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Role of (1 →3)-β-D-glucan in the diagnosis of invasive aspergillosis

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Measurement of serum (1 →3)-β-D-Glucan (BG) is an aid in the diagnosis of fungemia and deep-seated mycoses, including invasive aspergillosis (IA). BG is present in the cell wall of most pathogenic fungi (including *Pneumocystis jiroveci*) in significant amounts with some notable exceptions such as *Cryptococcus neoformans* and *Zygomycetes*. Commercially available assays can detect serum BG concentrations as low as 1 pg/mL. Published validation studies have included patients with IA and other invasive fungal diseases (IFD). BG detection appears to be more sensitive than galactomannan detection in patients with IA, but BG's intrinsic lack of mycological specificity requires the integration of clinical, radiological, and microbiological data for proper interpretation. BG assay test characteristics can be used, for example, to exclude IA in some clinical scenarios, to increase the certainty of IA in the presence of an isolated positive galactomannan result or when testing follows initiation of antifungal treatment. BG may be falsely elevated in the serum in the absence of IFD in patients undergoing hemodialysis with cellulose membranes, in patients treated with immunoglobulin, albumin, or other blood products filtered through cellulose filters containing BG, and in patients with serosal exposure to glucan-containing gauze or to certain intravenous antimicrobials. These potential sources of false positivity should be considered when interpreting BG results. BG may be useful as a sensitive screening tool for surveillance of IA and other IFD in populations at risk. Stratified IFD screening and diagnostic strategies using both galactomannan and BG should be explored. Factors affecting the production and clearance of BG during IA and other IFD need additional study to further refine its diagnostic utility.

Keywords glucan, galactomannan, invasive aspergillosis, diagnosis, transplantation

Introduction

There have been impressive advances in the management of invasive aspergillosis since the turn of the millennium. Considered until recently a death-defining infection [1,2], advances in diagnosis and treatment have made it possible to establish a timely diagnosis and provide effective and curative treatment for many patients [3]. Key advances have been the development and approval of voriconazole in 2002 for primary treatment of invasive aspergillosis [4], the validation and availability of high-resolution chest computed tomography (CT) [5–7], and non-invasive testing for circulating fungal cell wall components such as galactomannan [8–10] and (1 →3)-β-D-glucan (BG) [11,12], which allow systematic screening and prompt identification of significant fungal infections.

BGs are heterogeneous molecules that constitute a major carbohydrate fraction of cell walls of most fungi,

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algae and plants [13]; curdlan is a BG molecule, obtained as a fermentation product of a strain of *Alcaligenes fecalis*, but not a cell wall component of bacteria [13]. There is a growing and fascinating body of knowledge regarding the interaction of fungal BGs with different elements of the immune system and effector pathways [14]. BG binds to several receptors including Dectin-1, macrophage scavenger receptors, and TLR-2 [14]. *In vitro*, modulation of BG exposure during antifungal treatment may have an impact on polymorphonuclear activity [15].

This manuscript reviews the history and current knowledge of BG testing, with emphasis on its utility in the diagnosis and management of invasive aspergillosis [IA].

History of (1 →3)-β-D-glucan testing

Lehmann and Reiss were the first to report the measurement of circulating *Aspergillus fumigatus* antigen in rabbits and humans with IA in 1978 [16]. Their strategy involved the development of an antiserum against serum from rabbits experimentally infected with *A. fumigatus*. They detected a single antigenic moiety that circulated in the blood of infected rabbits and humans with proven IA [16]. They later determined that galactomannan was the molecule being detected by the rabbit antiserum [17]. During these experiments, BG was isolated and characterized from *A. fumigatus* cell walls, but found to be non-antigenic [17].

In 1968, Levin and Bang developed an assay for bacterial endotoxin using the amebocytes of *Limulus polyphemus* [the American horseshoe crab] [18]. During pyrogenicity testing of carboxy-methylated BG that was being studied as an anti-tumor agent, Kakinuma and colleagues noted that BG consistently turned the *Limulus* test positive, despite confirmation of non-pyrogenicity in inoculated animals [19]. Morita and colleagues, by studying *Tachypleus tridentatus* [Japanese horseshoe crab] amebocyte lysate fractions, demonstrated that BG triggered the *Limulus* test coagulation cascade via a separate proenzyme, which was termed Factor G (Fig. 1) [20]. Obayashi and colleagues developed a chromogenic test based on the recombination of the different amebocyte lysate fractions and proposed that use of recombined fractions containing Factor G, but not Factor C, which recognizes endotoxin, could be used for non-invasive testing for the diagnosis of invasive fungal diseases (IFD) [21]. Following proof of principle studies [22] and after confirmation that BG was indeed the substrate that bound to Factor G to trigger the reaction cascade of their chromogenic test [23], Obayashi and colleagues published the first multicenter validation study in 1995 [11]. A similar BG test was developed using amebocyte lysate fractions of the American horseshoe crab and approved by the FDA in 2004 as an aid in the diagnosis of fungemia and deep-seated mycoses [24] following a prospective validation study [12].

Fungal spectrum of BG detection

BG is a major component of the cell wall of most fungal species and is readily detected in supernatants of most fungal cultures, with the exception of *Cryptococcus neoformans* and *Zygomycetes* [25,26]. Data from validation trials and case series consistently demonstrate elevated BG levels in the blood of patients with systemic infections caused by all species of pathogenic *Candida* and *Aspergillus* [11,12,27–30]. BG has also been detected in the serum of patients with infections caused by *Fusarium* sp., *Acremonium* sp., *Trichosporon* sp., *Saccharomyces cerevisiae*, and *Histoplasma capsulatum* [11,12,29,31–34]. In our experience [35], BG has been detected in patients with confirmed invasive infections caused by the fungi listed above. In addition, BG has been positive in invasive infections caused by *Scedosporium prolificans*, by agents of pheohyphomycosis such as *Acremonium*, *Phaeoacremonium,* and *Fonsecaea*, in patients with invasive dermatophytosis, and in a patient with fungemia caused by *Blastoschizomyces capitatus*.

The cumulative published experience is consistent in the finding that BG is rarely detectable in the setting of cryptococcosis and zygomycosis [11,28,29,36]. BG testing is consistently negative in patients with mucosal candidiasis in the absence of systemic disease [27]. On the other hand, BG testing has proven to be a particularly sensitive marker of *Pneumocystis jiroveci* pneumonia in patients with HIV infection and other predisposing conditions [37–39].

False positive BG results

There are several clinical circumstances in which BG is detected in patients without evidence of fungal infection (Table 1). In these instances, BG is truly present in the blood specimens, but introduced into the system by certain medical interventions.

BG positivity in blood samples from patients undergoing hemodialysis was noted early in the development of the test [22,28,40]. Further study determined that cellulose [41] or modified regenerated cellulose [42] dialysis membranes contain BG and consistently elevate BG levels in patient who are dialyzed with these membranes; this phenomenon was not observed in patients dialyzed with synthetic polysulfone [42],

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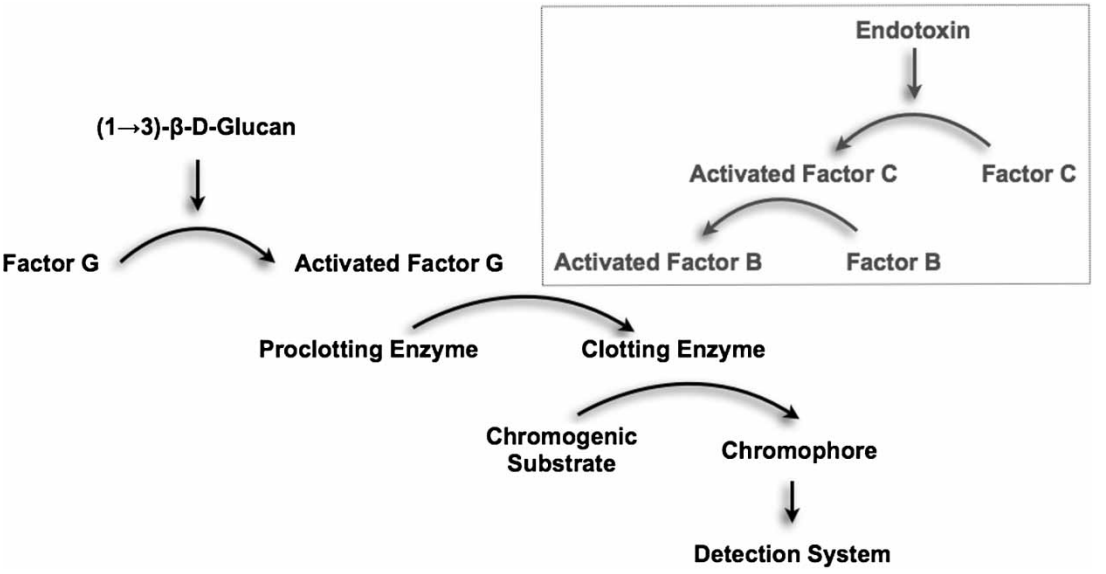

Fig. 1 Schema of BG detection by use of horseshoe crab lysate fractions. In gray are the enzymatic steps triggered by endotoxin, which are removed or neutralized during the manufacturing of BG-specific assays [13].

cellulose triacetate [41], or polymethyl methacrylate [41] membranes. Consideration of the type of dialysis membrane used in patients in whom BG testing is being considered is necessary for proper interpretation of the assay. As the type of dialysis membranes used for renal replacement therapy tends to be institution specific, local validation of the BG assay in patients undergoing dialysis is advisable.

BG has been consistently detected in the serum of patients who receive intravenous immunoglobulin [43,44], albumin, or other commercial blood components [45–47]. BG is released from cellulose filters used during the manufacturing process [45–47]. Not all manufacturers use BG-containing filters, and knowledge of the processing protocols or batch testing of lots used in the clinical care of patients in whom BG monitoring may be employed is essential to interpret BG assay results properly in this context. Otherwise, BG testing in patients who have received these products should not be performed.

Investigating sources of false positive serum *Limulus* assay positivity in patients after abdominal surgery, Kimura and colleagues discovered that gauze used intraoperatively was the source of BG. They confirmed this finding by inoculating gauze-saline intraperitoneally in rats [48]. This phenomenon has been observed by other authors [49]. Similarly, Mohr and colleagues have presented data on the use of BG surveillance in surgical ICU patients and noted frequent false-positive results in the first 3 days following surgery [50]. In our experience, BG detection following surgery is not limited to intraabdominal gauze exposure, but also seen in patients with intrathoracic gauze packings in the pleural space. Whether gauze packing or dressings of other non-serosal surfaces (extremities, burn patients) can increase serum BG levels has not been characterized.

The use of certain intravenous antimicrobials, especially piperacillin-tazobactam, is a well-recognized source of false positive galactomannan results [51,52]. Galactomannan is likely introduced during the manufacturing process, and does not imply fungal contamination of the sterile product. In the case of BG, *in vitro* testing of colistin, ertapenem, cefazolin, trimethoprim-sulfamethoxazole, cefotaxime, cefepime, and ampicillin-sulbactam was positive for BG at reconstituted-vial concentrations, but not when diluted to usual maximum plasma concentrations [53]. No BG was detected in several lots of piperacillin-tazobactam [53]. The clinical relevance of these observations warrants further evaluation, but these *in vitro* findings do not appear to be clinically significant in our experience. Although not available in United States, intravenous amoxicillin-clavulanic acid has been reported to cause false positive BG results [54]. In

Table 1 Causes of false positive BG results

Hemodialysis using cellulose membranes
Albumin
Intravenous immune globulin
Use of cellulose depth filters for intravenous administration
Gauze packing of serosal surfaces
Intravenous amoxicillin-clavulanic acid

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addition, azithromycin and pentamidine solutions have been described to inhibit the BG assay [53], and we have observed inhibitory BG test results in patients who have recently received pentamidine alone. Thus, it appears prudent to analyze lots of intravenous antimicrobials commonly used in patients at risk of fungal infection, especially if false positive results become common in a particular population.

There has been a suggestion in some reports that some bacteremias, especially with Gram-positive organisms, could be a source of false positive BG assay results. Digby and colleagues collected a single specimen in 46 patients with known bacterial or fungal infections being treated in two intensive care units (ICU), seven control ICU patients and eight healthy blood donors [55]. They reported that BG levels were elevated on average in all patients with infections, whether bacterial or fungal, when considered as discrete subgroups. No details of the patients studied, the nature and speciation of the fungal or bacterial infections observed, the proportion of positive results, nor the timing of sampling were provided. No systematic evaluation of known sources of false-positivity discussed above was attempted. Pickering and colleagues reported the results of BG testing of discarded serum samples available 5 days prior to 5 days following a positive blood cultures for bacteria or yeast, or from patients who had samples submitted for testing of galactomannan or *Histoplasma* antigen [31]. Among 15 patients with Gram-positive bacteremias, 11 patients had discarded samples with BG levels >80 pg/mL: six with *Staphylococcus aureus*, three with coagulase-negative staphylococci, one with *Streptococcus mitis*, and one with *Enterococcus faecium*. Of these 11 patients, one had confirmed synchronous invasive candidiasis on biopsy, one had received intravenous immunoglobulin and one patient was on hemodialysis. No clinical characteristics of patients sampled were provided. No information on receipt of albumin or intraabdominal gauze packing was sought. Given the discarded nature of the samples obtained, the authors admit that BG contamination during several manipulations may have occurred. No crossreactivity of the BG assay in patients experiencing bacteremias has been observed in the prospective validation assays published to date or in our experience [11,12,35,56]. Curdlan is a linear BG molecule produced by *Alcaligenes faecalis* that activates factor G [12,57,58]. It is not known whether bacteremia with *A. faecalis* would be a source of a positive BG test, but *A. faecalis* bacteremia is a rare event, even in oncologic populations [59]. In our opinion, positive BG results in patients with a concomitant or recent bacteremia should not be interpreted as a false positive test, but, in the absence of sources of false positivity discussed above, potential sources and nature of IFD should be considered and evaluated.

Validation studies and diagnostic performance

Prospective studies

Odabasi and colleagues systematically studied 283 patients undergoing initial induction chemotherapy for treatment of acute myelogenous leukemia or myelodysplastic syndrome by collecting serum samples twice weekly and blindly analyzing BG test results against clinical outcomes [12]. Sample collection was independent of any clinical syndrome and was limited to one episode per patient. An average of 7.3 specimens per patient was obtained and all patients received either intravenous itraconazole or caspofungin as antifungal prophylaxis. They observed 16 proven and 4 probable IFD [60] among the study patients, 4 of whom had probable or proven IA. Using the *T. polyphemus*-based assay (Associates of Cape Cod, Falmouth, MA) and a predefined cutoff value of 60 pg/ml, all subjects with a proven or probable IFD had at least one positive BG test (sensitivity 100%, specificity 90%). When two sequential BG assays ≥ 60 pg/ml were used as the criteria for test positivity, the sensitivity of the test was 65%, with a specificity of 96%. They noted that positive results preceded the clinical diagnosis of IFD by a median of 10 days.

Obayashi and colleagues' multicenter validation study involved the collection of blood samples during 202 episodes of fever among 179 patients in nine Japanese hospitals [11]. The population tested was more heterogeneous; 67% of patients had a hematological malignancy or AIDS. Only 32% of the patients had more than one blood sample tested. Forty-one patients had proven or microbiologically confirmed IFD, including 4 cases of autopsy-confirmed IA, and 59 patients had confirmed alternative diagnoses [11]. The BG assay cutoff value using the *T. tridentatus*-based assay was 20 pg/ml. The sensitivity of the BG assay using any positive result was 90%, and no patient with a confirmed alternative diagnosis had a positive BG assay result. When only the initial BG assay was evaluated, the sensitivity of the test was 76% [11]. In both studies, all patients with proven IA had elevated serum BG levels.

Most recently, Senn and colleagues [61] published a prospective study of 95 patients with acute leukemias undergoing myelosuppressive chemotherapy between 2002 and 2006. They collected serum samples twice

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weekly during 190 neutropenic episodes. They diagnosed 30 IFD episodes, including five cases of proven and 10 cases of probable IA. Each episode was considered independent in their analysis, but they did exclude 14 subsequent episodes in patients previously diagnosed with IFD. Using a single positive assay as the criteria for test positivity, they found optimal test performance at a cutoff value of 11 pg/ml using the *T. tridentatus*-based assay (Wako Pure Chemical Industries, Osaka, Japan), with a sensitivity of 50% (95% CI, 32–68%) and specificity of 89% (95% CI, 0.82–0.94). Diagnostic performance was improved by considering two consecutive results of $\geq$7 pg/ml as a positive test: the sensitivity was 63% (95% CI, 44–79%), and the specificity was 96% (95% CI, 89–98%). Using two consecutive positive BG results as the criteria for test positivity, the receiver operating characteristic (ROC) curve had an area of 0.87 [61].

Comparative studies of diagnostic tests for invasive aspergillosis

A few studies have attempted to compare the performance of the BG assay with other diagnostic modalities in patients with IA. Kami and colleagues [62] compared a *T. tridentatus*-based assay (Fungi-Tec, Seikagaku Corporation, Tokyo, Japan) with the latex agglutination (LA) galactomannan assay and thoracic computed tomography. Plasma samples were obtained once weekly from 215 patients with hematological malignancy receiving chemotherapy. In a patient-based analysis, LA galactomannan testing had a sensitivity of 44% and a specificity of 94%, whereas BG testing was 63% sensitive and 76% specific for the diagnosis of IA. They concluded that chest CT was a superior diagnostic modality for the diagnosis of IA, but suggestive CT abnormalities without histologic or culture confirmation were considered probable IA cases, making their conclusion problematic [62].

Kawazu and colleagues [63] compared the performance of the BG assay with real-time PCR and double-sandwich EIA galactomannan for the screening and diagnosis of IA in 96 patients with hematological malignancies during 149 consecutive neutropenia-inducing treatment episodes. They diagnosed 11 cases of IA [9 proven, 2 probable]. They used the *T. tridentatus*-based assay (Wako). In this study, using two consecutive positive galactomannan results with a cutoff value EIA index of 0.6 had a better diagnostic performance (ROC 0.97) than two consecutive BG results [ROC 0.79] [63]. In our initial clinical experience [35], among 21 patients with probable or proven IA, galactomannan EIA index was $\geq$0.5 OD in 9 (42.9%) and BG was $\geq$80 pg/ml in 16 (76.2%) on initial testing; five patients did not have elevated galactomannan or BG. No patient had an elevated galactomannan without an elevated BG [35].

BG diagnostic thresholds

Although both horseshoe crab-based assays have limits of detection around 1 pg/ml BG, validation studies using the Japanese horseshoe crab assay have consistently used lower BG thresholds for test positivity [11,61]. Kinetic analyses comparing both BG assays against standard concentrations of several polysaccharides containing BG show that the *L. polyphemus*-based assay is $\sim$2.5-fold less reactive than the *T. tridentatus*-based assay [12]. It is unclear whether this phenomenon translates into a higher sensitivity of the *T. tridentatus*-based assay, as no clinical studies have compared the two assays. The selection of a cutoff value for any quantitative assay is a tradeoff between sensitivity and specificity and depends on the intended use of the test [64]. The suggested manufacturer's cutoff for the *T. tridentatus*-based assay is 20 pg/ml, based on Obayashi's data which was derived from a clinically heterogeneous patient population [11]. The data presented by Senn and colleagues suggest that a single sample cutoff of 11 pg/ml, or two consecutive samples with values $\geq$7 pg/ml, may instead be optimal for the *T. tridentatus-based* assay in patients with hematological malignancies undergoing myelosuppresive chemotherapy [61].

Odabasi and colleagues derived a cutoff of 60 pg/ml for the *L. polyphemus*-based assay by evaluating serum BG levels in 30 candidemic patients [12]. They did not publish a receiver-operating characteristic [ROC] curve of the BG assay in their study population [12]. The multicenter validation study published by Ostrosky-Zeichner and colleagues suggested a cutoff value of 80 pg/ml as the optimal discriminatory value [29]; this is the current manufacturer's recommended cutoff value for a positive assay (values $<$60 pg/ml are considered negative, values between 60 and 79 pg/ml are reported as indeterminate). Of note, this study had a case-control design, and patients and controls had significantly different baseline characteristics, which was not optimal [65].

Kinetics and metabolism of BG

The serum concentration of BG in patients with IFD depends on several factors, many of which have not been well studied to date. Experimental models of IA [66–68] demonstrate that BG concentration parallels

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the progression of untreated infection and burden of infected tissue; effective treatment leads to a decrease of BG levels over time. BG levels likely also depend on the infecting fungal species, its angioinvasive properties, and the structure and molecular weights of BG released during infection [69]. At the host level, factors that influence presence and levels of BG include fungal tissue burden, organs infected, degree of neutropenia, and other determinants of the net state of immunosuppression [70]. Beyond the production and release of BG during infection and its suppression with effective antifungal treatment, the clearance of BG *in vivo* is poorly understood. Humans do not possess beta-glucanases, and clearance of BG likely depends on the glomerular filtration of low molecular weight BG [69], while larger BG molecules appear to be retained in the liver and degraded by Kupffer cells [69,71]. We have observed a few patients with proven IA with persistently elevated BG and negative galactomannan levels months to years following successful antifungal treatment: in these patients, chronic liver dysfunction due to cystic fibrosis or chronic hepatic graft-versus-host disease has been present.

Conclusions

BG is emerging as a useful adjunct in the diagnosis and management of IFD, including IA. Given its intrinsic lack of mycologic specificity, its optimal use requires an integrated consideration of host risk factors for fungal infection, the clinical presentation of the patient, and radiologic patterns of various fungal infections, including IA. As with any diagnostic test, its proper use is based on the pretest probability for IFD. In several studies where BG has been used in systematic surveillance for IFD [12,61], BG appears to rise before infection becomes clinically apparent, and may allow for early evaluation and preemptive initiation of appropriate antifungal treatment. BG testing may be particularly useful in excluding most IFD given its consistent high negative predictive value. It has been suggested that combined use of BG and galactomannan testing may be superior to either test alone for diagnosing IA [30], but this strategy deserves further study. Preemptive fungal risk management strategies [72] using BG are appealing and should be studied systematically.

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Potential conflict of interests

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Declaration of interest: The authors report no conflict of interest. The authors alone are responsible for the content and writing of the paper.

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References

1 Patterson TF, Kirkpatrick WR, White M, *et al*. Invasive aspergillosis. Disease spectrum, treatment practices, and outcomes. I3 Aspergillus Study Group. *Medicine* (Baltimore) 2000; **79**: 250–260.
2 Lin SJ, Schranz J, Teutsch SM. Aspergillosis case-fatality rate: systematic review of the literature. *Clin Infect Dis* 2001; **32**: 358–366.
3 Upton A, Kirby KA, Carpenter P, Boeckh M, Marr KA. Invasive aspergillosis following hematopoietic cell transplantation: outcomes and prognostic factors associated with mortality. *Clin Infect Dis* 2007; **44**: 531–540.
4 Herbrecht R, Denning DW, Patterson TF, *et al*. Voriconazole versus amphotericin B for primary therapy of invasive aspergillosis. *N Engl J Med* 2002; **347**: 408–415.
5 Caillot D, Casasnovas O, Bernard A, *et al*. Improved management of invasive pulmonary aspergillosis in neutropenic patients using early thoracic computed tomographic scan and surgery. *J Clin Oncol* 1997; **15**: 139–147.
6 Caillot D, Couaillier JF, Bernard A, *et al*. Increasing volume and changing characteristics of invasive pulmonary aspergillosis on sequential thoracic computed tomography scans in patients with neutropenia. *J Clin Oncol* 2001; **19**: 253–259.
7 Kuhlman JE, Fishman EK, Siegelman SS. Invasive pulmonary aspergillosis in acute leukemia: characteristic findings on CT, the CT halo sign, and the role of CT in early diagnosis. *Radiology* 1985; **157**: 611–614.
8 Maertens J, Verhaegen J, Demuynck H, *et al*. Autopsy-controlled prospective evaluation of serial screening for circulating galactomannan by a sandwich enzyme-linked immunosorbent assay for hematological patients at risk for invasive Aspergillosis. *J Clin Microbiol* 1999; **37**: 3223–3228.
9 Maertens J, Verhaegen J, Lagrou K, Van Eldere J, Boogaerts M. Screening for circulating galactomannan as a noninvasive diagnostic tool for invasive aspergillosis in prolonged neutropenic patients and stem cell transplantation recipients: a prospective validation. *Blood* 2001; **97**: 1604–1610.
10 Pfeiffer CD, Fine JP, Safdar N. Diagnosis of invasive aspergillosis using a galactomannan assay: a meta-analysis. *Clin Infect Dis* 2006; **42**: 1417–1427.
11 Obayashi T, Yoshida M, Mori T, *et al*. Plasma (1→3)-beta-D-glucan measurement in diagnosis of invasive deep mycosis and fungal febrile episodes. *Lancet* 1995; **345**: 17–20.
12 Odabasi Z, Mattiuzzi G, Estey E, *et al*. Beta-D-glucan as a diagnostic adjunct for invasive fungal infections: validation, cutoff development, and performance in patients with acute myelogenous leukemia and myelodysplastic syndrome. *Clin Infect Dis* 2004; **39**: 199–205.

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13 Finkelman MA, Tamura H. Detection and measurement of (1→3)-beta-D-glucan with Limulus amebocyte lysate-based reagents. In: Young S-H, Castranova V (eds). *Toxicology of 1–3-beta-Glucans: Glucans as a Marker for Fungal Exposure*. Boca Raton: CRC Press/Taylor & Francis, 2005: 179–197.
14 Williams DL, Lowman DW, Ensley HE. Introduction to the chemistry and immunobiology of beta-glucans. In: Young S-H, Castranova V (eds). *Toxicology of 1–3-beta-Glucans: Glucans as a Marker for Fungal Exposure*. Boca Raton: CRC Press/Taylor & Francis, 2005: 1–34.
15 Lamaris GA, Lewis RE, Chamilos G, *et al*. Caspofungin-mediated beta-glucan unmasking and enhancement of human polymorphonuclear neutrophil activity against Aspergillus and non-Aspergillus hyphae. *J Infect Dis* 2008; **198**: 186–192.
16 Lehmann PF, Reiss E. Invasive aspergillosis: antiserum for circulating antigen produced after immunization with serum from infected rabbits. *Infect Immun* 1978; **20**: 570–572.
17 Reiss E, Lehmann PF. Galactomannan antigenemia in invasive aspergillosis. *Infect Immun* 1979; **25**: 357–365.
18 Levin J, Bang FB. Clottable protein in *Limulus*; its localization and kinetics of its coagulation by endotoxin. *Thromb Diath Haemorrh* 1968; **19**: 186–197.
19 Kakinuma A, Asano T, Torii H, Sugino Y. Gelation of *Limulus* amoebocyte lysate by an antitumor (1 leads to 3)-beta-D-glucan. *Biochem Biophys Res Commun* 1981; **101**: 434–439.
20 Morita T, Tanaka S, Nakamura T, Iwanaga S. A new (1→3)-b-D-glucan-mediated coagulation pathway found in *Limulus* amebocytes. *FEBS Lett* 1981; **129**: 318–321.
21 Obayashi T, Tamura H, Tanaka S, *et al*. A new chromogenic endotoxin-specific assay using recombined *Limulus* coagulation enzymes and its clinical applications. *Clin Chim Acta* 1985; **149**: 55–65.
22 Obayashi T, Tamura H, Tanaka S, *et al*. Endotoxin-inactivating activity in normal and pathological human blood samples. *Infect Immun* 1986; **53**: 294–297.
23 Obayashi T, Yoshida M, Tamura H. Determination of plasma (1→3)-beta-D-glucan: a new diagnostic aid to deep mycosis. *J Med Vet Mycol* 1992; **30**: 275–280.
24 Federal Drug Agency. Evaluation and Safety Center for Devices and Radiological Health. Glucatell (1-3-Beta-D-Glucan Serological Assay). Updated 21 May 2004. Accessed 16 March 2006 from: http://www.fda.gov/cdrh/pdf3/K032373.pdf
25 Odabasi Z, Paetznick VL, Rodriguez JR, *et al*. Differences in beta-glucan levels in culture supernatants of a variety of fungi. *Med Mycol* 2006; **44**: 267–272.
26 Miyazaki T, Kohno S, Mitsutake K, *et al*. (1→3)-beta-D-glucan in culture fluid of fungi activates factor G, a *Limulus* coagulation factor. *J Clin Lab Anal* 1995; **9**: 334–339.
27 Mori T, Ikemoto H, Matsumura M, *et al*. Evaluation of plasma (1→3)-beta-D-glucan measurement by the kinetic turbidimetric *Limulus* test, for the clinical diagnosis of mycotic infections. *Eur J Clin Chem Clin Biochem* 1997; **35**: 553–560.
28 Miyazaki T, Kohno S, Mitsutake K, *et al*. Plasma (1→3)-beta-D-glucan and fungal antigenemia in patients with candidemia, aspergillosis, and cryptococcosis. *J Clin Microbiol* 1995; **33**: 3115–3118.
29 Ostrosky-Zeichner L, Alexander BD, Kett DH, *et al*. Multicenter clinical evaluation of the (1→3) beta-D-glucan assay as an aid to diagnosis of fungal infections in humans. *Clin Infect Dis* 2005; **41**: 654–659.
30 Pazos C, Ponton J, Del Palacio A. Contribution of (1→3)-beta-D-glucan chromogenic assay to diagnosis and therapeutic monitoring of invasive aspergillosis in neutropenic adult patients: a comparison with serial screening for circulating galactomannan. *J Clin Microbiol* 2005; **43**: 299–305.
31 Pickering JW, Sant HW, Bowles CA, Roberts WL, Woods GL. Evaluation of a (1→3)-beta-D-glucan assay for diagnosis of invasive fungal infections. *J Clin Microbiol* 2005; **43**: 5957–5962.
32 Yoshida M, Obayashi T, Iwama A, *et al*. Detection of plasma (1→3)-beta-D-glucan in patients with *Fusarium*, *Trichosporon*, *Saccharomyces* and *Acremonium* fungaemias. *J Med Vet Mycol* 1997; **35**: 371–374.
33 Reiss E, Obayashi T, Orle K, Yoshida M, Zancope-Oliveira RM. Non-culture based diagnostic tests for mycotic infections. *Med Mycol* 2000; **38**: 147–159.
34 Iwama A, Yoshida M, Miwa A, *et al*. Improved survival from fungaemia in patients with haematological malignancies: analysis of risk factors for death and usefulness of early antifungal therapy. *Eur J Haematol* 1993; **51**: 156–160.
35 Koo S, Bryar J, Page JH, Baden LR, Marty FM. Clinical utility of (1→3)-β-D-Glucan Assay (BG) in the diagnosis of invasive fungal infections (Abstract M-1600). 46th Annual Interscience Conference on Antimicrobial Agents & Chemotherapy; 2006; San Francisco, CA.
36 Mitsutake K, Miyazaki T, Miyazaki H, *et al*. [Evaluation of (1-3)-beta-D-glucan in aspergillosis and cryptococcosis]. *Nihon Kyobu Shikkan Gakkai Zasshi* 1994; **32**: 37–41.
37 Yasuoka A, Tachikawa N, Shimada K, Kimura S, Oka S. (1→3) beta-D-glucan as a quantitative serological marker for *Pneumocystis carinii* pneumonia. *Clin Diagn Lab Immunol* 1996; **3**: 197–199.
38 Marty FM, Koo S, Bryar J, Baden LR. (1→3)beta-D-glucan assay positivity in patients with *Pneumocystis (carinii)* jiroveci pneumonia. *Ann Intern Med* 2007; **147**: 70–72.
39 Tasaka S, Hasegawa N, Kobayashi S, *et al*. Serum indicators for the diagnosis of pneumocystis pneumonia. *Chest* 2007; **131**: 1173–1180.
40 Yoshioka T, Ikegami K, Ikemura K, *et al*. A study on *Limulus* amebocyte lysate (LAL) reactive material derived from dialyzers. *Jpn J Surg* 1989; **19**: 38–41.
41 Kanda H, Kubo K, Hamasaki K, *et al*. Influence of various hemodialysis membranes on the plasma (1→3)-beta-D-glucan level. *Kidney Int* 2001; **60**: 319–323.
42 Kato A, Takita T, Furuhashi M. Elevation of blood (1→3)-beta-D-glucan concentrations in hemodialysis patients. *Nephron* 2001; **89**: 15–19.
43 Ikemura K, Ikegami K, Shimazu T, Yoshioka T, Sugimoto T. False-positive result in *Limulus* test caused by Limulus amebocyte lysate-reactive material in immunoglobulin products. *J Clin Microbiol* 1989; **27**: 1965–1968.
44 Ogawa M, Hori H, Niiguchi S, Azuma E, Komada Y. False-positive plasma (1→3)-beta-D-glucan test following immunoglobulin product replacement in an adult bone marrow recipient. *Int J Hematol* 2004; **80**: 97–98.
45 Usami M, Ohata A, Horiuchi T, *et al*. Positive (1→3)-beta-D-glucan in blood components and release of (1→3)-beta-D-glucan from depth-type membrane filters for blood processing. *Transfusion* 2002; **42**: 1189–1195.
46 Nagasawa K, Yano T, Kitabayashi G, *et al*. Experimental proof of contamination of blood components by (1→3)-beta-D-glucan caused by filtration with cellulose filters in the manufacturing process. *J Artif Organs* 2003; **6**: 49–54.

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47 Ohata A, Usami M, Horiuchi T, Nagasawa K, Kinoshita K. Release of (1→3)-beta-D-glucan from depth-type membrane filters and their in vitro effects on proinflammatory cytokine production. *Artif Organs* 2003; **27**: 728–735.
48 Kimura Y, Nakao A, Tamura H, Tanaka S, Takagi H. Clinical and experimental studies of the *Limulus* test after digestive surgery. *Surg Today* 1995; **25**: 790–794.
49 Nakao A, Yasui M, Kawagoe T, *et al*. False-positive endotoxemia derives from gauze glucan after hepatectomy for hepatocellular carcinoma with cirrhosis. *Hepatogastroenterology* 1997; **44**: 1413–1418.
50 Mohr J, Paetznick VL, Rodriguez JR, *et al.* A prospective pilot survey of B-glucan (BG) seropositivity and its relationship to invasive candidiasis (IC) in the surgical ICU (SICU) (Abstract M-168). 45th Annual Interscience Conference on Antimicrobial Agents & Chemotherapy; 2005; Washington, DC.
51 Sulahian A, Touratier S, Ribaud P. False positive test for *Aspergillus* antigenemia related to concomitant administration of piperacillin and tazobactam. *N Engl J Med* 2003; **349**: 2366–2367.
52 Viscoli C, Machetti M, Cappellano P, *et al*. False-positive galactomannan platelia Aspergillus test results for patients receiving piperacillin-tazobactam. *Clin Infect Dis* 2004; **38**: 913–916.
53 Marty FM, Lowry CM, Lempitski SJ, *et al*. Reactivity of (1→3)-beta-d-glucan assay with commonly used intravenous antimicrobials. *Antimicrob Agents Chemother* 2006; **50**: 3450–3453.
54 Mennink-Kersten MA, Warris A, Verweij PE. 1,3-beta-D-glucan in patients receiving intravenous amoxicillin-clavulanic acid. *N Engl J Med* 2006; **354**: 2834–2835.
55 Digby J, Kalbfleisch J, Glenn A. Serum glucan levels are not specific for presence of fungal infections in intensive care unit patients. *Clin Diagn Lab Immunol* 2003; **10**: 882–885.
56 Senn L, Robinson JO, Schmidt 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**: 878–885.
57 Tanaka S, Aketagawa J, Takahashi S. Inhibition of high-molecular-weight-(1→3)-beta-D-glucan-dependent activation of a Limulus coagulation factor G by laminaran oligosaccharides and curdlan degradation products. *Carbohydr Res* 1993; **244**: 115–127.
58 Zhang GH, Baek L, Buchardt O, Koch C. Differential blocking of coagulation-activating pathways of *Limulus* amebocyte lysate. *J Clin Microbiol* 1994; **32**: 1537–1541.
59 Aisenberg G, Rolston KV, Safdar A. Bacteremia caused by *Achromobacter* and *Alcaligenes* species in 46 patients with cancer (1989–2003). *Cancer* 2004; **101**: 2134–2140.
60 Ascioglu S, Rex JH, de Pauw B, *et al*. Defining opportunistic invasive fungal infections in immunocompromised patients with cancer and hematopoietic stem cell transplants: an international consensus. *Clin Infect Dis* 2002; **34**: 7–14.
61 Senn L, Robinson JO, Schmidt 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**: 878–885.
62 Kami M, Tanaka Y, Kanda Y, *et al*. Computed tomographic scan of the chest, latex agglutination test and plasma (1AE3)-beta-D-glucan assay in early diagnosis of invasive pulmonary aspergillosis: a prospective study of 215 patients. *Haematologica* 2000; **85**: 745–752.
63 Kawazu M, Kanda Y, Nannya Y, *et al*. Prospective comparison of the diagnostic potential of real-time PCR, double-sandwich enzyme-linked immunosorbent assay for galactomannan, and a (1→3)-beta-D-glucan test in weekly screening for invasive aspergillosis in patients with hematological disorders. *J Clin Microbiol* 2004; **42**: 2733–2741.
64 Haynes RB. *Clinical Epidemiology: How to do Clinical Practice Research*, 3rd ed. Philadelphia: Lippincott Williams & Wilkins, 2006.
65 Upton A, Leisenring W, Marr KA. (1→3) beta -D-glucan assay in the diagnosis of invasive fungal infections. *Clin Infect Dis* 2006; **42**: 1054–1056; author reply 6.
66 Hashimoto A, Yamakami Y, Kamberi P, *et al*. Comparison of PCR, (1→3)-beta-D-glucan and galactomannan assays in sera of rats with experimental invasive aspergillosis. *J Clin Lab Anal* 1998; **12**: 257–262.
67 Wiederhold NP, Najvar LK, Vallor AC, *et al*. Assessment of serum (1→3)-{beta}-D-Glucan as a measure of disease burden in a murine model of invasive pulmonary aspergillosis. *Antimicrob Agents Chemother* 2007; **52**: 1176–1178.
68 Mitsutake K, Kohno S, Miyazaki T, *et al*. Detection of (1-3)-beta-D-glucan in a rat model of aspergillosis. *J Clin Lab Anal* 1995; **9**: 119–122.
69 Brown GD, Gordon S. Fungal beta-glucans and mammalian immunity. *Immunity* 2003; **19**: 311–315.
70 Marty FM, Rubin RH. The prevention of infection post-transplant: the role of prophylaxis, preemptive and empiric therapy. *Transpl Int* 2006; **19**: 2–11.
71 Suda M, Ohno N, Hashimoto T, *et al*. Kupffer cells play important roles in the metabolic degradation of a soluble antitumor (1→3)-beta-D-glucan, SSG, in mice. *FEMS Immunol Med Microbiol* 1996; **15**: 93–100.
72 Maertens J, Theunissen K, Verhoef G, *et al*. Galactomannan and computed tomography-based preemptive antifungal therapy in neutropenic patients at high risk for invasive fungal infection: a prospective feasibility study. *Clin Infect Dis* 2005; **41**: 1242–50.

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