International Journal of Infectious Diseases 16 (2012) e2–e4

Contents lists available at SciVerse ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Perspective

# Is elevation of the serum β-D-glucan level a paradoxical sign for Trichosporon fungemia in patients with hematologic disorders?

Kazunori Nakase [a,b,*], Kei Suzuki [b], Taiichi Kyo [c], Tadahiro Kohara [d], Yumiko Sugawara [b], Naoyuki Katayama [b]

[a] Cancer Center, Mie University Hospital, 2-174 Edobashi, Tsu, Mie 514-8507, Japan
[b] Department of Hematology and Oncology, Mie University Graduate School of Medicine, Mie, Japan
[c] Fourth Department of Internal Medicine, Hiroshima Red Cross and Atomic-Bomb Survivors Hospital, Hiroshima, Japan
[d] Department of Clinical Laboratory, Hiroshima Red Cross and Atomic-Bomb Survivors Hospital, Hiroshima, Japan

ARTICLE INFO

*Article history:*
Received 16 June 2011
Received in revised form 19 August 2011
Accepted 26 September 2011

**Corresponding Editor:** Andy Hoepelman, Utrecht, the Netherlands

*Keywords:*
1,3-β-D-glucan
Trichosporon fungemia
Hematologic disorder

SUMMARY

The detection of serum 1,3-β-D-glucan (BDG) has been reported to be useful for the diagnosis and therapeutic monitoring of various invasive fungal infections. Although Trichosporon fungemia is increasingly recognized as a fatal mycosis in immunocompromised patients, the utility of this assay for Trichosporon fungemia is still unknown. In our experience (28 cases), the level of BDG rose in about half of the patients with hematologic disorders who developed Trichosporon fungemia. Among them, early death from this infection was more frequently seen in BDG-negative patients than in BDG-positive patients. In addition, overall survival was also significantly worse in BDG-negative patients than in BDG-positive patients. There were no significant differences between these two patient groups in terms of clinical background. Unlike for other invasive fungal infections, elevation of BDG level may indicate a paradoxical sign for Trichosporon fungemia in patients with hematologic disorders.



## 1. Introduction

1,3-β-D-glucan (BDG) is a polysaccharide component specific to the fungal cell wall and it circulates in the blood of patients with invasive fungal infections (IFIs).[1] Serum BDG is commonly elevated in Candida and Aspergillus infections, and may also be elevated, though this is rare, in infections due to cryptococci and zygomycetes.[2] Therefore, BDG assays have been reported to be useful for the early diagnosis and therapeutic monitoring of various IFIs.[1–3]

Invasive trichosporonosis is an extremely rare mycosis, but has been increasingly recognized in immunocompromised patients over the past three decades.[4–6] Trichosporon fungemia (TF) is particularly noted to be a fulminant and highly lethal infection.[4–7] However, little data are available on the usefulness of measuring serum BDG levels in such patients.[1,6] We retrospectively evaluated the clinical significance of BDG detection in patients with TF and hematologic disorders.

## 2. Patients and methods

### 2.1. Patients

We retrospectively reviewed the medical records of 28 consecutive patients with hematologic disorders and TF who were treated and examined for serum BDG levels at five hematology divisions (four belonging to tertiary care hospitals and one to the Mie University Hospital in Japan) during 2000–2010. These data were collected and analyzed at the Mie University Hospital. Patient age ranged from 13 to 85 years (mean 55 years), and all but one of the patients were male. The underlying diseases in these patients included acute myeloid leukemia (20 patients), acute lymphoblastic leukemia (two patients), chronic myeloid leukemia (two patients), non-Hodgkin's lymphoma (two patients), macroglobulinemia (one patient), and aplastic anemia (one patient). The *Trichosporon* species was identified on the basis of morphological characteristics, determined from slide cultures, and biochemical features, determined using the Vitek yeast biochemical card (bioMérieux Vitek Inc., Hazelwood, MO, USA) with or without API 20C Aux (bioMérieux, Marcy l'Etoile, France). In most patients, a surveillance culture for fungi was performed twice a week using throat swab, urine, and stools specimens. The serum levels of BDG were assayed using the beta-glucan test WAKO kit (Wako Pure Chemical Industries Ltd,

* Corresponding author. Tel.: +81 59 231 5296; fax: +81 59 231 5348.
*E-mail address:* k2nakase@clin.medic.mie-u.ac.jp (K. Nakase).

1201-9712/$36.00 – see front matter © 2011 International Society for Infectious Diseases. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.ijid.2011.09.017

**Table 1**
Comparison of clinical background in β-D-glucan-positive and β-D-glucan-negative patients

| Clinical background | BDG-positive ($n$ = 16), $n$ (%) | BDG-negative ($n$ = 12), $n$ (%) | $p$-Value |
|---|---|---|---|
| Age >60 years | 9 (56) | 4 (33) | 0.28 |
| Acute leukemia | 12 (75) | 10 (83) | 0.67 |
| Intensive chemotherapy | 14 (88) | 12 (100) | 0.49 |
| Allogeneic HSCT | 3 (19) | 2 (17) | 1.0 |
| Neutropenia | 14 (88) | 10 (83) | 1.0 |
| Neutrophil recovery | 5 (31) | 1 (8) | 0.2 |
| Hyperglycemia | 5 (31) | 7 (58) | 0.25 |
| Candida colonization | 6 (38) | 8 (67) | 0.25 |
| Bacteremia | 2 (13) | 1 (8) | 1.0 |
| Pneumonia | 11 (69) | 7 (58) | 0.45 |
| CVC removal | 2 (13) | 0 (0) | 0.49 |
| G-CSF administration | 15 (94) | 11 (92) | 1.0 |
| Breakthrough infection | 14 (88) | 11 (92) | 1.0 |
| Micafungin related | 11 (69) | 6 (50) | 0.44 |
| Antifungal treatment | | | |
| Amphotericin B only[a] | 4 (25) | 2 (17) | 0.67 |
| Azole only | 4 (25) | 3 (25) | 1.0 |
| Amphotericin B + azole | 6 (38) | 4 (33) | 1.0 |
| Attributable mortality | 11 (69) | 11 (92) | 0.2 |
| Early death within 10 days | 6 (38) | 11 (92) | 0.006 |

BDG, β-D-glucan; HSCT, hematopoietic stem cell transplantation; CVC, central venous catheter; G-CSF, granulocyte colony-stimulating factor.
[a] Two patients who received liposomal amphotericin B were included.

Osaka, Japan). In most patients, BDG was examined routinely once a week.

### 2.2. Infection prophylaxis and empiric therapy

When the neutrophil count was less than $0.5 \times 10^9$/l, most patients received prophylactic oral antibiotics (fluoroquinolone) and oral antifungals (fluconazole 200 mg/day, or itraconazole capsule 100–200 mg/day with amphotericin B 1200–2400 mg/day), and were housed in rooms equipped with a high-efficiency particulate air filtration system. For patients with a fever higher than 38 °C, broad-spectrum antibiotics (β-lactam with or without aminoglycoside, occasionally combined with glycopeptides) were given as an empiric antibacterial therapy. When high fever persisted despite the use of these antibiotics for 3 to 5 days, patients received an empiric antifungal therapy with intravenous micafungin (100–300 mg/day), amphotericin B (0.5–1 mg/kg per day), fluconazole (400 mg/day), or miconazole (600 mg/day), or a combination of these agents (amphotericin B plus a certain azole).

### 2.3. Definitions and statistical analysis

A patient was considered to have TF if at least one *Trichosporon* species was detected in the blood culture. We detected *Trichosporon beigelii* in 27 samples and *Trichosporon pullulans* in one sample. An elevation of serum BDG level was defined as a serum level higher than 11 pg/ml during the clinical course. Hyperglycemia was defined as a serum glucose level higher than 200 mg/dl, and renal dysfunction was defined as a serum creatinine level higher than 2 mg/dl. The manifestation of jaundice was regarded as liver dysfunction.

Correlations between the two groups were evaluated using Fisher's exact test. Patient survival was evaluated by Kaplan–Meier method, and the difference was evaluated by log-rank test. As all deaths caused by this infection occurred within 91 days from the date of diagnosis, the patients who were alive were censored at 100 days after diagnosis; the patients who died from an unrelated cause, including underlying disease progression, were censored at the time of death. The statistical significance level was defined as a $p$-value of $<0.05$. Data were analyzed using STATISTICA software (StatSoft, Tulsa, OK, USA).

## 3. Results

Serum BDG was positive in 16 of 28 patients (57%) during the clinical course. The mean value of its maximum in each of the BDG-positive patients was 182.5 pg/ml (range 15.0–1471.0 pg/ml). Clinical features of BDG-positive and the BDG-negative patients were compared (Table 1). There were no statistically significant differences between the two patient groups in terms of clinical background, including age, underlying disease, previous intensive chemotherapy, neutropenia at the onset, neutrophil recovery, hyperglycemia,[6] Candida colonization confirmed by surveillance cultures,[8] bacteremia,[9] pneumonia, removal of central venous catheter, administration of granulocyte colony-stimulating factor, breakthrough fungal infection, antifungal treatment, and mortality due to this infection.

Among BDG-positive patients, we assessed the presence of factors causing false-positive results, such as hemodialysis with cellulose membrane,[10] intravenous albumin, immunoglobulin,[11] or certain antibiotics (amoxicillin–clavulanate or piperacillin–tazobactam),[12] or use of glucan-containing gauze.[13] One patient with macroglobulinemia (BDG 75.4 pg/ml) received immunoglobulin, and one with aplastic anemia (BDG 1471.0 pg/ml) received albumin and was treated with hemodialysis. As for renal and/or liver failure possibly relating to the elevated BDG levels, renal dysfunction was observed in one patient with acute myeloid leukemia (creatinine 2.4 mg/dl, BDG 37.8 pg/ml) and in the patient with aplastic anemia (creatinine 5.6 mg/dl), who also had liver failure (total bilirubin 7.2 mg/dl). Breakthrough infection in patients receiving micafungin,[6,7] which is a BDG synthesis inhibitor,[14] was seen in 11 of 16 BDG-positive patients (69%) and in six of 12 BDG-negative patients (50%). This result did not indicate that BDG negativity was related to the previous use of micafungin. The responsiveness to azoles in the treatment of TF has been reported,[6,15,16] but therapy including azoles was performed at similar frequencies in both BDG-positive patients (10/16, 63%) and BDG-negative patients (7/12, 58%). However, early death within 10 days after the onset of TF was more frequently seen in BDG-negative patients (11/12, 92%) than in BDG-positive patients (6/16, 38%) ($p$ = 0.006). The overall survival was also noted to be significantly shorter in BDG-negative patients than in BDG-positive patients ($p$ = 0.029, Figure 1).

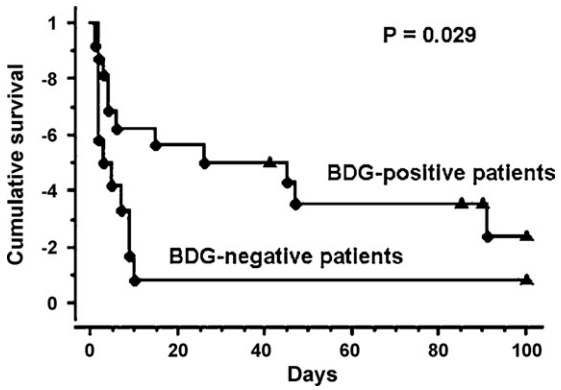


**Figure 1.** Overall survival in patients with Trichosporon fungemia according to the serum β-D-glucan level. Overall survival was significantly longer in patients who tested positive for β-D-glucan ($n = 16$) than in those who tested negative for β-D-glucan ($n = 12$) ($p = 0.029$).

## 4. Discussion

Invasive trichosporonosis is increasingly recognized as a mycosis with a poor prognosis in patients with hematologic disorders.[4–6] However, there have only been a few reports on the use of the BDG assay for patients with TF,[1,6] and its clinical significance is yet to be ascertained. It has been reported that the serum concentration of BDG is usually parallel to the amount of fungal burden in IFIs.[1–3,17] However, of note in this study is that BDG-negative patients had a higher mortality rate (within 10 days) ($p = 0.006$) and a worse survival than BDG-positive patients ($p = 0.029$). There were no significant differences between these two patient groups in terms of clinical background, supporting a close relationship between BDG positivity and patient prognosis. These results are paradoxical findings, unlike those for other IFIs, especially for invasive aspergillosis and candidiasis. In trichosporonosis, the fungal BDG synthetic system and the relevant host defense mechanism may differ from those in other IFIs. Additional studies involving more cases are needed to investigate this issue in more detail.

As for the possibility of false BDG positivity, the data of two patients (one with macroglobulinemia receiving immunoglobulin and one with aplastic anemia receiving albumin and hemodialysis) may be considered as such results. However, it is unlikely that the extremely high BDG level (1471.0 pg/ml) in the aplastic anemia patient could be explained only by factors other than the fungal burden. Since renal and/or liver function is related to the clearance and metabolism of the BDG molecule, such organ dysfunction appears to cause the elevated BDG level. In our series, apart from the aplastic anemia patient who was treated with dialysis and had liver dysfunction, one with acute myeloid leukemia displayed a renal insufficiency. However, the above mentioned macroglobulinemia patient and this acute myeloid leukemia patient died 3 days and 4 days after the onset of TF, respectively. If these two patients were included in the BDG-negative group, the survival difference between the two groups would become more distinct.

Some investigators have reported that BDG is recognized by the innate immune system and plays important roles in host defense.[18,19] Tissue macrophages and dendritic cells are known to express several BDG receptors including compliment receptor 3, lactosylceramide, scavenger receptors, and dectin-1;[19,20] these cells resist cytotoxic chemotherapy and retain functional capacity to protect the body against infection.[21,22] In addition, the administration of BDG has been shown to prevent infection due to various pathogens.[23] Therefore, the anti-infective properties of BDG may be associated with the better outcome, even in patients with hematologic disorders. Clarification of the role of BDG linked to the host protective system against *Trichosporon* species may contribute to the improvement of patient prognosis in this fatal fungal infection.

## Acknowledgements

The authors thank Drs E. Usui, A. Fujieda, K. Ohishi, T. Tsukada, and Y. Watanabe for providing important information, and Y. Matsushima and A. Nakamura for providing patient data.

*Ethical approval:* This study was approved by the institutional ethics review board at Mie University Hospital.

*Conflict of interest:* No conflict of interest to declare.

## References

1. Obayashi T, Yoshida M, Mori M, Goto H, Yasuoka A, Iwasaki H. Plasma (1→3)-beta-D-glucan measurement in diagnosis of invasive deep mycosis and fungal febrile episodes. *Lancet* 1995;**345**:17–20.
2. Koo S, Bryar JM, Page JH, Baden LR, Marty FM. Diagnostic performance of the (1→3)-beta-D-glucan assay for invasive fungal disease. *Clin Infect Dis* 2009;**49**: 1650–9.
3. Karageorgopoulos DE, Vouloumanou EK, Ntziora F, Michalopoulos A, Rafailidis PI, Falagas ME. β-D-glucan assay for the diagnosis of invasive fungal infections: a meta-analysis. *Clin Infect Dis* 2011;**52**:750–70.
4. Walsh TJ. Trichosporonosis. *Infect Dis Clin North Am* 1989;**3**:43–52.
5. Girmenia C, Pagano L, Martino B, D'Antonio D, Fanci R, Specchia G, et al. Invasive infections caused by *Trichosporon* species and *Geotrichum capitatum* in patients with hematological malignancies: a retrospective multicenter study from Italy and review of the literature. *J Clin Microbiol* 2005;**43**:1818–28.
6. Suzuki K, Nakase K, Kyo T, Kohara T, Sugawara Y, Shibazaki T, et al. Fatal Trichosporon fungemia in patients with hematologic malignancies. *Eur J Haematol* 2010;**84**:441–7.
7. Matsue K, Uryu H, Koseki M, Asada N, Takeuchi M. Breakthrough trichosporonosis in patients with hematologic malignancies receiving micafungin. *Clin Infect Dis* 2004;**42**:753–7.
8. Ellis M, Al-Ramadi B, Finkelman M, Hedstrom U, Kristensen J, Ali-Zadeh H, et al. Assessment of the clinical utility of serial β-D-glucan concentrations in patients with persistent neutropenic fever. *J Med Microbiol* 2008;**57**:287–95.
9. Albert O, Toubas D, Strady C, Cousson J, Delmas C, Vernet V, et al. Reactivity of (1→3)-β-D-glucan assay in bacterial bloodstream infections. *Eur J Clin Microbiol Infect Dis* 2011;**30**:1453–60.
10. Kato A, Takita T, Furuhashi M, Takahashi T, Maruyama Y, Hishida A. Elevation of blood (1→3)-beta-D-glucan concentration in hemodialysis patients. *Nephron* 2001;**89**:15–9.
11. Usami M, Ohta A, Horiuchi T, Nagasawa K, Wakabayashi T, Tanaka S. 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**: 1189–95.
12. Marty FM, Lowry CM, Lempitski SJ, Kubiak DW, Finkelman MA, Baden LR. Reactivity of (1→3)-beta-D-glucan assay with commonly used intravenous antimicrobials. *Antimicrob Agents Chemother* 2006;**50**:3450–3.
13. Nakao A, Yasui M, Kawagoe T, Tamura H, Tanaka S, Takagi H. False-positive endotoxemia derives from gauze glucan after hepatectomy for hepatocellular carcinoma with cirrhosis. *Hepatogastroenterology* 1997;**44**:1413–8.
14. Chandrasekar PH, Sobel JD. Micafungin: a new echinocandin. *Clin Infect Dis* 2006;**42**:1171–8.
15. Anaissie E, Gokaslan A, Hachem R, Rubin R, Griffin G, Robinson R. Azole therapy for trichosporonosis: clinical evaluation of eight patients, experimental therapy for murine infection, and review. *Clin Infect Dis* 1992;**15**:781–7.
16. Fournier S, Pavageau W, Feuillhade M, Deplus S, Zagdanski AM, Verola O. Use of voriconazole to successfully treat disseminated *Trichosporon asahii* infection in a patient with acute myeloid leukemia. *Eur J Clin Microbiol Infect Dis* 2002;**21**: 892–6.
17. Wiederhold NP, Najvar LK, Vallor AC, Kirkpatrick WR, Bocanegra R, Molina D. Assessment of serum (1→3)-β-D-glucan concentration as a measure of disease burden in a murine model of invasive pulmonary aspergillosis. *Antimicrob Agents Chemother* 2008;**52**:1176–8.
18. Goodridge HS, Wolf AJ, Underhill DM. Beta-glucan recognition by the innate immune system. *Immunol Rev* 2009;**230**:38–50.
19. Brown GD, Gordon S. Fungal β-glucans and mammalian immunity. *Immunity* 2003;**19**:311–5.
20. Cramer DE, Allendorf DJ, Baran JT, Hansen R, Marroquin J, Li B, et al. Beta-glucan enhances complement-mediated hematopoietic recovery after bone marrow-injury. *Blood* 2006;**107**:835–40.
21. Golde DW, Finley TN, Cline MJ. The pulmonary macrophage in acute leukemia. *N Engl J Med* 1974;**290**:875–8.
22. Nakase K, Tsuji K, Miyanishi E, Shirakawa S. Pathogenesis of fever of unknown origin in patients with acute leukemia and granulocytopenia. *Med Hypotheses* 1990;**33**:235–7.
23. Tzianabos AO. Polysaccharide immunomodulators as therapeutic agents: structural aspects and biologic function. *Clin Microbiol Rev* 2000;**13**:523–33.