RESEARCH ARTICLE

Evaluation of galactomannan and beta-d-glucan assays for the diagnosis of invasive aspergillosis in clinically suspected cases

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Abstract

Objective: To assess the utility of galactomannan and beta-D-glucan assays in the diagnosis of invasive aspergillosis in clinically suspected cases, and to compare their diagnostic potential to determine whether a combination of the two may result in an early and specific diagnosis.

Methods: The descriptive cross-sectional case-control study was conducted at the Armed Forces Institute of Pathology, Rawalpindi, Pakistan, from April 1, 2017, to March 31, 2018, and comprised serum samples from clinically suspected invasive aspergillosis patients and healthy controls. The sera were tested for galactomannan and beta-D-glucan detection. Proven, probable and possible categories of invasive aspergillosis according to European Organisation for Research and Treatment of Cancer / Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group criteria. Galactomannan antigen was detected using a one-stage immunoenzymatic sandwich microplate assay. Beta-D-Glucan antigen was detected using a protease zymogen-based colorimetric assay. Sensitivity and positive / negative likelihood ratio of both the cases and the controls were calculated and compared.

Results: Of the 178 subjects, 119(67%) were cases and 59(33%) were controls. Beta-D-glucan assay was more sensitive than galactomannan assay (91.6% versus 80.67%) whereas galactomannan assay was more specific than beta-D-glucan assay (86.44% versus 76.27%) in the diagnosis of invasive aspergillosis. The sensitivities of both assays decreased with decreasing probability of invasive aspergillosis, i.e., maximum sensitivities of both beta-D-glucan and galactomannan assays were for proven cases (100% versus 87.5%), followed by probable cases (89.29% versus 85.71%), and possible cases (91.57% versus 78.31%).

Conclusion: Both beta-D-glucan and galactomannan assays seemed to play an encouraging role in the diagnosis of invasive aspergillosis in high-risk clinically suspected cases, with the former assay being more sensitive and the latter assay being more specific.

Keywords: Invasive aspergillosis, Galactomannan, Beta-D-glucan.

(JPMA 70: 442; 2020). **https://doi.org/10.5455/JPMA.1476**

Introduction

Invasive Aspergillosis (IA) is an opportunistic mycosis with high mortality,[1] especially among patients of haematological malignancies, haematopoietic stem cell transplant (HSCT) and solid organ transplant (SOT) recipients.[2-4] Early diagnosis and prompt initiation of treatment are critical for IA management in these patients since it is a major cause of death in such populations.[5] Delay in the reliable diagnosis is usually associated with fatal outcome as fungal proliferation becomes overwhelming or therapy may no longer remain successful.[3] The invasive nature of the gold standard for diagnosis i.e. histopathology and culture are often precluded due to cytopenia or critical condition of the patients. This emphasises the need for non-invasive diagnostic procedures allowing easy and early diagnosis of IA. High mortality rates and limitations in diagnostic methods lead to a requirement for improved detection techniques for IA diagnosis[6]. Serological biomarkers like galactomannan (GM) and (1,3)-beta-D-glucan (BDG), therefore, can play a significant role for establishing a convenient diagnosis of this potentially fatal clinical

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condition.

Over the past decades, patient population having IA risk factors has expanded significantly, and IA, since it s associated with high morbidity and mortality, requires improved diagnostic modalities. GM is currently a commonly used method for IA diagnosis and has high specificity, whereas BDG has a high negative predictive value (NPV), making it quite useful to rule out IA rather than to confirm it.[7] GM and BDG assays can also play an important role in IA diagnosis in non-neutropenic patients with underlying respiratory diseases without haematologic malignancy or previous SOT.[8]

GM, is a major aspergillus cell wall component released during the invasive disease. A double-sandwich enzyme-linked immunosorbent GM assay has been approved and is widely used for IA surveillance in immunocompromised patients.[2] This diagnostic modality has a threshold for detection as low as 1.0 ng/mL of GM in serum compared to latex agglutination test with a threshold of 15ng/mL.[9,10] The mean time of detection of serum GM can often be 7-14 days before other diagnostic clues become apparent. It also offers monitoring of GM levels to allow the initiation of pre-emptive antifungal therapy before life-threatening infection occurs. Although initial studies assessed the performance characteristics of this assay with high sensitivity and specificity, more recent studies show significant performance variations.[11]

BDG is the most important and abundant polysaccharide component of the cell wall of most fungi. While incorporated within the fungal cell wall, BDG typically exists as an insoluble structure. In the presence of blood or other body fluids, it transforms into single helix, triple helix (most commonly), or random coil forms and is rendered soluble.[12] The assays developed specifically to measure fungal beta-glucan typically use serum and rely on the activation of the Limulus Amoebocyte Lysate (LAL) clotting cascade as the principle of detection.

The current study was planned to assess the effectiveness of GM and BDG detection in sera for IA diagnosis in clinically suspected cases. Both the assays were planned to be compared to study the diagnostic potential of both markers in various clinical cases and the utility of a combination of the two tests for an early and specific IA diagnosis.

Patients and Methods

The descriptive cross-sectional case-control study was conducted at the Armed Forces Institute of Pathology (AFIP), Rawalpindi, Pakistan, from April 1, 2017, to March 31, 2018, and comprised serum samples from clinically suspected IA patients and healthy controls. After permission from the institutional ethics review board, samples were collected from suspected IA patients (with or without radiological evidence) of either gender aged 4-82 years. Serum samples were collected after obtaining informed consent from all the patients and the healthy controls.

The patient group had all three IA categories - proven, probable and possible - described by the European Organisation for Research and Treatment of Cancer / Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) criteria.[13]

Blood samples were collected in sterile clot-activator tubes. Serum was separated, divided into aliquots and stored at 2°C to 8°C for up to 48 hours until testing.

GM antigen was detected using a one-stage immunoenzymatic sandwich microplate assay (Platelia Aspergillus: Bio-Rad, Marnes-la-Coquette, France), according to manufacturer's instructions. Positive, negative and cut-off controls were included in each assay. The serum samples and controls were treated with the addition of sample treatment solution, vortexed, heated for 3 minutes at 100°C using a water bath, and centrifuged at 10,000xg for 10 minutes. The supernatant was then tested with an enzyme immunoassay (EIA), reading the optical density of each well at 450nm (reference filter of 620/630nm). A result was considered positive with index value of ≥ 0.5, and <0.5 index value was considered negative.

BDG antigen was detected using a protease zymogen-based colorimetric assay (Fungitell assay: Associates of Cape Cod), based upon a modification of the LAL pathway. According to manufacturer's instructions, serum samples were vortexed, treated with serum pre-treatment reagent (0.25 molarity [M] potassium hydroxide [KOH] and 1.2 M potassium chloride [KCl]) and incubated for 10 min at 37°C. A kinetic colorimetric assay was performed and read at 405nm minus 490nm for 40 minutes at 37°C. The concentration of BDG in each sample was automatically calculated using a calibration

Table-3: Galactomannan (GM) and beta-D-glucan (BDG) statistics.

Category of Cases (EORTC/MSG) criteria	GM statistics		BDG statistics	
	Sensitivity	Positive likelihood ratio	Sensitivity	Positive likelihood ratio
Proven cases	87.5%	0.88	100%	1.00
Probable cases	85.71%	0.86	89.29%	0.89
Possible cases	78.31%	0.78	91.57%	0.92
Total cases	80.67%	0.81	91.6%	0.92
Control cases	Specificity: 86.44%	Negative likelihood ratio: 1.16	Specificity: 76.27%	Negative likelihood ratio: 1.31

EORTC/MSG: European Organisation for Research and Treatment of Cancer / Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group.

curve prepared with standard solutions ranging from 6.25 to 100 pg/mL. The assays were performed in duplicate. BDG levels of >80 pg/mL were considered positive results, <60 pg/mL were considered negative, 60-79 pg/mL were considered indeterminate.

Serological assays were performed among clinical cases with a spectrum of evidence of mycological infections.

Statistical analysis was done, using SPSS 16.0 as the statistical tool, to determine sensitivity and positive likelihood ratio of all the three IA categories and specificity and negative likelihood ratio of the controls. Results were also evaluated to determine the significance of both serological assays done in combination.

Results

Of the 178 subjects, 119(67%) were cases and 59(33%) The median age of the patients was 33 years with an IQR of 32 years. Among the patients, 82(69%) were male and 37(31%) were female. Among the controls, 33(56%) were male and 26(44%) were female. The median age of controls was 30 years with an IQR of 35 years. The clinical history of the patients was noted (Table 1). Among the patients, 8(6.7%) were in the proven category with histopathological evidence of IA, 28(23.5%) were in the probable category with growth on culture or fungal hyphae on direct microscopy, and 83(69.7%) were in the possible category with clinical features suggestive of IA with or without radiological features. Both GM and BDG assays were tested on patients in all the three categories as well as on the controls (Table 2).BDG was more sensitive than GM assay (91.6% versus 80.67%) whereas GM was more specific than BDG (86.44% versus 76.27%). The sensitivities of both assays decreased with decreasing IA probability (Table 3).Among the patients, 96(80.7%) were GM-positive, 23(19.3%) were GM-negative, while 109(91.6%) were BDG-positive, and 10(8.4%) were BDG-negative (Table 4).

Table-1: Distribution of patients (n=119).

Patient Population/Category	Number of patients
Bone marrow transplant recipients	17
Hematologic malignancy/Chemotherapy	26
Intensive care patients	59
Liver transplant recipients	4
Solid organ malignancy/Chemotherapy	8
Miscellaneous	5

Table-2: Results of galactomannan (GM) and beta-D-glucan (BDG) assays.

IA cases	GM results		BDG results		Total
	Positive	Negative	Positive	Negative	
Proven	7 (87.5%)	1 (12.5%)	8 (100%)	0	8
Probable	24 (85.7%)	4 (14.3%)	25 (89.3%)	3 (10.7%)	28
Possible	65 (78.3%)	18 (21.7%)	76 (91.6%)	7 (8.4%)	83
Control	8 (13.6%)	51 (86.4%)	14 (23.7%)	45 (76.3%)	59

Table-4: Overview of both diagnostic assays' results (n=119).

	BDG Positive	BDG Negative
GM Positive	89	7
GM Negative	20	3

GM: Galactomannan, BDG: Beta-D-glucan

Discussion

The presence of risk factors and clinical suspicion with other evidence of IA can be supported by either GM or BDG assay, but the diagnostic potential is improved if both assays are performed / interpreted simultaneously. Several studies have reported variable sensitivities and specificities of GM and BDG assays. One such study compared the two assays and found the GM assay to be more specific than BDG (97% versus 82%), and BDG more sensitive than GM (81% versus 49%), concluding that a combination of both tests could strengthen the diagnosis of IA.[12] Another study also reported higher sensitivity

associated with BDG assay and higher specificity associated with GM assay for the diagnosis of IA.[13]

BDG assay is convenient to perform and is more sensitive, making it valuable as a screening tool. However, compared to GM, BDG bears higher cost and requires purchase of expensive glucan-free disposable materials. From a lab stand-point, BDG requires a batch of test requests, which poses limitations for the lab to entertain a single emergency request made by the clinicians.[14] BDG assay can also yield false positive (FP) results, attributed to several factors other than invasive fungal infections (IFIs), including intravenous (IV) use of antibiotics like amoxicillin-clavulanate and tazobactum-piperacillin,[15] use of haemodialysis membranes in end-stage renal disease (ESRD) patients,[16] immunoglobulin (IGs) or albumin administration,[17] blood cultures positive for bacteria,[18] contamination of blood products by filtering through cellulose filters during manufacturing process,[19] use of surgical gauzes containing glucan,[20] or mucositis etc.[21]

GM assay is easy to perform, gives early results, but it has a lesser sensitivity and higher specificity than the BDG assay FP results are also associated with the GM assay by release of GM antigen into circulation from factors other than IA, including foods, IV antibiotics such as tazobactum-piperacillin, dysfunction of gastrointestinal (GI) mucosal barrier releasing faecal GM into circulation,[22] blood transfusion components etc.[23]

Conclusion

GM was found to have high diagnostic specificity, while BDG displayed better sensitivity. Either test used alone carried certain level of diagnostic limitation. A combination of both assays would improve the diagnostic capacity.

Disclaimer: None.
Conflict of Interest: None.
Source of Funding: None.

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