

Contribution of the (1→3)-β-D-Glucan Assay for Diagnosis of Invasive Fungal Infections[∇]

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Diagnosis of invasive fungal infection (IFI) remains a challenge. A retrospective study was performed on 279 patients at three French university hospitals to evaluate the performance of the (1→3)-β-D-glucan assay (BG assay; Fungitell; Associates of Cape Cod, Inc.) for the diagnosis of IFI. The results of one serum per subject were analyzed for 117 patients who had probable or proven IFI according to the European Organization for Research and Treatment of Cancer criteria (70 invasive pulmonary aspergilloses [IPA], 27 fungal bloodstream infections, and 20 *Pneumocystis jiroveci* pneumonias), 40 blood donors, and 122 patients who were hospitalized in hematology wards or intensive care units and were at risk for IFI but in whom IFI had not been diagnosed. For the overall IFI diagnosis, the BG assay had 77.8% sensitivity and specificities of 92.5 and 70.5% for blood donors and patients at risk, respectively. The assay was positive in 48 patients with IPA (68%), in 23 with bloodstream infections (85.2%), and in all who had *P. jiroveci* pneumonias (100%), and the false-positive rate varied depending on the controls used. It allowed a higher rate of detection among IPA patients compared to the galactomannan enzyme-linked immunosorbent assay (ELISA) (48 versus 39 patients, respectively) and among candidemia patients compared to the mannan ELISA (20 versus 11 patients, respectively). This assay therefore appears to be useful in the diagnosis of IFI, particularly for serum analysis of pneumocystosis pneumonia patients, but further studies are needed to evaluate false-positive rates and its future role in IFI diagnosis.

The incidence of invasive fungal infections (IFI) has increased over the last two decades, especially among immunocompromised patients, and is associated with a high mortality rate of 30 to 70% for aspergillosis (10, 22) and 40% for candidemia (21). Conventional microbiological, histological, and radiological techniques that have been used for the diagnosis of IFI are relatively insensitive, time-consuming, and not generally accessible (4). Intensive research is currently aimed at improving the diagnosis of IFI. Recently, particular emphasis was placed on the detection of fungal molecules within biological samples.

Several fungal detection kits have been commercialized during the past few years. Two enzyme-linked immunosorbent assays target fungal cell wall antigens: Platelia *Aspergillus* (27) detects *Aspergillus* galactomannan (GM) antigens and Platelia *Candida* (24) detects mannan (M) antigens. The *Aspergillus* antigen detection assay has been used most frequently, and the results of this assay are included in the IFI diagnosis criteria of the European Organization for Research and Treatment of Cancer (EORTC) (1). The combined detection of M antigen and anti-M antibodies has been advocated to distinguish col-

onization and invasive infections with *Candida* spp. (24, 25). However, all of these methods have limitations.

Recently, colorimetric assays have been commercialized (15) to detect (1→3)-β-D-glucans (BG), which are major cell wall components of various medically important fungi. The Fungitell test (Associates of Cape Cod, Inc., Cape Cod, MA) is a chromogenic kinetic test that was approved in 2003 by the U.S. Food and Drug Administration for the presumptive diagnosis of IFI.

A retrospective study of this BG assay was conducted on patients at three French university hospitals who had or were at risk for IFI. The primary aim was to evaluate the performance of this assay in the diagnosis of IFI, and in particular of aspergillosis, candidemia, and pneumocystosis. The secondary aim was to compare the assay to either the GM or M assay for the diagnosis of invasive pulmonary aspergillosis (IPA) or fungal bloodstream infections (BSI), respectively.

MATERIALS AND METHODS

Patients. A total of 279 patients from five groups were included in the study. One hundred and seventeen patients who had an IFI according to the EORTC criteria (1) were included from three groups: (i) 70 who had probable or proven IPA, (ii) 27 who had a positive hemoculture (26 *Candida* spp. and 1 *Geotrichum capitatum*), and (iii) 20 who had *Pneumocystis jiroveci* pneumonia with trophozoites and/or cysts detected microscopically in the bronchoalveolar lavage fluid after staining with DiffQuick and/or Gomori-Grocott methenamine silver or toluidine blue.

Two control groups were also included (i) 40 healthy blood donors and (ii) 122 patients from hematology wards or intensive care units, who were at risk for developing an IFI but who had no identified IFI according to the EORTC criteria (1). All sera tested in routine for GM and/or M antigens were stored at

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TABLE 1. BG assay results (and efficiency parameters) for the diagnosis of IFI in patients, in populations at risk for IFI, and in subgroups at risk for IPA and/or bloodstream infection

Parameter ^a	IFI patient groups and subgroups/control groups			
	Total IFI patients/ blood donors	Total IFI patients/ patients at risk	Pulmonary aspergillosis/ corresponding patients at risk	Bloodstream infections/ corresponding patients at risk
No. of patients	117/40	117/122	70/100	27/101
No. of patients with a BG \geq 80 pg/ml	91/3	91/36	48/27	23/36
Sensitivity (95% CI)	77.8 (70.2–85.3)	77.8 (70.2–85.3)	68.6 (57.7–79.5)	85.2 (71.8–98.6)
Specificity (95% CI)	92.5 (84.3–100)	70.5 (62.4–78.6)	73.0 (64.3–81.7)	64.4 (55.0–73.7)
LR ⁺ (95% CI)	10.4 (3.5–30.9)	2.64 (1.97–3.53)	2.54 (1.77–3.64)	2.39 (1.76–3.24)
LR ⁻ (95% CI)	0.24 (0.17–0.34)	0.32 (0.22–0.45)	0.43 (0.30–0.62)	0.23 (0.09–0.57)
Yule Q	0.95	0.79	0.71	0.82

^a LR, likelihood ratio. The Yule Q coefficient measures the strength of the association between the test results and the disease (0, null; 0.01 to 0.09, negligible; 0.10 to 0.29, light; 0.30 to 0.49, moderate; 0.50 to 0.69, strong; 0.70 to 1, very strong).

–20°C until the BG assay. For each patient, detection for BG was performed on the same serum sample. When several serum samples were available for one patient, we chose the one closest to the time of fungal infection diagnosis.

BG assay. BG levels were measured by using the Fungitell test kit as recommended by the manufacturer. A kinetic colorimetric assay performed at 37°C was read at 405 nm for 25 min. The concentration of BG in each sample was automatically calculated by using a calibration curve with standard solutions ranging from 6.25 to 100 pg/ml. A BG level of \geq 80 pg/ml was considered to be positive; no gray zone was considered. Serum assays were performed in duplicate.

GM *Platelia Aspergillus* assay. The GM *Platelia Aspergillus* assay was performed as recommended by the manufacturer. An index of \geq 0.5 was considered positive (8). Each of the 70 IPA patients had been routinely tested for the presence of GM antigen by using this assay. The control group consisted of 100 sera from hematology patients who had IFI, all of whom had been tested for the presence of GM; 21 sera gave documented false-positive GM results.

Platelia Ag *Candida* assay. The *Platelia Ag Candida* assay was performed as recommended by the manufacturer. The M concentration was calculated by using a calibration curve with a standard solution ranging from 0.25 to 2 μ g/ml. A concentration of \geq 0.5 μ g/ml was considered positive. A total of 24 of the 27 fungemia patients had been tested for the presence of M. Their sera had been collected between 8 days before and 14 days after the positive hemoculture (median, 1.5 days; mean, 2 days). The control group comprised 101 patients at risk, 46 of whom were tested for the presence of M (including 12 sera that gave false-positive M results).

Statistical analysis. The following indices of diagnostic performance and their 95% confidence intervals were computed: sensitivity, specificity, likelihood ratio test (LR) positivity (LR⁺), LR negativity (LR⁻), the Yule Q coefficient, and Cohen's kappa. LR⁺ was calculated as Se/(1 – Sp), LR⁻ was calculated as (1 – Se)/Sp (10), where Se is the sensitivity and Sp is the specificity. The probability of the presence or absence of the disease increased with the LR values. In contrast to the predictive values, LR⁺ and LR⁻ are independent of prevalence, as are sensitivity and specificity, and are therefore increasingly used to evaluate diagnostic test performances. The Yule Q coefficient measures the strength of the association between the test results and the disease as follows: 0, null; 0.01 to 0.09, negligible; 0.10 to 0.29, light; 0.30 to 0.49, moderate; 0.50 to 0.69, strong; and 0.70 to 1, very strong. Cohen's kappa estimates the accuracy of the test beyond the agreement by chance; kappa values of 0.40 indicate an accurate diagnostic test.

RESULTS

The BG levels determined in 279 sera from patients and controls are summarized in Table 1. The test parameters in the first column were calculated for all IFI patients compared to blood donors. In the other columns, both all and various subgroups of IFI patients were compared to patients at risk. The BG levels were very strongly associated to IFI with Yule Q values of 0.95 (compared to blood donors) and 0.79 (compared to patients at risk).

IPA patients were compared to patients at risk for IPA. In the patients with IPA, 31 of 35 (88.6%) true GM-positive sera and 13 of 25 (52%) false GM-negative sera were BG positive. The test results were very strongly associated with the disease (Yule Q = 0.71). Ten patients with IPA were treated with caspofungin, an antifungal drug that interferes with BG synthesis. Of these, four tested as positive for BG, three of whom were also GM positive, while another serum tested as GM positive but BG negative.

Patients with fungal BSI were compared to patients at risk of fungal BSI. A total of 23 of 27 of the fungal BSI patients yielded BG-positive results (Table 1). The causative agents (22 *Candida* spp. and one *G. capitatum*), the time from serum sampling to the first positive hemoculture, and the BG and M levels are summarized in Table 2. Again, the test results were very strongly associated with the disease (Yule Q = 0.82). Among the patients who had candidemia (Table 2), four of seven patients from whom sera had been collected before the first positive bloodstream culture were BG positive. All except 1 of the 19 sera collected from candidemia patients after the first positive bloodstream culture were BG positive. The patient with a negative BG test had a positive bloodstream culture with *C. albicans* 5 days before the serum sampling. The median glucan level in the candidemia patients was 300 pg/ml (range, 21 to 3,006 pg/ml). The serum of the patient with a *G. capitatum* fungemia tested as BG positive 4 days before the positive bloodstream culture.

All 20 patients who had *P. jiroveci* pneumonia tested as BG positive, 13 of whom (65%) had BG levels higher than 500 pg/ml. The features of these patients are summarized in Table 3. In seven patients, the BG assay was positive in sera taken before the day of microscopic diagnosis from a bronchoalveolar lavage fluid sample. The median BG level was 945 pg/ml (range, 103 to 3,892 pg/ml).

All 70 sera from patients who had IPA were tested for the presence of GM, and 23 of 26 candidemia patients were also tested for the presence of M. A comparison of these tests with the BG assay yielded the results shown in Table 4. In 19 patients with IPA, the BG and GM tests gave discordant results: 5 were BG negative and GM positive and 14 were BG positive and GM negative. For the patients with candidemia, 11 discordant results with the BG and M tests were found: one

TABLE 2. Hemoculture and quantitative BG results for candidemia and geotrichosis patients

Patient no.	Fungus isolated in hemoculture	Days from serum sampling to first positive hemoculture	BG level (pg/ml)	M level (μg/ml)
1	<i>C. parapsilosis</i>	-8	77	<0.250
2	<i>C. glabrata</i>	-4	49	<0.250
3	<i>C. guilliermondii</i>	-4	>500	1.47
4	<i>C. albicans</i>	-3	>500	>2
5	<i>C. albicans</i>	-2	>500	>2
6	<i>C. glabrata</i>	-1	>500	1.121
7	<i>C. krusei</i>	-1	46	1.071
8	<i>C. albicans</i>	0	247	<0.250
9	<i>C. guilliermondii</i>	0	358	<0.250
10	<i>C. albicans</i>	1	>500	<0.250
11	<i>C. albicans</i>	1	>500	>2
12	<i>C. glabrata</i>	1	181	<0.250
13	<i>C. lusitaniae</i>	1	>500	1.026
14	<i>C. guilliermondii</i>	2	289	<0.250
15	<i>C. tropicalis</i>	2	>500	1.436
16	<i>C. guilliermondii</i>	3	151	1.2
17	<i>C. guilliermondii</i>	3	282	<0.250
18	<i>C. albicans</i>	4	160	<0.250
19	<i>C. albicans</i>	5	21	NA
20	<i>C. tropicalis</i>	5	>500	NA
21	<i>C. glabrata</i>	5	312	0.62
22	<i>C. krusei</i>	6	>500	<0.250
23	<i>C. albicans</i>	7	83	<0.250
24	<i>C. glabrata</i>	7	198	1.650
25	<i>C. krusei</i>	8	210	<0.250
26	<i>C. albicans</i>	14	>500	NA
27	<i>G. capitatum</i>	-4	>500	<0.250*

^a NA, not available; *, GM test = 0.68.

was M positive and BG negative, and 10 were M negative and BG positive. The patient with a *G. capitatum* fungemia had a negative M test, while both the GM and BG tests were positive.

Among the patients who had no IFI, only 3 of the 40 blood donors had BG-positive results. Among the 100 patients at risk for IPA, all of whom were routinely tested for GM antigen, the test analysis showed a specificity of 55.7%. Among the 101 patients at risk for BSI, 44 were also tested for M antigen. Compared to the M test, the BG test gave a significantly higher number of positive results in patients with candidemia, with a sensitivity of 87% versus 47.8%. The specificities of both the BG and the M tests were similar (70.4 and 72.7%, respectively). Agreement between the BG and GM assays was moderate (kappa = 0.43) and that between the BG and M tests was poor (kappa = 0.07) (Table 4). Six of the twenty-one patients with a false-positive GM result and three of the twelve patients with a false-positive M result were also BG positive (28.6 and 25%, respectively), whereas twenty-one of the seventy-nine sera that tested negative for GM were also BG positive (26.6%). Among the false-positive GM tests, 16 were related to cross-reactivity with β-lactam antibiotic treatment (28), 12 of which tested as negative for BG (75%).

DISCUSSION

The aim of this French multicenter study was to evaluate the performance of the BG test in the diagnosis of IFI among

TABLE 3. Quantitative BG results for *P. jiroveci* pneumonia patients

Patient no.	HIV status	Days from serum sampling to microscopic diagnosis in BAL ^a fluid	BG level (pg/ml)
1	Positive	-7	>500
2	Positive	-5	156
3	Positive	-5	455
4	Positive	-2	>500
5	Positive	-1	>500
6	Positive	-1	>500
7	Positive	-1	>500
8	Positive	0	>500
9	Positive	0	>500
10	Positive	1	>500
11	Positive	2	>500
12	Positive	3	203
13	Positive	4	176
14	Positive	5	397
15	Positive	6	103
16	Positive	22	>500
17	Negative (two lung grafts)	0	115
18	Negative (cerebral lymphoma)	0	>500
19	Negative (dermatomyositis ARDS) ^a	1	>500
20	Negative (pulmonary neoplasia)	2	>500

^a BAL, bronchoalveolar lavage.

^bARDS, acute respiratory distress syndrome.

various control groups: blood donors, well-defined patients, and patients at risk for IFI encountered in daily practice.

The results of the Fungitell BG assay were analyzed by using a 80-pg/ml cutoff. Different cutoffs have been used previously: 60 (16), 80 (18), and 120 pg/ml (19). If a 120-pg/ml cutoff had been applied to our data, a lower detection rate would have been observed: 11 patients with IFI would have been classified as negative. Studies that used a similar 80-pg/ml cutoff for the diagnosis of IFI showed sensitivities and specificities that ranged from 78.0 to 93.3% and 77.2 to 92.6%, respectively (18, 23; S. Koo, J. Bryar, J. H. Page, L. R. Baden, and F. M. Marty, 46th Intersci. Conf. Antimicrob. Agents Chemother., poster M-1600, 2006; K. Yoshida and Y. Niki, 16th Cong. Int. Soc. Hum. Anim. Mycol., poster 0-434, 2006). Our study gave similar results for sensitivity (77.8%). The specificity was high when IFI patients were compared to blood donors (92.5%), even inferior to the previously reported 100% (23), and showed a good discrimination of the test between well-defined patients. The specificity, as expected, decreased (70.5%) when the control patients were at risk for IFI; one explanation for this may be underdiagnosis of IFI. Similarly, the LR⁺ largely decreased. However, as shown by Yule Q coefficients higher than 0.70, the BG results remained very strongly associated

TABLE 4. Comparison of BG assay versus GM and M ELISA tests for the IPA and bloodstream infection patients

BG status	IPA patients (n = 70)			Candidemia patients (n = 26)		
	GM +	GM -	Kappa (95% CI)	M +	M -	Kappa (95% CI)
BG +	34	14	0.43 (0.22-0.65)	10	10	0.07 (0.32-0.47)
BG -	5	17		1	2	

with IFI sensu lato, as well as with subgroups of both IPA and BSI.

In a recent review, BG tests for the diagnosis of IPA yielded a 55 to 100% sensitivity and 52 to 100% specificity (2). In our study, BG tests were positive in 85.7% of patients with proven and 52.4% of patients with probable aspergillosis. In a prospective study of 40 neutropenic adults, BG tests were positive in 100 and 66% of the patients with proven or probable invasive aspergillosis, respectively (19). BG test performance for the diagnosis of IPA was, however, within the range of that reported elsewhere since another prospective study reported 55% sensitivity for patients with hematological disorders (6). Two other BG studies conducted in hematology patients yielded similar sensitivities of 63 and 80%, respectively (5, 18).

Our findings for the diagnosis of IPA showed that the BG test had a higher sensitivity than the GM test (68.6% versus 55.7%). Moreover, among the seven proven IPA patients, one was GM negative and BG positive and another was GM positive and BG negative. Nineteen patients were thus diagnosed by only one of the two tests.

These results contrast with those of Koo et al. (46th Intersci. Conf. Antimicrob. Agents Chemother.), who found that all patients with invasive aspergillosis and elevated GM levels had elevated BG results. In another study (23), 31 of 32 GM-positive sera tested as positive for BG, while 9 of 32 GM-negative sera were positive for BG; two of the latter patients were found to have an IFI after reanalysis. Our results for the two tests suggest that their combined use would lead to more widespread detection of IPA.

A recent review showed that the sensitivity and specificity of the GM test for IFI diagnosis varied between patient populations (22). Although the GM test proved useful in patients with hematological malignancies and in hematopoietic stem cell transplant recipients, the limitations of this test were a relatively high false-positive rate (in neonates or children) and cross-reactions with different antibiotics, particularly β -lactams (including piperacillin-tazobactam) (28). In our study, only 5 (31.2%) of the 16 patients who had a false-positive GM assay due to cross-reaction with β -lactams were BG positive, a finding which indicates a lesser degree of cross-reaction of the BG test with these antibiotics. In line with these findings, BG was not detected in vitro at concentrations up to the theoretical maximal plasma concentration for 45 different intravenous antibiotics, including piperacillin-tazobactam (13).

Several studies have also indicated lower GM levels in patients undergoing antifungal treatment, which is an important issue in view of the increasing number of patients who receive antifungal prophylactic or preemptive treatment (9, 11). Our findings with IPA patients who were treated with caspofungin indicate a potential interaction of echinocandins with the GM and the BG test results. However, further studies are needed with a larger sample size to confirm the effect of caspofungin on BG levels.

The BG assay appeared to be of particular relevance for the diagnosis of *P. jiroveci* pneumonia, since all of our patients presented positive BG levels. In seven patients, the serum had been sampled before the time of direct diagnosis. The BG test would thus be the first valuable noninvasive serodiagnostic tool for *P. jiroveci* pneumonia and would be particularly useful for patients for whom bronchoscopy was sometimes problematic.

In line with our findings, positive BG levels have recently been reported in 31 of 32 AIDS patients (3) who had pneumocystosis. Strikingly, most of our pneumocystosis patients had very high BG levels (mean, $1,299 \pm 1,151$ pg/ml; median, 945 pg/ml) compared to patients with candidemia (BG-positive results: mean, 700 ± 779 pg/ml; median, 447 pg/ml). High levels of BG in the context of pneumocystosis have been reported previously (15, 31).

Among these patients, the four human immunodeficiency virus (HIV)-negative patients had positive serum BG levels. This group had a lower fungal burden that was more difficult to diagnose microscopically. Similar result was reported for nine cancer patients with pneumocystosis, all of whom tested as BG positive (12). Elevated BG levels ($>1,400$ pg/ml) were also found in three of the four HIV-negative patients, which indicates, as reported previously, that high BG levels also occur in HIV-negative patients (12, 30). This noninvasive test might prove particularly useful for monitoring the treatment of pneumocystosis patients. A recent study indicated that BG levels decreased during treatment with cotrimoxazole (12), which suggests that antipneumocystis drugs may alter BG levels. In keeping with this hypothesis, a lower BG level was found in our study in one HIV-negative patient who was under long-term cotrimoxazole prophylaxis.

In the present study, we obtained a sensitivity of 84.6% with the BG assay compared to sensitivities of 77.6% (18), 86.7% (23), and 100% (20) in other reported diagnoses of candidemia. Four of the six samples (66.6%) that were collected in our study before the day of the first positive hemoculture tested positive for BG. In one follow-up study, elevated BG levels were detected before the onset of clinical signs (20). Further studies are needed to explain this observation.

BG-positive results have been obtained for candidemia of different *Candida* species (*C. albicans*, *C. glabrata*, *C. tropicalis*, and *C. krusei*) (18). Similar results were also obtained with *C. guilliermondii* and *C. lusitanae*. BG was not detected in one patient infected with *C. parapsilosis*, which was probably related to the 8-day delay between the time of sampling and the positive hemoculture. However, low BG levels have already been reported in patients infected with this *Candida* species, for which the caspofungin MICs are generally elevated (18).

Some sera from patients who had candidemia or were at risk for candidiasis were routinely tested for the presence of M antigens, and this allowed a comparison between the M and BG assay results. The detection rate for candidemia was noticeably higher when the BG assay (20 patients, 76.9%) was used than when the M assay (11 patients, 42%) was used. Our present data show that the BG assay is of particular value for the diagnosis of candidemia.

False-positive results were observed with the M test for no definite reason. Among the 12 false M-positive sera, only 3 were BG positive, which indicates less cross-reaction. However, false-positive BG results have been described after hemodialysis on cellulose membrane (18), after contamination by environmental BG (7), after surgery (contamination by gases), after treatment with immunoglobulin or antitumoral polysaccharides such as lentinan and schizophyllan (29), or during some bacterial infections (23). We observed four positive BG results among six hemodialysis patients and seven other "false BG-positive" results in the group at risk for candidemia. This

phenomenon should be evaluated carefully in further studies; a serial monitoring would probably clarify these false-positive results.

Our findings highlight the value of the BG assay in the serodiagnosis of a wide range of opportunistic IFI such as aspergillosis, candidiasis, geotrichosis, and pneumocystosis. It was also reported to be useful for the diagnosis of histoplasmosis (23) and fusariosis (18). However, BGs are known to be an unreliable marker of zygomycosis and cryptococcosis (14, 18). In our study, the BG assay appears to be slightly superior to the GM assay for the diagnosis of IPA and markedly superior to the M assay for the diagnosis of fungal BSI. However, some patients with IFI showed negative BG results and positive GM and/or M results. The combined use of these assays would enhance diagnostic performance but would also increase its cost. Two critical issues need to be assessed with regard to the use of the BG assay in a diagnostic strategy for IFIs: (i) the incidence of false-positive BG results in patients with no IFI, which is a well-recognized issue in fungal antigen assays, and (ii) the benefit of monitoring BG levels (17, 19, 26, 31) in repeated samples from selected patients at risk for IFI, as advocated for the other fungal antigen assays.

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