Difficulties in using 1,3-β-D-glucan as the screening test for the early diagnosis of invasive fungal infections in patients with haematological malignancies – high frequency of false-positive results and their analysis

Zdenek Racil,[1] Iva Kocmanova,[2] Martina Lengerova,[1] Barbora Weinbergerova,[1] Lucie Buresova,[3] Martina Toskova,[1] Jana Winterova,[1] Shira Timilsina,[4] Isa Rodriguez[4] and Jiri Mayer[1]

Correspondence
Zdenek Racil
zracil@fnbrno.cz

[1]Department of Internal Medicine Hemato-Oncology, Masaryk University and University Hospital Brno, Brno, Czech Republic

[2]Department of Microbiology, University Hospital Brno, Brno, Czech Republic

[3]Institute of Biostatistics and Analyses, Faculty of Medicine and Faculty of Science, Masaryk University, Brno, Czech Republic

[4]Faculty of Medicine, Masaryk University, Brno, Czech Republic

We have evaluated the contribution of the 1,3-β-D-glucan (BG) assay for the screening of invasive fungal infections (IFIs) in patients with haematological malignancies. Serum samples from patients at risk of IFI were collected twice a week and retrospectively tested using the BG assay. BG screening was performed on 1143 samples from 91 patients during 104 anticancer treatment cycles. Proven and probable cases of IFI occurred in 9 (8.7 %) treatment cycles. Depending on the criterion of positivity used (1× >60 pg ml^{-1}, 1× >80 pg ml^{-1}, 2× >60 pg ml^{-1} or 2× >80 pg ml^{-1}) the sensitivity and specificity were 89, 89, 67 and 44 %, and 20, 48, 33 and 56 %, respectively. Although the test was marked as positive in 82, 68, 54 and 45 % of all the treatment cycles, in the majority of cases, these positivities were probably false. The major limit of the BG test was an extremely low positive predictive value (10 to 12 %). We have analysed mucositis, candida colonization, bacteraemia, use of antimicrobials, erythrocyte and thrombocyte filtered blood products, collecting tubes or sampling via venous catheters. Even though no factor is a major source of BG, it could at least partially influence BG assay performance. Thus, BG detection has a limited usefulness as a screening method for IFIs in patients with haematological malignancies.

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INTRODUCTION

Invasive fungal infections (IFIs) are serious and often a life-threatening complication in patients with haematological malignancies (Marr *et al.*, 2002; Pagano *et al.*, 2006). Invasive aspergillosis (IA) and invasive candidiasis (IC) represent the majority of IFIs with an attributable mortality of 30–40 % (Pagano *et al.*, 2006). Early diagnosis and the initiation of efficacious antifungal treatments are major factors in contributing to the prognosis of patients with IFIs (Garey *et al.*, 2006; von Eiff *et al.*, 1995). Since conventional microbiological methods often lack sensitivity in the diagnostics of IFI, non-culture-based tests are used for an early noninvasive diagnosis of these infections. While galactomannan (GM) has an undoubted role in the diagnosis of IA (Maertens *et al.*, 2002, 2004, 2005), the utility of mannan and antimannan detection is somewhat limited (Sendid *et al.*, 2002), and PCR methods have not yet been standardized (Mengoli *et al.*, 2009).

1,3-β-D-glucan (BG) is a fungal-cell-wall polysaccharide that is released into the bloodstream of patients with IC, IA and some other IFIs, except for invasive zygomycosis and cryptococcosis (Kedzierska *et al.*, 2007). Thus, the detection of BG seems to be a very interesting tool for IFI

Abbreviations: AUC, area under the curve; BG, 1,3-β-D-glucan; CVC, central venous catheter; EORTC/MSG, European Organization for Research and Treatment of Cancer/Mycosis Study Group; GM, galactomannan; HSCT, haematopoietic stem cell transplantation; IA, invasive aspergillosis; IC, invasive candidiasis; IFI, invasive fungal infection; NPV, negative predictive value; PPV, positive predictive value; ROC, receiver operating characteristic; WHO, World Health Organization.

diagnostics in haemato-oncological patients – making early diagnosis possible for the two major IFIs in one test. Several commercial kits with different reactivity have now been made available for BG detection (Obayashi *et al.*, 2008). Most of the available studies have used the detection of BG in samples from patients with proven or probable IFIs (Hachem *et al.*, 2009; Obayashi *et al.*, 2008; Ostrosky-Zeichner *et al.*, 2005; Persat *et al.*, 2002, 2008; Pickering *et al.*, 2005) and there are only limited data about the use of BG detection in IFI screening (Ellis *et al.*, 2008; Koo *et al.*, 2009; Odabasi *et al.*, 2004; Pazos *et al.*, 2005; Senn *et al.*, 2008). This should be the main role of all tests used for the guiding of the pre-emptive antifungal treatment – therapy of a suspected or presumed fungal infection in advance of confirmation. We therefore conducted a study to evaluate the clinical usefulness of BG detection for the screening and early diagnosis of IFI in routine clinical settings in patients with haematological malignancies.

METHODS

Patients. Between September 2004 and June 2007, 91 patients who had undergone 104 anticancer treatment cycles with an intermediate [high dose chemotherapy with autologous haematopoietic stem cell transplantation (HSCT)] and high risk (induction/reinduction for acute myelogenous leukaemia or allogenic HSCT) of IFI were enrolled in our study. Furthermore, in 37 patients, paired blood samples were collected for excluding the central venous catheter (CVC) as a source of BG contamination. All patients signed their written informed consent for sample and data collection, and the study was approved by the Institutional Review Board.

Clinical management. Patients were hospitalized in standard air-controlled rooms or in high-efficiency particular air-filtered single rooms. All patients received antifungal prophylaxis (89 % of treatment cycles with fluconazole and 11 % with oral voriconazole). Regular (twice a week) monitoring of *Candida* spp. colonization (in the throat, rectum and urine) and GM antigenaemia (Platelia *Aspergillus* EIA; Bio-Rad) was performed on all patients. If febrile neutropenia not responding to antibiotics occurred, the patient received empirical antifungal treatment mostly with conventional amphotericin B, and then a routine full diagnostic work up for IFI was performed.

Definitions. The new anticancer treatment cycle has been defined as the period from beginning a distinct cycle of chemotherapy until discharge, to the beginning of a new chemotherapy cycle or until day +100 after transplantation (in the case of patients after allogenic HSCT). The diagnosis of IFI was assessed according to the European Organization for Research and Treatment of Cancer/Mycosis Study Group (EORTC/MSG) criteria and definitions published in 2002 (the criteria available at the time of the study) (Ascioglu *et al.*, 2002). The BG results have not been used for diagnostic categorization of IFI. If mucositis was present, the severity was scored at the time of the serum sample collection according to the World Health Organization (WHO) scale (WHO, 1979).

Blood sampling. Blood samples for BG analysis were taken twice a week. Collection was performed mostly through venous catheters using routine plastic test tubes (7.5 ml Monovette; Sarstedt). Samples were allowed to clot for at least 1 h, and then the serum was harvested by aspiration and stored frozen at −20 °C (2004–2006) or at −80 °C (starting in 2006). Sterile storage tubes (Sarstedt) and sterile pipette tips (Eppendorf) were used.

BG detection assay. BG was detected with the Fungitell test kit as recommended by the manufacturer (Associates of Cape Cod). A total of 5 µl of serum were briefly pretreated with 20 µl alkaline reagent (0.125 M KOH/0.6 M KCl) for 10 min at 37 °C and then 100 µl reconstituted Fungitell reagent was added. The reaction was incubated for 40 min at 37 °C and the optical density was measured at 405/490 nm every 20 s (iEMS MF reader; Labsystems). Correlation coefficients were >0.98 in all reactions. All specimens were tested in duplicate. The concentration of BG in each sample was calculated using a calibration curve. BG results were not used for the management or classification of IFI.

Tests for false positivity. Plastic blood collection tubes (Monovette 7.5 ml; Sarstedt) were analysed for BG contamination. Each tube was shaken for 60 min with 2 ml reagent grade water (Associates of Cape Cod) that had been tested. Antibiotics were tested as a routinely prepared intravenous solution and then after dilution to a common serum concentration by reagent grade water. Erythrocytes and platelet concentrates were centrifuged and acellular liquid supernatant was tested in the same way as the serum.

Statistical analysis. Per test analysis was performed as published elsewhere (Maertens *et al.*, 2007; Marr *et al.*, 2004). Fisher's exact test was used for binary outcomes, the Mann–Whitney test was used for the comparison of cycles with and without IFI in continuous variables, and for pair wise comparison the Wilcoxon test was used. A two-sided P value <0.05 was considered statistically significant. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated when cycles without IFI were considered as being true negatives, and cycles with proven and probable IFI as true positives. The receiver operating characteristic (ROC) curve and the area under the curve (AUC) were used to estimate the discriminatory capability of the BG assay for IFI screening in haemato-oncological patients. PPV was estimated for different values of prevalence using the obtained value of sensitivity and specificity. For statistical analysis, the software Statistica, version 8.0 (StatSoft), and R (R Development Core Team) were used.

RESULTS AND DISCUSSION

BG in the screening of IFI

During 104 anticancer treatment cycles (44 %, treatment of acute myelogenous leukaemia; 39 %, allogenic HSCT; 16 %, autologous HSCT; 1 %, other) in 91 patients (63 % male, 37 % female) 9 cases of possible IAs, 8 probable IAs and 1 proven IC occurred. The incidence of all IFIs was 17.3 %; proven and probable IFIs occurred in 8.7 % of cycles. BG was measured in 1143 blood samples – median 9 samples per treatment cycle (minimum 3, maximum 46) – and in 422 (37 %) and 308 (27 %) BG values were >60 and >80 pg ml^{-1}, respectively.

The test detected 8/9 (89 %) of proven and probable cases of IFIs if the criterion one/single samples >60 or >80 pg ml^{-1} were used, and only 6/9 (67 %) and 4/9 (44 %) when criterion two/consecutive samples >60 and >80 pg ml^{-1}, respectively, were used. Interestingly, the BG values in the only case of proven IFI (candidaemia caused by *Candida krusei*) did not reach any of these cut-offs and the test

remained negative. However, the BG detection was not performed with the same blood sample as the one that was *Candida* culture positive, although the sample used was collected at the same time.

The sensitivity, specificity, PPV and NPV of the assay in our study population for different criteria of positivity are shown in Table 1. There could be several factors affecting the sensitivity of the assay in our study: relatively low incidence of proven and probable IFIs; using GM as part of the criteria for probable IA (possibility of false-positive results – but all known causes were eliminated); administration of antifungal prophylaxis (however, fluconazole was used in 89 % of cycles, thus probably only sensitivity for IC cases could be decreased); and finally early and prompt administration of pre-emptive antifungal treatment when the criteria for IFI were fulfilled.

However, the major limitation of using BG detection for IFI screening in our study was an extremely low PPV (varying from 10 to 12 %), regardless of the cut-off and single or consecutive positivity that was used. PPV depends on the prevalence of IFIs in the study and this was only 8.7 % in our analysis. This could be one of the major limitations in our study but this number is appropriate for a combined group of patients with an intermediate and a high risk of IFI. Moreover, the PPV remained low, even if the results were recalculated for a hypothetical higher prevalence of IFIs. For cut-off 2 values >60 pg ml^{-1} and a prevalence of IFI 10, 15 and 20 %, the corresponding PPV would be 12, 18 and 24 %, and for cut-off one positivity >80 pg ml^{-1} PPV 14, 21 and 27 %.

The other explanation for a high number of BG-positive cycles could be the high sensitivity of the test. It detects IFI at a very early stage before being detected clinically and could be covered and treated by empirical antifungal treatment. However, we did not confirm this hypothesis of cryptic IFI. The percentage of anticancer treatment cycles with systemic antifungal treatment (empirical, pre-emptive or specific) were not different between BG-positive and BG-negative cycles, irrespective of the criterion of a positive cycle used. Using the criterion for the BG-positive treatment cycle of one sample with a BG value >60 pg ml^{-1}, the frequency of systemic antifungal therapy was 32 % in BG positive as well as 32 % in BG-negative treatment cycles ($P=1.0$). Similarly, for the criterion of one sample with BG value >80 pg ml^{-1} these frequencies were 35 and 26 %, respectively ($P=0.53$).

Thus, the major reason for the extremely low PPV in our study [also described by other authors (Digby *et al.*, 2003; Mattiuzzi *et al.*, 2007)] was a high frequency of clinically false-positive results (positive test results without clinical correlates). A total of 76 % out of 422 and 75 % out of 308 BG-positive samples with a BG level >60 and >80 pg ml^{-1}, respectively, were clinically false positive. Similarly, approximately only 20 % of all BG-positive cycles (irrespective of the criterion used) had any sign of IFI. Frequent false positivities are an explanation for the absence of any statistically significant differences in mean, median and peak BG levels, as well as in the number of BG-positive samples between the treatment cycles with and without IFI (Table 2) and for the ROC curve showing with AUC 0.562 minimal discriminatory capability of the BG assay for IFI screening (Fig. 1).

Analysis of the possible causes of clinically false-positive results in the BG assay

We have performed subsequent analysis of data obtained from our patient group together with further *in vitro* tests, to identify the influence of all the major known factors described in the literature as a possible source of BG causing clinical false positivity in the BG assay. Mucositis, possibly leading to cross-over of *Candida* spp. or its antigens through damaged mucosa (Ellis *et al.*, 2008), and candida colonization (Pazos *et al.*, 2005) as the cause of clinically false-positive results of the BG assay are not likely. Considering only cycles without IFI, there wasn't a statistically significant difference in the frequency of mucositis, severe mucositis (WHO grade III and IV) or candida colonization between BG-positive and BG-negative cycles. (The criterion for a BG-positive treatment cycle for one value >60 pg ml^{-1}: the incidence of mucositis – 48 % in BG-positive cycles vs 47 % in BG-negative cycles, $P=1.0$; the incidence of severe mucositis – 23 % vs 29 %, $P=0.753$; the incidence of candida colonization – 46 % vs 41 %, $P=0.789$. The criterion for a BG-positive cycle for one value >80 pg ml^{-1}: the incidence of mucositis – 48 vs 48 %, $P=1.0$; the incidence of severe mucositis – 24 vs 25 %, $P=1.0$; the incidence of candida colonization – 47 % vs 43 %, $P=0.789$).

Table 1. Performance of the BG assay – per cycle analysis

	One value >60 pg ml^{-1} (%)	Two values >60 pg ml^{-1} (%)	One value >80 pg ml^{-1} (%)	Two values >80 pg ml^{-1} (%)
Sensitivity	88.89	66.67	88.89	44.44
Specificity	19.77	47.67	32.56	55.81
PPV	10.39	11.76	12.12	9.52
NPV	94.44	93.18	96.55	90.57

Table 2. Differences in BG levels and the number of BG-positive samples in cycles with and without IFI (Mann–Whitney test)

	Cycles without IFI	Cycles with proven and probable IFI	*P*
Median (minimum–maximum) BG concentration in positive samples (pg ml^{-1})			
Cut-off of 60 pg ml^{-1}	115 (60–1380)	97 (62–1000)	0.521
Cut-off of 80 pg ml^{-1}	141 (80–1380)	143 (80–1000)	0.276
Median (minimum–maximum) of peak BG concentrations (pg ml^{-1}) during cycles	143 (0–1380)	177 (44–1000)	0.552
Median no. of BG-positive samples per cycles			
Cut-off of 60 pg ml^{-1}	3.0	5.5	0.224
Cut-off of 80 pg ml^{-1}	3.0	4.0	0.825

During six cycles, without signs of IFI, false GM positivity (two samples with the index of positivity >0.5) occurred [four – administration of Plasmalyte solution (Baxter Czech), one – administration of piperacillin/tazobactam, one – unknown]. Except for the patient with unknown GM positivity, all patients were BG negative in this period.

Antigens of some bacterial strains could react with the test and lead to false positivity (Digby *et al.*, 2003; Mennink-Kersten *et al.*, 2007; Pickering *et al.*, 2005). Thus we have analysed 10 episodes of bacteraemia (excluding positive blood culture for coagulase-negative *Staphylococcus*) that occurred in 8 out of 104 treatment cycles during our study. However, only in six of these bacteraemias, BG positivity could be possibly considered as related. Three infections were caused by *Enterococcus* spp., two by *Pseudomonas* spp. and one by *Klebsiella* sp. infection. We did not identify any case of *Pneumocystis jiroveci* pneumonia in our patient population during the study (Desmet *et al.*, 2009; Watanabe *et al.*, 2009).

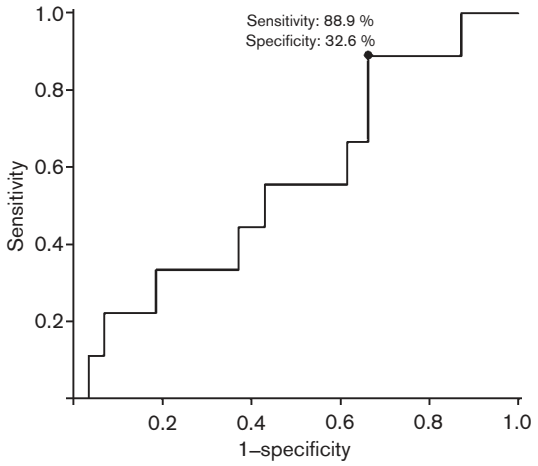

Fig. 1. ROC curve of sensitivity (true-positive results) versus 1–specificity (false-positive results) using different peak values of BG during the treatment cycle as cut-off values to define positivity. For a ROC curve with good discriminatory ability the value of the AUC should be ⩾0.75; however, for the ROC curve presented in this study this value is only 0.562. Thus the peak value of BG during the treatment cycle is not a useful indicator for detecting IFI. It is impossible to estimate a good cut-off value (with high sensitivity and specificity) for the test because in our ROC curve the increase in sensitivity means almost the same decrease in specificity.

We have tested several batches of four of the most commonly used antibiotics in our department as a known possible source of BG – cefepime, piperacillin/tazobactam, meropenem, amoxicillin/clavulanate (Marty *et al.*, 2006). All antibiotics except cefepime had a BG value <80 pg ml^{-1} when the vials of a stock solution were analysed. All five different batches of cefepime were strongly BG positive before dissolving (median 602 pg ml^{-1}), but after dilution to the common serum concentration the solution was negative in the BG assay (median 18 pg ml^{-1}). During 16 out of 38 BG-positive treatment cycles (defined as two consecutive samples with a BG level >80 pg ml^{-1}) without IFI, cefepime was administered. In 14 out of these 16 cycles, cefepime could possibly have contributed to false-positive results. Thus, its administration could have at least partially contributed to false positive BG results significantly frequently (Table 3), especially if the serum sample was obtained shortly after the drug administration (Marty *et al.*, 2006). The use of intravenous immunoglobulin and albumin was very rare in our study population (Usami *et al.*, 2002) and none of our patients underwent dialysis during the period of BG monitoring (Koo *et al.*, 2009).

Leukocyte removing filters could be the source of BG (Kedzierska *et al.*, 2007; Nagasawa *et al.*, 2003). We have tested thrombocyte and erythrocyte concentrates, with and without using these filters. Only 1 out of 11 filtered transfusions had a BG level >60 pg ml^{-1} (median 27 pg ml^{-1}, minimum 7 pg ml^{-1}, maximum 72 pg ml^{-1}). Interestingly 3 out 11 non-filtered blood products had BG >60 pg ml^{-1} (median 50 pg ml^{-1}, minimum 21 pg ml^{-1}, maximum 80 pg ml^{-1}).

We hypothesized that the inserted CVC could potentially be contaminated by a fungal species (mainly *Candida* spp.) and thus any blood samples obtained through CVCs could

Table 3. Details of 25 out of 38 treatment cycles without signs of IFI and with a BG level >80 pg ml^{-1} in two consecutive samples where a possible explanation for BG positivity was identified

BG-positive cycles defined as two consecutive samples with BG level >80 pg ml^{-1}.

Patient initials	Patient's anticancer treatment cycle	BG results (pg ml^{-1})	Possible influence of BG performance
J. A.	2	8, 130, 111*, 51*, 80*, 136	(*) Cefepime administration
Z.D.	1	0, 129*, 184*, 163*, 22, 51, 101	(*) Bacteraemia (*Enterobacter* sp.)
L.H.	1	0, 212, 76, 97, 97, 67, 0*, 49*, 90*	(*) Cefepime administration
F.H.	1	0, 353, 23, 162, 41, 176*, 101*, 174*, 190*, 139*,	(*) Bacteraemia (*Enterococcus* sp.)
A. J.	1	62, 0*,189*, 611*, 31, 117*, 51*, 71, 45, 23, 36, 118, 33	(*) Cefepime administration
M.L.	1	82, 65, 74, 63, 43, 12, 84, 21, 14, 69, 104, 62, 33, 110, 28, 0, 40, 6, 45, 33, 0, 56, 76, 85, 0, 43, 36, 0, 91, 37, 149*†, 87*, 75*	(*) Intestinal GvHD and (†) cefepime administration
M.M.	1	0*, 1380*, 96*, 94*, -*, 205*, 924*, 392*, 88*	None – however, (*) all the period also false positivity of GM of unknown origin
J.M.	2	46, 74, 75, 17, 17, 52, 21, 0, 10, 248*, 124*, 103*, 207*, 71*, 232*, 0, 64	(*) Very severe intestinal GvHD with severe mucosal damage; BG decrease after empirical antifungal treatment
A.M.	1	75, 48, 90, 75, 186, 71, 141, 120, 311, 216, 0, 64, 61, 55, 118*, 696*, 101*, 159*, 58, 161, 44, 20, 278, 69, 79, 63, 52, 0	(*) Administration of intravenous immunoglobulin
M.N.	1	508*, 139*, 98*, 1209, 49†, 101†, 67†, 118†, 224, 190*	(*) False GM positivity caused by Plasmalyte administration and (†) administration of cefepime
M.P.	1	217, 302, 214, 115*	(*) Cefepime administration
M.R.	1	20, 19, 0, 26*, 160*, 155, 0, 0, 44, 141, 44	(*) Cefepime administration
M.S. 1	1	9, 129, 13*, 130*, 91*, 0, 0, 0, 131, 41, 12, 31, 31, 100	(*) Cefepime administration
M.S. 2	1	0, 0, 0, 0, 0*, 132*, 297*, 104	(*) Cefepime administration
D.S.	1	26, 80, 33, 74, 1072*, 301*, 14*, 140*, 0, 12, 0, 36	(*) Febrile neutropenia with clinical improvement after empirical antifungal therapy
E.S.	1	405*, 274*, 131*, 53, 47, 117, 0, 48, 0, 12, 176, 4, 27, 203†, 278†, 59†, 209†, 213†, 59†, 113†, 55, 68, 24, 62,	(*) Bacteraemia (*Enterobacter* sp.) and (†) paraoesophageal abscess (culture positive for *Enterococcus* sp., *Enterobacter* sp. and *Candida* sp.)
F.S.	1	120, 266, 115*, 72*, 127*, 61*, 145, 149, 321	(*) Cefepime administration
J.S. 1	1	50, 22, 0, 26, 44, 66, 61, 34, 0, 0, 111*, 110*, 260*, 8, 32, 0	(*) Bacteraemia (*Pseudomonas* sp.)
J.S. 2	1	62†, 68†, 47†, 125*, 222, 209, 88, 90, 107, 224, −, 203, −, 79, −, 133, 127†	(*) Bacteraemia (*Klebsiella* sp.) and (†) cefepime administration
K.S.	1	13, 182, 168, 184*, 372*	(*) Cefepime administration
M.S.	1	0, 0, 0, 66, 219, 339*, 742*, 72	(*) Cefepime administration
J.V. 1	1	47, 161, 74, 19, 80*, 128*, 94*, 14*, 137*, 167*, 82*, 78*, 21, 46, 77, 0, 61, 26, 0, 130, 25, 0, 177, 15, 0, 68, 75, 69, 28, 95, 120, 74, 73, 55, 0, 53, 0, 131, 65, 486, 9, 64, 82, 64	(*) Intestinal GvHD
D.V.	1	58, 0, 133, 61, 0, 114*, 223*, 78, 165, 751, 169, 93	(*) Cefepime administration
Z.W.	1	18, 380*, 305*, 415*, 193*, 202*, 20*, 638*, 550*, 4*, 0*, 384*, 72, 670	(*) Severe typhlitis; also mannan repeatedly positive

GvHD, Graft versus host disease.

* Important samples in the BG column and the corresponding possible explanation for the BG positivity as indicated on the right.

† Important samples in the BG column and the corresponding possible explanation for the BG positivity as indicated on the right.

contain BG. To test this hypothesis, paired blood samples (one sample from CVC and one from a peripheral venepuncture) were taken from 37 patients out of the presented study – 20 patients without any signs of infection and 17 with uncomplicated febrile neutropenia. However, only three blood samples obtained via CVC had BG >80 pg ml^{-1} and in two of them the venepuncture samples were also positive with this cut-off.

Because BG could contaminate blood collection tubes, 40 plastic collection tubes (Monovette 7.5 ml; Sarstedt) used in our department were tested for BG. The BG concentration was >80 pg ml^{-1} in only three (7.5 %) cases. Thus, the vast majority of collecting tubes can be marked as BG negative. Furthermore, any kind of clustering of false-positive results into specific collection or processing dates have not been identified, hence the important factor of contamination of the storage tubes or pipette's tips can be excluded.

All *in vitro* tests mentioned above and performed for the reason of identification of possible sources of BG contamination were performed after the end of the study. However, all production batches of antibiotics, filtered blood products, CVCs and tubes available at the time of analysis were tested. Thus the probability of large differences in results between these two periods, if the same supplier and brand were used, is relatively small.

Although none of the studied factors was identified as the major reason for clinically false-positive results of the BG test, each of them could at least partially influence some patient results. Individual patient data were reviewed in 38 BG-positive treatment cycles (defined as two consecutive samples with a BG level >80 pg ml^{-1}) without IFI (clinically BG-false-positive cycles) in order to find a possible reason for BG positivity during the period. There were only 13 cycles where no clear explanation was found. In the remaining 25 (Table 3), at least some clinically false-positive results during the cycle could be explained.

Conclusions

In summary, although the BG assay can correctly identify patients with IFI, when it is used for screening, the sensitivity of the assay is relatively limited. Moreover, the major limitation of this test is the high frequency of false-positive results. With its extremely low PPV, the positive result of the test in the majority of cases does not mean the presence of IFI. Moreover, in contrast to the GM assay, there wasn't a single or even a small number of causes for these false positivities that could be easily eliminated after their identification. On the contrary, practically all factors and situations that were analysed in our study could partially contribute to the positivity of the test in patients without signs of IFI. Thus, in summary, a combination of all the possible sources of BG that were analysed in our study and possibly others yet unknown lead to such a high rate of false-positive results in often severely ill patients with haematological malignancies. Hence, according to our results, BG positivity cannot be used as a marker for the beginning of a pre-emptive antifungal treatment in this group of patients, and also the inclusion of BG positivity into the new EORTC/MSG criteria for IFI, published in 2008 (De Pauw *et al.*, 2008), thus may need to be reconsidered. The only contribution of the assay in our study was its high NPV. However, this raises the question of the value of a relatively expensive test requiring specific laboratory equipment. Hence, in the described group of high-risk hosts where IA is until now a major pathogen with double incidence compared to IC, we propose that the BG assay used as a part of the surveillance strategy does not bring any major advantage compared to the GM ELISA test.

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