MAJOR ARTICLE

1,3-β-D-Glucan Antigenemia for Early Diagnosis of Invasive Fungal Infections in Neutropenic Patients with Acute Leukemia

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(See the editorial commentary by Herbrecht and Berceanu on pages 886–9)

Background. Invasive fungal infections (IFIs) are life-threatening complications in neutropenic patients with hematological malignancies. Because early diagnosis of IFI is difficult, new noninvasive, culture-independent diagnostic tools are needed to improve clinical management. Recent studies have reported that detection of 1,3-β-D-glucan (BG) antigenemia may be useful for diagnosis of IFI. The aim of the present prospective study was to evaluate the usefulness of monitoring BG in patients undergoing chemotherapy for acute leukemia.

Methods. BG antigenemia was measured by a colorimetric assay twice weekly in the absence of fever and daily in the presence of fever. IFIs were classified according to the criteria of the European Organization for Research and Treatment of Cancer/Mycoses Study Group.

Results. During 190 consecutive neutropenic episodes (median duration, 22 days; range, 7–113 days) in 95 patients, 30 proven or probable IFIs (13 aspergillosis, 15 candidiasis, and 2 mixed IFIs) were diagnosed. Sensitivity, specificity, positive predictive value, negative predictive value, and efficiency of 2 consecutive BG values ⩾7 pg/mL for diagnosis of proven or probable IFI was 0.63 (95% confidence interval, 0.44–0.79), 0.96 (95% confidence interval, 0.89–0.98), 0.79 (95% confidence interval, 0.57–0.92), 0.91 (95% confidence interval, 0.84–0.95), and 0.89, respectively. The time interval between onset of fever as first sign of IFI and BG antigenemia was significantly shorter than the time to diagnosis of IFI by clinical, microbiological, radiological, and/or histopathological criteria ($P < .001$). BG values >50 pg/mL were observed in only 2 patients, both of whom experienced failure of antifungal therapy.

Conclusion. Monitoring of BG antigenemia is a useful noninvasive method for early diagnosis of IFI in patients with acute leukemia.

Diagnosis of invasive aspergillosis (IA) and invasive candidiasis (IC) in acute leukemic patients is challenging because of nonspecific clinical presentation, poor diagnostic yield of cultures, difficulty in obtaining samples of infected tissues, and nonspecificity and delay of radiological imaging (e.g., CT scan). Late diagnosis and antifungal therapy are associated with severe morbidity and high mortality [1, 2]. Prophylaxis may prevent IFI but implies the exposure of large numbers of patients to antifungals [3–5]. Empirical antifungal therapy is the standard of care for suspected IFI in neutropenic patients with persistent fever not responding to antibacterial therapy [6]. Experts have challenged the appropriateness of this strategy, because it is based on a nonspecific clinical sign that cannot differentiate IFI from other conditions [7, 8]. New culture-independent, rapid, and specific tools for early noninvasive diagnosis of IFI are needed to promptly identify patients who need antifungal therapy [9–11]. Studies of tests detecting *Aspergillus* antigens (galactomannan) or *Candida* antigens and/or antibodies (mannan and/or antimannan) have reported variable results in neutropenic patients [12–16].

1,3-β-D-Glucan (BG) is a fungal cell wall component circulating in the blood of patients with IA, IC, and

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Table 1. Patient demographic characteristics, clinical characteristics, and febrile episodes.

Characteristic	Finding
No. of patients	95
Sex, male/female	58/37
Age, years	57 (19–77)
No. of hematological malignancies, AML/ALL	80/15
Neutropenic episodes	
Total no. of episodes	190
Chemotherapy, induction-reinduction/consolidation	109/81
Median episodes per patient (range)	2 (1–4)
Duration of neutropenia, median days (range)	22 (7–113)
Febrile episodes	
Total no. of episodes	337[a]
Microbiologically documented infection with bacteremia	103 (31)
Microbiologically documented infection without bacteremia	22 (7)
Clinically documented infection	123 (36)
Unexplained fever	89 (26)
IFI	
Total no. of episodes	62[b]
Proven IFI	9 (15)
Aspergillosis, no. of episodes	5
Candidiasis, no. of episodes	4
Probable IFI	23 (37)
Aspergillosis, no. of episodes	10
Candidiasis, no. of episodes	13
Possible IFI	30 (48)

NOTE. Data are no. (%) of episodes, unless otherwise specified. ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; IFI, invasive fungal infection.

[a] Median of 2 febrile episodes per neutropenic episode (range, 0–4 febrile episodes).

[b] Includes 2 patients with 2 consecutive IFIs during the same neutropenic episode and 2 patients with 2 different IFIs during 2 consecutive neutropenic episodes.

other IFIs [17–19]. BG assays based on activation of factor G of the coagulation cascade of horseshoe crabs have been studied in hematological patients [19–23]. These observations have shown promising diagnostic performances in proven and/or probable IFI. False-positive results have been reported in patients exposed to products contaminated by BG or after environmental contaminations during analytical processing [24–28]. We prospectively assessed the usefulness of monitoring BG in leukemic patients by a colorimetric assay.

PATIENTS AND METHODS

Patients. Ninety-five of 100 consecutive adult patients with acute myeloid or lymphoblastic leukemia hospitalized for myeloablative chemotherapy in a single hospital during 2002–2006 gave written informed consent for inclusion in the study. The patients were studied throughout each hospital stay. Demographic characteristics, clinical characteristics, investigations, and antimicrobial therapy were prospectively recorded. The study was approved by the institutional ethics committee.

Clinical management. Patients were hospitalized in positive-pressure, high-efficiency particulate air–filtered isolation rooms. Semiquantitative cultures of mouth, stool, and urine specimens were obtained twice weekly for monitoring *Candida* colonization (i.e., detection of any growth) [29]. Patients with mucositis and documented oral and/or gastrointestinal tract colonization received fluconazole prophylaxis (400 mg/day) during neutropenia. The initial diagnostic work-up of febrile neutropenic patients included blood and urine cultures and chest radiography. Empirical antibacterial treatment was promptly initiated according to international guidelines [6]. Follow-up included daily clinical examination and microbiological and other clinically indicated investigations (e.g., CT scan and tissue biopsy). Modifications of antibacterial therapy and addition of antifungal therapy were based on clinical course (persistent fever >72 h and site of infection) and the results of diagnostic procedures [6].

Definitions. Neutropenia was defined as a neutrophil count <500 cells/mm^3 [30]. In an individual patient, a new neutropenic episode was defined as a period of neutropenia after a distinct cycle of chemotherapy. A febrile episode was defined

Table 2. Assessment of different 1,3-β-D-glucan (BG) cutoff values for either a single positive test result or 2 consecutive positive test results in patients with and without invasive fungal infections (IFIs).

BG cutoff value, pg/mL	Positive test results, no. (%) of samples (n = 3156)	No. (%) of patients					
		With ⩾1 positive BG measurement			With ⩾2 positive BG measurements		
		Proven or probable IFI (n = 30[a])	Proven or probable or possible IFI (n = 60[a])	No IFI (n = 113[a])	Proven or probable IFI (n = 30[a])	Proven or probable or possible IFI (n = 60[a])	No IFI (n = 113[a])
⩾3	977 (31)	30 (100)	58 (96)	81 (72)	29 (97)	52 (87)	55 (49)
⩾5	499 (16)	25 (83)	44 (73)	45 (40)	23 (77)	37 (62)	19 (17)
⩾7	335 (11)	20 (67)	34 (57)	28 (25)	19 (63)	22 (37)	5 (4)
⩾9	234 (7)	16 (53)	24 (40)	15 (13)	15 (50)	18 (30)	3 (3)
⩾11	177 (6)	15 (50)	19 (32)	12 (11)	12 (40)	15 (25)	1 (1)

[a] Includes 2 patients with 2 consecutive IFIs during the same neutropenic episode and 1 patient with *Pneumocystis jiroveci* pneumonia and proven invasive aspergillosis during the same neutropenic episode.

as 2 measurements of temperature ⩾38°C over 12 h or 1 measurement ⩾38.5°C [31, 32]. A period of ⩾72 h with temperature <38°C was required to distinguish a new febrile episode [31, 32]. The etiology of fever was classified as microbiologically documented infection with or without bacteremia, clinically documented infection, or unexplained fever, according to the definitions of the International Immunocompromised Host Society [30]. Diagnosis of proven, probable, or possible IFI was based on clinical, microbiological (including galactomannan assay), radiological, and histopathological criteria, according to European Organization for Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) definitions [33]. Two independent senior radiologists who were blind to clinical diagnosis reviewed all high-resolution CT scans: (1) major radiological signs were halo sign, air-crescent sign, or cavity within area of consolidation; (2) minor radiological signs were any new infiltrate not fulfilling major criteria or pleural effusion; and (3) probable hepatosplenic IC was defined by small, target-like abscesses of liver and/or spleen in intravenous contrast-enhanced CT [33]. Response to antifungal therapy was defined by defervescence, clinical resolution or improvement, and radiological stabilization or improvement.

Blood sampling. Blood samples were collected twice weekly as long as the patient remained afebrile. For each febrile episode, blood was sampled at fever onset and on days 1–4, 7, 10, and 14. Follow-up included twice-weekly blood sampling until occurrence of a subsequent febrile episode or hospital discharge. Blood samples were collected in sterile tubes containing lithium-heparin (Monovette) and were centrifuged (2000 *g* for 10 min at 4°C). Plasma specimens were frozen at −80°C.

BG assay. BG was measured by a colorimetric assay (Wako) [34]. Five-microliter plasma samples were pretreated (for 15 min at 75°C) with 45 μL of a solution containing 0.01% Triton X-100, 4 mM sodium periodate, and 2 mM NaOH in a 96-well microplate. Fifty microliters of the reagent solution containing the endotoxin-insensitive ameboecyte lysate of the horseshoe crab *Tachypleus tridentatus* and the substrate for the horseshoe crab's clotting enzyme—*t*-Boc-Thr-Gly-Arg-(N-ethyl-N-hydroxyethyl-phenylene-diamine)—were added and incubated (for 30 min at 37°C). One hundred microliters of coloring reagent (2.7% sodium dodecyl sulfate, 4.5 mM N,N-diethylaniline hydrochloride, and 91 mM orthoperiodic acid) were added; absorbance was measured at 730–650 nm. The plasma BG concentration was calculated by a linear calibration curve over the analytical range 1.2–120 pg/mL [34]. Intra-assay and interassay accuracy and coefficient of variation were 85%–115% and <7%, respectively [34]. BG was stable for >36 months in plasma stored at −80°C. BG values <3 pg/mL were measured in plasma of 72 (96%) of 75 healthy control subjects. No analytical interference with BG measurement was detected while testing commonly used antifungal and antibacterial agents [34]. Laboratory measurements were performed by technicians blind to diagnosis of IFI. BG results were not available for clinical management or classification of IFI.

Analysis of BG results. The primary analysis of the diagnostic performance of BG was performed per neutropenic episode in proven or probable IFI (true positive) versus no IFI (true negative). The performance was assessed by calculation of the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), likelihood ratio (LR) of positive [sensitivity/(1 − specificity)] and negative [(1 − sensitivity)/specificity] results, and efficiency [(true positives + true negatives)/all tests]. A receiver operating characteristic (ROC) curve was obtained by plotting the false-positive rate (1−specificity) against the true-positive rate (sensitivity) over the range of cutoff values 3–11 pg/mL. The performances of a single or 2 consecutive BG values above a given cutoff were compared. Secondary analyses of BG performance were done (1) in neu-

tropenic episodes with proven, probable, or possible IFI, according to the standard methodology used in recent studies of new diagnostic markers in hematological patients [12, 13, 21], (2) per febrile episode (on the basis of BG obtained within 72 h before fever onset), and (3) in patients with documented bacterial bloodstream infection without concomitant IFI.

The time elapsed between fever onset as first sign of IFI and positive BG, diagnosis of IFI by EORTC/MSG criteria, and minor or major radiological criteria of IFI were compared with the paired Friedman rank test (multigroup comparison) and the paired Wilcoxon signed-rank test (2-group comparison). Nonpaired continuous variables were compared by the Mann-Whitney rank sum test. The level of 2-sided statistical significance was .05. The BG kinetics according to the response to antifungal therapy were described in patients with a >10-day follow-up.

RESULTS

Clinical characteristics. One hundred ninety episodes of neutropenia (median duration, 22 days; range, 7–113 days) were studied in 95 adult patients undergoing myeloablative chemotherapy for acute leukemia. An IFI was diagnosed in 60 neutropenic episodes (9 proven, 21 probable, and 30 possible), no IFI occurred in 113, and 17 neutropenic episodes were excluded from analysis (14 follow-up cases of IFI diagnosed during a previous neutropenic episode and 3 with *Pneumocystis jiroveci* pneumonia, which is not considered to be an IFI in EORTC/MSG definitions). Patient demographic characteristics, clinical characteristics, febrile episodes, and classification of IFI are shown in table 1. Proven cases included 5 pulmonary IA (4 *A. fumigatus* and 1 *Aspergillus* species) and 4 IC (3 with hepatosplenic involvement and skin dissemination, including 2 with *C. tropicalis* and 1 with *C. humicola,* and 1 with *C. norvegensis* candidemia). Probable cases included 10 with pulmonary IA and 13 with hepatosplenic IC. Possible cases included 30 pulmonary infections. *Candida* colonization was present in 70% (21 of 30) of the neutropenic episodes involving proven or probable IFI, 67% (40 of 60) of those involving proven, probable, or possible IFI, and 60% (68 of 113) of those that did not involve IFI. Fluconazole prophylaxis was used in 20% (6 of 30), 17% (10 of 60), and 26% (29 of 113) of episodes, respectively.

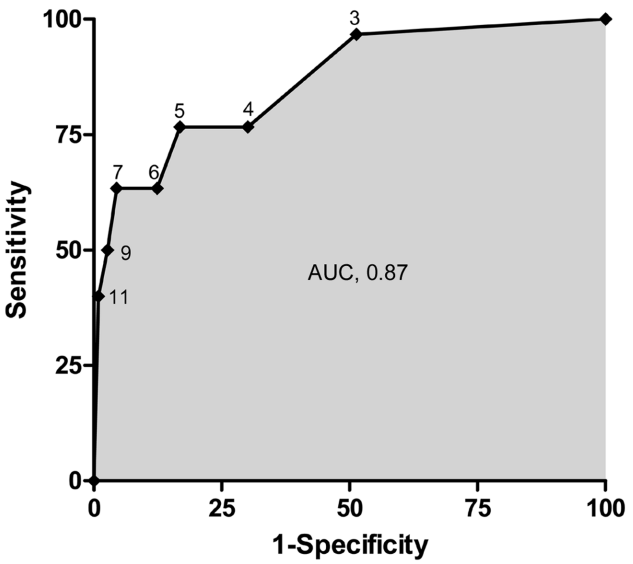

Figure 1. Receiver operating characteristic curve of 1,3-β-D-glucan (BG) antigenemia for diagnosis of invasive fungal infections with use of 2 consecutive positive BG values in proven or probable IFI. BG cutoff values are indicated at each point of the curve. The diagnostic performance is summarized in table 3. AUC, area under the curve.

Diagnostic performance of BG. BG was measured in 3156 serum samples (median samples per neutropenic episode, 16; range, 3–49). BG results in neutropenic episodes with proven or probable IFI, proven, probable, or possible IFI, and without IFI are shown in table 2. With use of a single BG value for the primary analysis per neutropenic episode in proven or probable IFI, the highest test efficiency (81%) was obtained with a cutoff value ⩾11 pg/mL; the sensitivity, specificity, PPV, and NPV were 0.50 (95% CI, 0.32–0.68), 0.89 (95% CI, 0.82–0.94), 0.56 (95% CI, 0.36–0.74), and 0.87 (95% CI, 0.79–0.92), respectively. The diagnostic performance of BG with use of 2 consecutive values above different BG cutoff values in proven or probable IFI is shown in table 3. The corresponding ROC curve is il-

Table 3. Diagnostic performance of 2 consecutive 1,3-β-D-glucan (BG) values above different cutoff values in proven and probable invasive fungal infections (IFIs).

BG cutoff value, pg/mL	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	Positive LR (95% CI)	Negative LR (95% CI)	Efficiency, %
⩾3	0.97 (0.81–1)	0.51 (0.42–0.61)	0.35 (0.25–0.46)	0.98 (0.9–1)	1.99 (1.62–2.43)	0.06 (0.01–0.45)	60.8
⩾5	0.77 (0.57–0.89)	0.83 (0.75–0.89)	0.55 (0.39–0.7)	0.93 (0.86–0.97)	4.56 (2.89–7.19)	0.28 (0.15–0.54)	81.8
⩾7	0.63 (0.44–0.79)	0.96 (0.89–0.98)	0.79 (0.57–0.92)	0.91 (0.84–0.95)	14.31 (5.82–35.17)	0.38 (0.24–0.61)	88.8
⩾9	0.50 (0.32–0.68)	0.97 (0.92–0.99)	0.83 (0.58–0.96)	0.88 (0.81–0.93)	18.83 (5.83–60.83)	0.51 (0.36–0.73)	87.4
⩾11	0.40 (0.23–0.59)	0.99 (0.94–1)	0.92 (0.62–0.1)	0.86 (0.79–0.91)	45.2 (6.12–333.94)	0.61 (0.45–0.81)	86.7

NOTE. See the receiver operating characteristic curve in figure 1. LR, likelihood ratio; NPV, negative predictive value; PPV, positive predictive value.

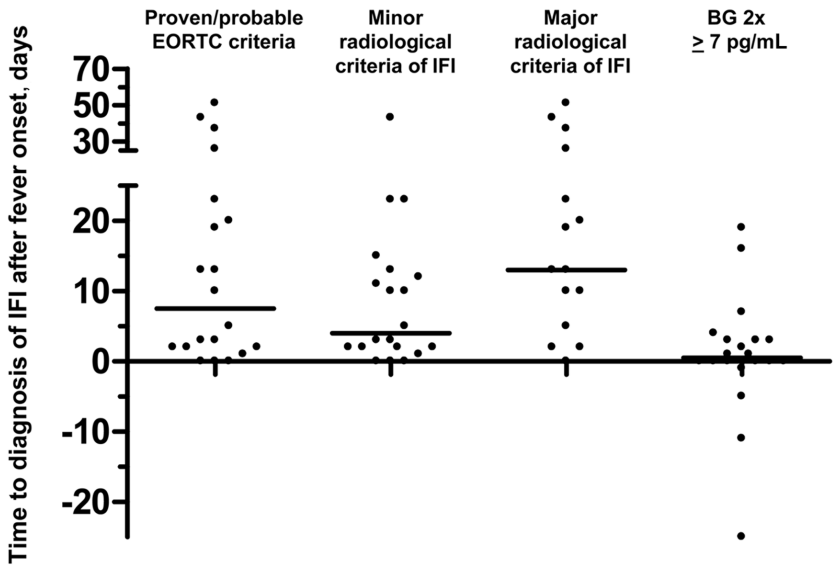

Figure 2. Time elapsed between onset of fever as first sign of invasive fungal infections (IFIs) and diagnosis of proven or probable IFIs, according to European Organization for Research and Treatment of Cancer and Mycoses Study Group (EORTC) criteria, documentation of radiological criteria of IFI, and 2 consecutive 1,3-β-D-glucan antigenemia measurements $\geq$7 pg/mL (2 $\times$ $\geq$7 pg/mL). Each time point represents a single IFI episode. *Horizontal bars,* median values.

lustrated in figure 1. The best diagnostic performance of BG was obtained with 2 consecutive samples with BG values $\geq$7 pg/mL; sensitivity, specificity, PPV, NPV, and efficiency were 0.63 (95% CI, 0.44–0.79), 0.96 (95% CI, 0.89–0.98), 0.79 (95% CI, 0.57–0.92), 0.91 (95% CI, 0.84–0.95), and 0.89, respectively. The performance of 2 BG values $\geq$7 pg/mL was similar for IA and IC (sensitivity, specificity, PPV, and NPV were 0.60, 0.96, 0.64, and 0.95, respectively, for IA and 0.59, 0.96, 0.67, and 0.94, respectively, for IC). Negative results (absence of $\geq$2 BG measurements $\geq$7 pg/mL) were observed in 11 (37%) of 30 cases of proven or probable IFI, as follows: 2 cases of proven IC (1 case of *C. humicola* disseminated IC and 1 case of *C. norvegensis* candidemia), 5 cases of probable IC (hepatosplenic lesions), 2 cases of proven pulmonary IA (histopathologically proven without signs of vascular invasion), and 2 probable cases of pulmonary IA (in patients with major radiological signs). Of 113 neutropenic episodes in patients without IFI, 28 (25%) had a single sample with BG $\geq$7 pg/mL, and only 5 (4%) had $\geq$2 samples with BG $\geq$7 pg/mL (all patients were colonized by *Candida* species, 2 with oral mucositis, and 3 with enterocolitis; all patients received empirical antifungal therapy for persistent fever).

In the secondary analysis per neutropenic episodes with proven, probable, or possible IFI, the sensitivity, specificity, PPV, and NPV of 2 consecutive BG values $\geq$7 pg/mL were 0.37 (95% CI, 0.25–0.50), 0.96 (95% CI, 0.89–0.98), 0.81 (95% CI, 0.61–0.93), and 0.74 (95% CI, 0.84–0.95), respectively. In the secondary analysis per febrile episodes in patients with proven or probable IFI, the sensitivity, specificity, PPV, and NPV of 2 consecutive BG values $\geq$7 pg/mL were 0.60 (95% CI, 0.29–0.65), 0.97 (95% CI, 0.93–0.99), 0.74 (95% CI, 0.49–0.90), and 0.91 (95% CI, 0.85–0.95), respectively. Of note, only 2 (2%) of 93 febrile episodes with documented bacteremia without concomitant IFI had 2 BG measurements $\geq$7 pg/mL.

Time to diagnosis of IFI. In patients with proven or probable IFI and 2 BG measurements $\geq$7 pg/mL, the median time elapsed between fever onset as first sign of IFI and first BG level $\geq$7 pg/mL was 0.5 days (range, $-$25 to 19 days) (figure 2). The BG assay produced positive results sooner than any

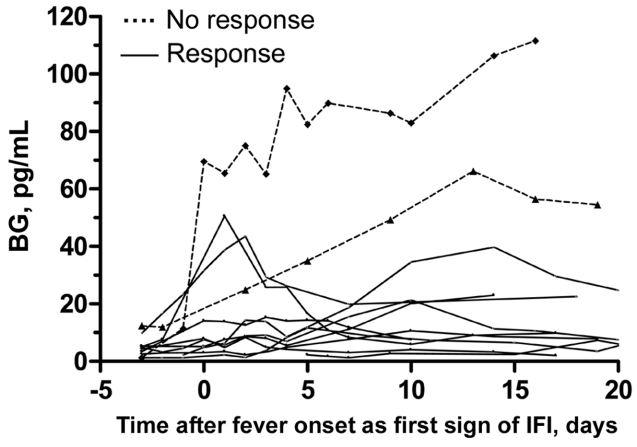

Figure 3. Kinetics of 1,3-β-D-glucan (BG) antigenemia in patients with 2 BG measurements $\geq$7 pg/mL and >10-day follow-up who responded or did not respond to antifungal therapy.

other conventional diagnostic method ($P < .001$, by multigroup comparison). BG positivity preceded positive culture and/or histopathology results in 4 (80%) of 5 cases with positive BG and positive microbiology or histopathology results (median time between fever onset and positive culture or histopathology results, 1 day; range, 0–31 days; $P = .12$). It preceded minor radiological CT signs in 14 (70%) of 20 cases with positive BG assay results and minor radiological criteria of IFI (median time between fever onset and positive radiological findings, 4 days; range, 0–43 days; $P = .005$). It preceded major radiological signs in 15 (100%) of 15 cases with positive BG assay results and major radiological criteria of IFI (median time between fever onset and positive radiological findings, 13 days; range, 0–51 days; $P < .001$). It preceded diagnosis by EORTC/MSG criteria in 18 (90%) of 20 cases with positive BG assay results and EORTC/MSG criteria of proven or probable IFI (median time between fever onset and EORTC/MSG criteria, 7.5 days; range, 0–51 days; $P < .001$). Empirical antifungal therapy was initiated at a median time between fever onset and positive radiological findings of 1.5 days (range, 0–10 days) after fever onset ($P =$ not significant [NS], vs. BG assay result). When IA and IC were analyzed separately, the median time from fever onset to first positive BG assay result was 0 days (range, −25 to 19 days) and 2 days (range, −1 to 16 days), respectively; to minor radiological signs was 2.5 days (range, 0–43 days; $P =$ NS, vs. BG assay result) and 10.5 days (range, 0–23 days; $P = .03$, vs. BG assay result), respectively; to major radiological signs was 9 days (range, 0–43 days; $P = .03$, vs. BG assay result) and 19 days (range, 2–51 days; $P = .004$, vs. BG assay result), respectively; and to diagnosis by EORTC/MSG criteria was 3 days (range, 0–43 days; $P = .004$, vs. BG assay result) and 16 days (range, 0–51 days; $P = .004$, vs. BG assay result) days, respectively.

Kinetics of BG antigenemia. In the 19 proven or probable IFI cases with 2 BG measurements ⩾7 pg/mL, the BG peak value (median result value, 22 pg/mL; range, 8–111.5 pg/mL) was reached a median of 10 days (range, −25 to 46 days) after fever onset as first sign of IFI. In patients with IA, the BG peak value (median peak value, 18 pg/mL; range, 8–51 pg/mL) was measured a median of 3 days (range, −25 to 25 days) after fever onset. In patients with IC, the BG peak value (median peak value, 24 pg/mL; range, 10–111 pg/mL; $P =$ NS, vs. the IA group) was measured a median of 12 days (range, 1–46 days; $P =$ NS, vs. the IA group) after fever onset. Of note, recovery from neutropenia occurred within a median of 11 days (range, −6 to 54 days) after fever onset. Among 12 patients with a follow-up of BG measurements >10 days after initiation of antifungal therapy, BG antigenemia became negative or remained <50 pg/mL in 10 patients who responded to treatment and increased and remained >50 pg/mL in 2 patients who did not respond to therapy (figure 3).

DISCUSSION

Investigations using different BG assays have reported sensitivity of 55%–100%, specificity of 87%–93%, PPV of 40%–84%, and NPV of 75%–100% [19–22]. Differences in study design (case-control vs. population studies, number of cases with proven or probable IFI [8–20 cases in population studies], proportions of IA/IC and other IFIs, case-mix of neutropenic and nonneutropenic patients, and antifungal prophylaxis) highlight the need for further investigations.

We investigated the usefulness of a systematic monitoring of BG antigenemia in neutropenic adult patients with acute leukemia who did not routinely receive antifungal prophylaxis. During 190 prolonged neutropenic episodes, 32 proven or probable and 30 possible IFIs occurred. The best diagnostic performance in proven or probable IFI was obtained with 2 consecutive positive BG assay results (ROC area under the curve, 0.87); a cutoff of 2 BG measurements ⩾7 pg/mL provided the best efficiency (89%). Per neutropenic episode and per febrile episode analyses showed similar results. The specificity of the BG assay in documented bacteremia was excellent (96%). This result contrasts with the high rates of false-positive BG assay results (14 [56%] of 25) reported elsewhere among bacteremic patients [23].

Of interest, all of the false-positive BG assay results (4%) were observed in patients with gastrointestinal fungal colonization and/or mucositis who received empirical antifungal therapy for persistent fever, suggesting an occult IFI at an early stage. A case report has described false-positive BG assay results in 6 patients receiving treatment with amoxicillin–clavulanic acid [27]. A recent in vitro investigation suggests that the amounts of BG detected in in vivo achievable concentrations of contaminated antibiotics are negligible [28]. The low rate of false-positive BG assay results in our study does not allow us to draw any conclusion about the possible role of contamination with BG of antibacterials given at time of blood sampling. False-negative results were observed in 11 patients with proven or probable IFIs. Three of these patients were receiving antifungal prophylaxis, and 3 were receiving empirical antifungal therapy. Prompt initiation of antifungal therapy may result in false-negative results of circulating fungal markers, as reported elsewhere for galactomannan [35]. The test reactivity reflects the species-specific BG content of the cell wall in different fungi, which might explain false-negative results—for example, in *Candida* species other than *Candida albicans* [36].

In the secondary analysis including proven, probable, or possible IFI, the efficiency of 2 consecutive BG values ⩾7 pg/mL was 75%. Major issues about the accuracy of the EORTC/MSG classification of possible IFI as a diagnostic gold standard have been raised [33, 37]. The revision of EORTC/MSG criteria may provide an improved reference standard for the evaluation of new diagnostic tests [37].

The study of the BG kinetics in patients with proven or probable IFI showed that BG levels were ⩾7 pg/mL very early (in the time window during which empirical antifungal therapy is recommended) and preceded other diagnostic criteria, as reported elsewhere [20, 21]. Because radiology cannot be performed as frequently as blood sampling can, it is possible that the value of radiology for the early diagnosis of IFI is underestimated. In patients with proven or probable IFI and >10-day BG follow-up after initiation of antifungal therapy, BG levels were <50 pg/mL in those responding to antifungal therapy, whereas they remained >50 pg/mL in those not responding. However, the limited data set does not allow any firm assessment of the role of BG in the follow-up of IFI.

The small number of proven or probable IFIs is a common limitation of population studies of consecutive patients with similar risk profiles (i.e., prolonged neutropenia). However, only this experimental design provides an estimation of the prevalence of IFI in the target population and allows an assessment of PPV and NPV by use of a diagnostic test. Moreover, major methodological issues have been recently raised about case-control studies for the assessment of new fungal markers [38]. The close monitoring of BG levels and the balanced proportions of IA and IC episodes occurring in the absence of routine antifungal prophylaxis are strengths of our study, which is aimed at evaluating a test that detects both types of IFI. However, the lack of data on the performance of the BG assay in candidemia without dissemination, in IFI due to fungi other than *Aspergillus* and *Candida* species and in allogeneic hematopoietic stem cell transplant recipients are limitations. Moreover, this observational study was not designed to assess the cost-effectiveness of the high number of BG tests required for systematic monitoring.

In summary, monitoring BG antigenemia in neutropenic patients with acute leukemia is useful for the diagnosis of IFI. Early positivity and excellent specificity suggest that detection of BG antigenemia may support the decision to promptly start empirical antifungal therapy in persistently febrile neutropenic patients. However, the high proportion of single false-positive results highlights the need to immediately confirm a positive result by testing a second sample. Because the sensitivity of the BG assay is limited, a test result negative for antigenemia needs to be combined with the usual diagnostic tools (cultures, imaging, and histopathology) in the consideration of whether to postpone empirical antifungal therapy. A noncomparative pilot study has shown that a preemptive strategy combining measurement of circulating galactomannan, imaging, and microbiological examination may improve the efficiency of management by reducing the empirical use of antifungal agents [39]. The combination of BG assessment with species-specific tests might further improve the diagnostic performance [20, 40]. The inclusion of BG assessment in the revised EORTC/MSG definitions is being considered [37]. Studies are needed to confirm BG interpretation criteria (e.g., 2 BG measurements ⩾7 pg/mL), performance in different clinical settings, and usefulness for follow-up. The assessment of the efficiency of combining BG monitoring with traditional diagnostic tools in preemptive management strategies needs further investigation.

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