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Summary *Objectives:* The ß-D-glucan assay (BDG) has been added to the EORTC/MSG criteria for the diagnosis of invasive fungal infections (IFI), but data from pediatric populations is scarce. The aim of this study was to evaluate performance of BDG in a cohort of hemato-oncological children with hematological malignancy at risk for IFI.
Methods: 113 patients were included through an 18-month period. In addition to routine IFI screening, BDG was assayed once a week. IFIs were classified using EORTC/MSG criteria

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without including the BDG results. Performances were assessed after a ROC analysis for optimization and multivariate analysis to detect the causes of false positivity.
Results: 8 proven and 4 probable IFIs, and 7 possible IFIs were diagnosed in 9 and 7 patients, respectively. Sensitivity and specificity increased from 75% and 56% to 100% and 91.1%, respectively when considering the whole population and patients not having received any antifungals prior to the test. Multivariate analysis revealed that being younger than 7, severe colitis/mucositis, recent administration of polyvalent immunoglobulins and digestive colonization with *Enterococcus* sp were independent risk factors for false positivity.
Conclusions: BDG is a valuable test to detect IFI in pediatric patients not previously treated with antifungals and to detect the occurrence of chronic infection.

Introduction

Patients suffering from hematological malignancy are considered a population at high risk of developing invasive fungal infections (IFI). Nevertheless, epidemiological data in the particular setting of pediatrics is still limited. In a recent study including 244 high-risk children (acute leukemia, stem cell transplant), 55 patients (22.5%) developed a probable or proven IFI, consisting predominantly of invasive candidiasis (IC) (42%), followed by proven and probable invasive mold infections (33%).[1] It is well known that these infections are typically associated with high mortality rates, 38%, in the above study. This poor prognosis is partly due to the weak performance of the currently available diagnostic instruments, highlighting the requirement for developing fungal biomarker tests.[2] Indeed, conventional diagnostic methods are both poorly sensitive and require a period of incubation, leading to a delay in initiating an adequate antifungal therapy. Currently, specific biomarker commercial kits are available to diagnose invasive candidiasis, cryptococcosis and aspergillosis and the European Conference on Infections in Leukaemia ECIL has recently proposed recommendations for their use.[3]

In addition to these specific tests, the β D-glucan (BDG) assay is becoming widely used for diagnosing IFI in various clinical contexts, including hematology,[4,5] solid organ transplantation[6] and intensive care unit.[7] BDG is a cell wall polysaccharide produced by a wide range of fungi and can be detected in the patient's serum during the infectious process of IFI. Preliminary data led the EORTC—MSG to include this test as part of the biological criteria used for the definition of IFI.[8] Recently, two studies focusing on hematology—oncology adult patients reported a high sensitivity of 92—98% and a high specificity of 90—96%[9,10] and the ECIL group confirmed that this test is be useful in diagnosing IFI in adult patients with leukemia.[11] On the contrary, data on the use of this test in pediatric populations remains very limited and led the fourth ECIL to conclude that no specific recommendation could be proposed for BDG in the diagnosis management of IFI in children.[12]

The objective of this study was to evaluate the performances of BDG for the diagnosis of IFI in a hematology—oncology cohort of pediatric patients. Furthermore, BDG kinetics during the course of IFIs, causes of false positive results, and the impact of the use of different cut-offs were analyzed.

Patients and methods

Patients

Patients of 18-year old or less, admitted in the hematology—oncology pediatric ward of our institution between 01/01/2013 to 30/06/2014 (18 months), with either Acute Lymphoblastic Leukemia (ALL), Acute Myeloblastic Leukemia (AML), Burkitt leukemia, aplastic anemia, or admitted for an Autologous Hematopoietic Stem Cell Transplantation (AHSCT), were included. For each patient, demographic data (age, sex) and the type of hematological disease and other underlying conditions, were collected. Antifungal treatments received were also recorded, as well as the use of total parenteral and enteral nutrition, the administration of polyvalent immunoglobulins, and the occurrence and severity of colitis and/or mucositis, and of bacteremia and viral infections.

Antimicrobial management

The patients were handled according to the standard procedures of the ward. Systemic antifungal prophylaxis with 1 mg/kg/day micafungin was restricted to high-risk patients with expected prolonged neutropenia (>10 days) and the demonstration of *Candida* colonization in at least two stool samples. When fever occurred during the neutropenic phase, empiric antimicrobial therapy consisted in a combination of tazocillin (300 mg/kg/day of piperacillin, maximum 12 g/day) plus amikacin (15 mg/kg/day, maximum 1 g/injection). If fever persisted after 48 h, vancomycin (45 mg/kg/day, maximum 2 g/day) was added. According to the patient's condition, an empirical antifungal treatment was added between the 48th and the 96th hours of fever, or in the case of a new febrile episode after initial apyrexia, consisting in liposomal amphotericin B (3 mg/kg/day), or caspofungin (70 mg/m2 day 1, then 50 mg/m2).

Diagnosis of IFIs

Routine procedures were applied for the diagnosis of IFIs. Multisite sampling for bacterial and fungal cultures was performed once a week during the neutropenic phase. All patients were routinely screened twice a week for *Aspergillus* galactomannan (GM) (Platelia Aspergillus, BioRad, Marnes la

Coquette, France). A single positive GM test result (index ≥ 0.5) that was not associated with clinical symptoms and that did not lead to additional diagnostic procedures or change in antifungal medication, was considered as false positive. *Candida* Mannan antigenemia (Platelia *Candida* Antigen Plus, Biorad, Marnes la Coquette, France) were performed on demand according to the manufacturer' recommendations.

Specimens from samples targeted by symptoms were subject to direct examination to detect fungal elements and cultures on fungal media. Diagnosis of *Pneumocystis* pneumonia relied on the results of a home-made qPCR test.[13]

During the neutropenic phase, a BDG seric assay was routinely performed once a week, according to the manufacturer's recommendations (Fungitell, Capecode, East Falmouth, USA). Determination was performed on a thermostat-controlled spectrophotometer (ELX 808, Biotek Instruments SAS, Colmar). All the sera were tested in duplicate, and the mean was assigned as the final result for the specimen. According to manufacturer's criteria, result was interpreted as negative for values lower than 60 pg/ml, as positive for values higher than 80 pg/ml, and as equivocal for values between 60 and 80 pg/ml. Values lower than 8 pg/ml and higher than 523 pg/ml were considered as being 8 and 523 pg/ml.

Performance evaluation and statistical analysis

To assess the performance of the BDG test, fungal infections were classified as proven, probable, and possible, according to the 2008 EORTC-MSG criteria without including the results of the BDG assays.[8] In addition, patients presenting small target abscesses in the liver and/or the spleen, which were detected using either ultrasonography or CT-scan, were considered as having possible or probable chronic disseminated infection if they had developed fungemia within the previous month or if they had positive mannan antigenemia assay, accordingly. Day 0 of the IFI diagnosis was defined either as the date of the first positive blood culture or as the date of the first positive GM seric assay or as the date when organ lesions were confirmed by imaging.

Sensibility, specificity, predictive positive value and predictive negative value were calculated using the cut-off levels recommended by the manufacturer in a first instance, i.e. negative assay for concentrations $<$60 pg/ml, positive when $>$80 pg/ml. Performance was also assessed using concentration rate highlighted by a ROC curve analysis. Finally, we compared the performance of the test when considering the first positive result of BDG and using two successive positive results.

Statistical analysis

Statistical analysis was performed using the Stata/IC 13.0 for Mac software (StataCorp, College Station, TX 77845, USA). For the descriptive analysis, the percentage rate was used to describe the characteristics of the population studied. A Chi-square test was used to test the associations between the variables. We also analyzed the causes of false positive results among the sera collected from patients considered free of IFI. In the univariate analysis, the dependent variable was the occurrence of a false positive result while the independent variables were gender, age class (0–6, 7–12, 13–18 years old), polyvalent immunoglobulin infusion in the previous 96 h, enteral nutrition, total parenteral nutrition, digestive colonization with *Candida* or *Enterococcus*, bacteremia, viral infection, severe digestive mucosal inflammation (colitis or mucositis grade III and IV). All variables associated with false positive BDG result with a p value < 0.25 in the univariate model were included in a binary logistic regression analysis. For each statistically significant factor, an odds ratio (OR) and 95% confidence interval (CI) were computed. The level of statistical significance was set as $p < 0.05$.

Ethical considerations

All samples and data were collected as part of routine diagnostic procedures. The study was retrospective and the results of the analysis did not influence the management of the patients. The database was declared to the French Data Protection Authority (Commission Nationale Informatique et Liberté) (no.1949795 v 0).

Results

Description of the pediatric population and IFIs diagnosed

Between January 2013 and June 2014, 126 children were enrolled in the study. Thirteen had a single BDG assay during their follow-up and were excluded from further analysis (Fig. 1). One hundred and thirteen children were retained corresponding to 785 sera collected, with an average of 7

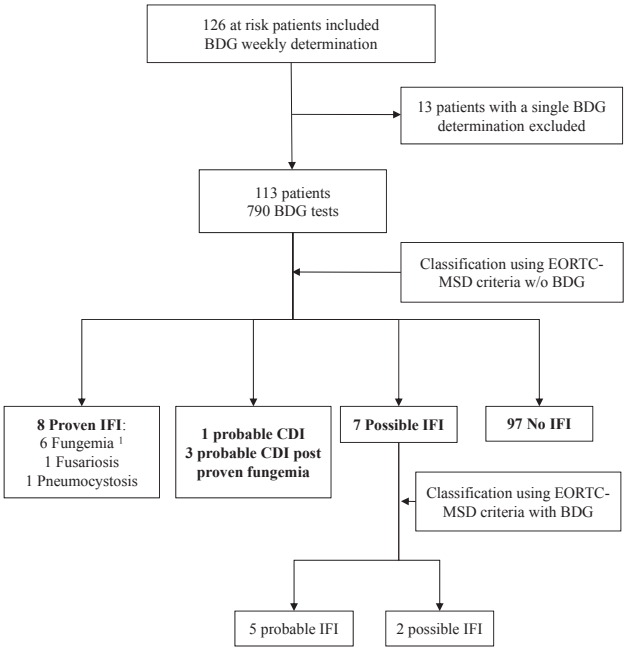

Figure 1 Flow chart of patient's inclusion and classification of IFI according EORTC-MSG criteria. BDG: β D-glucan, CDI: Chronic Disseminated Infection, IFI: Invasive Fungal Infection. [1]Among the 6 cases of fungemia, 3 evolved towards of probable chronic disseminated infection.

assays per patient (median: 10, range 2–22). Demographics charts and underlying diseases are summarized in Table 1. Eight episodes of proven and 4 of probable IFI were diagnosed in 9 patients. Six patients presented fungemia due to *Candida parapsilosis* (n = 2), *Candida kefyr* (n = 1), *Candida lusitaniae* (n = 1), *Candida tropicalis* (n = 1), and *Trichosporon asahii* (n = 1) (Table S1, Supplementary data). Three of the patients, including the one with *Trichosporon* fungemia, developed probable chronic disseminated infection (CDI), which were considered independent episodes of infection. The other two presented *Fusarium verticillioides* invasive infection and pneumocystosis. There was also a case of probable chronic disseminated candidiasis. The overall incidence of probable and proven IFI was thus calculated at 10.6% in our cohort. In addition, 7 patients were classified as possible IFI (Table S1). Ninety-seven patients (617 sera) were considered free of IFI.

Performance of the β D-glucan *assay*

We first used the positivity criteria proposed by the manufacturer to evaluate the performance of the test. BDG glucan was found positive at the time of diagnosis in 6 cases among the 12 episodes of proven and probable infection (Fig. 2). Among the 4 with probable CDI, 2 patients had highly positive BDG test, while the other 2 (including the patient infected with *Trichosporon*) remained negative.

Among the 7 patients with possible IFI, 5 had positive BDG and consequently turned into probable Invasive Aspergillosis (IA) or probable CDI using the EORTC-MSG criteria, including the BDG test in the definition. Four of them had several consecutive positive tests (≥3) supporting the diagnostic value of the test. On the other hand, 43 patients, considered free of infection, had at least one positive assay during their follow-up.

Taking into account these results and the cut-off levels recommended by the manufacturer (considering equivocal results as positive), the sensitivity for the detection of proven and probable episodes of IFI was calculated at 75%

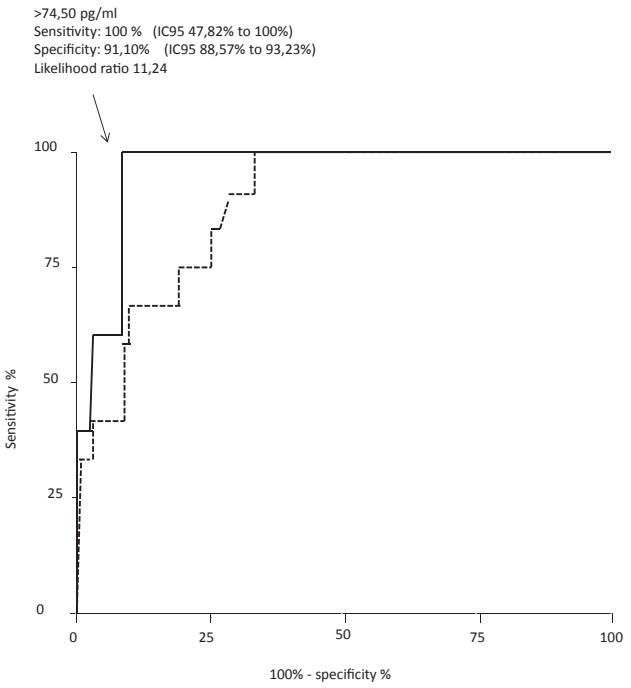

Figure 2 ROC curves analysis of BDG for the diagnosis of IFI. Analysis comparing values of sera collected from patients free of IFI and (i) in dotted line, sera from patients with probable and proven IFI; AUC 0.8869 95% confidence interval (0.8209–0.9529) p-value = 0.0004 (ii) in full line, sera from patients with probable and proven IFI having not been treated with antifungal therapy for more than 5 days prior to the BDG assay; AUC 0.9584 95% confidence interval (0.9222–0.9947) p-value = 0.0001. IFI: Invasive Fungal Infection, AUC: Area Under Curve, BDG: β D-glucan.

IC95 (69%–0.81%) with a specificity of 56% IC95 (50%–62%) (Table 2). When considering two consecutive positive tests, the specificity increased to above 90% but sensitivity decreased to below 50%.

Table 1 Demographics and underlying diseases of 113 patients tested for BDG.

	All patients	Proven + Probable IFI	Possible IFI	No IFI	P value
Patients (serum)	113 (785)	9 (93)	7 (75)	97 (617)	
Median age (years or weeks) (range)	7.6 y (6 w–17 y)	8.66 y (1.51–12.57 y)	8.53 y (3.88–16.09 y)	6.92 y (6 w–17 y)	NS
Gender, Male/Female	64/49	5/4	4/3	55/42	NS
Underlying diseases					NS
AML	25	4	1	20	
ALL	84	5	5	74	
Aplastic anemia	4	0	1	3	
Primo diagnosis/ Relapse + refractory	96/17	9/0	3/4	83/14	NS
Chemotherapy course	162				NS
LAL induction/consolidation/ intensification/salvage	67/35/7/11	4/5/0/0	2/2/0/3	61/28/7/8	
LAM induction/consolidation/ intensification/salvage	18/14/0/6	4/4/0/0	0/1/0/1	14/9/0/5	
Horse ATG	4	0	1	3	NS

AML: Acute myeloid leukemia, ALL: Acute lymphoid leukemia, ATG: Anti-Thymocyte Globulin, Y: years, W: weeks.
Some patients received more than one chemotherapeutic course during the 18 months period.

Table 2 Accuracy of BDG assay for the diagnosis of proven/probable IFIs in 106 children suffering hematological disease (corresponding to 109 episodes) considering a single positive result (1×) or 2 consecutive values (2×).

Cut off serum BDG	Sensitivity (%)	Specificity (%)	PPV	NPV	Accuracy (%)
BDG ≥ 60 pg/ml 1×	75 (69–81)	56 (50–62)	0.17 (0.11–0.23)	0.95 (0.89–1.01)	84
BDG ≥ 75 pg/ml 1×	75 (69–81)	70 (64–76)	0.24 (0.18–0.3)	0.96 (0.9–1.02)	85
BDG ≥ 80 pg/ml 1×	42 (36–48)	71 (65–77)	0.15 (0.09–0.21)	0.91 (0.85–0.97)	88
BDG ≥ 60 pg/ml 2×	50 (44–56)	90 (84–96)	0.38 (0.32–0.44)	0.94 (0.88–1)	92
BDG ≥ 75 pg/ml 2×	50 (44–56)	91 (85–97)	0.4 (0.34–0.46)	0.94 (0.88–1)	93
BDG ≥ 80 pg/ml 2×	42 (36–48)	93 (87–99)	0.42 (0.36–0.48)	0.93 (0.87–0.99)	92

NPV: Negative Predictive Value, PPV: Positive Predictive Value, numbers in parentheses represent 95% confidence intervals.

We then hypothesized that previous antifungal therapy may negatively impact the assay. Indeed, among the 12 episodes of proven and probable infection, 5 of them that had been treated previously for more than 6 days with antifungal drugs, presented a negative BDG result at the time of the IFI diagnosis (Table S1, Supplementary data). We thus compared ROC-curves obtained when using results of all sera sampled from patients free of IFI, and the first sample collected from patients with proven and probable IFI, either independently from any previous antifungal therapy, or limited to patients not having been treated for more than 6 days with antifungals. The area under the curve was at 0.8869 (p-value < 10-4) and 0.9584 (p-value < 0.0004), respectively, a difference at the limit of significance ($p = 0.06$, Hanley method) (Fig. 2). In the second case, a seric concentration of 75 pg/ml offered a sensitivity at 100% (IC95 47.82%–100.0%) and a specificity at 91.1% (IC95 88.57%–93.2%). The use of this cut-off level on a single sample gave the best performance of the test at the patient's level. In particular, the negative predictive value reached 96% and the accuracy 93%.

Potential factors leading to false positive results in the β D-glucan assay

Because specificity appeared limited, we tried to decipher the causes of false positive results by reviewing the data of the 613 BDG assays obtained from the 97 patients considered free of IFI. Using the cut-off value of 75 pg/ml (see above), we found, from the univariate analysis, that belonging to the youngest class of age, administration of polyvalent immunoglobulins within the 96 h preceding the BDG test, concomitant severe mucositis/colitis, ongoing enteral nutrition and *Enterococcus* colonization significantly increased the rate of false positive results (Table 3). In the multivariate analysis, belonging to the youngest class of age, digestive colonization with *Enterococcus*, severe colitis/mucositis and administration of polyvalent immunoglobulins were independent risk factors for false positive BDG results.

Timeline and kinetics of β -D-glucan in children with IFI

Fig. 3 summarizes the time interval between day 0 of diagnosis of proven and probable IFI and the positivity of the BDG assays. Excluding the episodes of CDI complicating the course of fungemia, among the 7 episodes assessable, only one had a positive test before the diagnosis of IFI (episode 13). It should be further noted that this patient could not benefit from precocious imagery, and the precocity of positive BDG was somewhat relative.

Looking at the 5 patients with a positive test at the time of diagnosis and several tests performed during the follow-up, one can note that in 2 cases of probable non pre-treated chronic disseminated infections (cases 2.2 and 4.2) the BDG assay persisted to be positive, at a high level for weeks, while no clinical improvement occurred. On the contrary, BDG tests rapidly turned negative in 3 cases that had a favorable outcome under treatment (patients 7, 11, 12).

Discussion

The incidence of IFI has significantly increased over the last decades.[14] In addition, there is now a wider spectrum of etiologic agents causing these infections.[15] Indeed, some previously rare yeast species or filamentous fungi such as *Fusarium*, *Scedosporium* and *Mucormycetes* are now commonly reported as responsible for IFI, mainly in hematological wards.[16] This is illustrated in our analysis whereby the incidence of proven and probable IFI was of 10.6% but with no less than 7 different species recovered as causative agents.

It is largely admitted that the precocity of antifungal therapy is a key prognosis factor for these infections. Thus, a reliable and anticipated diagnosis is crucial. However, most often the clinical signs of IFI are nonspecific and the culture-based diagnostic tools have a limited sensitivity, which is even lower in hematologic patients who frequently receive prophylactic or empiric therapy. With the exception of those targeting cryptococcosis, the biomarkers available today for the diagnosis of IFI also exhibit limited performance. Notably, *Aspergillus* GM is subject to false positive and false negative results.[12,17] Indeed, while still debated, the incidence of false-positive results could be more frequent in children as compared to adults.[18,19] Thus the availability of any new biomarker assay, such as BDG, is considered as a potential breakthrough in the field of diagnosis.

The wide spectrum of targeted fungal pathogens with BDG is attractive regarding the increasing diversity of fungal pathogens. However, it should be remembered that mucormycetes and *Cryptococcus neoformans* do not produce enough BDG to be detected. It has also been suggested that some *Candida* species are less prone to

Table 3 Potential predictors of false positive BDG (>75 pg/ml) results in univariate and multivariate analysis.

	No. of sera	Incidence of false-positive BGD assay	p		Odds ratio (95% CI)
			Univariate analysis	Multivariate analysis	
Sex			NS		
Female	276	9.51			
Male	288	7.69			
Age			0.011	0.023	2.25 (1.11—4.53)
<7 years	302	11.59			
>7 years	315	6.03			
TPN			NS		
Yes	116	10.34			
No	501	8.18			
Enteral nutrition			0.05	NS	
Yes	99	14.14			
No	463	7.78			
Severe mucositis/colitis			0.011	0.024	2.85 (1.15—7.07)
	41	19.51			
	515	7.57			
Ig perfusion			<0.0001	0.000	80.68 (8.89—732.54)
Yes	7	85.71			
No	610	7.70			
Candida colonisation			NS		
Yes	268	8.21			
No	277	8.66			
Enterococcus colonisation			0.005	0.006	3.20 (1.40—7.30)
Yes	480	18.87			
No	53	7.08			
Bacteriemia			NS		
Yes	18	16.67			
No	84	16.67			

produce BDG (Bougnoux ME personal communication). A low sensitivity for the BDG test, calculated at 50%, has already been mentioned for children suspected of IA.[20] However, our study points out that the limited sensitivity of the test may be due mainly to previous antifungal therapy. Indeed, after defining an appropriate cut-off level using a ROC analysis, we show that the sensitivity increases from 75% to 100% when we limit the study to patients who have been treated only for a limited period before the first BDG test. A number of patients treated with prolonged antifungal therapy prior to the BDG test received echinocandin antifungal drugs, which act through inhibition of BDG synthesis. This phenomenon has already been mentioned for both the detection of *Aspergillus* galactomannan antigen and the BDG test.[21,22] In adult hematological populations, controversial results have been published, some reporting that pretreated patients have a greater chance of false negative results,[21,23] while other not.[24]

From these data, a recommendation could be to perform the BDG test before the initiation of any antifungal therapy. If a control is needed, it is best that it is performed before the 6th day of antifungal treatment.

In our study, 5 patients among 7 with a possible diagnosis of infection, turn into probable infection when integrating the BDG result in the definition criteria. This occurred in 2 patients with possible IA but with negative galactomannan antigen and 3 patients with possible CDI. While the positivity of the BDG test does not enable an etiologic diagnosis, this is an important result to comfort the fungal etiology and thus begin an antifungal rather than an antibacterial treatment.

In addition to its potential value in detecting IFI, the test appeared valuable in predicting the clinical outcome. Indeed, patients with an affected organ, notably in the case of CDI, had their BDG assays remaining high for weeks; whereas typically, patients with a single episode of candidemia rapidly cleared their seric BDG. Similar findings have been previously reported in adult patients.[25—27] Thus persistence of elevated BDG seric levels should prompt one to detect potential deep-seated infections.

However, the frequency of false positive results appeared as an important drawback of the BDG test. This limitation has already been mentioned by Badiee et al., who reported a 46% specificity in 62 pediatric patients suffering from hematological malignancy.[20] A better specificity at 78% was obtained when considering two successive samples in children undergoing allogeneic hematopoietic stem cell transplantation.[28] Among the cause of false positivity, previous administration of polyvalent immunoglobulins has been incriminated; as filtration on cellulose filters is part of their manufacturing process, that may release glucan components.[29] Intestinal barrier integrity also seems to be of major importance in false positives. Mucosal injury associated with severe mucositis/colitis due to

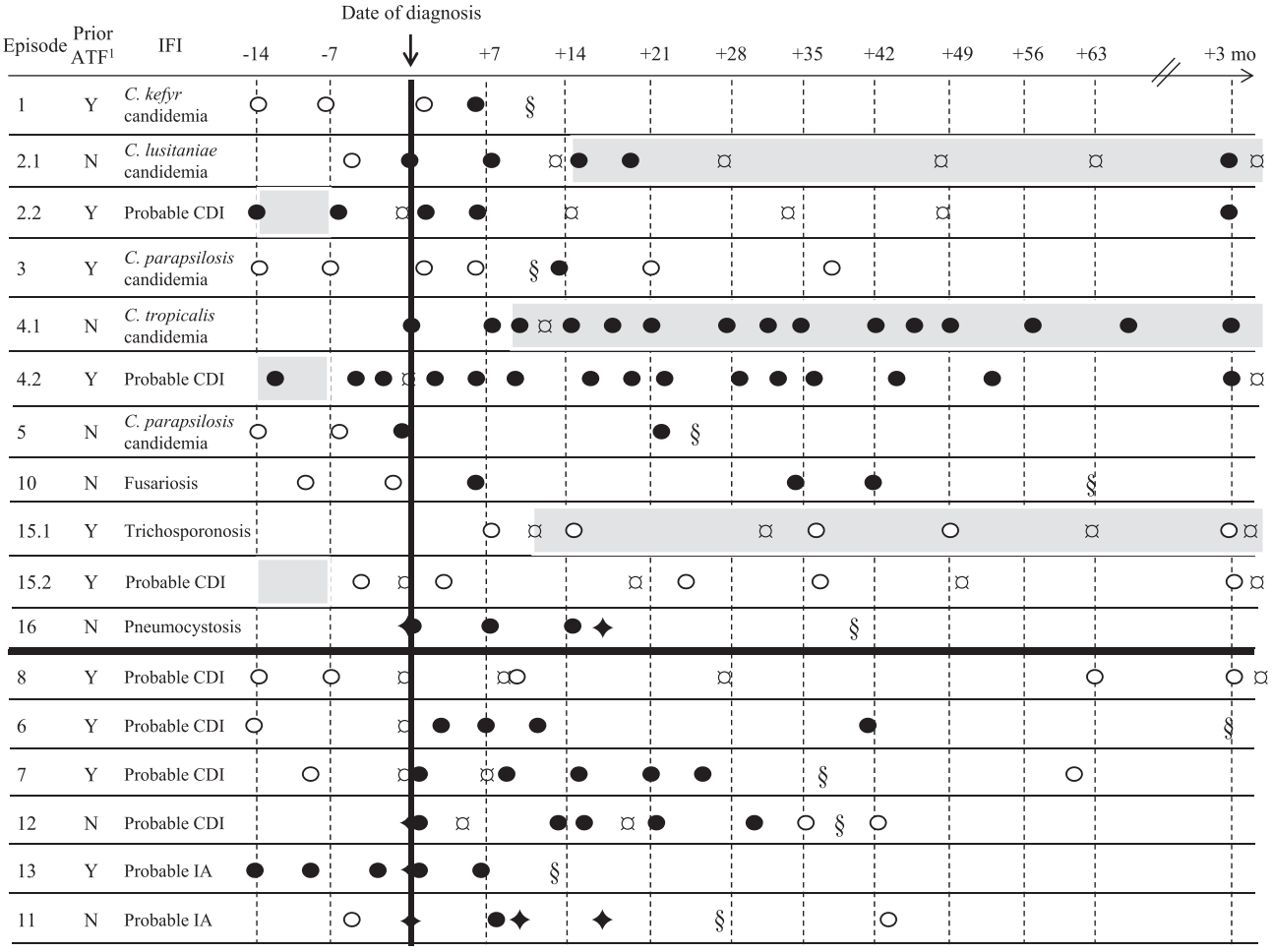

Figure 3 Time interval between the day 0 of diagnosis of IFI (see Patients and methods for definition) and the day of the first positive result by BDG. Data from 17 episodes occurred in 14 patients (patients 2, 4 and 15 developed probable chronic disseminated infection following fungemia). Cases 6, 7, 8, 11, 12, 13 are probable IFI including the BDG result in the EORTC-MSG criteria. ATF: Antifungal treatment, mo: months, BDG: β D-glucan, CDI: Chronic Disseminated Infection, IA: Invasive Aspergillosis, IFI: Invasive Fungal Infection, N: no, Y: yes. ● positive BDG (>75 pg/ml), ○ negative BDG, ✦ positive pulmonary CT scan; ¤ positive abdominal echography; § IFI cure.

chemotherapy has been shown to favor false positive BDG result,[30] while *Enterococcus* digestive colonization and youngest age are known to increase the intestinal permeability.[31,32]

Thus, while the diagnosis of IFI in pediatric patients remains challenging, the availability of the BDG test offers a new possibility to detect these infections. Optimal sensitivity would be obtained for patients who have not been previously treated or at least treated for no more than 6 days. Careful interpretation is required in the case of a positive result as false positivity may be due to the infusion of polyvalent immunoglobulins or intestinal barrier damage. Further studies are required to confirm the usefulness of the test to guide maintenance therapy, as the kinetics of BDG seems to correlate with the clinical outcome.

Conflict of interest

No.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jinf.2016.07.007.

References

1. Hale KA, Shaw PJ, Dalla-Pozza L, MacIntyre CR, Isaacs D, Sorrell TC. Epidemiology of paediatric invasive fungal infections and a case-control study of risk factors in acute leukaemia or post stem cell transplant. *Br J Haematol* 2010; **149**(2):263—72.
2. Clancy CJ, Nguyen MH. Finding the "missing 50%" of invasive candidiasis: how nonculture diagnostics will improve understanding of disease spectrum and transform patient care. *Clin Infect Dis* 2013;**56**(9):1284—92.
3. Marchetti O, Lamoth F, Mikulska M, Viscoli C, Verweij P, Bretagne S, et al. ECIL recommendations for the use of biological markers for the diagnosis of invasive fungal diseases in leukemic patients and hematopoietic SCT recipients. *Bone Marrow Transpl* 2012;**47**(6):846—54.
4. Karageorgopoulos DE, Vouloumanou EK, Ntziora F, Michalopoulos A, Rafailidis PI, Falagas ME. beta-D-glucan assay for the diagnosis of invasive fungal infections: a meta-analysis. *Clin Infect Dis* 2011;**52**(6):750—70.
5. Reischies FM, Prattes J, Woelfler A, Eigl S, Hoenigl M. Diagnostic performance of 1,3-beta-d-glucan serum screening in patients receiving hematopoietic stem cell transplantation. *Transpl Infect Dis* 2016;**18**(3):466—70.
6. Levesque E, El Anbassi S, Sitterle E, Foulet F, Merle JC, Botterel F. Contribution of (1,3)-beta-D-glucan to diagnosis of invasive candidiasis after liver transplantation. *J Clin Microbiol* 2015;**53**(3):771—6.
7. Posteraro B, De Pascale G, Tumbarello M, Torelli R, Pennisi MA, Bello G, et al. Early diagnosis of candidemia in intensive care unit patients with sepsis: a prospective comparison of (1->3)-beta-D-glucan assay, *Candida* score, and colonization index. *Crit Care* 2011;**15**(5):R249.
8. De Pauw B, Walsh TJ, Donnelly JP, Stevens DA, Edwards JE, Calandra T, et al. Revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. *Clin Infect Dis* 2008;**46**(12):1813—21.
9. Ceesay MM, Desai SR, Berry L, Cleverley J, Kibbler CC, Pomplun S, et al. A comprehensive diagnostic approach using galactomannan, targeted beta-d-glucan, baseline computerized tomography and biopsy yields a significant burden of invasive fungal disease in at risk haematology patients. *Br J Haematol* 2015;**168**(2):219—29.
10. Hammarstrom H, Kondori N, Friman V, Wenneras C. How to interpret serum levels of beta-glucan for the diagnosis of invasive fungal infections in adult high-risk hematology patients: optimal cut-off levels and confounding factors. *Eur J Clin Microbiol Infect Dis* 2015;**34**(5):917—25.
11. Lamoth F, Cruciani M, Mengoli C, Castagnola E, Lortholary O, Richardson M, et al. Beta-glucan antigenemia assay for the diagnosis of invasive fungal infections in patients with hematological malignancies: a systematic review and meta-analysis of cohort studies from the Third European Conference on Infections in Leukemia (ECIL-3). *Clin Infect Dis* 2012;**54**(5):633—43.
12. Groll AH, Castagnola E, Cesaro S, Dalle JH, Engelhard D, Hope W, et al. Fourth European Conference on Infections in Leukaemia (ECIL-4): guidelines for diagnosis, prevention, and treatment of invasive fungal diseases in paediatric patients with cancer or allogeneic haemopoietic stem-cell transplantation. *Lancet Oncol* 2014;**15**(8):e327—40.
13. Louis M, Guitard J, Jodar M, Ancelle T, Magne D, Lascols O, et al. Impact of HIV infection status on interpretation of quantitative PCR for detection of *Pneumocystis jirovecii*. *J Clin Microbiol* 2015;**53**(12):3870—5.
14. Martin GS, Mannino DM, Eaton S, Moss M. The epidemiology of sepsis in the United States from 1979 through 2000. *N Engl J Med* 2003;**348**(16):1546—54.
15. Trick WE, Fridkin SK, Edwards JR, Hajjeh RA, Gaynes RP. Secular trend of hospital-acquired candidemia among intensive care unit patients in the United States during 1989—1999. *Clin Infect Dis* 2002;**35**(5):627—30.
16. Auberger J, Lass-Florl C, Aigner M, Clausen J, Gastl G, Nachbaur D. Invasive fungal breakthrough infections, fungal colonization and emergence of resistant strains in high-risk patients receiving antifungal prophylaxis with posaconazole: real-life data from a single-centre institutional retrospective observational study. *J Antimicrob Chemother* 2012;**67**(9): 2268—73.
17. Herbrecht R, Letscher-Bru V, Oprea C, Lioure B, Waller J, Campos F, et al. *Aspergillus* galactomannan detection in the diagnosis of invasive aspergillosis in cancer patients. *J Clin Oncol* 2002;**20**(7):1898—906.
18. Choi SH, Kang ES, Eo H, Yoo SY, Kim JH, Yoo KH, et al. *Aspergillus* galactomannan antigen assay and invasive aspergillosis in pediatric cancer patients and hematopoietic stem cell transplant recipients. *Pediatr Blood Cancer* 2013;**60**(2): 316—22.
19. Leeflang MM, Debets-Ossenkopp YJ, Visser CE, Scholten RJ, Hooft L, Bijlmer HA, et al. Galactomannan detection for invasive aspergillosis in immunocompromized patients. *Cochrane Database Syst Rev* 2008;(4). CD007394.
20. Badiee P, Alborzi A, Karimi M, Pourabbas B, Haddadi P, Mardaneh J, et al. Diagnostic potential of nested PCR, galactomannan EIA, and beta-D-glucan for invasive aspergillosis in pediatric patients. *J Infect Dev Ctries* 2012;**6**(4):352—7.
21. Abe M, Kimura M, Araoka H, Taniguchi S, Yoneyama A. Serum (1,3)-beta-D-glucan is an inefficient marker of breakthrough candidemia. *Med Mycol* 2014;**52**(8):835—40.
22. Marr KA, Balajee SA, McLaughlin L, Tabouret M, Bentsen C, Walsh TJ. Detection of galactomannan antigenemia by enzyme immunoassay for the diagnosis of invasive aspergillosis: variables that affect performance. *J Infect Dis* 2004;**190**(3): 641—9.
23. Obayashi T, Negishi K, Suzuki T, Funata N. Reappraisal of the serum (1->3)-beta-D-glucan assay for the diagnosis of invasive fungal infections—a study based on autopsy cases from 6 years. *Clin Infect Dis* 2008;**46**(12):1864—70.
24. Azoulay E, Guigue N, Darmon M, Mokart D, Lemiale V, Kouatchet A, et al. (1, 3)-Beta-D-glucan assay for diagnosing invasive fungal infections in critically ill patients with hematological malignancies. *Oncotarget* 2016. http://dx.doi.org/10.18632/oncotarget.7471.
25. Jaijakul S, Vazquez JA, Swanson RN, Ostrosky-Zeichner L. (1,3)-Beta-D-glucan as a prognostic marker of treatment response in invasive candidiasis. *Clin Infect Dis* 2012;**55**(4): 521—6.
26. Senn L, Robinson JO, Schmidt S, Knaup M, Asahi N, Satomura S, et al. 1,3-Beta-D-glucan antigenemia for early diagnosis of invasive fungal infections in neutropenic patients with acute leukemia. *Clin Infect Dis* 2008;**46**(6):878—85.
27. Sims CR, Jaijakul S, Mohr J, Rodriguez J, Finkelman M, Ostrosky-Zeichner L. Correlation of clinical outcomes with beta-glucan levels in patients with invasive candidiasis. *J Clin Microbiol* 2012;**50**(6):2104—6.
28. Koltze A, Rath P, Schoning S, Steinmann J, Wichelhaus TA, Bader P, et al. Beta-D-glucan screening for detection of invasive fungal disease in children undergoing allogeneic hematopoietic stem cell transplantation. *J Clin Microbiol* 2015; **53**(8):2605—10.
29. Pini P, Bettua C, Orsi CF, Venturelli C, Forghieri F, Bigliardi S, et al. Evaluation of serum (1-> 3)-beta-D-glucan clinical performance: kinetic assessment, comparison with

galactomannan and evaluation of confounding factors. *Infection* 2016;**44**(2):223—33.
30. Ellis M, Al-Ramadi B, Finkelman M, Hedstrom U, Kristensen J, Ali-Zadeh H, et al. Assessment of the clinical utility of serial beta-D-glucan concentrations in patients with persistent neutropenic fever. *J Med Microbiol* 2008;**57**(Pt 3):287—95.
31. Maharshak N, Huh EY, Paiboonrungruang C, Shanahan M, Thurlow L, Herzog J, et al. *Enterococcus faecalis* gelatinase mediates intestinal permeability via protease-activated receptor 2. *Infect Immun* 2015;**83**(7):2762—70.
32. Siemann M, Koch-Dorfler M, Gaude M. False-positive results in premature infants with the Platelia *Aspergillus* sandwich enzyme-linked immunosorbent assay. *Mycoses* 1998; **41**(9—10):373—7.