MINIREVIEW

(1→3)-β-D-Glucan – A New Marker for the Early Serodiagnosis of Deep-seated Fungal Infections in Humans

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Abstract

Until recently, the diagnosis of systemic mycoses was mainly based on traditional methods producing late and inconclusive results. Currently used methods of serological diagnostics are generally based on detection of cell wall components of selected pathogenic fungal species – mannoproteins, functioning as a antigenic markers. There are big hopes for adaptation of commercially available assays to detect (1→3)-β-D-glucan because of the fact that its presence in blood and normally sterile body fluids should lead to initiation of the diagnostic workup of invasive fungal infection. Monitoring (1→3)-β-D-glucan antigenemia is useful in predicting the therapeutic outcome of patients with invasive aspergillosis and in combination with galactomannan detection to identify false-positive reactions. The simultaneous use of both tests is probably more pertinent for the differentiation between yeast and mould infections.

K e y   w o r d s: antigenemia, (1→3)-β-D-glucan, invasive aspergillosis, serological diagnostic methods, systemic candidiasis

Introduction

Early and precise diagnosis of invasive fungal infections (IFI) is still difficult because of lack of specific symptoms, and late appearance in the course of the infection of clinical and radiological signs. Differentiation between IFI and colonization still remains a problem. Classic mycological examination that includes histopathological assessment, microscopic methods, and cultures of clinical material samples also have limited diagnostic value (Horvath and Dummer, 1996; Verweij and Heis, 2000; Yeo and Wong, 2002). Because of low sensitivity of culture methods as well as to necessity shorten the waiting period for mycological exam results, the use of more sensitive non-culture and non-invasive methods is recommended (Ruhnke *et al.*, 2003). New techniques of diagnosing invasive mycoses allow for detection of substitute markers like soluble *Candida* and *Aspergillus* antigens and/or metabolites, as well as molecular detection of fungal DNA from body fluid samples using conserved or specific genome sequences. These techniques, when combined with risk stratification permit the early diagnosis of IFI and the implementation of pre-emptive therapeutic strategies (Wingard and Leather., 2004).

A number of published data have focused on currently available assays, which allow detection of highly immunogenic components of fungal cell wall: galactomannan (*Aspergillus*), and mannan (*Candida*) (Maertens *et al.*, 2001; Sendid *et al.*, 1999). Yet another, more and more widely used antigenic marker in the diagnose of systemic mycoses is (1→3)-β-D-glucan (BDG) (Odabasi *et al.*, 2004; Ostrosky-Zeichner *et al.*, 2005; Pazos *et al.*, 2005; Takebayashi *et al.*, 2005). These are (1→3)-β-D-glucopyranosyl polymers with randomly dispersed single β-D-glucopyranosyl units held by (1→6)-β linkages, giving a comb-like structure. β-glucans are widely distributed in nature in, for example, fungi, yeast, algae, bacteria and higher plants. (1→3)-β-D-glucan is the part of the outer cell wall of saprophytic and pathogenic fungi

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Abbreviations: BDG, (1→3)-β-D-glucan; GM, galactomannan; IA, invasive aspergillosis; IFI, invasive fungal infections; IPA, invasive pulmonary aspergillosis

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with the exception of those belonging to Zygomycetes (*Mucor* and *Rhizopus* species) and *Cryptococcus* species. Viruses, as well as human cells, do not contain BDG (Miyazaki *et al.*, 1995; Yeo and Wong, 2002; Kondori *et al.*, 2004).

Biological role of (1→3)-β-D-glucan

Fungal BDG exist both in soluble and in particulate forms. Conformation of the soluble BDG was classified into three types: triple helix, single helix and random coil. The mechanisms responsible for mediating biological activity of the BDG, especially in soluble form, are still not clearly demonstrated. Soluble yeast BDG upregulated leukocyte activity both on its own and in concert with LPS. In experiments conducted by Engstad *et al.* (2002) soluble yeast BDG had a strong synergistic effect on the LPS-induced secretion of IL-8, IL-10, and on monocyte tissue factor (TF) activity, but not on TNF-α and IL-6. Recently it was found that soluble BDG from the mycelial and yeast forms of *Candida* induced the production of a large amount of macrophage inflammatory protein-2 (MIP-2), a chemotactic factor from mouse peritoneal exudate cells *in vitro* (Miura *et al.*, 2003). Soluble form of BDG may also inhibit the phagocytosis by monocytes, by blocking the receptors for BDG present on phagocytes that according to Miyazaki *et al.* (1995) could explain the possible mechanism of development of fungal infection.

It has been postulated that cell wall glucans may serve as a pattern recognition molecules for the innate immune system (Mueller *et al.*, 2000). In mammals, glucans are thought to induce biological activity through interaction with receptors on macrophages, neutrophils, NK cells, and nonimmune cells (Mueller *et al.*, 1996; Vetvicka *et al.*, 1996; Kougias *et al.*, 2001). Many species have pattern recognition receptors or binding proteins which recognize BDG. Binding of BDG in human and murine monocytes and macrophages is specific, saturable, and susceptible to displacement by other BDG (Mueller *et al.*, 1996; Battle *et al.*, 1998). More recently, the human β-glucan receptor DECTIN-1 was cloned and found to be expressed on monocytes, macrophages, dendritic cells, and NK cells. It function as a pattern recognition receptor recognizing a variety of (1→3)-β- and/or (1→6)-β-linked glucans (Willment *et al.*, 2001). Pattern recognition receptors recognizing of BDG also exist on human fibroblasts. Interaction of fungal glucan with fibroblast membrane receptors increases NF-kB activity and proinflammatory cytokine gene expression IL-6 (Kougias *et al.*, 2001).

Fungal 1→3-β-glucans exhibit a variety of biological and immuno-pharmacological activities, and the significance of these activities is dependent on the structure, such as solubility in water, molecular weight, degree of branching, and conformation (Miura *et al.*, 2003). These activities could be beneficial and pharmacologically useful (Cheung *et al.*, 2002; Hong *et al.*, 2003), while some are strongly related to the allergic and inflammatory adverse reactions (Engstad *et al.*, 2002; Miura *et al.*, 2003). The role of glucans in infection is unknown. Recently an inverse correlation between serum glucan levels and interleukin-2 (IL-2), IL-4, TNF-α and granulocyte-macrophage-colony stimulating factor (GM-CSF) levels was shown in patients with infections from Intensive Care Unit. This correlation was only observed at glucan concentration <40 pg/ml. According to Gonzalez *et al.* (2004) th is may represent an adaptive response to septic injury. Recent data indicate that glucans are released from fungal cell walls into the systemic circulation of patients with proven (is defined by positive microbiological culture and histology) or probable (is defined as at least one microbiological criterion and one or two major/minor clinical criteria) IFI who were/or not receiving antifungal medication. Its presence in blood or other body fluids may be a serological marker of fungal sepsis in patients having fungemia, aspergillosis, candidiasis, fusariosis, and other deep-seated mycoses (Yasuoka *et al.*, 1996; Yuasa *et al.*, 1996; Yoshida *et al.*, 1997; Odabasi *et al.*, 2004; Ostrosky-Zeichner *et al.*, 2005).

BDG-specific assays as a diagnostic adjunct for IFI

Excellent complementary tests and in the future, maybe, an alternative to the methods for detection of *Aspergillus* galactomannan, are assays directed to detection of BDG. Determination of BDG plasma concentration is another useful screening method detecting deep mycoses, because (i) the result can be obtained in a short time period, and (ii) many species of pathogenic fungi have BDG as a cell wall component (Kami *et al.*, 2000; Ishizuka *et al.*, 2004). Use of these methods was beneficial especially in: (a) adult hematological cancer patients with acute myelogenous leukemia, chronic lymphocytic leukemia, acute lymphocytic leukemia, myelodysplastic syndrome, non-Hodkin's lymphoma (Odabasi *et al.*, 2004; Pazos *et al.*, 2005); (b) suspected to have endogenous fungal endophthalmitis (Takebayashi *et al.*, 2005); (c) critically ill surgical patients (Takesue, 2004); (d) heat-stroke patients (Aibiki *et al.*, 2005); (e) Farmer's lung cases (Imai *et al.*, 2004), (f) patients with pulmonary aspergillosis (Yuasa *et al.*, 1996); (g) subjects with *Pneumocystis carinii* pneumonia (Yasuoka *et al.*, 1996), and (h) other groups of patients with proven or probable IFI (Ostrosky-Zeichner *et al.*, 2005).

All commercially available diagnostics tests (Fungi-Tec G; Test Wako-WB003; B-G Star; β-glucan Test Maruha; FungiTec G Test MK; Glucatell (Fungitell) are subject of prospective studies, so far only performed in Japan. The diagnostic tests which are being verified show different sensitivity and specificity (Hossain et al., 1997; Martino and Subira, 2002; Ostrosky-Zeichner et al., 2005; Upton et al., 2006). In the first one mentioned, based on calorimetric method, the affinity of limulus factor G to glucan is used (Fungitec-G test; Seikagaku Kogyo Corp., Tokyo, Japan). (1→3)-β-D-glucan can be detected by its ability to activate factor G of the horseshoe crab coagulation cascade (Uchiyama et al., 1999). It specifically binds to the a subunit of factor G, activating its serine protease zymogen β subunit. In this test, the chromogenic substrate Boc-Leu-Gly-Arg-p-nitroanilide is cleaved by the activated clotting enzyme, followed by the release of p-nitroanilide (pNA). The released p-nitroanilide is determined at an absorbance at 450 nm (Obayashi et al., 1995; Digby et al., 2003). In vitro experiments proved that the limulus factor G activation ability of soluble β-glucan from the mycelial form of Candida (M-CSBG) is comparable to that of the yeast form (Y-CSBG). Miura et al. (2003) reported that M-CSBG from the mycelial form of C. albicans also has high limulus factor G activation ability, further demonstrating that BDG assay in human blood is a promising way for the early diagnosis of deep seated mycoses (Miura et al., 2003).

Few reports show that Fungitec-G is highly specific and sensitive test detecting 1 pg of glucan in 1 ml blood (Miyazaki et al., 1995; Hossain et al., 1997; Martino and Subira, 2002; Takebayashi et al., 2005). In the analysis of 202 episodes of fever of unknown origin, presence of BDG was show in 37 of 41 patients with post mortem or microbiologically confirmed IFI. Sensitivity of the test was estimated to be 90%, specificity in the range of 84–100%. Serum samples with BDG concentration of ≥20 pg/ml were recognized as positive (Obayashi et al., 1995). Miyazaki et al. (1995) confirmed the usefulness of the test in serodiagnostic of invasive candidiasis. Average BDG concentration in plasma was 2207.4 pg/ml with the exception of a case where the etiological factor of bloodstream infection was C. krusei. Interestingly, fungal colonization of the oral cavity, urine and bronchi did not cause an increase of the antigen concentration above the mentioned value (Obayashi et al., 1995). Also was no benefit shown for detection of (1→3)-β-glucan in patients with cryptococcosis (group of 10 patients), as they had the antigen concentration lower than 16.5 pg/ml (Miyazaki et al., 1995). The benefit of detecting BDG by using Fungitec-G test was confirmed in patients suspected to having endogenous fungal endophthalmitis (EFE). The presence of BDG was demonstrated in 95% of patients with EFE, its blood concentration exceeded 20 pg/ml. Sensitivity of the test was estimated as 90.2% when the presence of Candida albicans was confirmed beforehand in blood and/or other specimens (urine, sputum, stool, catheter tip). Presence of BDG in serum (>20 pg/ml) was considered as one of the main factors predisposing to the development of EFE (Takebayashi et al., 2005). A different example is shown in the study of Digby at al. (2003) performed on 46 patients from intensive care ward with confirmed fungal and bacterial infections. Results of this study give a high negative predictive value of approximately 78% and a low specificity of Fungitec-G test. According to researchers, this assay may be useful as a negative predictor of infection. The reasons for little use of the test in serodiagnostic of mycoses may be related to low cut-off value adopted by these authors (20 pg/ml), clinical profile of patients, measuring of the concentration of BDG in only a single serum sample as well as unclearly defined criteria for diagnosing invasive fungal infection.

Data that suggest the use of BDG as an early marker of IFI also come from the report of Pazos et al. (2005). Antigen detected by a new, chromogenic Glucatell test turned out to be a good prognostic factor of invasive aspergillosis (IA) and valuable marker for monitoring antifungal therapy. Similarly as in Fungitec-G Glucatell reagent uses enzymes from Limulus polyphemus amebocyte lysate. This assay is manufactured by removing bacterial endotoxin-sensitive factor C (an endotoxin-activated serine protease zymogen) from LAL (the Limulus amebocyte lysate), making this reagent specific for BDG. The reagent is used in a quantitative assay that detects BDG in the serum of patients with symptoms or medical conditions predisposing to IFI. For the Glucatell assay, the suggested positive serum cut-off level for BDG is defined as 60 or 80 pg/ml (Odabasi et al., 2004; Ostrosky-Zeichner et al., 2005). In recent reports, authors point to the high sensitivity and specificity of the Glucatell test in diagnostics of invasive aspergillosis, candidiasis and fusariosis in onco-haematological patients. In multi-center trials performed by Ostrosky-Zeichner et al. (2005) on a group of 163 patients with proven or probable IFI and on 170 fungal-infection-negative persons, the sensitivity of the test was 69.9% and the specificity 87.1%. The high sensitivity of Glucatell test was demonstrated for Candida species (with the exception of C. parapsilosis), and fungi from Aspergillus and Fusarium genera, amounting to 82.6%, 80% and 100%, respectively. Similar results were obtained by Pazos at al. (2005). In IA patients, tested for the presence of BDG twice weekly, the sensitivity and specificity of the test were 87.5% and 89.6%, respectively. In their study, these authors also emphasized the fact that parallel detection of GM and BDG raises the specificity of the

test to 100%. Odabasi *et al.* (2004) reported comparative outcomes, indicating that specificity of the test was 90% for a single positive sample to 96% and 99% for ≥2 sequential positive results. Testing was performed with serial serum samples obtained from 283 neutropenic subjects (7.3 specimens per subject) with acute myeloid leukaemia or myelodysplastic syndrome who received antifungal prophylaxis, and who were undergoing initial induction chemotherapy. At the same time, in a study on 142 patients with proven IFI, no statistically differences were noted (P = 0.9) in the sensitivity of the test when comparing the group of patients undergoing antifungal therapy (N = 118; 83.3%) with the non-treated patients (N = 24; 72.9%) for a cut-off value of 60 pg/ml (Ostrosky-Zeichner *et al.*, 2005).

Wako-WB003 is another assay for (1→3)-β-D-glucan that determines the change in turbidity of the gelatin reaction of limulus factor G with BDG. In a study where animals were intravenously administered *C. albicans* at a concentration of 1×10^7 CFU, the concentration of BDG in Wako-WB003 test was only 42.225 ± 41.275 ng/ml contrary to the high level of this antigen (660.9 ± 427.9 pg/ml) measured parallel with Fungitec G test. Low usefulness of the Wako-WB003 test in detection of BDG was also demonstrated in patients with candidemia, suspected candidemia, invasive pulmonary aspergillosis (IPA), aspergilloma, and with pulmonary cryptococcosis (Hossain *et al.*, 1997).

Clinical usefulness of BDG in screening for IFI

Many experimental data has been gathered as evidence showing the possible use of BDG in the diagnostics of IFI, especially the ones caused by *Aspergillus* and *Candida* (Kondori *et al.*, 2004; Pazos *et al.*, 2005). Detection of BDG also has the potential to detect IFI in patients including fusariosis, trichosporonosis, and those caused by *Pneumocystis jiroveci*, *Acremonium* spp. and *Saccharomyces* spp. (Yoshida *et al.*, 1997; Odabasi *et al.*, 2004, Ostrosky-Zeichner *et al.*, 2005). Measurement of BDG it is unable to identify the species of infectious fungi. Invasive fungal infection is suspected at a blood BDG concentration over a cut-off value of 20 pg/ml used for the Fungitec-G assay (Obayashi *et al.*, 1995; Miura *et al.*, 2003; Odabasi *et al.*, 2004). Average concentration of BDG in serum samples collected from 163 patients with proven aspergillosis, candidiasis and fusariosis were 1103 pg/ml, 755 pg/ml and 1652 pg/ml, respectively. Determination of BDG by Glucatell test was of limited use in patients with IFI of *Mucor* spp., *Rhizopus* spp. and *Cryptococcus* spp. etiology. The level of this marker in samples collected only once was lower than 60 pg/ml. (Ostrosky-Zeichner *et al.*, 2005). Finally, an elevated levels of BDG has also been

reported in bronchoalveolar lavage fluids (BALF$_s$) from patients with *Pneuocystis carinii* pneumonia (Yasuoka *et al.*, 1996) and in Farmer's lung cases (Imai *et al.*, 2004). In the Farmer's lung cases an immunoprecipitation positive reactions against *Aspergillus fumigatus*, *A. terreus* and *Nocardiopsis alba* were also reported. It is worth to mention that BDG concentration in serum of the same patients was not exceeding levels typical for healthy people. In clinical trials it was shown that the concentration of BDG in the blood of healthy people is <10 pg/ml with the average of 2.7 pg/ml. In another multi-centre studies in the United States a concentration of BDG in sera of 170 individuals without proven IFI (control group) was ranging between 0 and 212 pg/ml and average level was 47.5 pg/ml. (Ostrosky-Zeichner *et al.*, 2005).

Although BDG are detected in the blood at the time of fungal infection, it is not clear what effects these β-glucans have on the body. In patients with confirmed IA significant rise of BDG concentration in serum was shown within 5 days, therefore the observed kinetics of liberated antigen *in vivo* points to the necessity of examining serum samples twice a week (Pazos *et al.*, 2005). In 100% of patients with proven and possible IA the presence of BDG was detected on average 9.3 days before detectable radiological changes in high resolution computed tomography (HRCT). Odabasi *et al.* (2004) reported that BDG was positive at a median of 10 days before the clinical diagnosis (range 32 days before and 2 days after) in patients with acute myelogenous leukemia (AML) or myelodysplastic syndrome (MDS) with proven or probable IFI including candidiasis, aspergillosis, fusariosis, and trichosporonosis (Odabasi *et al.*, 2004). On the other hand Kami *et al.* (2000) demonstrated that positive findings in chest CT scan preceded those of BDG assay by 11.5 days (range 7 to 40), and by 6.8 days (range 0 to 11) in patients with definite and suspected IPA, respectively (Kami *et al.*, 2000).

Kami *et al.* (2000) reported that the sensitivity of BDG assay was 88% in case of patients with disseminated IA, but it decreased to 38% in those with IA localised to the lung. With respect to this, detection of antigenemia in localised form of aspergillosis may be of little value in the early stage of diagnosing IPA (Kami *et al.*, 2000). In the same study at early stage the detection of circulating *Aspergillus* antigen in the blood revealed 10 and 100-fold higher sensitivity of the β-glucan assay in comparison with other commercial available immunoenzymatic (Platelia *Aspergillus*, Diagnostic Pasteur, France) and latex agglutination (LA; Pastorex *Aspergillus*, BioRad) assays. In patients with definite IPA (n = 16) BDG in plasma was positive at a median of 20.2 ± 12.9 days (range 0 to 53) after the first fever episode, while in patients with suspected IPA the presence of antigen was detected on average

26.2 ± 24.4 days (range 8 to 69). Similarly, Ostrosky-Zeichner *et al.* (2005) demonstrated significantly lower sensitivity of the Glucatell assay in 21 patients with probable IFI in comparison to 142 patients with proven IFI which was 42.9% and >80%, respectively.

BDG as a serological marker for monitoring of IFI during treatment

Recent clinical trials proved that the concentration of BDG in serum of adult hematological cancer patients with IFI, changes in relation to the patient's response to antifungal treatment with amphotericin B and/or caspofungin. Results of these observations are particularly interesting in patients with proven IA. In five patients with proven IA the monitored BDG antigen level corresponded with the response to the antifungal therapy. In patients with regression of the illness a decrease in serum BDG was noted with continuously high level of antigen in patients not responding to therapy. In the same study it was shown that the presence of BDG in the serum of 71.4% of patients was observed 4 to 25 days before the initiation of antifungal therapy. According to Pazos *et al.* (2005) BDG value reflects the extend of illness and it is useful for the judgement of cure effect. Another example is a study of Yasuoka *at al.* (1996) encompassing a group of 7 patients with *Pneumocistis carinii* (*jiroveci*) pneumonia and AIDS. In this study all follow up samples showed decreased BDG levels after antipneumocystis therapy.

Non-specific cross reactions

Detection of soluble antigens is a faster method than culture, but as in all immunological methods, there may be non-specific cross reaction. The final result of the serodiagnostic tests for the BDG presence may be influenced by several factors shown in Table I. The use of any of these products may lead to a false-positive BDG assay result. Lastly, Ishizuka *et al.* (2004) reported that administration of antitumor BDG preparations interfered with the BDG measurement for a long time, and the degree of interference differed among BDG measuring methods, so patients undergoing treatment with antitumor polysaccharides may present false-positive results.

In patients with haematological malignancies diagnosed as having IPA, the majority of false-positive results for BDG occurred during neutropaenia (65 of 865 samples; 7.5%) rather than during a period of normal neutrophil level (25 of 949 samples 2.6%). This may suggest the presence of a transient fungemia or *Aspergillus* antigenemia following cytotoxic chemotherapy and spontaneous clearance from the bloodstream with the recovery of the immune system (Kami *et al.*, 2000).

False positive results for the presence of BDG were noted in patients with *Escherichia coli* bloodstream infection (1 case) and superficial colonization with *Candida albicans* and *C. glabrata* (2 cases). The combined assessment of BDG and extent of colonization with *Candida* spp. is believed to have the advantage of lessening the likelihood of a false positive reaction of BDG (Takesue, 2004). This author suggests that the mono-utilization of BDG for the assessment of fungal infection should therefore be avoided. However, it has been reported that isolation of a number of bacteria in blood, as well as colonization by yeasts, did not produce BDG positive results by the FungiTec G test (Obayashi *et al.*, 1995). Pazos *et al.* (2005) and coworkers have presented data, which indicated that analysis of the kinetics of BDG levels helped in the identification of false-positive data since in these patients BDG levels showed abrupt rises and falls. In the opinion of authors the results obtained may be

Table I

Some reasons of false-positive and false-negative reactions

False positive results – probable causes:	References
● Presence of endotoxins in examined samples → affinity to glucan	
● Antitumor BDG preparation (lentinan and schizophyllan) → contain BDG	Ishizuka *et al.*, 2004
● Treatment of patients with immunological preparations (albumins or globulins)	Usami *et al.*, 2002
● [a]Use of cellulose membranes and filters made from cellulose in haemodialysis → BDG from cellulose filters had been eluted into blood components by filtration in the manufacturing process	Kanda *et al.*, 2001; Nagasawa *et al.*, 2003; Takesue, 2004
● Use of cotton gauze swabs/packs/pads and sponges during surgery	Nakao *et al.*, 1997
● β-glucans react with the factor G-mediated coagulation pathway in the limulus amebocyte lysate (LAL) method → activation of the LAL system	Vassallo, 1999
False negative results – probable cause:	
● Presence of gluconase enzyme in systemic fluids → breakdown of BDG in positive samples	Kawai *et al.,* 2005

[a] The use of cellulose membranes but not of cellulose triacetate or polymethyl-methacrylate membranes

questionable especially with no confirmation of IFI and exclusion of other causative factors of false positive reactions. In the same study it was shown that simultaneous detection of GM and BDG is evidently more effective in diagnosing IA than detection of each marker separately. In their study authors showed, that the presence of both antigens in the bloodstream at the same time allowed confirming IA. On the other hand, detection of only GM or BDG identified false positive results.

Conclusions

- Determination of $(1\rightarrow3)$-β-D-glucan concentration is an effective method of screening for invasive fungal infection caused by *Candida* spp., *Aspergillus* spp., *Fusarium* spp., *Trichosporon* spp., *Acremonium* spp., *Saccharomyces* spp.
- Limitation of screening for IFI by measurement of $(1\rightarrow3)$-β-D-glucan concentration are rare fungal infections of *Mucor* spp., *Rhizopus* spp., and *Cryptococcus* spp. etiology
- Repeated measurement serum or other body fluids level of $(1\rightarrow3)$-β-D-glucan are useful for evaluation of disease progression and therapeutic efficacy
- In order to improve sensitivity and specificity of the assay, parallel determination of both $(1\rightarrow3)$-β-D-glucan and galactomannan is recommended
- Further studies of the relationship between clinical data and measurements of $(1\rightarrow3)$-β-D-glucan should be continue.

Literature

Aibiki M., Ohtsubo, S. Nishiyama, T. Oka, H. Dote and Y. Shirakawa. 2005. Elevated serum $(1\rightarrow3)$-β-D-glucan level and depressed neutrophil phagocytosis in a heatstroke patient. *Resuscitation* 65: 115–117.

Battle J., T. Ha, C. Li, V. Della Beffa, P. Rice, J. Kalbfleisch, W. Browder and D. Williams. 1998. Ligand binding to the $(1\rightarrow3)$-β-D-glucan receptor stimulates NFkB activation, but not apoptosis in U937 cells. *Biochem. Biophys. Res. Commun.* 249: 499–504.

Cheung N-K.V., S. Modak, A. Vickers and B. Knuckles. 2002. Orally administered β-glucans enhance anti-tumour effects of monoclonal antibodies. *Cancer Immunol. Immunother.* 51: 557–564.

Digby J., J. Kalbfleisch, A. Glenn, A. Larsen, W. Browder and D. Williams. 2003. Serum glucan are not specific for presence of fungal infections in intensive care unit patients. *Clin. Diagn. Lab. Immunol.* 10: 882–885.

Engstad C.S., R.E. Engstad, J.O. Olsen and B. Osterud. 2002. The effect of soluble beta-1, 3-glucan and lipopolysaccharide on cytokine production and coagulation activation in whole blood. *Int. Immunopharmacol.* 2: 1585–1597.

Gonzalez J.A., J.D. Digby, P.J. Rice, K.F. Breuel, W.K. DePonti, J.H. Kalbfleisch, I.W. Browder and D.L. Williams. 2004. At low serum glucan concentrations there is an inverse correlation between serum glucan and serum cytokine levels in ICU patients with infections. *Int. Immunopharmacol.* 4: 1107–1115.

Hong F., R.D. Hansen, J. Yan, D.J. Allendorf, J.T. Baran, G.R. Ostroff and G.D. Ross. 2003. β-Glucan as an adjuvant for monoclonal antibody immunotherapy by recruiting tumoricidal granulocytes as killer cells. *Cancer Res.* 63: 9023–9031.

Horvath J.A. and S. Dummer. 1996. The use of respiratory-tract cultures in the diagnosis of invasive pulmonary aspergillosis. *Am. J. Med.* 100: 171–178.

Hossain M.A., T. Miyazaki, K. Mitsutake, H. Kakeya, Y. Yamamoto, K. Yanagihara, S. Kawamura, T. Otsubo, Y. Hirakata, T. Tashiro and S. Kohno. 1997. Comparison between Wako-WB003 and Fungitec G tests for detection of $(1\rightarrow3)$-β-D-glucan in systemic mycosis. *J. Clin. Lab. Anal.* 11: 73–77.

Imai K., Ashitani, J. Imazu, Y. Yanagi, S. Sano, A. Tokojima and M. Nakazato. 2004. Farmer's lung cases of a farmer and his son with high BAL fluid β-D glucan levels (in Japanese). *Nihon Kokyuki Gakkai Zasshi.* 42: 1024–1029.

Ishizuka Y., H. Tsukada and F. Gejyo. 2004. Interference of $(1\rightarrow3)$-β-D-glucan administration in the measurement of plasma $(1\rightarrow3)$-β-D-glucan. *Int. Med.* 43: 97–101.

Kami M., Y. Tanaka, Y. Kanda, S. Ogawa, T. Masumoto, K. Ohtomo, T. Matsumura, T. Saito, U. Machina and T. Kashima. 2000. Computed tomographic scan of the chest, latex agglutination test and plasma $(1\rightarrow3)$-β-D-glucan assay in early diagnosis of invasive pulmonary aspergillosis: A prospective study of 215 patients. H*aematologica* 85: 745–752.

Kanda H., K. Kubo, K. Hamasaki, Y. Kanda, A. Nakao, T. Kitamura, T.Fujita, K. Yamamoto and T. Mimura. 2001. Influence of various hemodialysis membranes on the plasma $(1\rightarrow3)$-β-D-glucan level. *Kidney Int.* 60: 319–323.

Kawai R., K. Igarashi, M. Yoshida, M. Kitaoka and M. Samejima. 2005. Hydrolysis of β-1,3/1,6-glucan by glycoside hydrolase family 16 *endo*-1,3(4)-β-glucanase from the basidiomycete *Phanerochaete chrysosporium. Appl. Microbiol. Biotechnol.*: X 1–9 (Epub ahead of print).

Kondori N., L. Edebo and I. Mattsby-Baltzer. 2004. Circulating β (1–3) glucan and immunoglobulin G subclass antibodies to *Candida albicans* cell wall antigens in patients with systemic candidiasis. *Clin. Diagn. Lab. Immun.* 11: 344–350.

Kougias P., D. Wei, P.J. Rice, H.E. Ensley, J. Kalbfleisch, D.L. Williams and W. Browder. 2001. Normal human fibroblasts express pattern recognition receptors for fungal $(1\rightarrow3)$-D-glucans. *Infect. Immun.* 69: 3933–3938.

Maertens J., J. Verhaegen, K. Lagrou, J. Van Elderle and M. Boogaerts. 2001. Screening for circulating galactomannan as a non-invasive diagnostic tool for invasive aspergillosis in prolonged neutropenic patients and steam cell transplantation recipients: a prospective validation. *Blood* 97: 1604–1610.

Martino R. and M. Subirá. 2002. Invasive fungal infection in haematology: new trends. *Ann. Hematol.* 81: 233–243.

Miura N.N., Y. Adachi, T. Yadomae, H. Tamura, S. Tanaka and N. Ohno. 2003. Structure and biological activities of β-glucans from yeast and mycelial forms of *Candida albicans. Microbiol. Immunol.* 47: 173–182.

Miyazaki T., S. Kohno, K. Mitsutake, S. Maesaki, K. Tanaka, N. Ishikawa and K. Hara. 1995. Plasma $(1\rightarrow3)$-β-D-glucan and fungal antigenemia in patients with candidemia, aspergillosis, and cryptococcosis. *J. Clin. Microbiol.* 33: 3115–3118.

Mueller A., J. Raptis, P.J. Rice, J.H. Kalbfleisch, R.D. Stout, H.E. Ensley, W. Browder and D.L. Williams. 2000. The influence of glucan polymer structure and solution conformation on binding to (1→3)-β-D-glucan receptors in a human monocytes-like cell line. *Glycobiology* 10: 339–346.

Mueller A., P.J. Rice, H.E. Ensley, P.S. Coogan, J.H. Kalbfleisch, J.L. Kelley, E.J. Love, C.A. Portera, T. Ha, I.W. Browder and D.L. Williams. 1996. Receptor binding and internalization of water-soluble (1→3)-β-D-glucan biologic response modifier in two monocytes/macrophage cell lines. *J. Immunol.* 156: 3418–3425.

Nagasawa K., T. Yano, G. Kitabayashi, H. Morimoto, Y. Yamada, A. Ohata, M. Usami, T. Horiuchi and D. Eng. 2003. Experimental proof of contamination of blood components by (1→3)-β-D-glucan caused by filtration with cellulose filters in the manufacturing process. *J. Artif. Organs* 6: 49–54.

Nakao A., M. Yasui, T. Kawagoe, H. Tamura, S. Tanaka and H. Takagi. 1997. False-positive endotoxemia derives from gauze glucan after hepatectomy for hepatocellular carcinoma with cirrhosis. *Hepatogastroenterology* 44: 1413–1418.

Obayashi T., M. Yoshida, T. Mori, H. Goto, A. Yasuoka, H. Iwasaki, H. Teshima, S. Kohno, A. Horiuchi and A. Ito. 1995. Plasma (1→3)-β-D-glucan measurement in diagnosis of invasive deep mycosis and fungal febrile episodes. *Lancet* 345: 17–20.

Odabasi Z., G. Mattiuzzi, E. Estey, H. Kantarjian, F. Saeki, R.J. Ridge, P.A. Ketchum, M.A. Finkelman, J.H. Rex and L. Ostrosky-Zeichner. 2004. β-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.* 39: 199–205.

Ostrosky-Zeichner L., B.D. Alexander, D.H. Kett, J. Vazquez, P.G. Pappas, F. Saeki, P.A. Ketchum, J. Wingard. R. Schiff, H. Tamura, M.A. et al. 2005. Multicenter clinical evaluation of (1→3)-β-D-glucan assay as an aid to diagnosis of fungal infections in humans. *Clin. Infect. Dis.* 41: 654–659.

Pazos C., J. Pontón and A. Del Palacio. 2005. Contribution of (1→3)-β-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.* 43: 299–305.

Reichenberger F., J.M. Habicht, A. Gratwohl and M. Tamm. 2002. Diagnosis and treatment of invasive pulmonary aspergillosis in neutropenic patients. *Eur. Respir. J.* 19: 743–755.

Ruhnke M., A. Böhme, D. Buchheidt, K. Donhuijsen, H. Einsele, R. Enzensberger, A. Glasmacher, H. Gumbel, C.P. Heussel, M. Karthus et al. 2003. Diagnosis of invasive fungal infections in haematology and oncology-guidelines of the Infectious Diseases Working Party (AGIHO) of the German Society of Hematology and Oncology (DGHO). *Ann. Hematol.* 82 (Suppl 2): 141–148

Sendid B., M. Tabouret, J.L. Poirot, D. Mathieu, J. Fruit and D. Poulain. 1999. New enzyme immunoassays for sensitive detection of circulating *Candida albicans* mannan and antimannan antibodies: useful combined test for diagnosis of systemic candidiasis. *J. Clin. Microbiol.* 37: 1510–1517.

Takebayashi H., A. Mizota and M. Tanaka. 2005. Relation between stage of endogenous fungal endophthalmitis and prognosis. *Graefes Arch. Clin. Exp. Ophthalm*ol. 6 1–5.

Takesue Y. 2004. Strategy for the treatment of fungal infections in critically ill surgical patients. *Nippon Ishinkin Gakkai Zasshi* (in Japanese). 45: 217–221.

Uchiyama M., N. Ohno, N.N. Miura, Y. Adachi, M.W. Aizawa, H. Tamura, S. Tanaka and T. Yadomae. 1999. Chemical and immunochemical characterization of limulus factor G-activating substance of *Candida* spp. *FEMS Immunol. Med. Microbiol.* 4: 411–420.

Upton A., W. Leisenring and K.A. Marr. 2006. (13) β-D-glucan assay in the diagnosis of invasive fungal infections. *Clin. Infect. Dis.* 42: 1054–1056.

Usami M., A. Ohata, T. Horiuchi, K. Nagasawa, T. Wakabayashi and S. Tanaka. 2002. Positive (1→3)-β-D-glucan in blood components and release of (1→3) β-D-glucan from depth-type membrane filters for blood processing. *Transfusion* 42: 1189–1195.

Vassallo R. 1999. Fungal β-glucan can yield false-positive results with the Limulus Amebocyte Lysate Endotoxin Assay. *Chest* 116: 583–584.

Verweij P.E. and J.F. Meis. 2000. Microbiological diagnosis of invasive fungal infections in transplant recipients. *Transpl. Infect. Dis.* 2: 80–87.

Vetvicka V., B.P. Thornton and G.D. Ross. 1996. Soluble β-glucan polysaccharide binding to the lectin site of neutrophil or natural killer cell complement receptor type 3 (CD11b/CD18) generates a primed state of the receptor capable of mediating of iC3b-opsonized target cells. *J. Clin. Invest.* 98: 50–61.

Willment J.A., S. Gordon and G.D. Brown. 2001. Characterization of the human β-glucan receptor and its alternatively spliced isoforms. *J. Biol. Chem.* 276: 43818–43824.

Wingard J.R. and H.A. Leather. 2004. New era of antifungal therapy. *Biol. Blood Marrow Transplant.* 10: 73–90.

Yasuoka A., N. Tachikawa, K. Shimada, S. Kiura and S. Oka. 1996. (1→3)-β-D-glucan as a quantitative serological marker for *Pneumocystis carinii* pneumonia. *Clin. Diagn. Lab. Immunol.* 3: 197–199.

Yeo S.F. and B. Wong. 2002. Current status of nonculture methods for diagnosis of invasive fungal infections. *Clin. Microbiol. Rev.* 15: 465–484.

Yoshida M., T. Obayashi, A. Iwama, M. Ito, S. Tsunoda, T. Suzuki, K. Muroi, M. Ohta, S. Sakamoto and Y. Miura. 1997. Detection of plasma (1–3)-β-D-glucan in patients with *Fusarium, Trichosporon, Saccharomyces*, and *Acremonium* fungaemias. *J. Med. Vet. Mycol.* 35: 371–374.

Yuasa K., H. Goto, M. Iguchi, T. Okamura and R. Ieki. 1996. Evaluation of the diagnostic value of the measurement of (1→3)-β-D-glucan in patients with pulmonary aspergillosis. *Respiration* 63: 78–83.