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SERUM GALACTOMANNAN AND (1-3)-B-D-GLUCAN DETECTION FOR THE EARLY DIAGNOSIS OF INVASIVE ASPERGILLOSIS

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Abstract

Background

Invasive aspergillosis (IA) stands as one of the most common fungal infections among immunocompromised and high-risk patients. Early detection and swift antifungal treatment may enhance patient outcomes. The guidelines incorporated microbiological criteria using GM and BG tests to identify invasive fungal infections and probable IA in immunocompromised patients. The present study aimed to detect the presence of IA via GM and BDG tests in a plethora of patients.

Materials and methods

After the approval from the institutional review board, the specimens (BAL, serum, CSF, and sputum) were collected from various hospitals of the Karachi from patients irrespective of their age or gender. The BDG was evaluated using the lateral flow methodology and GM was detected via the chromogenic method.

Results

Of the total 820 samples, the frequency of various specimens revealed bronchoalveolar lavage (n=412) as the majority followed by serum (n=268), sputum (n=80), and cerebrospinal fluid (n=60). Results showed that BDG exhibited higher sensitivity (97.8%) compared to Galactomannan (91.8%). In BAL samples, GM identified 384 cases, with 93% (304/325) confirmed, while BDG detected 410 positive cases, of which 99.6% (324/325) were proven IA cases. For serum samples, GM identified 264 cases, with 82% (13/16) confirmed, whereas BDG detected 257 positive cases, all of which (16/16) were proven IA cases. In CSF samples, GM identified 52 cases, with 86.8% (33/38) confirmed, while BDG detected 57 positive cases, of which 94.7% (36/38) were proven IA cases. Lastly, in sputum samples, GM identified 71 cases, with 91.4% (48/51) confirmed, whereas BDG detected 78 positive cases, all of which (51/51) were proven IA cases.

Conclusion

Invasive aspergillosis is a fatal fungal infection that remains an important cause of illness and death in immunocompromised patients; the development of early and sound diagnostic strategies remains pivotal. It has been aimed in this research to explore the role of serum galactomannan and β-D-

glucan tests as diagnostic markers for the early diagnosis of this lethal fungal infection. This analysis shows that both biomarkers are useful in the assessment of the diagnostics, although they incorporate different strengths and weaknesses. Both approaches, when used simultaneously and in conjunction with clinical and radiographic examinations, present a viable system to enable timely diagnosis and monitoring of this challenging fungal disease.

Keywords: Invasive aspergillosis, Galactomannan, B-D-Glucan, bronchoalveolar lavage, sputum, serum, cerebrospinal fluid

Introduction

Invasive aspergillosis (IA) remains a serious risk to immunocompromised individuals, especially those undergoing haematopietic stem cell transplantation or chemotherapy for hematological malignancies.[1, 2] Early diagnosis of IA is needed as delayed treatment increases morbidity and death, which improves patient outcomes.[3] Yet clinical symptoms are non–specific and traditional diagnostic methods are limited, making IA extremely difficult to diagnose.

In recent years biomarker based diagnostic techniques have gained popularity due to their ability to diagnose fungal infections earlier and more accurately.[4] Two of these biomarkers, serum galactomannan (GM) and (1-3)-β-D-glucan (BDG) have been promising biomarkers in the identification of IA.[5, 6] GM is a polysaccharide part of the Aspergillus cell wall that is released into the culture during fungal growth, whereas BDG is a component of the Aspergillus cell wall and occurs in many fungi including Aspergillus species.[1, 7]

GM has been well studied and used into IA diagnostic methods based on detection of GM in serum.[5] The GM assay has been particularly sensitive and specific for the diagnosis of IA, particularly in hematological patients.[8] Nevertheless, the efficacy of the GM assay is affected by a number of conditions, including the use of mold-active antifungal prophylaxis and the presence of concomitant infections.[4]

However, while the detection of BDG has also shown promise as a diagnostic tool for IA, its specificity is lower, as BDG is present in a wider variety of fungal species than GM.[8] The introduction into clinical practice of GM and BDG tests may significantly improve the management of patients and their outcomes. If IA is detected early enough using biomarker-based methods, antifungal treatment may be started on time, reducing mortality and improving the overall prognosis.[9] They also may be useful to determine the duration of antifungal treatment, and to evaluate the effectiveness of treatment.[10]

Encouraging results have been seen in testing for GM and BDG but there are many impediments and restrictions. There is an ongoing discussion about the time and frequency at which biomarker testing should be performed and the cut off levels for positivity.[8, 10] Additionally, the performance of these biomarker is dependent upon the patient demographic, the existence of underlying diseases and presence of confounding factors.[2, 4]

These diagnostic instruments have extensive application, which is dependent on the development of standardized procedures for biomarker analysis and testing.[7, 8] Guidelines and standardization of testing methods for the use of GM and BDG for IA diagnosis is a current focus of ongoing initiatives.[11] They are these strategies which are directed to raising the consistency and comparative quality of findings in different research arrangements and therapeutic settings. In addition to GM and BDG, the possible role of other biomarkers and molecular diagnostic methods in IA diagnosis is being considered. These include polymerase chain reaction (PCR)-based techniques, Aspergillus specific lateral flow device testing and new generation technologies such as proteomics and metabolomics.[7] Method of diagnosing IA in the future may involve a more thorough way by the combination of several diagnostic modalities including imaging tests, molecular techniques and biomarkers.

In this study, we aim to evaluate the role of (1-3)-β-D-glucan and the serum galactomannan in the early diagnosis of invasive aspergillosis. The authors set out to determine the potential of these biomarkers to improve diagnostic precision and allow for early initiation of antifungal therapy based

upon sensitivity and specificity. Furthermore, the possible impact of the variables on biomarker performance and the resulting effect on patient outcomes and clinical practice.

Materials and methods

The present study commenced the collection of the specimens the patients after the attainment of an approval from the institutional review board (IRB:). The specimens included samples of bronchoalveolar lavage, serum, sputum, and cerebrospinal fluid collected from patients irrespective of age or gender from various hospitals of Karachi on convenient sampling basis. However, sputum and bronchoalveolar lavage samples with high quantity of sputum, presence of blood or clot in the cerebrospinal fluid samples, and serum samples which were lipemic or lipolyzed were excluded from the study.

Galactomannan detection was done using the lateral flow methodology which is based on the principle of colloidal gold immunochromatography. When testing positive samples, the Aspergillus galactomannan antigen in the samples combines with the gold-conjugated mouse anti-galactomannan monoclonal antibody to form an immune complex, the complex flow forward on the nitrocellulose membrane through chromatography effect. When crossing the test line (T) it will react with the test strip pre-coated with anti-galactomannan monoclonal antibody to form a sandwich structure and display red strip. When the excess gold-conjugated crossing the control line (C), it will combine with the pre-coated sheep anti-mouse IgG antibody and display a red stripe. Negative test results form only line (C). If the control line fails to develop then the test is not valid.

The detection of (1-3)-β-D-Glucan is based upon a modification of the Tachypleus tridentatus (TAL) pathway. BDG triggers factor G, a serine protease enzyme. The triggered factor G converts the inactive proclotting enzyme to the active clotting enzyme. The activated factor G facilitates the transformation of the inactive proclotting enzyme into its active form. This active clotting enzyme then separates pNA from the chromogenic peptide substrate, Boc-Leu-Gly-Arg=pNA, creating a chromophore that absorbs light at 405nm. The kinetic assay, as shown below, is based on measuring the rate at which optical density increases in each sample. This rate is then compared to a standard curve to determine the concentration of BDG in the sample. The interpretation of the findings is displayed in **Table 1**.

Table 1: Interpretation of the findings

Sample type	Result (pg/ml)	Interpretation
Serum	<60	Negative results
BAL fluid	<400	
Serum	60-79	Possible fungal infections, continuous detection is required
BAL fluid	400-449	
Serum	≥80	Positive results Positive result means the presence of (1-3)-β-D-Glucan, but it does not define the presence of the disease and should be used in conjunction with other clinical findings for the establishment of a diagnosis
BAL fluid	≥450	

Results

As per the inclusion criteria, a total of 820 patients were enrolled in the current study. The study had a male predominance i.e., 485 (59.2%) among a total of 820 samples. Almost half of all samples were obtained via bronchoalveolar lavage i.e. 432 (50.2%) followed by serum (n=268; 32.9%), sputum (n=80; 9.76%), and cerebrospinal fluid (n=60; 7.32%). The patients admitted to the ICU due to sepsis or mixed reasons were the most frequent condition observed in the study participants i.e. 330 (40.2%) followed by 180 (22.0%) of haematological disorders and 176 (21.5%) patients on immunosuppressive therapy cases **(Figure 1)**.

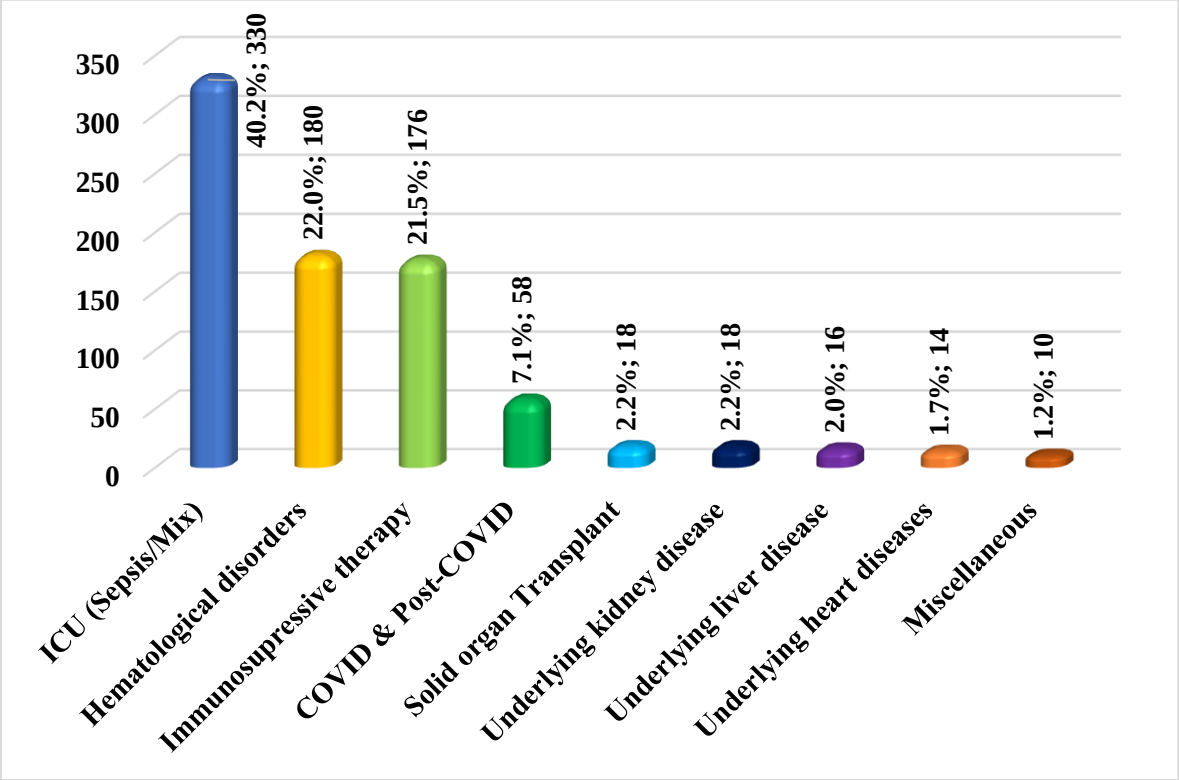

Figure 1: Frequency of the underlying conditions

Beta D Glucan was able to identify slightly more cases of Invasive aspergillosis i.e. 97.8% (n=802) in comparison to Galactomannan [91.8%; n=753]. The Beta D Glucan chromogenic test methods were the most sensitive and reliable method with 97.8% sensitivity, but the Galactomannan was the easiest and most rapid method to detect invasive aspergillosis within one hour with 91.8% sensitivity. The maximum number of patients identified for the presence of Invasive Aspergillosis via Galactomannan [289; 35.2%] and Beta D Glucan [312; 38.0%] were above the age of 60 years followed by second most frequent age category of 44-60 years with po**sitive** IA cases via Galactomannan [226; 27.6%] and Beta D Glucan [238; 29.0%] respectively **(Figure 2).**

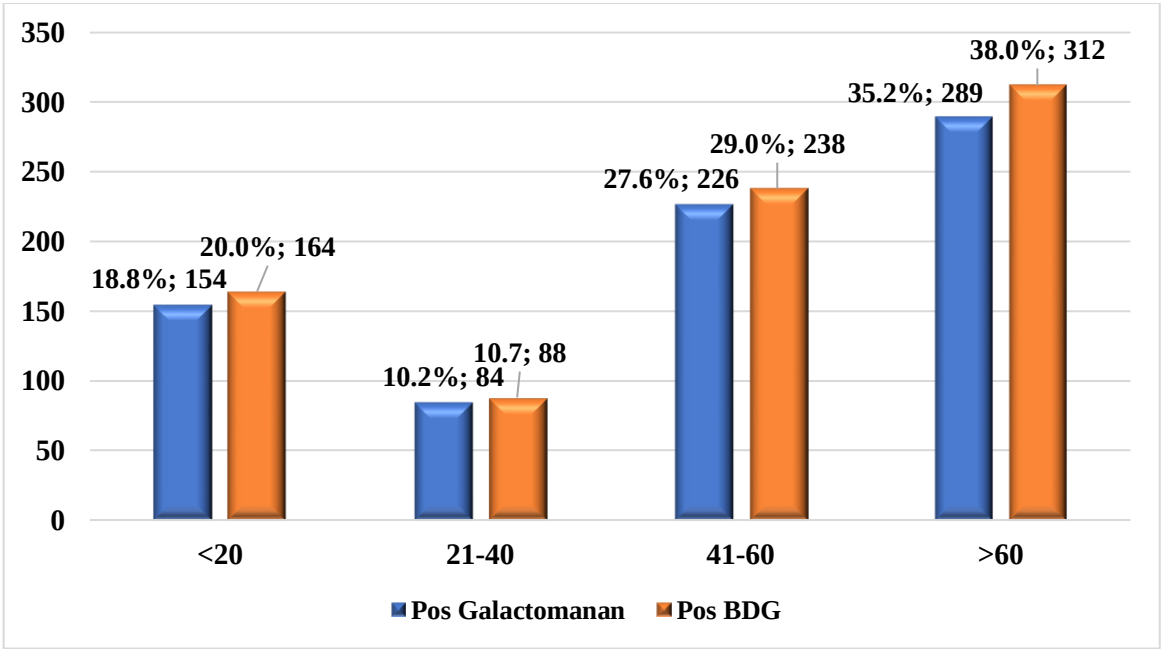

Figure 2: Age-wise distribution of positively identified Invasive Aspergillosis cases via Galactomannan and Beta D Glucan

Of all the cases identified via Galactomannan and Beta D Glucan, the proven cases were observed to be the most frequent i.e. 430 (52.4%) followed by probable [213; 26.2%], and possible [177; 22.0%] cases. In all 412 bronchoalveolar lavage samples [Proven (n=325), probable (n=73), and possible (n=14)] the number of positive samples for Galactomannan was 384 (93.2%) and negative 28 (6.80%) while for Beta D Glucan the number of Positive samples was 410 (99.5%) and negative were 2 (0.5%) **(Table 2)**. Of those identified, the proven cases were the most frequent in both groups i.e. 304 (93.0%) positive cases via GM and 324 (99.6%) cases via BDG. In all 268 Serum samples [Proven (n=16), probable (n=105), and possible (n=147)] the number of positive samples for S. Galactomannan was 246 (91.8%) and negative 22 (8.2%) while for Beta D Glucan the number of positive samples was 257 (95.9%) and negative were 11 (4.1%) **(Table 2)**. Of those identified, the possible cases were the most frequent in both groups i.e. 133(90.0%) positive cases via GM and 137 (93.0.%) cases via BDG. In all 60 CSF samples [Proven (n=38), probable (n=14), and possible (n=8)], the number of positive samples for S. Galactomannan was 52 (86.7%) and negative 8 (13.3%) while for Beta D Glucan the number of positive samples was 57 (95.9%) and negative were 3 (5.0%) **(Table 2)**. Of those identified, the proven cases were the most frequent in both groups i.e. 33 (86.8%) positive cases via GM and 36 (94.7%) cases via BDG. In all 80 Sputum samples [Proven (n=51), probable (n=21), and possible (n=8)] the number of positive samples for S. Galactomannan was 71 (87.8%) and negative 9 (11.3%) while for Beta D Glucan the number of positive samples was 78 (97.5%) and negative was 2 (2.5%) **(Table 2)**. Of those identified, the proven cases were the most frequent in both groups i.e. 48 (94.1%) positive cases via GM and 51 (100%) cases via BDG.

Table 2: Frequency of Beta D Glucan and Galactomannan in all specimens

		Galactomannan		**Beta D Glucan**	
	Broncolaveolar lavage				
		Positive (n=384; 93.2%)	**Negative (n=28; 6.8%)**	**Positive (n=410: 99.5%)**	**Negative (n=2; 0.5%)**
Proven	325 (78.8%)	304 (93%)	21 (7%)	324 (99.6%)	1 (0.4%)
Probable	73 (17.7%)	69 (94%)	4 (6%)	73 (100%)	0
Possible	14 (3.3%)	11 (78.5%)	3 (21.5%)	13 (92.8%)	1 (7.15%)
	Serum				
		Positive (n=246; 91.8%)	**Negative (n=22; 8.2%)**	**Positive (n=257; 95.9%)**	**Negative (n=11; 4.1%)**
Proven	16 (5.9%)	13 (82%)	3 (18%)	16 (100%)	0
Probable	105 (39.1%)	100 (95%)	5 (5%)	104 (99%)	1 (1%)
Possible	147 (54.9%)	133 (90%)	14 (10%)	137 (93%)	10 (7%)
	Cerebrospinal fluid				
		Positive (n=52; 86.7%)	**Negative (n=8; 13.3%)**	**Positive (n=57; 95.9%)**	**Negative (n=3; 5.0%)**
Proven	38 (63.3%)	33 (86.8%)	5 (13.2%)	36 (94.7%)	2 (5.3%)
Probable	14 (23.3%)	13 (92.8%)	1 (7.20%)	14 (100%)	0
Possible	8 (13.3%)	6 (75%)	2 (25%)	7 (87.5%)	1 (7.2%)
	Sputum				
		Positive (n=71; 88.8%)	**Negative (n=9; 11.3%)**	**Positive (n=78; 97.5%)**	**Negative (n=2; 2.5%)**
Proven	51 (63.8%)	48 (94.1%)	3 (5.9%)	51 (100%)	0
Probable	21 (26.3%)	17 (81%)	4 (19%)	20 (95.20%)	1 (4.8%)
Possible	8 (10%)	6 (75%)	2 (25%)	7 (87.50%)	1 (12.50%)

Discussion

This current work offers important findings on the use of beta-D-glucan and galactomannan, two important biomarkers, in the diagnosis of invasive aspergillosis (IA). The findings underscore the significance of these diagnostic instruments for detecting IA cases in a range of patient categories and specimens.

The results of the study are strengthened by the large sample size of 820 patients. We found that male predominance (59.2%) is consistent with earlier findings indicating male gender may be a risk factor for IA.[12, 13] To understand the probable causative biological or environmental factors that might contribute towards the grater vulnerability of the males, this gender gap requires more research.

The pattern of sample types shows the great variety of clinical manifestations of IA, with bronchoalveolar lavage (BAL) being the most frequent type (50.2%), followed by serum (32.9%) and sputum (9.76%). This diversity in sample type underscores the importance of a thorough diagnostic strategy given the fact that different sample types may generate different answers depending on the infection's stage and location.[14]

The most common causes of admission to the ICU were sepsis or mixed causes (40.2%), hematological illnesses (22.0%) and those on immunosuppressive medication (21.5%). The distribution is consistent with established IA risk factors, especially in patients who are severely ill and immunocompromised.[11, 15] Early diagnosis and careful screening are important in high-risk groups because of the high incidence of IA in different patient categories.

One important conclusion of the study was that BDG was more sensitive (97.8%) than GM (91.8%) in detecting IA patients. The variation is modest, but may have important clinical implications. BDG may be a better IA screening method due to its greater sensitivity, and since it is less sensitive the chances for false negative results would be lower.[4, 16] However, BDG is not restricted to Aspergillosis and can be grown in other fungal infections. It must therefore be interpreted carefully in conjunction with other diagnostic tests and clinical findings, as such.

The age distribution of IA cases detected by both BDG and GM show that patients above 60 evidently account for the majority and those in the age between 44 and 60 are the second largest. Such an age-related pattern is consistent with other studies which suggest that older individuals are more likely to develop IA, possibly due to immunological senescence and comorbidity.[9, 14, 17] The older patients' increased vulnerability to IA is highlighted by its significance in terms of age-specific procedures for screening and preventive measures in situations of high risk.

Developing a scheme for classifying IA cases into three categories—proven, probable, and possible—provides important information regarding the degree of diagnostic certainty reached. The majority of proven cases (52.4%) in the study achieved a high degree of diagnostic accuracy in both the BDG and GM tests. This is very important considering the difficulties in identifying IA conclusively.[1, 8]

Some intriguing trends emerge when performing test performance analysis on several sample types. BDG showed extraordinarily high sensitivity (99.5%) compared to GM (93.2%) in BAL samples, especially in proven cases. The ability of BDG to identify fungal cell wall components at the infection site could explain its advantage over the other reagents in BAL samples and could make BDG an important diagnostic tool for pulmonary IA.[9, 16] Serum sample sensitivities for BDG (95.9%) and GM (91.8%) were slightly lower but remained high. Blood based assays are more accessible and less invasive than BAL, which makes it reassuring that the serum samples have a relatively high sensitivity. However, the reduced sensitivity relative to BAL samples suggests a limitation of using serum indicators alone for the diagnosis of IA.[7, 8] The success of BDG and GM in CSF samples (95.9% and 86.7% sensitivity, respectively) considering difficulties in identifying aspergillosis of the central nervous system is very remarkable. BDG may be useful in situations of suspected cerebral aspergillosis in CSF samples because of its increased sensitivity. BDG again outperformed GM with sensitivities of 97.5% and 87.8% for both BDG and GM in sputum samples, respectively. This research suggests that sputum analysis of these biomarkers could be a useful non-

invasive screening method, particularly for patients who cannot have more invasive procedures done.[11]

As this field develops, the performance of GM and BDG tests must be rigorously investigated in diverse clinical contexts and patient demographic. Large scale prospective studies are needed to confirm the usefulness of these biomarkers as well as to improve their application in clinical practice. In addition, the use of biomarker-based diagnostics within current clinical algorithms and resource constrained environments would require cost effectiveness studies and studies of implementation.

Conclusion

This study demonstrates the potential of the serum galactomannan and (1-3)-β-D glucan as biomarkers for the early detection of invasive aspergillosis. Based on the present work, the use of two tests, BDG, with higher sensitivity, and GM, with higher specificity, in clinical care may yield improved patient outcomes, increased diagnostic accuracy, and an earlier initiation of antifungal treatment. The performance of biomarkers, however, may be affected by such things as concurrent infections and antifungal treatment, highlighting the need for careful interpretation in the clinical context. Finally, there are also remaining issues to optimize cut off values and standardize testing procedures across a variety of patient populations, and to apply these diagnostic tools more efficiently in clinical practice. As our knowledge of IA pathogenesis and host-pathogen interactions increases, biomarker-based diagnostics are anticipated to develop and offer a new approach to treating this deadly fungal infection.

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CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

AUTHOR CONTRIBUTIONS: MZ collected and analyzed samples and wrote the initial draft; AA and NA critically reviewed the manuscript.

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ETHICS STATEMENT: The study was carried out with the approval of the Board of Advanced Studies of the University of Sindh.

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