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(1-3)-β-D-Glucan vs Galactomannan Antigen in Diagnosing Invasive Fungal Infections (IFIs)

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Abstract: Invasive fungal infections (IFIs) are serious and often life-threatening complications in patients with haematological malignancies. Early diagnosis and the initiation of efficacious antifungal treatments could affect the prognosis of these patients. The detection of (1-3)-β-D-Glucan (BDG) could be a promising non-culture-based, noninvasive tool for IFI analyses in haemato-oncological patients, allowing the diagnosis of the two major IFIs, invasive aspergillosis (IA) and invasive candidiasis (IC), with a single test. The aim of this work was to evaluate and compare the use of the BDG in combination with the galactomannan antigen (GAL) assay in order to exclude or confirm suspected IFIs. Sera from 46 haemato-oncological patients (24 with proven/probable IFI and 22 without IFI symptoms) were evaluated retrospectively for the detection of GAL and BDG. In 24 patients, the serum BDG levels facilitated IFI diagnosis: 18 probable IA, 3 proven IA and 3 IC. In the remaining 22 patients, the BDG level helped exclude IFIs. The BDG was positive earlier than GAL in 5/24 cases [three of probable invasive aspergillosis (IA), one of proven IA and one case of proven invasive candidiasis (IC)] and was positive at the same time as GAL in 19/24 cases; in no case was GAL positive before BDG was. The BDG detection is useful, however, the test has a great limitation because it is a completely manual procedure.

Keywords: Aspergillosis, fungal infection, galactomannan, glucan, haematological patients, predictive factors.

INTRODUCTION

The diagnosis of invasive fungal infections (IFIs) is a problem due to the absence of significant signs and symptoms and because of the lack of specificity of the available non-invasive diagnostic tools. Most IFIs occur in patients with haematological malignancies, and the percentage of patients who develop an IFI has increased dramatically in recent decades [1, 2]. Although the true incidence of IFIs among patients with haematological malignancies remains unknown because the data in the literature are based largely on reports from single institutions or on analyses of selected subgroups of patients (e.g., those with acute leukaemia or following stem cell transplantation), the incidence of IFIs in patients with acute myeloid leukaemia is estimated to be generally lower than that reported among patients undergoing stem cell transplantation [2]. Unfortunately, a definitive diagnosis of IFI often cannot be made promptly, as this may require the use of invasive procedures [3]. Many patients with a clinical suspicion of the presence of an IFI are treated empirically with antifungal therapy, which may involve the unnecessary use of potentially toxic and costly drugs [4]. The early detection of diagnostic markers of a fungal infection, such as fungal nucleic acids, antigens, antibodies or cell wall components, is essential [5, 6]. For example, galactomannan antigen (GAL) is widely used in the diagnosis of invasive aspergillosis [7, 8]. Another serum marker for the presence of IFIs is (1-3)-β-D-Glucan (BDG), which has been included in the relevant diagnostic criteria of the European Organization for Research and Treatment of Cancer/ Mycoses Study Group (EORTC/MSG) [8]. There is an ongoing debate about the best diagnostic tools to use for patient monitoring. The introduction of BDG assay, in addition to the GAL, seems to be of interest. The aim of this study was to evaluate and compare two assays (BDG and GAL) for the evaluation of immunocompromised patients. A total of 46 patients, who were under follow-up monitoring in the haematology unit of Tor Vergata University Hospital from January 2010 to December 2011, were included in our retrospective comparison of the GAL and BDG assays. These patients were classified as proven or probable cases of invasive aspergillosis IA based on the EORTC⁄MSG criteria. The patients with IA had compatible radiological images, as described elsewhere [8-11].

Of the 46 patients, 18 had probable cases of IA, and 6 had proven cases of IA/IC (3 with IA due to *Aspergillus fumigatus* and 3 with candidemia). The haematological malignancies were as follows: acute myeloid leukaemia (n = 17), non-Hodgkin's lymphoma (n = 2), multiple myeloma (n =2) and autologous bone marrow transplant (n = 3). All of these patients were positive for the GAL/BGD in their sera and for risk factors for IA according to the EORTC⁄MSG criteria [8, 9]. For the proven cases of IA, the presence of *A. fumigatus* was determined by both positive pathological examination

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and positive culture. For the probable IA cases (n = 18), the computed tomographic (CT) findings were as follows: nodules (n = 17), halo (n = 1), and nodules and halo (n = 1). The haematological malignancies for each of 24 patients were reported in the Table **1**. The three proven IA were: one case of ocular infection (endophthalmitis, in a patient with decreased visual acuity) and two cases of invasive pulmonary infections. The three proven IC were all candidaemia. For each of the 46 patients, the following 3–4 serum samples were tested retrospectively (one serum a week): (i) the first serum sample that was positive for GAL and/or BGD+ and, if available, (ii) 1 serum samples before GAL/BGD positivity was observed and 1–2 serum samples after GAL/BGD positivity was observed. As negative controls, a selection of 12 GAL-negative and 10 GAL-positive serum samples from 22 adult Haematology Department patients without clinical signs of IFI (i.e., fever, pulmonary symptoms and thoracic pain) were tested for BDG. For these patients, the following causes of cross-reactions were checked: intravenous antibiotics, parenteral nutrition and intravenous human immunoglobulins [12,13]. The tests for galactomannan (Platelia Aspergillus®, Bio-Rad Laboratories, Marnes La Coquette, France) and BDG (Fungitell®, Associates of Cape Cod, Falmouth, MA, USA) were performed according to the manufacturer's recommendations for testing serum samples, as described elsewhere [3,4,10, 14]. The GAL, in particular, was performed in full automation using the Power-LAB system and the 0.5 index was the threshold of positive samples. The BDG assay was performed in duplicate, and the positive threshold was 80 pg ⁄mL. For each assay, a standard curve of five points (500, 250, 125, 62.5, 31.25, and 0 pg ⁄mL) was constructed and the assays were conducted using a series of 20 serum samples. The BDG assays requires the use of specific laboratory equipment and all of the disposable materials used needed to be glucan free.

Fungi and yeasts culture (blood culture as well as culture from any relevant body sites, including respiratory tract) was performed according to the good microbiological practice [15]. Blood cultures were incubated for 5 days using a BacTAlert 3D automated blood culture system (bioMérieux, Marcy l'Etoile, France). At the end of the incubation (for negative samples) or at the time of a positive signal (for positive samples), the blood cultures were subcultured onto specific media for fungal identification and maintained in culture at 25°C under ordinary atmospheric conditions until the growth of fungi was observed. In cases of no growth, the culture was monitored for 21 days and then concluded to be negative. *Aspergillus* spp. were identified following the guidelines of Larone [16,17]. All of the *Candida* isolates were identified to the species level using the YST panel (bioMérieux, Marcy l'Etoile, France).

The BDG serum levels of the 46 patients helped in diagnosing 24 cases of IFI: 18 patients with probable invasive aspergillosis (IA), 3 patients with proven IA and 3 patients with proven invasive candidiasis (IC). Among the remaining 22 patients (who did not exhibit signs of IFI: twelve GAL-negative and ten GAL-positive), the BDG assay, showing negative results, helped in excluding an IFI. The kinetic variations in the BDG serum levels of the 24 patients were compared with those of the galactomannan antigen and the sera value for each determination has been reported in the Table **1**. In 5/24 cases (one of proven IC, one of proven IA and three probable IA), BDG was positive earlier than GAL, with a mean time lapse of approximately 7 days, in 2/3 cases of proven IA (due to *A. fumigatus*), BDG was positive at the same time as GAL. In one case of proven IA (patient 24) the BDG was strongly positive while the GAL remained negative. In 1/3 cases of proven IC (caused by *C. albicans*), BDG was positive before GAL. In no case of probable or proven IA/IC was BDG positive after GAL was (Table **1**). For 10 patients (in the group of 22-negative control) who were GAL positive but negative for BDG, neither clinical evidence nor the culturing supported the existence of an IFI. Therefore, for these 10 patients the GAL positivity was attributed to interfering antibiotics, such as piperacillin/tazobactam, used in the prophylaxis regimen [18]. No false positive result was found using the BDG assay.

Also the microbiological data sustained the results of BDG assay. All the patients with proven IA were positive to the culture of *A.fumigatus*: two detected in bronchoalveolar lavage, in patients with pulmonary symptoms, and one positive to the culture of vitreous humor in a patient with endophthalmitis (patient no.24, see Table **1**). In particular, for the patient no.24 the starting localization of the fungal infection to the ocular district may account for the delay in GAL test positivity. While the three proven IC were all *C.albicans* fungemia. In the group of 18 patients with probable invasive aspergillosis the cultures remained negative. Therefore, the microbiological data were not be of help in confirming the nature of fungal infection and the definition of probable IA has been based on host factors, clinical criteria and of course on the antigen detection.

The BDG assay was easy to perform but required a well-trained staff, as the distribution is a critical element in the quality of the results. Although the use of the BDG kits was optimised (complete plates of 20 samples), the cost of the BDG assay was higher than that of the GAL assay because of the number of samples tested; 15 samples (15 of ~120 determinations = 12.5%) needed to be re-tested because one replicate was below the 80 pg ⁄mL threshold and the other replicate was above the threshold.

Our preliminary data emphasise the importance of pairing the two assays (GAL and BDG) in the early diagnosis of IFIs. In the case of IA, the GAL assay appears to be more specific but may be less effective during the early stage of infection. GAL results show a large number of false positive samples (10 on 22 negative patients) and a false negative specimens (patient 24), so the sensitivity, the specificity the positive and negative predictive values were: 95.83, 54.54, 69.7 and 92.3, respectively. On the contrary the BDG assay is less specific in the early stages, but it shows a good negative predictive value and so it could be of help in order to reduce the percentage of false positives. Even though the reported sensitivity of the BDG is low, our data, albeit preliminary, show that when the test, if performed on a selected group of patients, could exhibit good performances in terms of sensitivity, specificity, negative and positive

Table 1. Comparison between Galactomannan Antigen (GAL) and (1-3) Beta-D-glucan (BGD) in Presumptive Diagnosis of Fungal Infections

Haematological malignancies	IFI	Sera tested / value detected					Sera tested / value detected				
		GAL					BGD				
		1st week	2nd week	3rd week	4th week	SD	1st week	2nd week	3rd week	4th week	SD
	Proven IA										
AML	patient 7	0.503*	1.054	0,577	0,456	608,228985	324*	274	176	7	135,06418
AML	patient 4	0,98	1,068	0,807*	0.503	0,50819372	171	180	66*	7	122,32947
AML	Patient 24	0.06	0.133*	0.06	0.274	0,13160043	186	70*	65	54	7,7781746
	Proven IC										
ABMT	patient 1	0,535	0,77*	1	2	0,70710678	109	53*	83	85	1,4142136
AML	patient 6	0,3	0,766	1,219*	0.423	0,33393118	214	301	387*	159	100,40916
AML	patient 13	0.554*	0,545	0.389	0.152	0,22201641	89*	114	62	<7	36,769553
	Probable IA										
ABMT	patient 3	0,863	0,732	0.766	0.4	0,37678567	338	141	37	7	70,323064
AML	patient 8	0,514	0,77	0,429	0.084	0,24112341	116	28	7	7	12,124356
AML	Patient 5	0.766	0.5	0.501	-	0,243559	214	266	85	-	127,98633
NHL	patient 9	0,599	0,8	0.514	-	0,31328759	109	103	11	-	65,053824
NHL	patient 10	0,814	0,611	1,229	-	0,43699199	152	249	287	-	26,870058
AML	patient 2	0,637	0,538	1,005	0.736	0,27771035	147	523	159	47	248,86944
AML	patient 11	0,460	0,617	0.06	-	0,43333861	408	171	43	-	90,509668
AML	patient 12	0,410	0,41	0.098	0.06	0,2869675	532	71	52	20	25,774665
AML	patient 14	0,051	0,887	0.599	0.2	0,44310524	199	66	59	7	32,233523
AML	patient 15	0,66	0,62	0,06		0,3959798	146	186	54		93,338095
AML	patient 16	0,587	0.288	0.204	0.553	0,12649632	82	83	124	161	39,01709
AML	patient 17	0,767	0.056	0.118	0.179	0,02995383	84	82	77	61	10,969655
AML	patient 18	0,859	0.404	0.125	0.026	0,13700194	124	70	56	62	7,0237692
ABMT	patient 19	0,583	0.278	0.474	0.11	0,16139273	394	388	301	287	54,720502
MM	patient 20	1,172	0,523	0,06	-	0,32739044	114	62	<7	-	38,890873
AML	patient 21	0,913	0,424	0,233	-	0,1350574	523	7	7	-	297.91
MM	patient 22	0,613	0,5	0,3	-	0,14142136	180	24	<7	-	12,020815
AML	patient 23	0,859	0,5	0,423	-	0,05444722	124	79	70	-	6,363961

Legend for Table **1**:
* indicate the time of positive culture for *Aspergillus/Candida;*
AML: acute myeloid leukemia; NHL: non-Hodgkin's lymphoma; ABMT: autologous bone marrow transplant; MM: multiple myeloma
SD: standard deviation

predictive values (all of them were 100%, data not shown) [4,12]. The GAL assay becomes positive later but is more specific in the case of IA. On the other hand the BDG has significant negative predictive value and coupled with the GAL assay reduces the number of false positives. It is noteworthy that the 2/6 cases of IA were early diagnosed by the BDG assay and not the GAL assay. The fact that the BDG assay was positive for all of the cases of IA (probable and proven) confirms that this test could be a useful tool to screen for IA. However, the BDG assay is deficient with respect to its repeatability and the use of a manual procedure; automation may improve these inadequacies. Although the BDG test was as easy to use as the GAL assay, it was more expensive than the GAL assay. Furthermore, there are also other costs that are not reflected in the price: (i) the test platform does not include double testing when there are discrepancies between replicates (15 cases in this study); (ii) the plat-

form is based on a series of 20 samples (a complete plate), which may not be compatible with emergency requests made by physicians; and (iii) testing requires the purchase of specific, expensive laboratory equipment (e.g., glucan-free disposable materials). Of course, the economic value of BDG detection cannot be determined by the price of the analysis alone, and other aspects should be taken into consideration, including the possibility of reducing risks to the patient by beginning the antifungal therapy as soon as possible. In fact, given the extremely high cost of IFIs, in both financial terms and in terms of human life, the use of expensive diagnostic tests could be justified and so probably the test should only be performed in patients with suspected mould diseases.

In conclusion, our findings suggest that measuring the serum or plasma BDG levels has a high level of accuracy in the discrimination of patients with and without IFIs, mainly IFIs due to *Candida* or *Aspergillus*. The use of the BDG assay in combination with the GAL assay could be of great interest to clinicians who can use these assays to exclude or confirm suspected IFIs, particularly in patients with haematological malignancies [13,19]. In clinical practice, the proper use of the BDG test would require a good knowledge of its characteristics, especially with regard to the fungal pathogen-induced diseases that this test does not detect (such as fusariosis, zygomycosis, and many cryptococcoses) and the factors associated with a false-positive test result [10,19, 20]. Particularly important is the accurate selection of the type of patients on which the tests are used to diagnose IFI. Appling these assays on a non-selected patient population could cause misleading results and difficulty in interpreting both positive results and discrepancies between the two tests.

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CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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