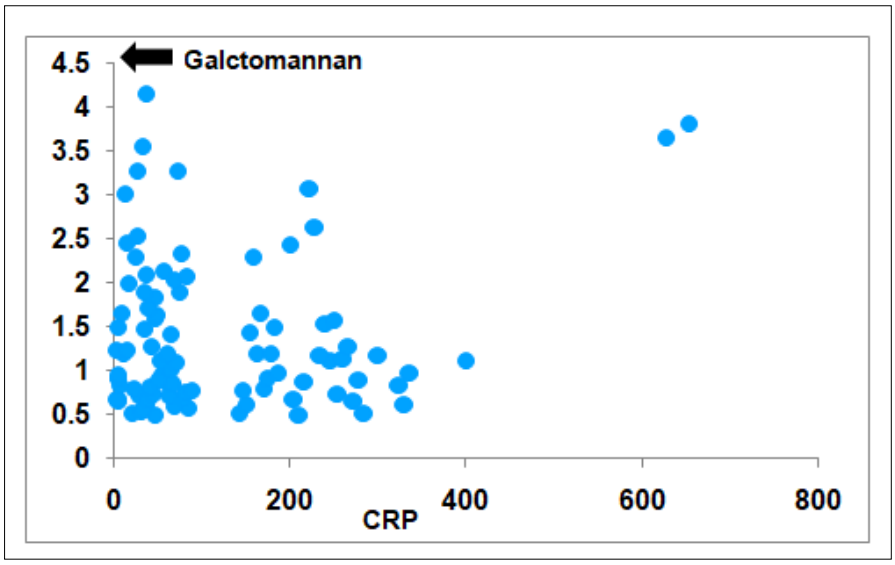

Figure 3: The scatter plot shows a weak positive correlation between CRP and GM Index (R= 0.1529, P= 0.1369), which is not statistically significant

6. Correlation between CRP and CT Value

The correlation analysis between CRP levels and PCR Ct values in patients showed a weak positive correlation with a correlation coefficient (r) of 0.3164. However, this relationship was not statistically significant, as reflected by a p-value of 0.1154 ($p>0.05$) (Figure 4).

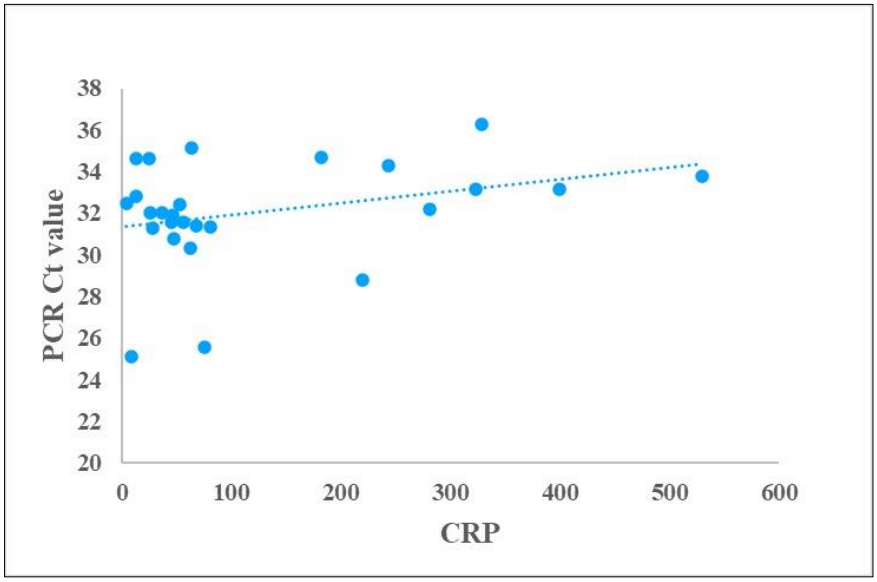

Fig. 4: A weak positive correlation between CRP levels and PCR Ct values (r = 0.3164, p = 0.1154) is shown in the scatter plot, which is not statistically significant

7. Clinical Findings

The most prevalent clinical manifestation was breathlessness, dyspnoea, respiratory distress, or chest pain, seen in 48.39% of cases, highlighting the pulmonary involvement typical of IA; while the less frequent findings included hypoxia (3.77%), syncope/convulsion (3.01%), bleeding (2.64%), and backache (3.01%). Regarding comorbidities, Hypertension (HTN) was the most common, found in 35.84% of patients. Diabetes mellitus (DM) followed closely at 34.33%, and chronic kidney disease (CKD) was observed in 16.98%. Chronic obstructive pulmonary disease (COPD) and ventricular septal defect (VSD) were present in 20.37% and 1.5% of cases, respectively (Figure 5).

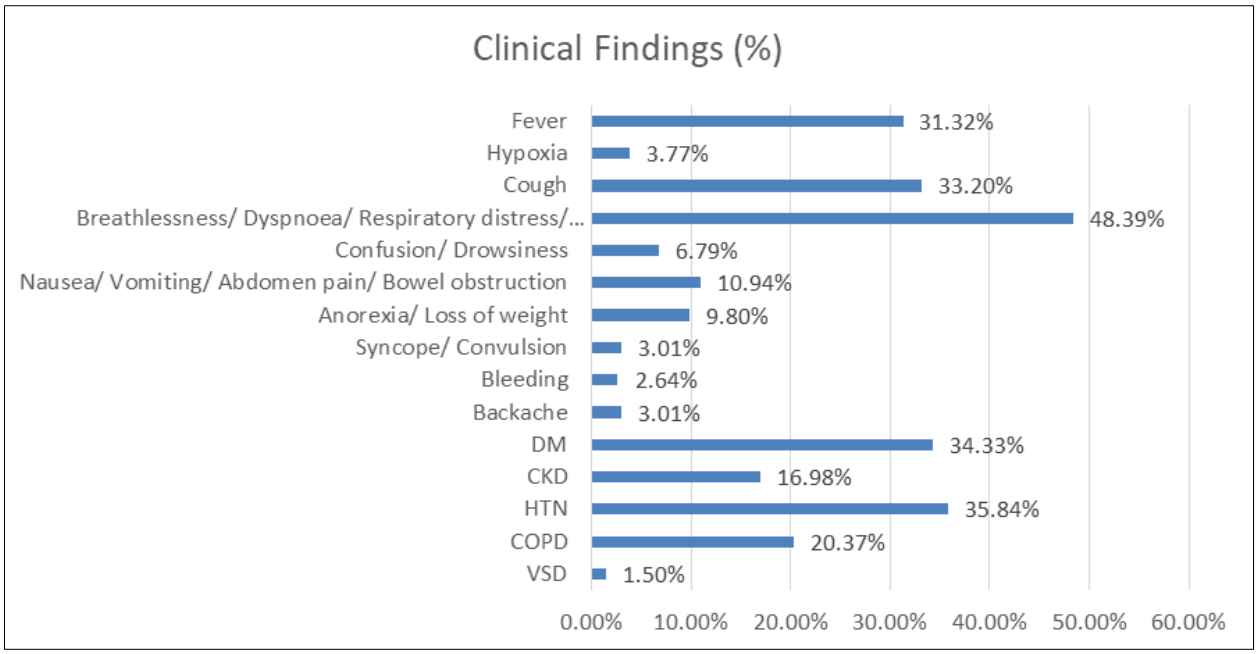

Fig. 5: Clinical Findings (%)- The graph illustrates that the most common clinical manifestation was breathlessness and related symptoms (48.3%), while the least frequent finding was bleeding (2.64%). Among comorbidities, hypertension (35.84%) and diabetes mellitus (34.33%) were most prevalent, followed by CKD (16.98%), COPD (20.37%), and VSD (1.5%).

8. Total Count (TC), Neutrophil Percentage (NP), Haemoglobin (HB), Erythrocyte Sedimentation Rate (ESR), C-reactive protein (CRP)

PCR CT Value:

None of the P-values for the parameters analysed fall below the typical threshold of 0.05, which means none of the differences in Ct values across these parameters are statistically significant (Table 5).

Table 5: None of the haematological parameters showed statistically significant differences in Ct values (p> 0.05).

Haematological parameters	PCR Ct value (Mean±SD±SEM)		P value
	PCR-positive cases	Both PCR and GM ELISA-positive cases	
TC	32.02±2.24±0.39	31.61±2.01±0.50	0.52
NP	32.22±2.06±0.39	31.52±2.21±0.61	0.33
HB	32.21±1.90±0.33	31.55±2.07±0.53	0.28
ESR	32.00±2.58±0.49	31.74±2.66±0.77	0.77
CRP	32.05±2.58±0.50	32.02±2.41±0.64	0.97

GM Index Value:

The GM index remains relatively consistent between the two patient groups, suggesting that variations in TC, NP, HB, ESR, and CRP do not significantly influence GM index values. All P-values are well above 0.05, indicating non-significant results (Table 6).

Table 6: The table shows no significant GM Index value differences across haematological parameters since all has p> 0.05

Haematological parameters	GM Index value (Mean±SD±SEM)		P value
	GM ELISA-positive cases	Both PCR and GM ELISA-positive cases	
TC	1.28±0.79±0.06	1.27±0.60±0.15	0.96
NP	1.33±0.83±0.08	1.31±0.65±0.18	0.93
HB	1.29±0.80±0.07	1.30±0.61±0.15	0.96
ESR	1.38±0.86±0.08	1.34±0.66±0.19	0.87
CRP	1.39±0.87±0.09	1.21±0.65±0.17	0.46

9. Radiological Findings

Chest X-Ray Findings

Chest radiographs of patients with invasive aspergillosis demonstrated a wide array of pulmonary changes, both acute and chronic. Bilateral abnormalities such as diffuse haziness, basal opacities, and patchy infiltrates were common. Lobar consolidations, pleural effusions, and cavitary lesions indicated advanced fungal involvement. Structural alterations like fibrotic bands, bronchiectasis, calcified plaques, and evidence of past tuberculosis pointed to underlying lung disease. Cardiomegaly and hilar prominence coexisted with parenchymal findings in some cases. Additional features included hyperinflation, vascular and fissural changes, and collapse-consolidation. One abdominal film showed bowel distension, suggesting extrapulmonary spread. These features reflect a mix of infectious, inflammatory, and structural pathology.

MRI Findings

MRI findings of the brain revealed a spectrum of neuroimaging abnormalities. Notably, features suggestive of normal pressure hydrocephalus (NPH) were observed, with ventriculosulcal disproportion. Several patients demonstrated evidence of chronic small vessel ischemic changes, including white matter ischemia and chronic lacunar infarcts predominantly in the bilateral gangliocapsular regions, thalamus, and basal ganglia, often accompanied by cerebral atrophy. Acute ischemic events were also noted, such as infarcts in the right basal ganglia and left thalamus. Additionally, multiple chronic infarctions and microbleeds in the right basal ganglia were observed. In one case, a brain abscess was localized in the thalamic region, suggesting fungal dissemination to central nervous tissue. These findings collectively highlight the propensity of invasive aspergillosis to cause both vascular and direct parenchymal brain involvement.

CT scan Findings

A detailed evaluation of thoracic and neurological CT scans in patients with invasive aspergillosis revealed diverse pulmonary and extrapulmonary abnormalities, primarily involving the lung parenchyma, pleura, and mediastinum. High-resolution thoracic imaging commonly showed bilateral ground-glass opacities, multifocal nodules, and lobar consolidations. Interstitial fibrotic changes, mosaic attenuation, and patterns were indicative of coexisting interstitial lung diseases. Bronchiolar involvement was evidenced by tree-inbud signs, traction bronchiectasis, and centrilobular nodules, while cavitations, halo signs, and mycetomatous lesions pointed to fungal invasiveness. Frequent pleural pathology included effusions, thickening, calcifications, and associated volume loss. Mediastinal lymphadenopathy, shifts, and pericardial effusions were also observed. Extrapulmonary findings included rib fractures, bowel wall thickening, and cerebral atrophy with lacunar infarcts. Overall, the CT findings highlighted widespread systemic involvement driven by invasive aspergillosis and compounded by chronic conditions.

10. Antifungal Treatment

During the treatment of IA, a variety of antifungal medications were administered based on clinical presentation, severity, and drug availability. Voriconazole was the most frequently used agent, prescribed in 54% of cases, aligning with its role as the first line therapy for invasive aspergillosis, while the least used was Anidulafungin (4%) (Figure 6).

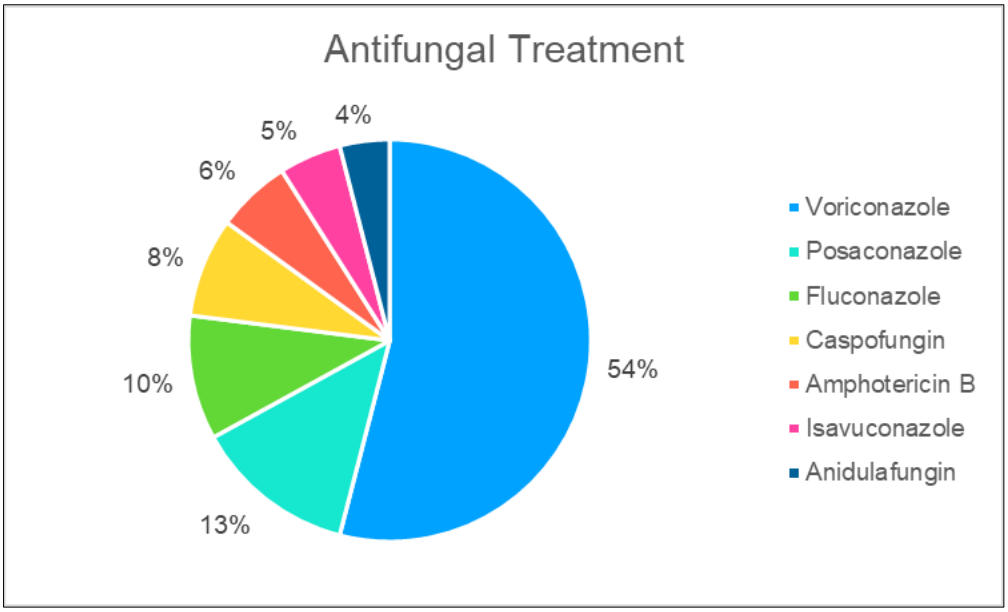

Fig. 6: Voriconazole was the most used antifungal (54%), while Anidulafungin was the least used (4%) in Invasive Aspergillosis treatment

DISCUSSION

This study aimed to compare the diagnostic performance and clinical utility of PCR and Galactomannan (GM) ELISA assays in detecting invasive aspergillosis (IA).

The GM index was significantly higher in BAL samples than in serum ($p = 0.001$), suggesting a greater antigen load in the respiratory tract [20]. In contrast, PCR Ct values showed no significant difference between BAL and serum ($p = 0.46$), indicating comparable DNA detection across sample types. A significant negative correlation ($r = -0.573$) was observed between GM index and PCR Ct values in BAL fluid, reinforcing that higher antigen loads are associated with lower Ct values (i.e., higher fungal burden) [15]. The p-value of 0.0053 confirms that this correlation is statistically significant, and the negative covariance (– 2.5402) further reinforces the inverse association. The test statistic ($t = -3.1265$) provides additional support for the strength of the relationship.

Species-wise analysis indicated that *A. flavus* had the highest Ct value and *A. fumigatus* had the highest GM index, with a significant difference in Ct values between *A. flavus* and other species ($p = 0.02$). Although patients with comorbidities like diabetes and CKD showed elevated GM indices, none of the differences in GM or Ct values across comorbidity groups reached statistical significance.

Additionally, CRP exhibited a weak, non-significant correlation with both GM index ($r = 0.1529$, $p = 0.1369$) and PCR Ct values ($r = 0.3164$, $p = 0.1154$), indicating limited utility of CRP as a marker for fungal burden. Haematological parameters (TC, NP, HB, ESR, CRP) also showed no significant influence on diagnostic values in either test.

In conclusion, GM ELISA appears more sensitive for detection, especially in BAL fluid, while PCR provides species-level detection and may be more specific [13]. Their complementary roles underscore the importance of a multimodal diagnostic approach for early and accurate detection of invasive aspergillosis.

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Author's Contributions

KR, AKD, SD helped in the collection of data, clinical testing and analyzation. BNC, PG, and SD guided the study design, advised on data interpretation and critically revised the manuscript. All the authors have reviewed and approved the final version of the manuscript.

Conflicts of Interest: The authors have declared no potential conflict of interest.

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