Rhinology Online

RESEARCH ARTICLE

OPEN ACCESS

Early and noninvasive diagnosis using serological antigen biomarkers in chronic invasive fungal rhinosinusitis*

Ayuko Oba[1], Shin Ito[1], Hiroko Okada[1], Takashi Anzai[1], Ken Kikuchi[2], Katsuhisa Ikeda[1]

[1] Department of Otorhinolaryngology, Juntendo University Faculty of Medicine, Tokyo, Japan

[2] Department of Infectious Diseases, Tokyo Women's Medical University, Tokyo, Japan

Rhinology Online, Vol 3: 117 - 122, 2020

http://doi.org/10.4193/RHINOL/20.041

***Received for publication:**

May 14, 2020

Accepted: August 4, 2020

Published: August 15, 2020

Abstract

Background: Chronic invasive form of fungal rhinosinusitis (FRS) is characterized by the invasion of fungal organisms into the sinonasal mucosa in the background of diabetes mellitus and corticosteroid treat---ment. Although the histopathology has traditionally been used to make a proven diagnosis of invasive fungal infections, the dependence on tissue samples and the slow turnaround time hamper the early confirmation of such infections.

Methodology: This is a retrospective case series conducted over 6 years. All patients with a chronic course and immunosuppressive background of FRS diagnosed by radiologic imaging and treated with endoscopic sinus surgery were eligible for inclusion. Data were collected through medical records, including basic characteristics, symptoms and signs, imaging findings, laboratory investigations, pathology, treatment, and outcomes.

Results: Fifteen patients with chronic course and immunosuppressive background of FRS diagnosed by radiologic imaging were identified. High values of 1,3-β-D-glucan (BDG) assay were recognized in 5 patients, whereas the other 10 patients with negative findings in the BDG assay showed sinus mycetomas. All the 5 patients showing significant elevations of serum BDG showed positive findings in the polymerase chain reaction (PCR), but only 2 patients were positive in the histopathology. The findings of the BDG assay were consistent with those of the PCR method, which was superior in sensitivity to the histopathology.

Conclusion: We first applied BDG assay as a diagnostic tool for chronic invasive FRS. The BDG assay may be useful to distinguish chronic invasive FRS, including its early stage, from noninvasive mycetoma, contributing to timely treatment.

Key words: chronic invasive fungal rhinosinusitis, 1,3-β-D-glucan assay, polymerase chain reaction, histopathology

Introduction

The invasive forms of fungal rhinosinusitis (FRS) comprise 3 groups: acute, chronic, and chronic granulomatous[1-3]. Acute invasive FRS is well described by a disease course of less than 1 month and is characterized as an acute life-threatening condition in which the fungal infection invades underlying mucosal tissue, especially progressive vascular components, as a result of an impaired immune response. Chronic types of invasive FRS show a long and indolent clinical course usually lasting for more than 12 weeks and even decades. Granulomatous invasive FRS is a rare form of chronic invasive FRS that has been most commonly reported in Sudan, India, Pakistan and Saudi Arabia.

Chronic invasive FRS is a slow growing invasive fungal infection that is sometimes associated with orbital apex syndrome. This entity is characterized by the invasion of numerous fungal organisms into the sinonasal mucosa in the background of diabetes mellitus and corticosteroid treatment[4]. Chronic invasive FRS can be distinguished from the other two forms by the clinical course and background, whereas the early stage of the chronic invasive form is likely to resemble a sinus mycetoma based on the dense accumulation of hyphae[1]. Namely, the condition may begin as

a fungal ball and become invasive, presumably as a result of the immunosuppression.

Early diagnosis of invasive fungal infections is critical for choosing the appropriate surgical intervention and effective antifungal therapies. Histopathological evidence of fungal hyphae within the sinus mucosa is the gold standard for invasive FRS diagnosis. Although histopathology and culture techniques have traditionally been used to make a proven diagnosis of invasive fungal infections, their dependence on tissue samples and slow turnaround times hamper early confirmation of such infections[5]. The serologic detection of circulating antigen fungal biomarkers shows promise for improving the early diagnosis of invasive fungal infections. 1,3-β-D-glucan (BDG) is a fungal-cell wall polysaccharide released into the blood stream of patients with invasive aspergillosis, invasive candidiasis, and other invasive fungal infections, except invasive zygomycosis, cryptococcosis, and *Blastomyces dermatitidis*[6,7]. Therefore, the detection of BDG is a very interesting tool for the diagnosis of invasive fungal infections.

In this study, we assessed the diagnostic accuracy of the BDG assay for diagnosing chronic invasive FRS on the basis of histopathology and molecular detection using polymerase chain reaction (PCR).

Patients and Methods

This is a retrospective observational study conducted from 2012 to 2018 at the Department of Otorhinolaryngology of Juntendo University Hospital. All patients with a chronic course and immunosuppressive background of FRS diagnosed by radiologic imaging and treated with endoscopic sinus surgery were eligible for inclusion. Radiologic imaging characteristic for FRS includes calcification in the sinus on computed tomography (CT) and decreased signal intensities on T1- and T2-weighted magnetic resonance imaging (MRI). Subjects with allergic FRS were excluded from the present study, which was confirmed by the absence of specific IgE antibodies against fungi, such as *Alternaria, Aspergillus, Candida, Penicillium, Mucor*, and *Cladosporium*, *Pityrosporium*. Data were collected through medical records, including basic characteristics, predisposing factors, symptoms and signs, imaging findings, laboratory investigations, pathological results, treatment, and patient outcomes.

Before operation, serum BDG assay (Fungitec-G-Test MK II, Nissui Pharmacy, Japan) was performed. The cutoff value of 20 pg/ml was defined as the best diagnostic accuracy with Fungitec-G-Test assay[8]. The potential causes of false-positive results such as cellulose membrane used in hemodialysis, exposure to glucan-containing gauze, administration of blood products, high triglycerides, and intravenous amoxiciline-clavulanic acid antibiotics[9], which contain cross-reactive components or interfere with the Fungitec-G-Test assay, were not recognized in the patients of this study. The sinus contents were collected for fungal smear and cultivation (Sabouraud's agar) during surgery. Simultaneously, pathological sinus mucosa was taken for histopathologic evaluation and PCR testing. The histopathologic diagnosis was determined from the morphology using hematoxylin and eosin, periodic acid-Schiff, and Gomori's methenamine silver staining. DNA was extracted from the paraffin-embedded tissues. PCR amplification of fungal-specific ribosomal DNA was performed using ITS-1F and ITS-4R primer, the PCR product covering the end of 18SrDNA gene to the start of 26SrDNA gene and NL-1 and NL-4 primer, the PCR product covering D1/D2 26SrDNA according to our previous paper[10]. A universal fungal primer set designed by the National Institute for Occupational Safety and Health, FF2 and FR1 primers specific for the amplification of 18S rDNA was also used. Amplification was performed in a TaKaRa PCR Thermal Cycler Dice Gradient (TAKARA BIO INC, Japan) according to the manufacturer's specifications. PCR products were purified by High Pure PCR Product Purification Kit (Roche, Indianapolis, IN, USA). These amplifications were sequenced using ITS1F, ITS4R, NL1, NL4, FF2 and FR1 primers by a Big Dye Terminator V3.1 Cycle sequencing kit and ABI sequence analyzer 3730×l (ABI). Species identification was determined by BLAST search of DDBJ (DNA Data Bank of Japan).

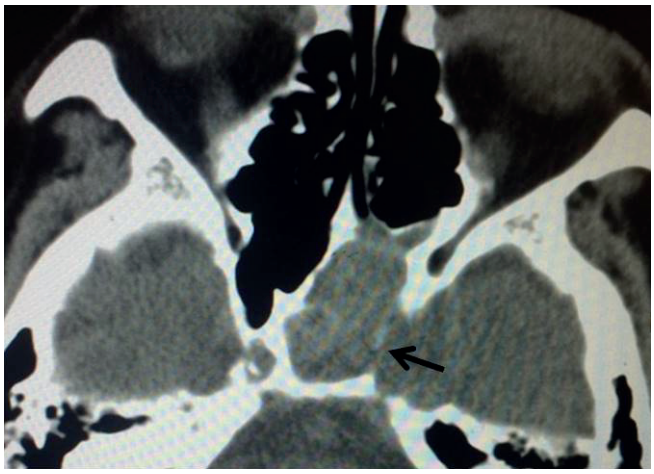

Figure 1. Axial view of computed tomography (case 1) showing extensive bone destruction of the lateral wall of left sphenoid sinus (an arrow).

Results

In total, 15 patients (10 females and 5 males, 50 to 85 years) were identified as having a chronic immunosuppressive background (10 patients with diabetes mellitus and 5 patients taking corticosteroid), and both the radiologic presentation and fungal material in the sinus were consistent with FRS. Corticosteroids administered to all the 5 patients were 2-10 mg predonisolone for more than 5 years, which is considered to have been a suf-

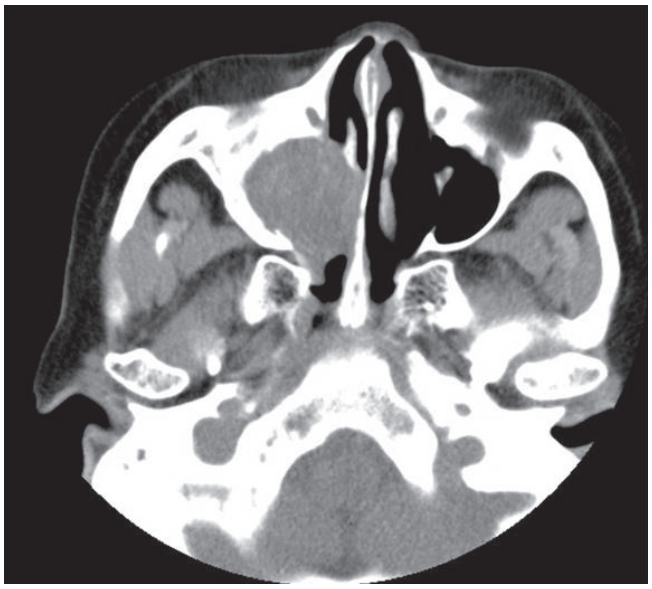

Figure 2. Axial view of computed tomography (case 2) showing bone erosion of the posterolateral and medial wall of right maxillary sinus.

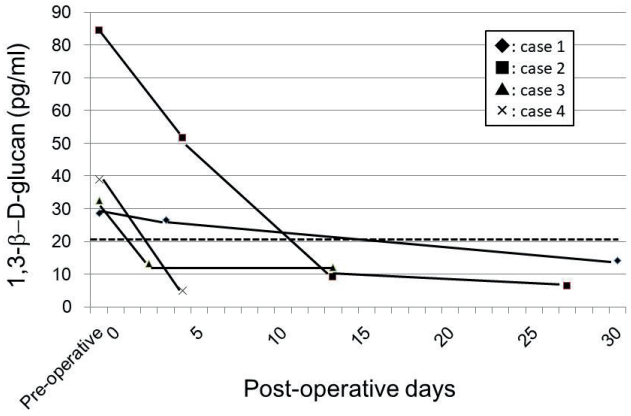

Figure 3. Time course of 1,3-β-D-glucan values before and after operation in cases 1 to 4. The dotted line shows the upper limit of the normal range.

ficient dosage to cause immunosuppression.

Two patients were suspected of chronic invasive FRS based on the clinical features and diagnostic imaging. The first patient (Case 1) showed unilateral visual disturbance, bone destruction by CT scan (Figure 1), and orbital involvement heralded by MRI finding of hypo-intense T2 signal, probably suggesting orbital apex syndrome. In the second patient (Case 2), CT scan showed bone erosion with extrasinus extension of the soft tissue (Figure 2).

Serum BDG levels > 20 pg/ml were detected in 5 out of the 15 patients. All 10 patients showing negative findings in the BDG assay were found to have sinus mycetomas (fungal balls), which were diagnosed by operative findings and histological evidence that there were no elements of invasion or granulomatous changes in the surrounding mucosa, bone or blood vessels. No fungi were recovered from the sinus content culture, whereas histologic preparations of sinus smears suggested the presence of the fungi *Aspergillus* sp. in all 15 patients. No PCR products of fungi were detected from inflamed mucosa of the mycetoma patients (n = 3).

Complete information of the 5 patients (cases 1-5) with positive findings and the 10 patients (cases 6-15) with negative findings in the BDG assay is summarized in Table 1. Case 1 was strongly suspected of orbital apex syndrome resulting from chronic invasive FRS. However, there were noticeable discrepancies among the histopathology, BDG data, and PCR results. Although there was no proven evidence for mucosal invasion of fungal hyphae in the histopathology, both the BDG assay and PCR method were compatible with the clinical features. Histopathology, BDG assay, and PCR test were consistent with the clinical features in case 2, suspected of chronic invasive FRS based on the CT findings. In case 3, no clinical features suggested chronic invasive FRS, whereas all three objective examinations supported the diagnostic criteria for invasive FRS. Cases 4 and 5, who showed no symptoms or signs of chronic invasive FRS without mucosal invasion of fungal hyphae, revealed positive findings of invasive FRS in the BDG assay and PCR. All the cases underwent surgical debridement of fungal hyphae and inflamed sinus mucosa under endoscopic sinus surgery; two cases received a broad-spectrum antifungal drug, oral voriconazole, for several weeks after the operation. There were no operative complications. Long-term outcome showed 4 cured cases, while one case died of a comorbidity. The serum BDG values returned to within the normal range about 4 to 16 days after the operation in 4 patients (Figure 3).

Discussion

Chronic invasive FRS is a relatively rare entity that is difficult to diagnose early and correctly due to low physician awareness of the disease, lack of specific symptoms, and the requirement of an invasive tissue biopsy[11]. Recent studies revealed a proclivity for occurrence in patients who are immunocompromised, often suffering from diabetes mellitus or taking corticosteroids long term[4,12]. In order to clarify unresolved problems related to chronic invasive FRS, we enrolled patients with chronic types of FRS associated with immunocompromised conditions.

The present study suggests i) that fungal biomarkers using BDG show promise for improving the early diagnosis of chronic invasive FRS, and ii) that certain sinus fungal balls progressively convert to chronic invasive FRS.

Table 1. Clinical features, mucosal histopathology, laboratory data, therapy, and outcome in fungal rhinosinusitis patients.

Comorbidities	Symptoms	Symptom duration (wk)	Affected sinuses	CT findings	BDG (pg/ml)	Histo pathology	PCR	Therapy	Outcome
DM	VD	40	SS	SO, IC, BD	26,7	(-)	*Tiletopsis albescens*	S+AFD	C
RA, PSL	PD	20	MS, ES	SO, IC, BE	84,3	(+)	*Scedosporium apiospernum*	S	C
DM	PD	16	MS, ES	SO, IC	32,6	(+)	*Aspergillus flavus*	S	C
RA, PSL	H	12	SS	SO, IC	39	(-)	*Candida parapsilosis*	S+AFD	C
IP, PSL	PD	100	MS	SO, IC	84,4	(-)	*Aspergillus fumigatus*	S	Died of IP
DM	PD, H	16	SS	SO, IC	<5.0	(-)	NP	S	C
SLE, PSL	PND	30	MS	SO, IC	<5.0	(-)	NP	S	C
DM	NC	25	MS	SO, IC	<5.0	(-)	NP	S	C
DM	NC	30	MS, ES, FS	SO, IC	19,5	(-)	Negative	S	C
ILD, PSL	NC, PND	22	MS	SO, IC	<5.0	(-)	Negative	S	C
DM	PD	35	MS. ES	SO, IC	<5.0	(-)	Negative	S	C
DM	PD	12	MS	SO, IC	5,6	(-)	NP	S	C
DM	PND	14	MS, ES	SO, IC	<5.0	(-)	NP	S	C
DM	PD	20	SS	SO, IC	16,7	(-)	NP	S	C
DM	PD	30	MS	SO, IC	<5.0	(-)	NP	S	C

CT = computed tomography; BDG = 1,3-β-D-glucan; PCR = polymerase chain reaction; F = female; DM = diabetes mellitus; RA = rheumatoid arthritis; PSL = predonisolone intake; IP = interstitial pneumonitis; VD = visual disturbance; PD = purulent nasal discharge; H = headache; SS = sphenoid sinus; MS = maxillary sinus; ES = ethmoid sinus; SO = sinus opacification; IC = intralesional calcification; BD = bone destruction; BE = bone erosion; (-) = no mucosal invasion of fungal hyphae; (+) = mucosal invation of fungal hyphae; NP = not performed; S = surgery; AFD = antifungal drug treatment; C = cured.

Although the results of multiple studies[9,13-15] have indicated that BDG assay is useful for diagnosing invasive fungal infections and is widely available for clinical use as a relatively noninvasive method, no reports have focused on the clinical use in invasive chronic FRS. In the present study, all 5 patients showing a significant elevation of serum BDG had positive findings by PCR indicating the mucosal invasion of fungi, but only 2 patients were positive in the histopathology. Therefore, the sensitivity of classic criteria using histopathology was only 40%, whereas that of the BDG assay was 100%. Despite the classification of FRS based on histopathological findings, approximately 40% of such cases were misdiagnosed and wrongly classified on the initial evaluation[16]. A recent study demonstrated that the PCR method was more sensitive than histopathology in diagnosing FRS, which was explained by the following reasons: i) tissue was not collected from the proper site, ii) the amounts of fungal elements were too low to be employed for special stains, iii) sparse fungal elements were missed during the formalin/wax processing for histopathology, and iv) inter-observer variability[17]. The findings of the BDG assay were consistent with those of the PCR method, which was superior to the histopathology in sensitivity. Thus, the BDG assay may be a promising tool for noninvasive diagnosis of chronic invasive FRS.

It is very interesting that in at least 2 patients (cases 4 and 5) there seemed to be fungal balls but were positive in both the BDG assay and PCR. These 2 patients may have had an early stage of the invasive form that had converted from the non-invasive form. Previous reports observed the co-existence of non-invasive and invasive foci in the same patients[16,18,19]. In other words, the BDG assay results might be interpreted as early signs of mucosal invasion of fungi in noninvasive FRS.

The BDG levels could be useful for monitoring the patient response to therapy; persistently high levels are associated with worse outcomes, which could facilitate the timely initiation of antifungal therapy[14,20]. We used antifungal drugs in 2 patients (cases 1 and 4) with fungal lesions in the sphenoid sinus. Case 1 was already accompanied by orbital apex syndrome, while case 4 was deeply concerned about worsening visual disturbance in the future. The restoration of the BDG levels to within a normal range after antifungal therapy in both patients was proof of a successful outcome. Furthermore, debridement surgery of inflamed sinus mucosa alone in cases 2 and 3 resulted in a reduction of the high BDG levels and good prognosis, suggesting that debridement surgery is sufficient to control the early stage of chronic invasive FRS.

Limitations of the present study include its retrospective design, small number of patients, lack of randomization, and limited clinical and laboratory data, which are shortcomings that limit the strength of our findings. However, we had 10 patients with chronic non-invasive FRS, which could serve as a kind of control group and would contribute to determining the specificity of the BDG assay. The BDG test is not organism-specific and does not detect several types of fungal infections, which require utilization in conjunction with other forms of fungal testing. Although serological antigen biomarkers of invasive fungal diseases are employed as part of the laboratory diagnostic criteria[5], the literature still relies strictly on tissue biopsy and analysis by traditional laboratory methods such as histopathology and fungal culture. The reliance on traditional methods hampers early diagnosis, preventing timely treatment. Thus, the use of available fungal biomarkers together with the application of molecular tests[21] in combination with clinical criteria may contribute to an earlier and more accurate diagnosis and improve the clinical outcome for chronic invasive FRS.

Conclusions

We first applied the BDG assay as a diagnostic tool for chronic invasive FRS. The BDG assay may be useful to distinguish chronic invasive FRS, including its early stage, from noninvasive mycetoma, contributing to early timely treatment.

Acknowledgments

None.

Conflict of interest

The authors have no conflict of interest to disclose.

Authorship contribution

Ayuko Oba, Ken Kikuchi, and Katsuhisa Ikeda designed the study, collected and analyzed the data, and drafted the manuscript. Shin Ito, Hiroko Okada, and Takashi Anzai collected and analyzed the data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

The study was approved by the ethics committee of the Juntendo University Faculty of Medicine. All patients provided written informed consent.

Availability of data and materials

The data of the pilot study is available within the manuscript.

Funding

The study has no funding.

References

1. deShazo RD, Chapin K, Swain RE. Fungal sinusitis. N Engl J Med. Jul 24 1997;337(4):254-259.
2. Montone KT. Pathology of Fungal Rhinosinusitis: A Review. Head Neck Pathol. Mar 2016;10(1):40-46.
3. Chakrabarti A, Denning DW, Ferguson BJ, et al. Fungal rhinosinusitis: a categorization and definitional schema addressing current controversies. Laryngoscope. Sep 2009;119(9):1809-1818.
4. D'Anza B, Stokken J, Greene JS, Kennedy T, Woodard TD, Sindwani R. Chronic invasive fungal sinusitis: characterization and shift in management of a rare disease. Int Forum Allergy Rhinol. Dec 2016;6(12):1294-1300.
5. Ambasta A, Carson J, Church DL. The use of biomarkers and molecular methods for the earlier diagnosis of invasive aspergillosis in immunocompromised patients. Med Mycol. Aug 2015;53(6):531-557.
6. Kedzierska A, Kochan P, Pietrzyk A, Kedzierska J. Current status of fungal cell wall components in the immunodiagnostics of invasive fungal infections in humans: galactomannan, mannan and (1-->3)-beta-D-glucan antigens. Eur J Clin Microbiol Infect Dis. Nov 2007;26(11):755-766.
7. Theel ES, Doern CD. β-D-glucan testing is important for diagnosis of invasive fungal infections. J Clin Microbiol. Nov 2013;51(11):3478-3483.
8. He S, Hang JP, Zhang L, Wang F, Zhang DC, Gong FH. A systematic review and meta-analysis of diagnostic accuracy of serum 1,3-beta-D-glucan for invasive fungal infection: Focus on cutoff levels. J Microbiol Immunol Infect. Aug 2015;48(4):351-361.
9. Tran T, Beal SG. Application of the 1,3-beta-D-Glucan (Fungitell) Assay in the Diagnosis of Invasive Fungal Infections. Arch Pathol Lab Med. Feb 2016;140(2):181-185.
10. Hirotsu M, Shiozawa A, Ono N, Miwa M, Kikuchi K, Ikeda K. Fungal extracts detected in eosinophilic chronic rhinosinusitis induced cytokines from the nasal polyp cells. Laryngoscope. Sep 2014;124(9):E347-353.
11. Li Y, Li Y, Li P, Zhang G. Diagnosis and endoscopic surgery of chronic invasive fungal rhinosinusitis. Am J Rhinol Allergy. Nov-Dec 2009;23(6):622-625.
12. deShazo RD, O'Brien M, Chapin K, Soto-Aguilar M, Gardner L, Swain R. A new classification and diagnostic criteria for invasive fungal sinusitis. Arch Otolaryngol Head Neck Surg. Nov 1997;123(11):1181-1188.
13. 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. Mar 15 2011;52(6):750-770.
14. Sims CR, Jaijakul S, Mohr J, Rodriguez J, Finkelman M, Ostrosky-Zeichner L. Correlation of clinical outcomes with beta-glucan levels in patients with invasive candidiasis. J Clin Microbiol. Jun 2012;50(6):2104-2106.
15. Tissot F, Lamoth F, Hauser PM, et al. beta-glucan antigenemia anticipates diagnosis of blood culture-negative intraabdominal candidiasis. Am J Respir Crit Care Med. Nov 1 2013;188(9):1100-1109.
16. Das A, Bal A, Chakrabarti A, Panda N, Joshi K. Spectrum of fungal rhinosinusitis; histopathologist's perspective. Histopathology. Jun 2009;54(7):854-859.
17. Singh AK, Gupta P, Verma N, et al. Fungal Rhinosinusitis: Microbiological and Histopathological Perspective. J Clin Diagn Res. Jul 2017;11(7):DC10-DC12.
18. Klapper SR, Lee AG, Patrinely JR, Stewart M, Alford EL. Orbital Involvement in Allergic Fungal Sinusitis. Ophthalmology. 1997;104(12):2094-2100.

19. Thakar A, Sarkar C, Dhiwakar M, Bahadur S, Dahiya S. Allergic fungal sinusitis: expanding the clinicopathologic spectrum. Otolaryngol Head Neck Surg. Feb 2004;130(2):209-216.
20. Koo S, Baden LR, Marty FM. Post-diagnostic kinetics of the (1 --> 3)-beta-D-glucan assay in invasive aspergillosis, invasive candidiasis and Pneumocystis jirovecii pneumonia. Clin Microbiol Infect. May 2012;18(5):E122-127.
21. Lamoth F, Calandra T. Early diagnosis of invasive mould infections and disease. J Antimicrob Chemother. Mar 1 2017;72(suppl_1):i19-i28.

Katsuhisa Ikeda, MD
2-1-1 Hongo
Bunkyo-ku
Tokyo 113-8421
Japan

E-mail: ike@juntendo.ac.jp