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Open Forum Infectious Diseases

MAJOR ARTICLE

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Background. Early diagnosis and treatment are crucial in invasive fungal diseases (IFD). Serum (1-3)-β-D-glucan (BG) is believed to be an early IFD marker, but its diagnostic performance has been ambiguous, with insufficient data regarding sensitivity at the time of IFD diagnosis (TOD) and according to outcome. Whether its clinical utility is equivalent for all types of IFD remains unknown.

Methods. We included 143 patients with proven or probable IFD (49 invasive candidiasis, 45 invasive aspergillosis [IA], and 49 rare IFD) and analyzed serum BG (Fungitell) at TOD and during treatment.

Results. (1-3)-β-D-glucan was undetectable at TOD in 36% and 48% of patients with candidemia and IA, respectively; there was no correlation between negative BG results at TOD and patients' characteristics, localization of infection, or prior antifungal use. Nevertheless, patients with candidemia due to *Candida albicans* were more likely to test positive for BG at TOD (odds ratio = 25.4, $P = .01$) than patients infected with other *Candida* species. In 70% of the patients with a follow-up, BG negativation occurred in >1 month for candidemia and >3 months for IA. A slower BG decrease in patients with candidemia was associated with deep-seated localizations ($P = .04$). Thirty-nine percent of patients with rare IFD had undetectable BG at TOD; nonetheless, all patients with chronic subcutaneous IFD tested positive at TOD.

Conclusions. Undetectable serum BG does not rule out an early IFD, when the clinical suspicion is high. After IFD diagnostic, kinetics of serum BG are difficult to relate to clinical outcome.

Keywords. (1-3)-β-D-glucan; diagnostic tool; invasive fungal diseases; kinetics.

The incidence of invasive fungal diseases (IFDs) has increased in the past decades as a consequence of the ever-growing population of immunocompromised patients (with a solid organ transplant [SOT] or hematopoietic stem cell transplant) or patients receiving advanced critical care [1–4]. Aside from *Pneumocystis jirovecii*, the most common threats are *Candida* spp and *Aspergillus* spp, but a large variety of other opportunistic fungal pathogens can cause severe diseases in vulnerable patients. A key to a favorable prognosis of these deadly infections is early initiation of an accurate antifungal therapy, which itself relies on early diagnosis. Nonetheless, diagnosis of both common and rare IFD remains challenging, partly due to the limited availability of sensitive early diagnostic markers.

Among possible markers, (1-3)-β-D-glucan (BG), an abundant cell wall polysaccharide found in a majority of fungi [5], has been used more and more frequently in the last 5 years [6, 7]. It has been proposed as an early biomarker of IFD and is included in diagnostic criteria of IFD in the 2008 version of European Organization for Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) [6]. However, this marker cannot discriminate the various fungi that can cause IFD.

Different studies have evaluated clinical performance of the BG assay. Some focused on specific target populations (ie, hematological patients [8], intensive care unit [ICU] patients [9, 10], or the pediatric population [11]) or on specific IFD types (candidemia [10, 12] or invasive aspergillosis [IA] [8]), whereas others included all kinds of patients with all kinds of IFD [13, 14]. They reported wide discrepancies on sensitivity (40%–100%) and specificity (45%–99%) of the assay [7, 15, 16], probably due to the heterogeneity of their designs. Other studies have analyzed diagnostic utility of serial BG sampling before diagnosis of IFD [17–20], with some of them emphasizing its value as an early IFD marker, especially in invasive candidiasis (IC) [10, 17, 18, 20]. Finally, a few

Received 3 April 2016; accepted 14 June 2016.

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DOI: 10.1093/ofid/ofw128

other research groups have attempted to interpret postdiagnosis kinetics of BG levels [18, 20–23]. Nonetheless, the use of this marker in clinical practice remains problematic [24].

In the present study, we sought to assess BG serum levels in patients who received a diagnosis of various types of IFD (proven or probable; including IC or IA, as well as a panel of rare IFD). To obtain useful clinical information, we evaluated the rate of BG positivity when the diagnosis was made (time of diagnosis [TOD]) and analyzed the kinetics of serum BG levels during antifungal treatment and its relation to prognosis.

PATIENTS AND METHODS

Hospital and Patients

Necker-Enfants Malades is a teaching hospital, where adults and children at high risk for IFD receive medical care. From January 2011 to July 2015, we carried out an observational study on BG testing performed in the mycology laboratory at the request of clinicians from various hospital wards. In the logs of the laboratory, we identified all patients (adults and children) with documented fungal infections (except for pneumocystosis) who had at least 1 sample analyzed for BG at the TOD (TOD being a time interval around the day of diagnosis, ie, the date of collection of the sample allowing diagnostic; see below).

From this list, we first selected a group of patients with IC, which included (1) patients with a positive culture from a normally sterile site (including blood cultures, joint fluid aspiration, vertebral or cardiac biopsy) and a BG sample collected between 1 day before and 7 days after the first positive culture was drawn and (2) patients with chronic disseminated candidiasis (ie, hepatosplenic candidiasis) diagnosed as possible IFD on the basis of a typical computed tomography (CT) scan, according to EORTC/MSG diagnostic criteria (6) and for whom a BG sample was taken between 7 days before and after the CT scan, which first showed microabscesses compatible with the diagnosis.

From the same original list, we then selected patients with probable or proven IA diagnosed according to the EORTC/MSG criteria (6), except that we extended the definition of probable IA to patients in whom real-time PCR detected deoxyribonucleic acid of *Aspergillus fumigatus* in a normally sterile sample (ie, serum, cerebrospinal fluid, or biopsy) [25, 26]. For IA, the patients selected had a BG sample taken between 3 days before and 7 days after the day of diagnosis. In case of slowly evolving IA, such as bone infections, the delay was extended to 15 days after the day of diagnosis.

Finally, from the same list, we selected patients with rare IFD defined as patients with deep-seated, invasive, or chronic infections due to fungi other than *Candida* and *Aspergillus*, with histopathological evidence or a positive culture of normally sterile material (biopsy or needle aspiration of a sterile site, or blood culture). For rare IFD, the patients selected had a BG sample taken between 7 days before and 10 days after the day of diagnosis.

For all 3 types of IFD cases, all BG test results (that were requested by the clinicians after the initial BG assay) were also analyzed and defined as "BG follow-up." Patients known to have another IFD diagnosed within 1 month before or after the diagnosis of the IFD under study, or those receiving intravenous immunoglobulins (IVIG), were excluded from the analysis. This study was approved by the local ethics committee (record 20160310).

(1-3)-β-D-Glucan Antigen Detection

All BG testing was performed at the request of treating physicians by means of the Fungitell assay (Associates of Cape Cod, Inc., Falmouth, MA). Each sample was tested in duplicate, and the mean of 2 values was considered a definitive result, except when duplicates differed by more than 20%, in which case the test was repeated. We used the recommended positive cutoff value of 80 pg/mL and recorded all positive results up to 523 pg/mL. Higher results were recorded as >523 pg/mL. None of the samples were diluted.

Data Collection and Analysis

Patients' charts were reviewed, and we collected patients' demographic and general data (hospital ward, immunocompromised status) and clinical data related to the IFD (use of antifungal drugs 2 weeks before IFD, antifungal treatment after diagnosis, neutropenia [ie, neutrophil count <1500 cells/μL], the presence of a central venous catheter, assessment of IFD extent, outcome, and medical procedures or treatments known as a possible cause of false-positive BG results [albumin use, a surgical procedure, mucositis, digestive graft-versus-host disease, or hemodialysis]).

We performed the statistical analyses using the R software (version 3.2.2; http://www.cran.r-project.org). Univariate analysis was conducted using 2-sided Pearson's, Fisher's exact, and Student's *t* tests ($\alpha = 0.05$). Variables with univariate *P* values <.25 were included in multivariate analyses, which were conducted using descending stepwise logistic regression. The logistic models were tested for statistical stability using the RVAideMemoire package (http://cran.r-project.org/web/packages/RVAideMemoire/). To analyze BG negativation kinetics, we performed survival analysis using Kaplan–Meier estimates.

RESULTS

Invasive Candidiasis

Forty-nine patients with candidiasis were included. As shown in Table 1, 41 had candidemia, with associated deep-seated localizations in 11. The remaining 8 had IC (endocarditis, osteoarthritis, or chronic disseminated candidiasis) without concomitant candidemia. Overall, cultures were positive in 43 of 49 patients, with *Candida albicans* being the most common (19 of 43, 44%) of the 11 species isolated (Table 1). Among the 41 cases of candidemia, 15 (36.5%) tested negative for BG (<80 pg/mL) at the TOD (Table 1). There were no differences between patients with negative or positive BG test results at the TOD in terms of the patients' category (see Table 1), time of BG sampling, antifungal drug use before candidemia, vascular catheterization at the TOD, outcome,

Table 1. Demographics, Clinical Characteristics, and Mycological Data of the Patients With Invasive Candidiasis at the Time of Diagnosis

								BG at TOD	
Patient ID	Age	Gender	Category of Patient	Sample Providing Diagnosis	*Candida* Species/ Diagnostic Element	Location of Candidiasis	Duration of Candidemia (Days)	Time Interval Between Sample Providing Diagnosis and BG Sample (Days)	BG Value (pg/mL)
1	23	M	Hematology	Blood culture	*Candida lusitaniae*	Blood	3	2	267
2	72	M	Hematology	Blood culture	*Candida krusei*	Blood	1	−1	>523
3	41	F	Hematology	Blood culture	*Candida glabrata*	Blood	3	3	<80
4	46	M	Hematology	Blood culture	*Candida tropicalis*	Blood	1	2	>523
5	59	M	Hematology	Blood culture	*C. glabrata*	Blood	4	3	<80
6	61	M	Hematology	Blood culture	*Candida guilliermondii*	Blood	5	−1	<80
7	76	M	Hematology	Blood culture	*C. glabrata*	Blood	2	0	471
8	64	F	Hematology	Blood culture	*Candida albicans*	Blood	1	0	156
9	62	F	Hematology	Blood culture	*C. guilliermondii*	Blood	1	−1	<80
10	51	F	Hematology	Blood culture	*C. krusei*	Blood	6	3	116
11	46	M	Hematology	Blood culture	*C. guilliermondii*	Blood	2	2	<80
12	68	M	Hematology	Blood culture	*Candida norvegensis*	Blood	5	−1	<80
13	74	M	Hematology	Blood culture	*C. albicans*	Blood	4	1	278
14	61	F	Hematology	Blood culture	*C. norvegensis*	Blood	3	2	<80
15	75	M	Hematology	Blood culture	*C. albicans*	Blood	1	3	<80
16	77	M	Hematology	Blood culture	*C. albicans*	Blood	1	2	233
17	30	M	Hematology	Blood culture	*C. albicans*	Blood	4	1	>523
18	56	M	SOT	Blood culture	*Candida parapsilosis*	Blood	1	2	131
19	65	M	SOT	Blood culture	*C. parapsilosis*	Blood	4	3	>523
20	70	F	SOT	Blood culture	*C. parapsilosis*	Blood	1	5	87
21	44	F	SOT	Blood culture	*C. tropicalis*	Blood	1	0	260
22	14	F	Pediatric ICU	Blood culture	*Kodamaea ohmeri*	Blood	3	6	<80
23	0	M	Pediatric ICU	Blood culture	*C. albicans*	Blood	1	2	372
24	17	F	Pediatric ICU	Blood culture	*C. albicans*	Blood	1	4	100
25	2	M	Pediatric ICU	Blood culture	*C. albicans*	Blood	2	5	>523
26	2	M	Pediatric ICU	Blood culture	*C. parapsilosis*	Blood	1	3	<80
27	6	F	Pediatric ICU	Blood culture	*Hyphopichia burtonii*	Blood	2	2	<80
28	1	F	Pediatric ICU	Blood culture	*C. lusitaniae*	Blood	2	7	<80
29	1	F	Pediatric ICU	Blood culture	*C. tropicalis*	Blood	1	3	216
30	2	M	Pediatric ICU	Blood culture	*C. guilliermondii*	Blood	1	2	<80
31	75	M	Hematology	Blood culture	*C. albicans*	Blood, Urine	1	2	428
32	12	M	Pediatric ICU	Blood culture	*C. lusitaniae*	Blood, Urine	1	3	<80
33	2	M	Pediatric ICU	Blood culture	*C. albicans*	Blood, Urine	4	2	179
34	7	M	Pediatric ICU	Blood culture	*C. krusei*	Blood, Ascites	1	7	<80
35	64	F	Hematology	Blood culture	*C. glabrata*; *C. krusei*	Blood, Abdominal abscess	11	−1	367
36	4	F	Pediatric ICU	Blood culture, Pericardial fluid	*C. albicans*	Blood, Sternum, Mediastinum,	3	7	97
37	42	F	Hematology	Blood culture	*C. albicans*	Blood, Brain	6	2	>523
38	66	M	Hematology	Blood culture	*C. albicans*	Blood, Brain	5	0	411
39	63	F	Hematology	Blood culture	*C. albicans; Candida kefyr*	Blood, Intestine	3	4	>523
40	34	M	Hematology	Blood culture	*C. albicans*	Blood, Liver, Spleen, Kidney	1	2	>523
41	31	M	Hematology	Blood culture	*C. albicans*	Blood, Liver, Spleen, Kidney	1	0	460
42	0	F	Preterm neonate	Joint fluid aspiration	*C. albicans*	Multifocal osteoarthritis, Eye	na	7	>523
43	81	M	Auto-immune disorder	Bone biopsy	*C. albicans*	Spondylodiscitis	na	4	274
44	27	M	Sickle Cell Anemia	Heart valve	*C. albicans*	Heart	na	−6[a]	117
45	55	M	Hematology	CT scan[b]	Microabscesses	Liver, Spleen	na	1	260

Table 1 continued.

Patient ID	Age	Gender	Category of Patient	Sample Providing Diagnosis	*Candida* Species/ Diagnostic Element	Location of Candidiasis	Duration of Candidemia (Days)	BG at TOD: Time Interval Between Sample Providing Diagnosis and BG Sample (Days)	BG at TOD: BG Value (pg/mL)
46	55	F	Hematology	CT scan[b]	Microabscesses	Liver, Spleen	na	3	>523
47	53	M	Hematology	Liver MRI	Microabscesses	Liver	na	–4	348
48	56	M	Hematology	CT scan[b]	Microabscesses	Liver, Spleen, Kidney	na	2	>523
49	23	F	Hematology	CT scan[b]	Microabscesses	Liver	na	1	373

Abbreviations: BG, (1-3)-β-D-glucan antigens; CT, computed tomography; ICU, intensive care unit; ID, identification; MRI, magnetic resonance imaging; na, not applicable; SOT, solid organ transplant; TOD, time of diagnosis.

[a] The diagnosis of endocarditis was made 2 months after fungemia due to *C. albicans* without deep-seated localization.

[b] Abdominal CT scan.

and a possible cause of false-positive BG (Table 2). By contrast, patients with *C. albicans* candidemia were significantly more likely to have a positive BG result at the TOD (15 of 16) than did patients with candidemia due to another *Candida* species (9 of 25; multivariate odds ratio = 25.4; 95% confidence interval, 3.6–560.3; P <.01) (Table 2). Of note, patients with *C. albicans* candidemia

Table 2. Comparison of Demographic and Clinical Characteristics of the Patients With Candidemia Who Tested Positive vs Negative for BG at the Time of Diagnosis

	Total (N = 41)	Patients With Negative BG at TOD (n = 15) (%)	Patients With Positive BG at TOD (n = 26) (%)	*P* Value[a]	Multivariate OR[b]	*Pa*[b]
Socio-demographic data						
Gender						
Female	16	6 (40.0)	10 (38.5)	1.00		
Male	25	9 (60.0)	16 (61.5)			
Average age (years) [range]	40.6 [0; 77]	34.5 [1–75]	44.2 [0–77]	.28		
Medical data						
Category of patient						
ICU pediatric patients	13	7 (46.7)	6 (23.1)	.17	1.0	0.09
Hematology or Renal transplant adult patients	28	8 (53.3)	20 (76.9)		4.7 [0.9–36.4]	
Candida species responsible for candidemia						
Non-albicans *Candida* species	25	14 (93.3)	11 (42.3)	<.01	1.0	0.01
Candida albicans	16	1 (6.7)	15 (57.7)		25.4 [3.6–560.3]	
Median time interval between blood culture sampling and yeast growth (hours) [range]	31 [10–67]	29 [15–60]	34 [10–67]	.67		
Median duration of candidemia (days) [range]	2 [1–11]	2 [1–5]	1.5 [1–11]	.64		
Use of catheter at time of 1st positive blood culture	39	14 (93.3)	25 (96.2)	1.00		
Positive culture of catheter	11	4 (26.7)	7 (28.0)	.75		
Systemic antifungal drugs before candidemia	17	6 (40.0)	11 (42.3)	1.00		
Outcome						
Positive	25	13 (86.7)	12 (46.2)	.02	—	
Worsening (invasive candidiasis and/or death within 30 d)	16	2 (13.3)	14 (53.8)			
Median time interval between candidemia and 1st BG sampling (day) [range]	2 [–3;7]	3 [–1;7]	2 [–3;7]	.21	—	
Neutropenia at time of candidemia	22	6 (40.0)	16 (61.5)	.21	—	
Albumin during 1 mo before candidemia	3	1 (6.7)	2 (8.0)	1.00		
Surgery within 15 d before candidemia	8	2 (13.3)	6 (23.1)	.69		
Mucitis or digestive GVH disease within 15 d before candidemia	15	5 (33.3)	10 (38.5)	1.00		
Hemodialysis within 15 d before candidemia	8	1 (6.7)	7 (26.9)	.22	—	

Abbreviations: BG, (1-3)-β-D-glucan antigens; GVH, graft-vs-host; ICU, intensive care unit; OR, odds ratio; TOD, time of diagnosis.

[a] Univariate analyses were performed using Pearson χ^2, Fisher exact, Wilcoxon, or Student t tests ($\alpha = 0.05$).

[b] All variables with P values <.25 were included for multivariate descending stepwise logistic regression.

had deep-seated localizations (8 of 16) more frequently than those with non-*C. albicans* candidemia (4 of 25), but the difference was not significant (data not shown).

In 21 of 41 (51%) patients with candidemia, BG were also tested 2 to 7 days before the first positive blood culture was drawn. Seven (33%) tested positive for BG, 5 of whom had an associated deep-seated localization (data not shown). (1-3)-β-D-glucan follow-up was available for 27 of 41 (66%) patients, for 1–9 weeks (Supplementary Table 1). Of those 27, 5 had persistently negative BG test results ("negative profile", Supplementary Table 1) and 6 alternating negative or low BG results ("unreliable kinetic profile"). Among the 16 of 27 (59%) remaining patients, who had positive BG kinetics, 5 (31%) had a rapid decrease in the BG level after the diagnosis (over half decrease in <7 days, and negativation in less than 1 month, "rapid BG decrease profile"), whereas 11 (69%) had persistently high BG test results (over half BG decrease in more than 15 days, and negativation in more than 1 month, "slow BG decrease profile"). Examples of these BG postdiagnosis kinetic profiles are presented in Supplementary Figure 1.

We observed a trend ($P = .04$) toward more frequent deep-seated localizations (central nervous system, kidney, liver, mediastinum, deep abscesses) associated with candidemia in patients with slow decrease profiles versus those with rapid decrease profiles (data not shown).

All 8 patients with IC without candidemia (1 case of endocarditis relapse, 2 osteoarthritis, and 5 chronic disseminated candidiasis) had positive BG at the TOD (Table 1). In 3 of the patients for whom a BG follow-up was available, BG values remained high for more than 6 weeks (Supplementary Table 1).

Invasive Aspergillosis

Forty-five patients with IA (32 [71%] probable and 13 [29%] proven IA) were included in the study. Thirty-two (71%) had a pulmonary IA, in 8 of them, associated with secondary locations (sinus, brain, skin, or pharynx), and 13 (29%) had extrapulmonary IA (ear, sinus, brain, meninges, cranium, skin, aorta, or vertebra). The patients' characteristics are given in Table 3.

At the TOD, 22 of 45 (48%) were BG negative (Table 3). When comparing the groups of patients with negative and positive BG test results at the TOD, we did not observe differences in the categories of the patients (see Table 3), the location or classification of IA, the time of BG sampling, the antifungal drug use before the diagnosis, neutropenia status of the patients, 6- or 12-week survival, or the possible cause of false-positive BG results (Supplementary Table 2).

In 14 of 45 (31%) IA patients, BG were measured 4 to 15 days before TOD, and the results were positive in 5 cases (data not shown). (1-3)-β-D-glucan follow-up was available for 37 of 45 (82%) patients for periods ranging from 1.5 to 29 weeks (Supplementary Table 3). Of the 37 patients with BG follow-up, 7 were persistently BG negative, 5 had variable positive/negative BG test results, and 25 were repeatedly BG positive (Supplementary Table 3). Among these 25, BG levels decreased rapidly in 7 (28%), with negativation within 1 month after the TOD, whereas in 14 (56%) patients, BG results were still positive after 3 months (Supplementary Figure 2). Examples of BG postdiagnosis kinetic profiles are presented in Figure 1. When comparing patients with rapid versus slow BG negativation in terms of type and location of IA and survival, we did not observe significant differences, except for a trend toward slower BG negativation in patients with sinus and/or brain IA ($P = .05$; data not shown).

Rare Invasive Fungal Diseases

The 49 patients with rare IFD were classified into 18 diagnoses including cryptococcosis (N = 10); mucormycosis (N = 6); fusariosis, scedosporiosis, and trichosporonosis (N = 4 each); eumycotic mycetoma (N = 5); and invasive subcutaneous phaeohyphomycosis (N = 3). The patients' characteristics are presented in Table 4.

Overall, 19 (39%) of the patients with rare IFD were BG negative at the TOD. We observed great variability of BG positivity at the TOD in almost every category of rare IFD, except for the 10 patients with chronic subcutaneous IFD (including phaeohyphomycosis, deep dermatophytosis, or eumycotic mycetoma) who were all BG positive at the TOD. A BG follow-up was available for 4 of these 10 patients, and their BG results remained positive for over 25 weeks after the TOD. Unexpectedly, 3 of 10 patients with cryptococcosis (1 pulmonary case and 2 meningeal cases) and 3 of 6 patients with mucormycosis (1 subcutaneous case, 1 intestinal, and 1 sinus case) were BG positive at the TOD.

DISCUSSION

Our study, which aimed at assessing BG serum levels at TOD and BG follow-up kinetics in patients treated for documented IFD, has resulted in several significant findings.

First, we observed a relatively low BG positivity rate at the TOD in the 2 most common IFD, candidemia and IA, as well as in rare IFD. Indeed, only 64%, 52%, and 61% of patients who received a diagnosis of candidemia, probable/proven IA, or rare IFD tested positive for BG at the TOD, respectively. These findings confirmed the results of other studies reporting low BG sensitivity in different IFD and various patient populations [13–15, 19]. For instance, Koo et al [13] and Ostrosky-Zeichner et al [14], who both analyzed the BG assay performance at the TOD regardless of the category of patients or type of IFD, observed an average sensitivity of 64%. Koo et al [13] reported even lower BG sensitivity at the TOD among patients receiving a hematopoietic stem cell transplant or patients with febrile neutropenia (43% and 38%, respectively) [13]. It is noteworthy that the BG positivity rate observed at the TOD in patients who received a diagnosis of candidemia in our study was somehow lower than the ones previously reported; other authors, including Ostrosky-Zeichner et al [14], reported sensitivity rates above 80% [27, 28]. This lack of positive BG test results at the TOD

Table 3. Demographics, Clinical Characteristics, and Mycological Data of the Patients With Invasive Aspergillosis at the Time of Diagnosis

Patient ID	Age	Gender	Category of Patient	Sample (Test) Providing Initial Diagnosis	*Aspergillus* Species	GM Ag at TOD (Index)	Location of IA	EORTC/MSG IA Classification	BG at TOD	
									Time Interval Between Sample Providing Diagnosis and BG Sample (Days)	BG Value (pg/mL)
1	28	F	Hematology	Serum (PCR)	*Aspergillus fumigatus*	0.55	Lung	Probable	0	80
2	38	M	Hematology	Serum (GM Ag)	*A. fumigatus*	0.72	Lung	Probable	0	<80
3	73	M	Hematology	Serum (GM Ag)	—	0.73	Lung	Probable	0	<80
4	80	F	Hematology	Serum (GM Ag)	—	1	Lung	Probable	1	<80
5	29	F	Hematology	Serum (GM Ag)	—	1.18	Lung	Probable	0	<80
6	63	F	Hematology	Serum (GM Ag)	*A. fumigatus*	0.56	Lung	Probable	−3	467
7	69	F	Hematology	Serum (GM Ag)	—	0.67	Lung	Probable	0	<80
8	30	F	Hematology	Serum (GM Ag)	—	0.54	Lung	Probable	0	<80
9	63	M	Hematology	Serum (GM Ag)	*A. fumigatus*	0.51	Lung	Probable	−3	<80
10	54	F	Hematology	Serum (PCR)	*A. fumigatus*	1.15	Lung	Probable	0	99
11	63	F	Hematology	BAL (Culture)	*A. fumigatus*	0.5	Lung	Probable	3	<80
12	20	M	Hematology	Serum (GM Ag)	*A. fumigatus*	0.64	Lung	Probable	0	131
13	23	M	Hematology	Serum (PCR)	*A. fumigatus*	0.27	Lung	Probable	−2	>523
14	0	M	Hematology	Lung biopsy (Culture)	*A. fumigatus*	Nd	Lung	Proven	−2	<80
15	51	M	Hematology	Serum (GM Ag)	—	1.69	Lung	Probable	4	<80
16	61	M	Hematology	Serum (GM Ag)	*A. fumigatus*	0.84	Lung	Probable	0	129
17	46	F	Hematology	Serum (GM Ag)	*A. fumigatus*	1.13	Lung	Probable	0	<80
18	76	M	Hematology	Serum (PCR)	*A. fumigatus*	5.63	Lung	Probable	0	<80
19	67	M	Hematology	Serum (GM Ag)	—	0.61	Lung	Probable	0	<80
20	73	F	Hematology	Serum (PCR)	*A. fumigatus*	1.9	Lung	Probable	0	176
21	53	M	SOT, Hematology	Bronchoaspiration (Culture)	*A. fumigatus*	0.06	Lung	Probable	−5[a]	113
22	60	M	SOT	Lung biopsy (Culture)	*A. fumigatus*	Nd	Lung	Probable	7	<80
23	71	F	SOT	Serum (GM Ag)	—	1.3	Lung	Probable	5	349
24	62	M	Hematology	Serum (PCR)	*A. fumigatus*	0.44	Lung	Probable	0	<80
25	22	M	Hematology	Uvula biopsy (Culture)	*A. fumigatus*	0.23	Lung, Pharynx	Proven	1	<80
26	47	F	Hematology	Serum (GM Ag)	—	1.8	Lung, Sinus	Probable	0	83
27	57	M	Hematology	Serum (GM Ag)	*A. fumigatus*	0.64	Lung, Sinus	Probable	0	257
28	55	F	Hematology	Serum (GM Ag)	—	4.61	Lung, Sinus	Probable	0	>523
29	42	M	SOT	Serum (PCR)	*A. fumigatus*	Nd	Lung, Brain	Probable	3	>523
30	74	M	SOT, Hematology	CSF (PCR)	*A. fumigatus*	Nd	Lung, Sinus, Brain	Probable	0	<80
31	65	M	Hematology	Serum (GM Ag)	—	2.04	Lung, Sinus, Brain	Probable	0	<80
32	51	M	SOT	Skin biopsy (Culture)	*A. fumigatus*	0.09	Lung, Brain, Skin	Proven	4	377
33	56	M	SOT	CSF (PCR)	*A. fumigatus*	Nd	Aorta, Skin, Brain	Probable	1	>523
34	61	M	Hematology	Serum (GM Ag)	—	1.44	Brain	Probable	0	95
35	63	F	Hematology	Sinus biopsy (Culture)	*Aspergillus flavus*	0.22	Sinus	Proven	0	<80
36	74	M	Non-IC	Sinus biopsy (Culture)	*A. fumigatus*	0.17	Sinus, Eye socket	Proven	2	<80
37	72	M	SOT	CSF (PCR)	*A. fumigatus*	0.11	Sinus, Brain	Probable	6	278
38	56	M	Non-IC	Sinus biopsy (Culture)	*A. fumigatus*	Nd	Sinus, Brain	Proven	−3	<80
39	19	M	Non-IC	Sinus biopsy (Culture)	*A. flavus*	0.1	Sinus, Cranium, Brain	Proven	11	>523
40	76	M	Diabetes	Sinus biopsy (Culture)	*A. fumigatus*	Nd	Ear, Meninges	Proven	7	328
41	71	M	Diabetes	Sinus biopsy (Culture)	*A. flavus*	0.3	Ear, Sinus, Cranium	Proven	14	<80
42	74	F	Diabetes	Clivus biopsy (Culture)	*A. flavus*	0.18	Ear, Cranium	Proven	12	160
43	68	F	Diabetes	Meningeal biopsy (Culture)	*A. flavus*	0.06	Ear, Cranium, Meninges	Proven	13	118

Table 3 continued.

Patient ID	Age	Gender	Category of Patient	Sample (Test) Providing Initial Diagnosis	*Aspergillus* Species	GM Ag at TOD (Index)	Location of IA	EORTC/MSG IA Classification	BG at TOD: Time Interval Between Sample Providing Diagnosis and BG Sample (Days)	BG at TOD: BG Value (pg/mL)
44	68	M	Non-IC	Vertebral biopsy (Culture)	*Aspergillus terreus*	1.06	Vertebra	Proven	3	>523
45	19	M	Non-IC	Vertebral biopsy (Culture)	*A. terreus*	0.18	Vertebra	Proven	0	440

Abbreviations: BG, (1-3)-β-D-glucan; CSF, cerebral spinal fluid; EORTC/MS, European Organization for Research and Treatment of Cancer/Mycoses Study Group; GM Ag, galactomannan antigens; IA, invasive aspergillosis; Nd, not determined; Non-IC, non-immunocompromised; PCR, polymerase chain reaction; SOT, solid organ transplant; TOD, time of diagnosis.

[a] An exception was made regarding the inclusion of this patient, even though his/her first BG was sampled 5 days before mycological diagnosis.

observed in every type of IFD is casting doubt on the usefulness of BG detection for early diagnosis of IFD. Thus, the BG serial sampling proposed in some works [17, 20, 29, 30] to help anticipate IFD diagnosis in at-risk patients might be effective only for a minority of patients, which remains to be determined.

Among patients with candidemia, we did not find medical risk factors associated with BG positivity at the TOD (eg, vascular catheterization, prior antifungal drug use, or an already known possible cause of false-positive BG results), but we observed differences strongly related to the *Candida* species involved. Indeed,

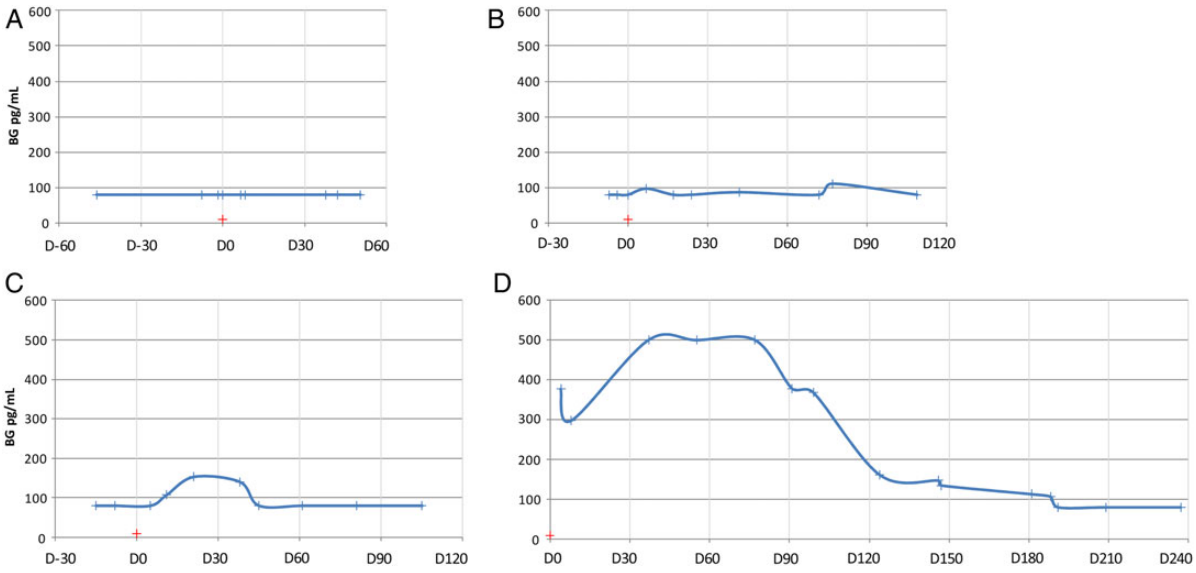

Figure 1. Examples of the 4 main categories of (1-3)-β-D-glucan (BG) postdiagnosis kinetic profiles observed after a diagnosis of invasive aspergillosis (IA). For each example, the blue curve represents the BG kinetics before, at the time, and after a diagnosis of IA. The red cross represents the day of collection (D0) of the first biological sample that led to the diagnosis of IA: y-axis, BG values in pg/mL; x-axis, time relative to the day (D0) of collection of the sample that led to the diagnosis of IA, expressed in weeks. (A) Negative BG profile (N = 8 of 37): patients showing persistently negative BG test results after IA diagnosis. The kinetics shown are those of Patient 19 (hematology patient), who received a diagnosis of probable pulmonary IA, for whom 3 BG assays were performed before diagnosis and 6 BG assays were performed within 12 weeks of diagnosis. The first mycological result allowing for the diagnosis of probable IA was the detection of serum galactomannan antigens. (B) Unreliable BG profile (N = 5 of 37): patients showing alternating negative or low BG test results after IA diagnosis. The kinetics shown are those of Patient 2 (hematology patient), who received a diagnosis of probable pulmonary IA, for whom 2 BG assays were performed before diagnosis and 8 BG assays were performed within 16 weeks of diagnosis. The first mycological result allowing for diagnosis of probable IA was the detection of serum galactomannan antigens. (C) Rapid BG decrease profile (N = 6 of 37): patients with positive BG kinetics after IA diagnosis, who showed a rapid decrease in the BG level, with BG negativation in less than 1 month. The kinetics shown are those of Patient 7 (hematology patient), who received a diagnosis of probable pulmonary IA, for whom 2 BG assays were performed before diagnosis and 8 assays were performed within 13 weeks of diagnosis. The first mycological result allowing for diagnosis of probable IA was the detection of serum galactomannan antigens. (D) Slow BG decrease profile (N = 14 of 37): patients with positive BG kinetics after IA diagnosis, who showed persistently elevated BG levels for more than 3 months. The kinetics shown are those of Patient 32 (solid organ transplant), who received a diagnosis of proven multisite IA, for whom 15 BG assays were performed within 33 weeks of diagnosis. The first mycological result allowing for diagnosis of proven IA was a culture of a skin biopsy that tested positive for *Aspergillus fumigatus*.

Table 4. Demographics, Clinical Characteristics, and Mycological Data of Patients With Rare Invasive Fungal Diseases

							BG at TOD	
IFD due to Rare Fungi	Age	Gender	Category of Patient	Sample Providing Diagnosis	Diagnostic Element	Location of Fungal Infection	Time Interval Between Sample Providing Diagnosis and BG Sample (Days)	BG Value (pg/mL)
Cryptococcosis	5	F	Non-IC	Blood culture	*Cryptococcus neoformans*	Blood, Meninges, Lymph nodes	6	<80
	55	F	Hematology	CSF	*C. neoformans*	Meninges	1	244
	57	M	HIV	CSF	*C. neoformans*	Blood, Meninges, Urine	4	<80
	56	F	SOT	Serum	Positive cryptococcal EIA antigen	Meninges, Skin, Urine	0	89
	43	M	SOT	Sputum	*C. neoformans*	Lung	5	<80
	48	F	SOT	CSF	*C. neoformans*	Meninges	1	<80
	60	M	HIV	Pulmonary biopsy	Encapsulated yeasts at Grocott staining	Lung	−1	342
	18	F	Hematology	Blood culture	*C. neoformans*	Blood	−1	<80
	62	M	SOT	CSF	*C. neoformans*	Meninges	0	<80
	17	F	SOT	CSF	*C. neoformans*	Meninges	8	<80
Zygomycosis	69	F	SOT	Cutaneous biopsy	*Mucor irregularis*	Skin and subcutaneous tissue	6	144
	4	F	SOT	Gastric biopsy	*Lichtheimia* sp	Gastrointestinal tract	1	158
	22	M	Hematology	Sinus biopsy	*Rhizopus* sp	Sinus	0	134
	66	F	Hematology	Sinus biopsy	*Lichtheimia ramosa*	Sinus	−1	<80
	8	F	SOT	Cutaneous biopsy; Serum	*Rhizopus oryzae*	Skin and subcutaneous tissue	10	<80
IFD due to *Fusarium* sp	70	F	Hematology	Blood culture	*Fusarium solanii*	Blood	2	99
	61	F	SOT	Sputum	*Fusarium* sp	Lung	0	<80
	42	M	Hematology	Blood culture	*Fusarium* sp	Blood	10	<80
	62	F	Hematology	Blood culture	*Fusarium proliferatum*	Blood	0	<80
IFD due to *Trichosporon* sp	33	M	Hematology	Blood culture	*Trichosporon* sp	Blood	1	>523
	67	F	Hematology	Blood culture	*Trichosporon* sp	Blood	9	>523
	10	F	ICU	Blood culture	*Trichosporon asahii*	Blood	6	<80
IFD due to *Rhodotorula* sp	3	M	ICU	Blood culture	*Rhodotorula mucilaginosa*	Blood	4	184
	2	M	Hematology	Blood culture	*R. mucilaginosa*	Blood	5	<80
IFD due to *Geotrichum* sp	72	M	Hematology	Blood culture	*Geotrichum capitatum*	Blood	0	287
IFD due to *Malassezia* sp	4	M	Hematology	Blood culture	*Malassezia furfur*	Blood	3	<80
IFD due to *Acremonium* sp	63	M	Hematology	Blood culture	*Acremonium strictum*	Blood	0	<80
Infection due to *Trichoderma* sp	67	F	SOT	BAL	*Trichoderma longibrachiatum*	Lung	3	489
IFD due to *Exophiala* sp	8	F	Hematology	CSF	*Exophiala dermatitidis*	Brain, Meninges	−3	>523
IFD due to *Cladophialophora* sp	6	M	Non-IC	CSF	*Cladophialophora bantiana*	Brain	6	145
IFD due to *Rasamsonia* sp	29	M	Hematology	Cutaneous abscess	*Rasamsonia argillacea*	Lung, Skin, Subcutaneous tissue	1	<80
Histoplasmosis	43	F	HIV	Bone marrow aspiration	*Histoplasma capsulatum*	Bone marrow, Lymph nodes, Skin	2	233
	36	F	HIV	Bone marrow aspiration	*H. capsulatum*	Disseminated	3	255
Coccidioidomycosis	32	M	Non-IC	Sputum	*Coccidioides immitis*	Lung (relapse)	0[a]	150
IFD due to *Scedosporium* sp	69	F	SOT	Bone biopsy	*Scedosporium apiospermum*	Lung, Bone, Eye, Blood	2	>523
	14	M	Hematology	Sputum	*Scedosporium prolificans*	Lung, Blood	1	<80
	32	M	Cystic fibrosis	Sputum	*Scedosporium* sp	Bone (relapse), Disseminated	7[b]	270
	52	F	SOT	Cutaneous sample	*S. apiospermum*	Subcutaneous tissue, Bone	0	<80

Table 4 continued.

IFD due to Rare Fungi	Age	Gender	Category of Patient	Sample Providing Diagnosis	Diagnostic Element	Location of Fungal Infection	BG at TOD: Time Interval Between Sample Providing Diagnosis and BG Sample (Days)	BG at TOD: BG Value (pg/mL)
Invasive cutaneous phaeohyphomycosis	62	M	SOT	Cutaneous biopsy	*Alternaria infectoria*	Skin, Subcutaneous tissue	0	>523
	58	F	SOT	Cutaneous biopsy	*Alternaria rosea*	Skin, Subcutaneous tissue	−4	298
	63	M	SOT	Cutaneous biopsy	*Phialemonium dimorphosporum*	Skin, Subcutaneous tissue	3	>523
Deep dermatophytosis	72	M	SOT	Cutaneous biopsy	*Trichophyton rubrum*	Skin, Subcutaneous tissue	−2	151
	56	M	SOT	Cutaneous biopsy	*T. rubrum*	Skin, Subcutaneous tissue	1	>523
Eumycotic mycetoma	34	M	Non-IC	Cutaneous biopsy	*Madurella mycetomatis*	Lumbar subcutaneous tissue, Lung, Pleura, Retroperitoneum	0[c]	254
	59	M	SOT	Cutaneous biopsy	Nonidentified black fungus	Subcutaneous tissue	1[d]	304
	69	M	Diabetes	Cutaneous biopsy	*Exophiala jeanselmei*	Subcutaneous tissue	0	305
	68	M	SOT	Cutaneous biopsy	*F. solanii*	Subcutaneous tissue	0	337
	54	M	SOT	Grains in seropurulent exudate	Nonidentified fungus	Subcutaneous tissue	0	237

Abbreviations: BG, (1-3)-β-D-glucan; BAL, bronchoalveolar lavage; CSF, cerebral spinal fluid; EIA, enzyme immunoassay; HIV, human immunodeficiency virus; ICU, intensive care unit; IFD, invasive fungal diseases; Non-IC, non-immunocompromised; SOT, solid organ transplant; TOD, time of diagnosis.

[a] The initial diagnosis of pulmonary coccidioidomycosis was performed 21 months before the current relapse.

[b] The initial diagnosis of spondylodiscitis due to *Scedosporium* sp was performed 4 years before the current relapse.

[c] The initial diagnosis of the eumycotic mycetoma was performed 13 years before the current onset.

[d] The initial diagnosis of the eumycotic mycetoma was performed 24 years before the current onset.

we found that BG is a more reliable diagnostic marker in cases of candidemia due to *C. albicans* than to other *Candida* species. To our knowledge, this finding has not been reported previously. Ostrosky-Zeichner et al [14] stated that the BG assay may be less sensitive for *Candida parapsilosis* than for other *Candida* species, but this notion was not confirmed by Martínez-Jiménez [27], who observed good sensitivity of BG testing in cases of candidemia due to *C. parapsilosis*, even better than for *C. albicans*. Because most *Candida* infections are caused by endogenous *Candida* strains colonizing the patients before the infection [31, 32], a procedure of BG serial sampling may thus be worthwhile (targeting patients at risk of candidemia and known to be colonized by *C. albicans* or hospitalized in wards reporting high prevalence of *C. albicans* infections). Regarding the BG evolution in patients with candidemia who tested positive for BG at the TOD, we observed very different patterns of BG dynamics. In one third of the cases, the BG levels decreased rapidly after initiation of treatment (a significant decrease in 1 week), whereas in the other cases (two thirds), the BG level was persistently elevated during at least 2 weeks after the IFD diagnosis. It is worth mentioning that patients with slow BG decrease profiles were more likely to have candidemia associated with deep-seated infected sites. Furthermore, patients with IC not associated with candidemia, in particular patients with chronic disseminated candidiasis, also showed persistently elevated serum BG levels. These findings are consistent with the results of other studies suggesting that a decrease in BG levels after treatment initiation is a marker of a favorable prognosis, especially in IC [20, 22, 23, 33]. Jaijakul et al [22] observed a decrease in BG levels among patients who received successful treatment of candidemia, whereas patients with tissue infection or treatment failure showed persistently elevated BG levels. Altogether, these results should encourage clinicians to evaluate BG kinetics in patients with positive BG test results at the TOD of candidemia or in patients with suspected deep-seated candidiasis, in particular chronic disseminated candidiasis. Complementary prospective studies focusing on BG kinetics during treatment would be useful in this regard.

As for IA, we did not find a significant association between the level of BG at TOD and the status of the patient (immunocompromised or not), the type of aspergillosis (location or EORTC/MSG classification), or the outcome. On the other hand, as in candidemia, only one third of the patients with positive BG at the TOD had a rapid BG decrease profile (negativation within 1 month), whereas two thirds showed a very slow BG decrease, with negativation often observed more than 100 days after the diagnosis. This very slow BG evolution, which

might be as lengthy as or even longer than the therapeutic protocol itself, may limit the use of BG serial sampling for monitoring the clinical course in cases of IA [18, 19]. Nevertheless, in the absence of a tangible improvement after treatment initiation, persistently elevated or rising BG levels 1 month after the diagnosis may warn clinicians about a possible treatment failure [18, 19]. Further studies in this area are necessary to evaluate the real efficacy of such a strategy.

Regarding BG detection in rare IFD, we observed a relatively low frequency of positive BG results at the TOD, but we noticed an exception. In cases of chronic subcutaneous IFD (phaeohyphomycosis, deep dermatophytosis, or eumycotic mycetoma), all patients tested positive for BG at the TOD, and we observed extremely slow BG decrease after the diagnosis (BG persistently positive for more than 25 weeks). This result may be linked to the chronic and relatively indolent progression of this type of infections, with often delayed diagnosis [34]. Thus, our findings suggest that BG may be a useful diagnostic marker of chronic subcutaneous IFD. Although we expected all patients with cryptococcosis and mucormycosis to test negative for BG at the TOD [6, 35], some of them tested positive. Such findings have already been reported regarding cryptococcosis [36, 37]. Rhein et al [37], who observed 89% sensitivity of a BG assay in cerebrospinal fluid of patients with cryptococcal meningitis, suggested that due to the low reactivity of *Cryptococcus* species with the BG test [38] (presumably due to BG's low concentration in the *Cryptococcus* cell wall), the success of BG detection during cryptococcosis may depend on the fungal burden during the infection. For mucormycosis, however, none of the studies involving patients with this type of IFD showed BG positivity at the TOD [13, 14, 19, 36]. Positive BG test results in our cases of mucormycosis may be associated, as suggested above, with the substantial fungal burden. These positive test results may also be a consequence of a yet unknown cross-reactivity of the BG assay. Indeed, if we had assessed the main causes of BG false-positivity in our study (IVIG, albumin injection, hemodialysis, or a recent surgical procedure), we might have missed less frequent causes, such as concomitant infection due to *Nocardia* [39] or even unknown causes.

CONCLUSIONS

Overall, our study, which included many categories of patients (hematology, SOT, ICU, and even non-immunocompromised patients), suggests that BG detection in the serum of at-risk patients is not as effective as an early diagnostic marker of IFD. Nonetheless, our findings also indicate that this marker may help to assess the risk of chronic infection after the initial IFD diagnosis, and, in particular, serial BG sampling may facilitate the evaluation of the risk of disseminated candidiasis associated with candidemia. Taken together, our results offer valuable information on clinical utility of the BG assay in various IFD and underscore the difficulties with interpretation of BG data in clinical settings.

Acknowledgments

Author contributions. All coauthors have contributed significantly to the work. C. A. and M.-E. B. wrote the first draft of the manuscript, and all of the coauthors participated in revision and approved the final version.

Financial support. This work was supported by the Association pour la Promotion de l'Enseignement et de la Recherche en Mycologie.

Potential conflicts of interest. C. A. received a research grant from Pfizer. P. F. received grants from SIDACTION and the French National Agency for Research on AIDS and Viral Hepatitis; consultancy, or lecture fees, and travel grants from ViiV Healthcare, Bristol-Myers Squibb, Janssen Cilag, Gilead Sciences, and MSD. D. C. has received fees for board membership of MSD. O. L. has received consultancy or lecture fees from Gilead Sciences, Pfizer, Astellas, and Merck. M.-E. B. has received institutional grant funding from MSD and Astellas and lecture fees or travel grants from MSD, Astellas, and Gilead Sciences. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

Supplementary Data

Supplementary material is available online at *Open Forum Infectious Diseases online* (http://OpenForumInfectiousDiseases.oxfordjournals.org/).

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