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Valuable Role of Beta-D-Glucan in The Diagnosis of Invasive Fungal Infections in Patients with Hematological Malignancies

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ABSTRACT

Background: Invasive fungal infections (IFIs) are a major cause of severe illness and death in immunocompromised patients, especially those with blood cancers. Diagnosing these infections is challenging due to nonspecific symptoms and the limited sensitivity of traditional culture-based methods. Recently, rapid non-culture-based diagnostics, such as the 1,3-beta-D-glucan (BDG) assay, have become important adjuncts to standard diagnostic tools.

Objective: To determine the diagnostic performance of BDG as an adjunct diagnostic strategy for IFIs in patients with hematological malignancies. **Subjects and Methods:** Fifty-one patients were enrolled from the Hemato-oncology Department of Ain Shams University Hospital in Egypt from May 2022 to April 2023. The enrolled subjects were classified into healthy controls (n= 13), pathological controls (n= 13), and IFIs cases group (n = 25), patients were subjected to BDG test, complete blood count (CBC) with differential, highly sensitive-C reactive protein (hs-CRP), and (Microbiological cultures and/or histopathological examination). **Results:** At a cut-off of 187.21 pg/ml the maximum efficacy of the BDG test was achieved concerning the cases versus total controls with a sensitivity of 84%, specificity of 84.6%, negative predictive value (NPV) of 84.6%, positive predictive value (PPV) 84%, and efficacy of 84.3%.

Conclusions: Identifying the BDG antigen can be a useful diagnostic approach for IFIs when applied in the appropriate clinical context, such as in patients with weakened immune systems, low white blood cell counts, or blood cancers, by a healthcare professional who is well-informed about the test's strengths and weaknesses and can customize its use for optimal clinical decision-making in each patient's case.

Keywords: Beta-D-glucan (BDG); Invasive fungal infections; Hematological malignancies.

INTRODUCTION

Patients with hematological malignancies (HMs) represent a vulnerable group of patients for invasive fungal infections (IFIs), which is attributed to the pathology of the disease itself, besides the side effect of the treatment regimen as most of the anti-neoplastic protocols are associated with major side effects including neutropenia which open the gate for such opportunistic infections [1]. Patients with blood cancers who are receiving chemotherapy, radiotherapy, or undergoing autologous or allogeneic hematopoietic stem cell transplantation (HSCT) are at a heightened risk of infection. In this context, *Aspergillus* spp. is the most commonly encountered microorganism [2].

In recent years, there has been increasing focus on rare molds like *Zygomycetes* and *Fusarium* spp., which can cause severe clinical complications. Although the widespread use of antifungal prophylaxis has reduced yeast infections such as candidemia, these infections still result in high mortality rates [2].

Diagnosing IFIs in immunocompromised patients is difficult due to non-specific symptoms and the low detection rate of traditional culture-based methods. Rapid diagnostic techniques that do not rely on cultures, such as 1,3-beta-D-glucan (BDG) and galactomannan (GM) assays, have shown promise as valuable supplements to standard diagnostic approaches [3].

Regarding BDG, it is a predominant and specific polysaccharide derived from the cell wall of most fungi except for the *Mucorales* and *Cryptococcus*. The mammalian cells, prokaryotes as well and viruses lack BDG. Thus, using this pan-fungal marker technique on blood samples and normally sterile body fluids could be ideal for the detection of IFIs in the human body [4]. There have been several reports showing the valuable role of BDG assay when used among hematological malignancy patients as a diagnostic marker for IFIs [4-8].

Lamoth *et al.*, *2021* [4] reported that since 2008, the use of 1,3-βDG detection has been considered for inclusion as a mycological criterion for diagnosing IFIs based on the definitions set by the European Organization for Research and Treatment of Cancer (EORTC) and the Mycoses Study Group (MSG). They further noted that BDG testing could be valuable in diagnosing both invasive aspergillosis and invasive candidiasis when interpreted alongside other clinical and radiological indicators and microbiological markers of IFI. In light of previously mentioned scientific data, our study aimed to determine the diagnostic performance of BDG as an adjunct diagnostic strategy for IFIs in HMs patients.

SUBJECTS AND METHODS

This study was conducted during the period from April 2022 to March 2023. Fifty-one patients were enrolled from the Hemato-oncology Department of Ain Shams University Hospital, Egypt. The study was a case-control prospective study conducted by simple randomization method.

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Subjected patients were classified into three groups: **Group 1a:** Healthy control group (n= 13), **Group 1b:** Pathological control group (n= 13), and **Group 2:** IFIs cases group (n=25). Both 1a and 1b groups were named together (control group).

Study Population

Inclusion Criteria: Patients with HMs who fulfilled the criteria of proven or probable IFIs according to EORTC/MSG definitions were included in the study [9].

Exclusion Criteria: Patients diagnosed with IFIs, but whose primary disease was not one of the HMs, also those patients with chronic fungal infections.

The control group included both healthy and pathological controls. The pathological controls (n= 13) included those who were diagnosed with hematological malignancy after ruling out IFIs clinical and laboratory criteria. The samples were taken from outpatient clinics during patients' attendance for receiving their chemotherapy regimen and from admitted HM patients after the infection has been rolled out

The healthy control group (n=13) samples were taken from healthy blood donors after their routine clinical and laboratory checkup excluding the presence of any chronic or interfering disease. The control group as a whole was age and sex-matched with the diseased group.

Enrolled patients were subjected to the following: detailed history taking, clinical examination, and sampling from peripheral blood for the following test examination; BDG test, CBC with differential, hs-CRP, and microbiological cultures and/or histopathological examination. BDG test was performed by Colorimetric Fungitell® assay utilizing a (1→3)-β-D-glucan -specific Limulus Amebocyte Lysate (LAL) reagent. The test was measured by ELISA technique, the kit was provided by Wuhan Fine Biotech Company (No. 818 Gaoxin Ave. East Lake High-Tech Development Zone, Wuhan, Hubei, China.). The assay was done in the laboratories of Clinical Pathology Department.

Ethical considerations:

The study was done after being accepted by the Research Ethics Committee, Ain Shams University (FMASU MS 337/2022). Study objectives and procedures were explained to the participants. All patients provided written informed consents prior to their enrolment. The consent form explicitly outlined their agreement to participate in the study and for the publication of data, ensuring protection of their confidentiality and privacy. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Statistical analysis

Statistical analysis was performed by using the SPSS statistical software package (V. 26.0, IBM Corp., USA, 2019). Data were expressed as numbers (percentage) for presenting qualitative data and median (interquartile range) for quantitative nonparametric data. The comparison between every two independent groups was done by the Wilcoxon rank sum test. The Kruskal-Wallis test was used for comparing more than 2 groups, and after using the Kruskal-Wallis test we used Wilcoxon to compare every 2 groups from the previous test. Regarding the correlation statistics, Spearman correlation was used for the possible associations between every two studied variables. The receiver operating characteristic (ROC) curve was used to assess the best cutoff point with sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and area under the curve (AUC) being calculated. The significance level was taken at P value ≤ 0.05 (P value at 0.05 or less was considered significant, while P values at 0.01 and 0.001 or less were highly significant).

RESULTS

A. Demographic and clinical data:

Comparative statistics between the studied groups: Table (1) demonstrates that compared to the control group, the disease group had more comorbidity, e.g., respiratory distress, they were more frequently admitted to hospital, and 52% died.

Concerning the cases group, IFIs were proven in ten cases while the remaining patients in this group (n = 15) were probable IFIs cases, the criteria of proven or probable IFIs were according to EORTC/MSG definitions [9].

Figure (1) illustrates the different diagnoses (concerning the type of HMs) among both cases and pathological control groups. It is evident from the figure that multiple myeloma (MM), lymphomas, and chronic lymphocytic leukemia (CLL) were more in the pathological controls than IFIs cases group, on the other side AML, acute lymphocytic leukemia (ALL), bone marrow aplasia and light chain disease were more predominant among IFIs cases group.

Table (1): Statistical Comparison between IFIs cases (Gr2) and the controls (Gr1a +Gr1b) as regards the demographic and clinical data using Wilcoxon Rank Sum Test as all the data were non-parametric.

Parameter	Control Group (n=26)	Disease Group (n=25)	P value	Sig
Age	*29 (40.5-51)	*47 (29-71)	0.113	NS
Male gender	15 (57.7%)	20 (80%)	0.139	NS
Respiratory distress	2 (7.6 %)	19 (76 %)	< 0.001	HS
Respiratory support	0 (0%)	15 (60%)	< 0.001	HS
ENT symptoms and/or proptosis	0 (0%)	5 (20%)	0.018	HS
CNS symptoms	0 (0%)	7 (28%)	0.005	HS
Neutropenia	2 (7.6%)	20 (80%)	< 0.001	S
HSCT	0 (0 %)	9 (36%)	<0.001	HS
IS diseases	1 (7.7%)	5 (20%)	0.082	NS
ICU admission	0 (0%)	12 (48%)	< 0.001	HS
LOH (days)	*5 (0-14)	*36 (22- 53.5)	< 0.001	HS
Deaths	0 (0 %)	13 (52%)	< 0.001	HS

Values are presented as median and interquartile range (IQR)* or number (%), P: probability value, Sig: significance, NS: not significant, S: significant, HS: Highly significant, IS diseases: immunosuppressive diseases, LOH: length of hospital stay, HSCT: hematopoietic stem cell transplantation, ENT: Ear-Nose-Throat, CNS: central nervous system, ICU: intensive care unit.

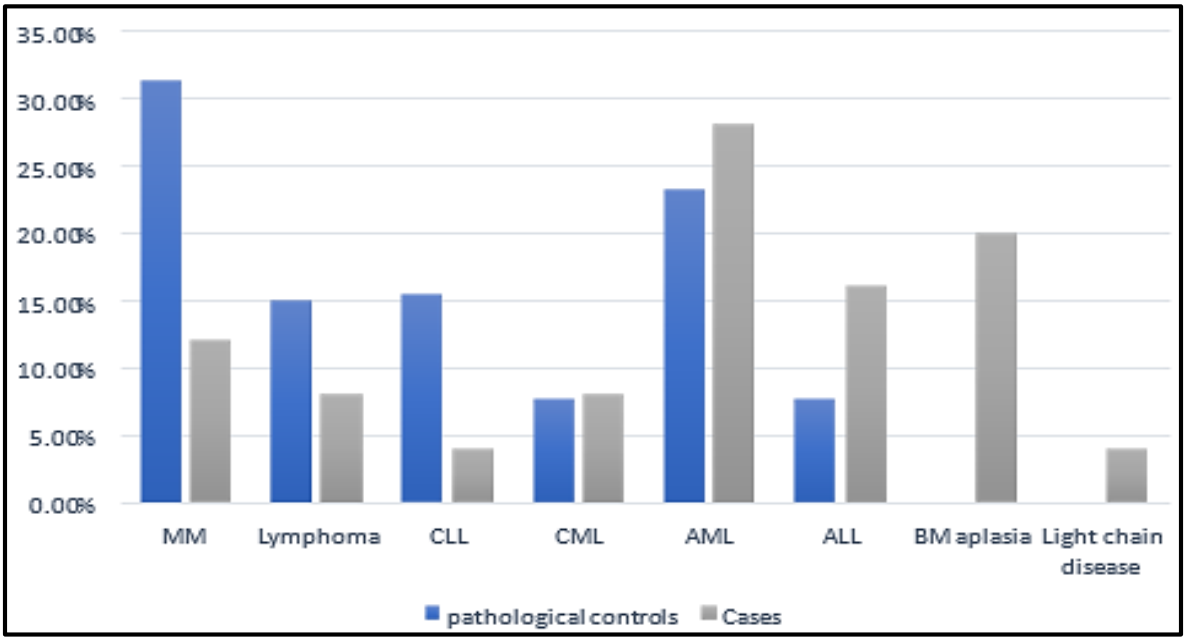

Figure (1): Diagnosis of patients regarding the type of their HMs for both IFI cases and pathological control groups. multiple myeloma (MM), Chronic lymphocytic leukemia (CLL), Acute lymphocytic leukemia (ALL), Acute Myeloid Leukemia (AML), Chronic Myeloid Leukemia (CML).

B- Beta -D-Glucan, CRP, and hematologic profiles:

1) Descriptive and comparative statistics:

Table (2) demonstrates that the pathological group were older, had more TLC, ANC, and platelet than the other 2 groups. While the IFI cases spent longer time hospitalized, had more ALC, CRP, and BDG than the 2 control groups.

Table (2): Statistical Comparison between IFI cases versus healthy controls, IFI cases versus pathological controls & IFIs cases versus total controls as regards the laboratory data using Wilcoxon Rank Sum Test as all the data were non- Parametric.

	Healthy controls	Pathological controls	IFIs cases	IFI cases versus healthy controls			IFI cases versus pathological controls			IFIs cases versus total controls		
	Median (IQR)	Median (IQR)	Median (IQR)	Z1	P1	Sig.1	Z2	P2	Sig.2	Z3	P3	Sig. 3
AGE	32(24 – 40.5)	50 (41.5 – 55)	29 (17 – 47)	0.216	0.829	NS	2.37	0.018	S	1.58	0.113	NS
DOH	0(0- 0)	5(0 – 14)	36 (22- 53.3)	4.385	< 0.001	HS	4.72	< 0.001	HS	4.72	< 0.001	HS
TLC (×10^9/L)	7.2 (5.1 – 9.2)	7.6 (5.3 – 9)	3.3 (0.8 - 6.35)	2.975	0.003	HS	2.97	0.003	HS	3.64	< 0.001	HS
ALC (×10^9/L)	2.4 (1.95 – 3.1)	1.1 (0.825- 2.165)	39.3 (2.4 – 64.4)	2.358	0.018	S	2.91	0.004	HS	3.20	0.001	HS
ANC (×10^9/L)	3.2 (2.1 – 4.25)	4.9 (3.37 – 5.71)	2 (0.6 – 3.1)	1.797	0.072	NS	2.98	0.003	HS	2.90	0.004	HS
Hb (g/dL)	13.5 (12.4 - 14.55)	11.7 (10.2 – 13	9.5 (7.65 - 11.65)	3.342	0.001	HS	2.11	0.035	S	3.33	0.001	HS
Plt (×10^9 /L)	247 (207.5 - 324.5)	257 (199.5 – 297)	72 (11.5 – 293)	2.388	0.017	S	2.20	0.028	S	2.80	0.005	HS
CRP (mg/dl)	4(4- 4)	4(4 – 5)	36 (12 – 81)	4.926	< 0.001	HS	4.45	< 0.001	HS	5.95	< 0.001	HS
BDG (pg/ml)	62.034 (50.956 - 124.725	109.17 (95.5765 - 249.525)	671.78 (221.75 – 1264	0	< 0.001	HS	3.55	< 0.001	HS	4.86	< 0.001	HS

HC: healthy controls, PC: pathological controls, Sig: significance, NS: not significant, S: significant, HS: Highly significant, Hb: hemoglobin, TLC: total leukocyte count, ANC: absolute neutrophil count, PLT: platelet, ALC: absolute lymphocyte count, DOH: duration of hospitalization, CRP: C reactive protein, BDG: Beta D glucan. , IFIs: invasive fungal infections, SD: standard deviation, IQR: interquartile range, Z = Wilcoxon's Rank Sum test.

Figure 2 demonstrates the box blot of BDG in the three studied groups, and it is evident that BDG **statistically highly significant**ly increased in IFIs cases (group 2), when compared to the healthy controls (group 1a) and pathological control (group 1b). Also, there was a **statistically highly significant increase** in BDG in group 1b when compared with group 1a.

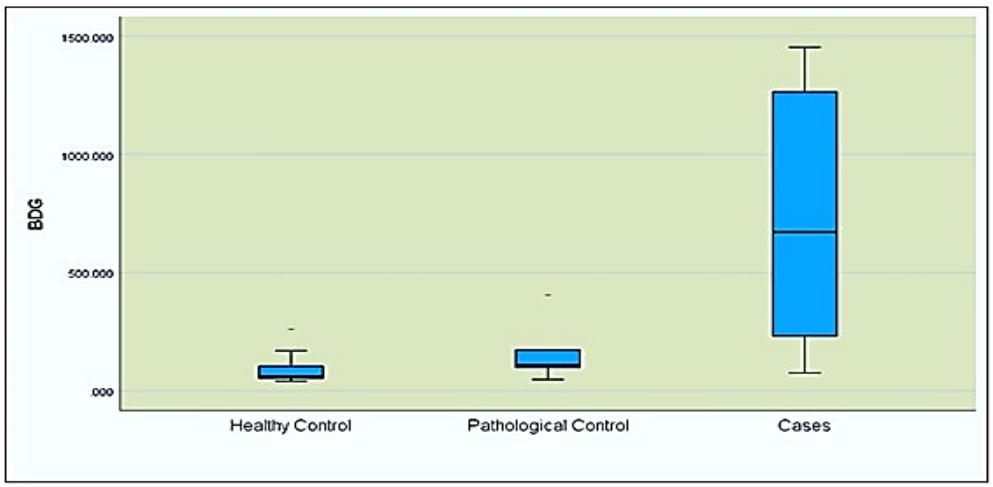

Figure 2: Box blot of BDG values in the three study groups.

2. *Diagnostic performance of BDG, and combined BDG and CRP measurement:*

Table (3) demonstrates that at the cut-off of 187.21 pg/ ml the maximum efficacy of the BDG test was achieved concerning the cases versus the total controls with sensitivity of 84% and specificity of 84.6%.

Table (3): The diagnostic performance of BDG test, CRP, and combination of (BDG and CRP) in the different studied groups

Name of the test and its cutoff	TN	FP	TP	FN	Specificity	Sensitivity	NPV	PPV	Eff
BDG (Cases versus HC) 92.127 pg/ ml	8	5	11	2	61.5	84.6	80.0	68.8	73.1
BDG (Cases versus PC) 75.758 pg/ ml	8	5	25	0	61.5	100.0	100.0	83.3	86.8
BDG (Cases Vs HC+PC) 187.21 pg/ ml	22	4	21	4	84.6	84.0	84.6	84.0	84.3
CRP 5 mg/dl	19	6	20	5	80	73.6	50	91.8	75.2
CRP at 5 mg/dl, And BDG at 187.21 pg/ ml	23	3	24	1	88.5	96.0	95.8	88.9	92.2

Eff.: Efficacy, NPV: Negative predictive value, PPV: Positive predictive value, HC: healthy controls, PC: pathological controls.

3. ROC-curve analysis of BDG test:

It was observed that there were highly significant differences between healthy controls, pathological controls, and case groups as regards many laboratory parameters, but by calculating the diagnostic performance for each one of these laboratory parameters separately, the BDG test achieved the highest univariant efficacy (84.3%) followed by CRP (75.2%). The best efficacy documented ever in the study (92.2%) was achieved by combined measurement of (BDG and CRP) with specificity of 88.5%, PPV of 88.9%, sensitivity of 96%, and NPV of 95.8%, respectively (Figure 3 & table 4).

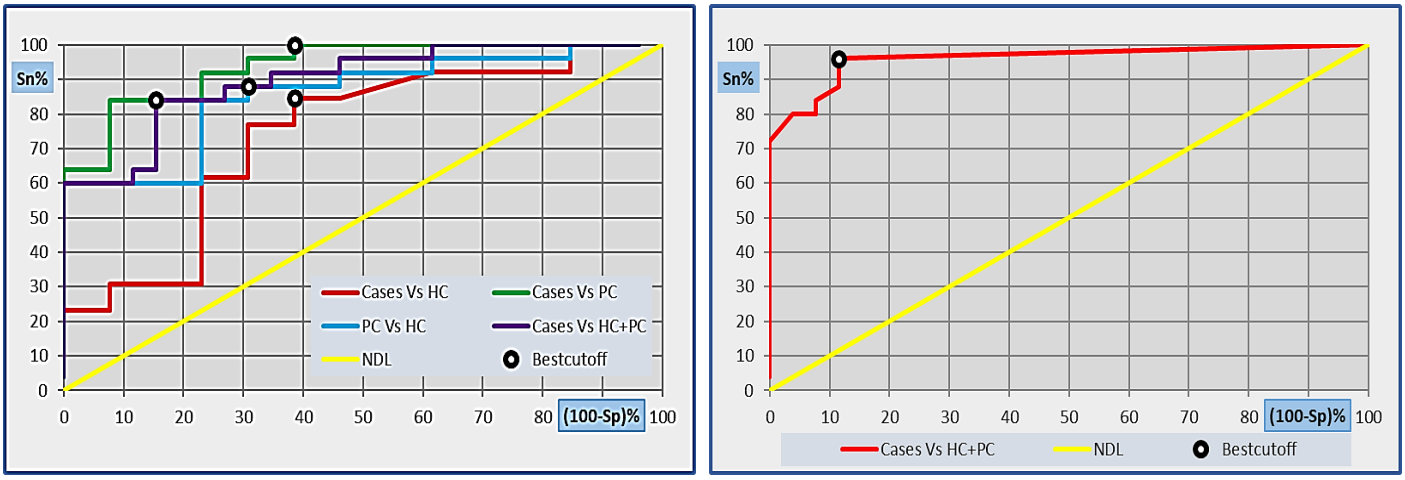

Figure (3): ROC curve analysis showing the diagnostic performance of BDG for discriminating patients with IFIs from those without, with the area under the curve 0.858 (left image). while the diagnostic performance of (the BDG and CRP) combination for discriminating patients with IFIs from those without achieved a better AUC value of 0.960 (right image).

Table (4): The (area under the curve) AUC, standard error (SE) & 95% confidence interval (95%CI) in the different studied groups

	AUC	SE	95CI	
Cases Vs HC	0.663	0.097	0.473	0.852
Cases Vs PC	0.862	0.071	0.723	1.000
Cases Vs HC+PC	0.858	0.053	0.754	0.963
Cases Vs HC+PC	0.960	0.028	0.904	1.016

2-Correlation statistics:

A correlation statistical analysis was conducted between the BDG test in each group of the three studied groups with the other studied parameters, and there were no significant correlations between BDG and any other studied parameters that could be documented (Table 5).

Table (5): Correlation between BDG vs. Different Studied Parameters Using Ranked Spearman's Correlation Test.

HC	BDG		
n= 13	r	p	Sig.
Age	0.295	0.328	NS
TLC	-0.303	0.315	NS
ALC	0.39	0.188	NS
ANC	-0.264	0.383	NS
Hb	-0.119	0.7	NS
Plt	-0.273	0.367	NS
CRP	-0.111	0.717	NS
Hosp.Sty	-0.127	0.678	NS

PC	BDG		
n= 13	r	p	Sig.
Age	0.122	0.692	NS
TLC	0.103	0.739	NS
ALC	-0.431	0.142	NS
ANC	0.022	0.943	NS
Hb	0.017	0.957	NS
Plt	0.243	0.424	NS
CRP	-0.111	0.717	NS
Hosp.Sty	-0.127	0.678	NS

Cases	BDG		
n= 25	r	p	Sig.
Age	-0.284	0.168	NS
TLC	-0.272	0.188	NS
ALC	-0.063	0.775	NS
ANC	-0.34	0.113	NS
Hb	-0.266	0.198	NS
Plt	-0.196	0.347	NS
CRP	-0.039	0.854	NS
Hosp.Sty	0.054	0.796	NS

rs: ranked spearman correlation P: probability Sig: significance S: significant HS: highly significant NS: non significant

Microbiological culture and histopathological examination results:

From a microbiological point of view and as regards the culture results (the gold standard diagnostic modality of IFIs), it was positive in three cases only (positive for *Candida* spp.) among the IFIs cases group (n= 25), which reflects its low diagnostic performance in our study (Table 6). On the other side, the histopathological examination results were positive in 7 cases (for *Zygomycetes* spp.) out of 25 cases with clinical features of IFIs (28%) (Table 7).

(Table 6): Microbiological culture diagnostic results.

			Cases	Total
Culture	No growth/ growth of normal flora (sputum samples)	Count	22	22
		%	88.0%	88.0%
	Positive culture results	Count	3	3
		%	12.0%	12.0%
Total		Count	25	25
		%	100.0%	100.0%

(Table 7): Histopathological examination diagnostic results

			Cases	Total
Biopsy	Positive results	Count	7	7
		%	28.0%	28.0%
	Negative results	Count	18	18
		%	72.0%	72.0%
Total		Count	25	25
		%	100.0%	100.0%

Patients with suspected fungal infections were subjected to an assessment of galactomannan test as a routine diagnostic workup, the test is more sensitive and specific for *Aspergillus* infections than any other fungal species, it was positive in the current study in 13 cases out of 25 (IFIs cases) (52%). The fungi claimed to be the causative micro-organisms in our study showed the following distribution: *Aspergillus* spp. (52%), *Zygomycetes* spp. (28%) and *Candida* (12%).

DISCUSSION

IFIs continue to be a significant source of illness and death in patients with hematological conditions, particularly those receiving HSCT. Despite their relatively high occurrence, diagnosing and treating these diseases is challenging because of their non-specific symptoms and the limited range of available antifungal treatments [10].

Beta-glucan is documented to be a valuable test to detect IFIs in high-risk groups of patients, especially those who have not previously been treated with antifungal therapy, and to detect the occurrence of chronic fungal infections as well [7]. In light of previously mentioned points, the current study aimed to determine the diagnostic performance of BDG as an adjunct diagnostic strategy for IFIs in patients with HMs. In the present study, the BDG test at a cut-off value of 187.21 pg/ ml was capable of discriminating between IFI cases and the controls with maximum efficacy 84.3 and achieving sensitivity of 84, specificity 84.6, NPV 84.6, and PPV of 84, respectively.

These current results come in line with many studies that documented that the BDG test has a valuable role in the detection of IFIs in both pediatric and adult age groups in the early stages of the disease especially in those patients who are not previously treated with antifungal therapy [4,7,8]. The novelty of our present study is that it is concerned with evaluating BDG assay in our population at the Hemato-oncology Department at a university hospital in Egypt, to decide its diagnostic validity for routine clinical implementation as a criterion for IFIs in HMs.

Various studies concerned with BDG diagnostic performance evaluation reported moderate values for both sensitivity and specificity in their studies ranging from 60% to 80% [11-13], this comes in line with the current study results. In our study, the AUC for the BDG test was calculated and it was 0.858, which is comparable to that achieved by other studies including **Karageorgopoulos *et al.*,** [11] who reported that BDG could detect IFIs with an AUC of 0.89. **White *et al.*,** [12] performed a meta-analysis on the diagnostic role of BDG in patients with HMs and solid organ transplants (SOT). They found that the overall sensitivity and specificity of BDG as a diagnostic test for IFIs were moderate, at 80% and 63%, respectively. However, there was significant variability between studies. When the analysis was limited to studies with a low risk of bias, variability decreased, but the overall specificity estimate also dropped. Furthermore, while the BDG assay is sensitive to IFIs, its specificity is lower among cancer patients. Therefore, BDG should be used alongside other clinical indicators when determining whether to administer antifungal therapy.

In the same line as these mentioned results, **He *et al.*,** [13] conducted their study on ICU patients, they reported that the BDG test at cut off value of 60 pg/ml could discriminate between IFI cases and the controls with a sensitivity of 78% and specificity 81%, respectively.

Additionally, **Karageorgopoulos *et al.*,** [11] reported that their analysis included 16 studies with a total of 2,979 patients, 594 of whom had proven or probable IFIs. The pooled sensitivity of BDG testing was 76.8% (95% confidence interval [CI], 67.1%–84.3%), and the specificity was 85.3% (95% CI, 79.6%–89.7%). The area under the summary receiver operating characteristic curve was 0.89, indicating good diagnostic accuracy. However, significant statistical heterogeneity was observed. BDG testing has good diagnostic accuracy for differentiating between proven or probable IFIs and no IFIs. It can be valuable in clinical practice when used in the appropriate context and interpreted with an understanding of its limitations.

Regarding the cutoff value calculated for the BDG test in our study, at a cutoff value of 187.21 pg/ml the maximum diagnostic performance was achieved concerning IFI cases versus the control group, while at

comparing IFI cases with healthy controls the best cutoff value was obtained at 92.127 pg/ ml and for IFIs cases versus the pathological controls the best cutoff was 75.7 pg/ml, respectively. A systematic review and meta-analysis focused on the diagnostic accuracy of serum BDG for IFIs, and its cutoff levels found that a BDG level of 60 pg/mL is the optimal threshold for distinguishing patients with IFIs from those without [13].

Regarding the question of whether incorporating BDG into the diagnostic process for IFIs can reduce antifungal treatment costs, **Hamilton *et al.*,** [14] noted that IFIs significantly increase mortality risk in critically ill patients. Therefore, treatment should commence before test results are available. The BDG test is quicker than standard blood culture tests, and it is estimated that using BDG tests could save approximately £1,643 per patient by improving the diagnostic capabilities of laboratory methods. On the other side, one of the main limitations of the BDG test was addressed by **Karageorgopoulos *et al.*,** [11] who reported that BDG has a limited role in diagnosing IA, especially in confined lesions; however, the combination with GM or The polymerase chain reaction (PCR) improves its diagnostic accuracy**.**

Additionally, **Fisher *et al.*,** [15] conducted a study involving 471 patients to evaluate the effectiveness of GM and BDG assays as diagnostic tools for Invasive fungal diseases (IFD) in patients with AML receiving fungal prophylaxis. They reported that neither GM nor BDG assays, alone or in combination, were effective in detecting IFIs during periods of neutropenia in children, adolescents, and young adults with AML receiving antifungal prophylaxis. They recommended against using these assays for surveillance in this clinical setting due to the low pretest probability for IFD.

Garnham *et al.*, [8] conducted a study comparing the diagnostic value of weekly screening with BDG to screening with *Aspergillus* PCR and *Aspergillus* GM in 57 at-risk episodes among Australian hematology patients for the diagnosis of IFIs. They concluded that while the BDG assay could offer speed and value in diagnosis, it was limited by low sensitivity and PPV. In a study that conducted a comparative meta-analysis to evaluate the accuracy of BDG and GM for diagnosing IFIs in pediatric patients, the findings were as follows: For proven and probable IFI, the GM assay had a sensitivity of 0.74, specificity of 0.76, pooled diagnostic odds ratio (DOR) of 13.25, and an AUC of 0.845. In comparison, the BDG assay had a sensitivity of 0.70, specificity of 0.69, pooled DOR of 4.3, and an AUC of 0.722 [16]. In summary, both GM and BDG assays show moderate performance in detecting IFIs in pediatric patients, with GM being more accurate than BDG. Combining both tests improves specificity but reduces sensitivity. These results indicate a limitation in the routine clinical use of the BDG test for diagnosing IFIs in suspected patients.

Wright *et al.*, [6] noted that current data indicate that a negative result from a BDG test does not completely exclude the possibility of an IFI, and a positive result on its own does not provide enough sensitivity and specificity for a conclusive diagnosis. The development of a quick and straightforward assay to act as an indirect indicator of fungal infections is being welcomed for testing populations at risk for BDG. However, additional studies are required to ascertain the most appropriate scope and regularity of testing that will ultimately be advised.

By comparing IFI cases in the current study with either healthy controls or pathological controls or both combined, many laboratory parameters including BDG, CRP, and hematological indices showed significant differences between the comparable groups, however by calculating the diagnostic efficacy and the statistical performance for each laboratory parameter, the highest efficacy in our study was registered by BDG test combined with CRP measurement followed by BDG test alone, followed by the other laboratory parameters.

These results reflect BDG's usefulness in our concerned diagnostic issue especially if combined with CRP as a routine laboratory investigation for IFIs with a sensitivity of 96%, specificity of 88.5%, PPV of 88.9%, and NPV of 95.8%, AUC of 0.960 and efficacy of 92.2%, respectively. This comes in line with other studies that reported that for best clinical management of IFIs, BDG test results should be interpreted in combination with other inflammatory parameters results and context with the patient clinical conditions [4,11,12]. Concerning the gold standard diagnostic modality for IFIs; the microbiological cultures and the histopathological examination, in the current study the fungal culture was positive in 12% of the cases while the histopathological examination was positive in 28%, respectively. Those cases were categorized as proven IFIs while the remaining (60%) of the diseased group were categorized as probable IFIs where clinical evidence supported the diagnosis in addition to positive serological markers and/or computed tomography (CT) results for IFI features.

Madney *et al.*, [17] support the present study result concerning the role of microbiological culture as they reported 45 cases positive for mucormycosis in their hospitals, seven cases from them were diagnosed by the culture result positive for *Zygomycetes* spp. while the remaining cases were diagnosed by the histopathological examination, so the culture results achieved a sensitivity of 15.5% comparable to that recorded in our present study which was 12%. On the other side concerning the distribution of the microorganisms claimed to be the causative pathogens for IFIs in the present study, it showed that *Aspergillus* species were the most common one (52%) followed by *Zygomycetes* species (28%), followed by *Candida* (12%). This distribution of the fungi attributed to IFIs in patients with HMs comes in line with **Valentine *et***

al., [18] study results which reported that *Aspergillus* spp. was the most common fungus to be documented in HMs with high risk for IFIs.

In recent years, there has been an increased focus on less common molds such as *Zygomycetes* and *Fusarium* spp., which can cause severe clinical outcomes. The widespread use of antifungal prophylaxis has decreased yeast infections like candidemia, but these infections still carry high mortality rates and have led to a shift towards other less frequently reported fungi [2]. Our current study findings support this observation, with *Zygomycetes* spp. being reported more frequently than *Candida* species.

By discussing the risk factors for IFIs in the current study, there were many risk factors could be documented in the selected patient group comparable to the controls where significant differences were present between both groups concerning HSCT, IS diseases, and neutropenia being higher among cases group (P values 0.001, 0.05 and 0.004, respectively).

This result comes in line with **Hansen *et al.,*** [19] ***and*** **Karkowska-Kuleta and Kozik.,** [20] who reported that many risk factors present in patients with HMs make them more prone to IFIs than any patients group including HSCT, IS diseases, ICU admission and neutropenia.

Concerning the demographic data of the studied patients, statistical analysis was conducted to evaluate if there is sex predilection can be documented for IFIs, there was no significant difference between the control and cases groups regarding male gender (P value 0.086), which comes in line with **Azoulay *et al.,*** [21] who reported no gender predilection between cases and control groups (male gender P value 0.083), on the other side **Egger *et al.,*** [22] reported that IFIs have male sex predilection over female gender especially for invasive aspergillosis, mucormycosis, and cryptococcosis.

In the present study, the clinical criteria for IFIs were evident in the case group comparable to the controls achieving a highly significant increase in the case group for respiratory criteria for invasive pulmonary aspergillosis mentioned earlier (referred to as respiratory distress in Table 1) (P value < 0.001), the need for ventilatory support (P < 0.001), ENT and CNS clinical manifestations (P value 0.018 & 0.005, respectively), this is supported by **Azoulay *et al.,*** [21] who reported similar study results.

Enrolled patients in our study had different diagnoses (concerning the type of HMs). The number of patients diagnosed with MM, Lymphomas, or CLL was higher in the pathological controls than IFI cases group, on the other side AML, ALL, bone marrow aplasia, and light chain disease were more commonly reported among the IFI cases group than in the pathological controls.

Biswal and Godnaik [23] reported that the frequency and severity of infections and sepsis vary among patients with HMs, depending on the type of HM. Patients with acute leukemia have higher rates of IFIs than those with other HMs, with an even higher incidence in patients with AML compared to those with ALL. Induction treatment in AML leads to more prolonged neutropenia, increasing the risk of infectious complications and early deaths, which are significantly more common in AML patients than in ALL patients. Concerning the mortality and morbidity among the studied patients; the death rate, the hospital length-of-stay, and the documented ICU admission, all were significantly higher among the cases group compared to the controls (P values < 0.001 for each).

In our study, the mortality rate for cases of IFIs was 52%, highlighting the severity of the disease and its significant impact. This finding is supported by **Ceesay *et al.,*** [24] who reported that patients with proven or probable IFD had notably lower survival rates compared to those without IFD, with 2-year survival estimates of 45% for IFD patients versus 87% for those without IFD. Furthermore, **Mueller *et al.,*** [25] reported that the mortality rate for IFIs is extremely high, estimated at around 40% for infections with susceptible fungal strains and rising to as much as 90% for infections with resistant strains.

CONCLUSION

In conclusion, identifying the BDG antigen can be a useful diagnostic tool when applied in the appropriate clinical context, such as in patients with weakened immune systems, low white blood cell counts, or hematological malignancies. It is important that the healthcare provider using the test is well-informed about its benefits and limitations to tailor its use for optimal clinical decision-making for each patient.

BDG detection cannot replace the current laboratory tests for IFI diagnosis, instead, its diagnostic performance is enhanced with the combination with other laboratory tests, especially CRP, and its result should be interpreted in context with the other radiological findings and in correlation with the patient clinical condition.

The simplicity of collecting samples using minimally invasive methods, the quick turnaround time (TAT) of BDG testing, and the valuable insights gained from repeated BDG evaluations all support and strengthen the argument for incorporating this assay as a diagnostic screening tool in various protocols for diagnosing IFIs.

RECOMMENDATIONS

IFIs are associated with significantly high morbidity and mortality in patients with HMs and HSCT, hence protocols for IFI management are highly recommended being guided by international guidelines and tailored according to our hospital facilities and statistical epidemiological results with the implementation of the new valuable diagnostic modalities like BDG assay to be included after full knowledge of its advantages and limitations. Further evaluation to determine BDG usefulness as a part of routine laboratory profile for the diagnosis of IFIs in patients with HMs with larger sample sizes of patients, standardization of specimen collection timing for serial assessment, determining its clinical

usefulness in specimens other than serum samples, and BDG evaluation in response to therapy and monitoring purposes is recommended before being routinely applicable at wide rang in the health care facilities.

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List of abbreviations:

ALC: absolute lymphocyte count, **ALL:** acute lymphocytic leukemia, **AML:** Acute Myeloid Leukemia, **ANC:** absolute neutrophil count, **AUC:** area under the curve, **BDG:** 1,3-beta-D-glucan, **CBC:** complete blood count, **CI:** confidence interval, **CLL:** Chronic lymphocytic leukemia, **CML:** Chronic Myeloid Leukemia, **CNS:** central nervous system, **CRP:** c-reactive protein, **CT:** computed tomography, **DOH:** duration of hospitalization, **DOR:** diagnostic odds ratio, **Eff.:** Efficacy, **ENT:** Ear-Nose-Throat, **EORTC:** European Organization for Research and Treatment of Cancer, **FN:** false negative, FP: false positive, **GM:** galactomannan, **Hb:** hemoglobin, **HC:** healthy controls, **HMs:** hematological malignancies, **HS:** highly significance, **hs-CRP:** highly sensitive-C reactive protein, **HSCT:** hematopoietic stem cell transplantation, **ICU:** intensive care unit, **IFD:** Invasive fungal diseases, **IFIs:** Invasive fungal infections, **IQR:** interquartile range, **IS** diseases: immunosuppressive diseases, **LAL:** Limulus Amebocyte Lysate, **LOH:** length of hospital stay, **MM:** multiple myeloma, **MSG:** Mycoses Study Group, **NA:** not available, **NPV:** Negative predictive value, **NS:** not significant, **P:** probability value, **PC:** pathological controls, **PCR:** polymerase chain reaction, **PLT:** platelet, **PPV:** positive predictive value, **ROC:** Receiver operating characteristic, **rs:** ranked spearman correlation, **S:** significance, **SD:** standard deviation, **TAT:** turnaround time, **TLC:** total leukocyte count, **TN:** true negative, **TP:** true positive, **Z:** Wilcoxon's Rank Sum test.

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