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ORIGINAL ARTICLE

Clinical features and cause analysis of false positive results of *Aspergillus* galactomannan assay in pulmonary cryptococcosis patients

Takahiro Takazono[1,2,3,4] · Tomomi Saijo[2] · Nobuyuki Ashizawa[2] · Kazuhiro Oshima[2] · Keitaro Nishimura[1] · Masato Tashiro[1] · Kazuko Yamamoto[2] · Yoshifumi Imamura[2] · Taiga Miyazaki[1,2] · Katsunori Yanagihara[5] · Hiroshi Mukae[2] · Koichi Izumikawa[1] · Donald C. Sheppard[3,4]

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Abstract

There have been conflicting reports of false positive galactomannan assay results in patients with systemic cryptococcosis. We sought to determine the frequency of GM positivity in patients with pulmonary cryptococcosis and confirm the source of this cross-reactivity in vitro. We conducted a retrospective study to elucidate the rate of galactomannan (GM) false positivity and cause in a cohort of 29 patients with pulmonary cryptococcal disease. The production of GM cross-reacting substances by clinical isolates and laboratory isolates of *C. neoformans* was tested in vitro. The mean serum GM index (Platelia *Aspergillus*) in patients with pulmonary cryptococcosis was 1.06, with 16 (55.2%) of patients having values above the positive cutoff value of 0.5. GM index values significantly decreased after treatment of cryptococcosis. There was no significant correlation between galactomannan and cryptococcal glucuronoxylomannan antigen (Eiken Latex test) results. Culture supernatants from clinical isolates and wild-type *C. neoformans* did not react in the GM assay; however, growth in the presence of 6% sodium chloride induced the production of cross-reacting GM antigens in culture supernatants from clinical isolates, wild type and a glucuronoxylomannan-deficient mutant of *C. neoformans*, but not in culture supernatants from a galactoxylomannan-deficient strain. Our results support the cross-reactivity of cryptococcal galactoxylomannan with the serum GM assay in vitro and in patients with pulmonary cryptococcal infection.

Keywords Galactomannan · Cryptococcosis · *Cryptococcus neoformans* · False positive · Aspergillus · Galactoxylomannan

Introduction

The Platelia™ *Aspergillus* assay is a commercially available enzyme-linked immunosorbent assay (ELISA) for the soluble *Aspergillus* galactomannan (GM) antigen that is used widely for the diagnosis of invasive Aspergillus infections [1]. However, a number of sources of false positivity have been identified. These include the use of antibiotics such as amoxicillin-clavulanate (AMP/CLAV) [2], cross-reaction with other lipoglycan from the bacterial species bifidobacterial lipoglycan [3], and the ingestion of soybean protein [4]. Although piperacillin-tazobactam (PIP/TAZ) use was historically associated with false positive GM assay results [5], recent studies have suggested that newer formulations of PIP/TAZ do not result in false positive GM results [6].

Cross-reaction of the GM assay with other pathogenic fungi has been reported including some isolates of the mold *Fusarium* and the dimorphic fungus *Histoplasma capsulatum* [7, 8]. There is conflicting evidence with respect to the cross-reactivity of [8] the GM assay with the pathogenic yeast, *Cryptococcus neoformans*. In an early study, a positive GM result was observed in a pulmonary cryptococcosis patient without evidence of *Aspergillus* infection. Further

✉ Takahiro Takazono
takahiro-takazono@nagasaki-u.ac.jp

1 Department of Infectious Diseases, Graduate School of Biomedical Sciences, Nagasaki University, Nagasaki, Japan

2 Second Department of Internal Medicine, Nagasaki University Hospital, Nagasaki, Japan

3 Departments of Medicine, Microbiology and Immunology, McGill University, Montréal, Québec, Canada

4 Infectious Diseases and Immunity in Global Health Program, Research Institute of the McGill University Health Centre, Montréal, Québec, Canada

5 Department of Laboratory Medicine, Nagasaki University Hospital, Nagasaki, Japan

investigations found that soluble antigens prepared from a range of clinical isolates of *Cryptococcus neoformans*, and purified capsular galactoxylomannan (GalXM), exhibited cross-reactivity in the *Aspergillus* GM assay [9]. However, a follow-up study not only failed to reproduce these findings with soluble antigen and purified GalXM but also found that serum and cerebrospinal fluid samples from patients with cryptotococcosis, and organ homogenates from mice with disseminated cryptococcosis also failed to react in the GM assay [10]. The reason underlying the discrepant results of these studies is not known.

To further explore the question of GM cross-reactivity during cryptococcal infection, we performed a single-center retrospective review of patients with pulmonary cryptococcosis who undergone GM testing.

Patients/materials and methods

A retrospective review of patients diagnosed with pulmonary cryptococcosis in the Second Department of Internal Medicine, Nagasaki University Hospital, between March 2008 and December 2014 was performed. Clinical information including diagnoses, culture results, antigen tests, and medication were collected from medical records. If the patients had undergone serial antigen tests, the specimens collected during diagnosis and the last post-treatment result were recorded. Platelia *Aspergillus* EIA (Bio-Rad, Tokyo, Japan) was used for GM assay testing. Briefly, patient samples were centrifuged at 10,000*g*, and an 100 μL of sample treatment solution (EDTA) was added to 300 μL of sample supernatant in 1.5-mL polypropylene tube and then heated in heat block at 100 °C for 3 min. The supernatant was used for the detection of the galactomannan antigen by enzyme immune assay, and the GM index was calculated using cutoff controls, according to the previous version of manufacturer's instruction. GM assays for in vitro experiments were performed according to the 2015 revised manufacturer's instructions, in which the sample heating step was changed to at 120 °C for 6 min.

Eiken Latex test (Eiken Kagaku Co., Tokyo, Japan) was used semi-quantitative detection of cryptococcal glucuronoxylomannan (GXM) antigen test in serum according to the manufacturers' instructions, and bronchoalveolar lavage (BAL) fluid samples (300 μL) were treated with pronase at 56 °C for 30 min, after which the reaction was stopped by heating at 100 °C for 5 min. Seventy-five microliters of this sample was mixed with anti-cryptococcal antibody-coated latex beads (25 μl) and mixed by rotation (125 ± 25 rev/min) for 10 min. A positive control (50 ng/ml of soluble capsular polysaccharide, isolated from the supernatant of cultured *C. neoformans* type A) and a negative control (sample diluent) were tested in each assay. All samples that tested positive were then subjected to twofold serial dilutions and assayed using the same procedures to determine the antigen titer. For BAL testing, fluid was centrifuged at 3000 rpm for 10 min. The cell-free supernatant was stored at − 80 °C and subsequently tested for GM assay and cryptococcal GXM antigen, and the pellet was used for culture and cytological examination. GM and GXM antigen testing were performed on the same day for all specimens.

Diagnosis of pulmonary cryptococcosis

Diagnosis of pulmonary cryptococcosis was confirmed by clinical background and compatible chest image findings with at least one of the three following criteria (1) positive serum GXM antigen, (2) positive culture in sputum or in BAL fluid, and (3) yeast cells exhibiting characteristic morphology of *C. neoformans* visualized on histopathological or cytological examination of lung biopsy tissue. Criteria described here matches proven or probable invasive fungal disease in definitions of Invasive Fungal Disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group [11].

Analysis of *Cryptococcus neoformans* capsule sizes

Capsule size was analyzed as previously reported [12]. Briefly, yeast cells were grown overnight for about 16 h in 10 mL of Sabouraud dextrose broth (Difco™ Cat. number 238230) at 30 °C, 150 rpm. Subsequently, they were centrifuged at 3500 rpm and pellets were washed twice using PBS. They were diluted to 5×10^6 cells/mL in Sabouraud dextrose broth and incubated for 24 h at 37 °C in tissue culture plates, stained with Indian ink, and observed by microscope. A minimum of five different fields were randomly chosen and photographed, and 40 to 60 cells were analyzed.

GM tests in *C. neoformans* culture supernatant

Cryptococcus neoformans clinical strains were incubated in Sabouraud dextrose broth alone or with sodium chloride (6.0%) for 48 h at 37 °C, 200 rpm. Experimental strains (H99, ACAP1, NE241, NE365) were incubated under 30 °C, as the NE365 does not grow under 37 °C. After centrifugation, supernatants were collected and measured with GM assay (Platelia *Aspergillus* EIA, Bio-Rad, Tokyo, Japan) in triplicate. The GXM-deficient ACAP1 (*Δcap59*) and its parent strain H99 were gifts from Dr. Jennifer K. Lodge, Washington University School of Medicine, USA [13]. The

Table 1 Summary of antigen, culture, and histological test results in pulmonary cryptococcosis cases

Case no.	Meningitis	GXM antigen in serum	GM antigen in serum	GXM antigen in BAL	GM antigen in BAL	BAL culture	BAL/TBLB cytology/pathology for *Cryptococcus*
1	–	64	0.538				
2	–	16	0.632	8	0.249	Negative	Positive
3	–	128	2.818	512	0.228	*Cryptococcus neoformans*	Positive
4	–	16	0.411				
5	–	8	1.871				
6	–	4	0.15	4	0.192	*Cryptococcus neoformans*	Positive
7	–	negative	1.07	1	0.151	*Cryptococcus neoformans*	Positive
8	–	256	2.391	4	0.091	*Cryptococcus neoformans*	Positive
9	–	512	1.296	16	0.193	Negative	Positive
10	–	8	0.347	1	0.094	Negative	Positive
11	–	64	1.487				
12	–	64	0.67				
13	–	negative	0.166	negative	0.188	*Cryptococcus neoformans*	Positive
14	–	512	2.257	128	0.075	*Cryptococcus neoformans*	Positive
15	–	32	6.255	8	1.561	*Cryptococcus neoformans*	Positive
16	–	64	2.033				
17	–	8	0.8	16	0.131	Negative	Positive
18	–	128	2.22				
19	–	512	0.062				
20	–	16	0.092				
21	–	64	0.532	16	0.213	*Cryptococcus neoformans*	Negative
22	–	8	0.327	4	0.091	Negative	Negative
23	+	256	0.57				
24	–	8	0.155	8	0.145	*Cryptococcus neoformans*	Positive
25	–	32	0.098	2	0.143	Negative	Positive
26	–	32	0.422	2	0.192	Negative	Positive
27	–	8	0.48				
28	+	> 1024	0.358				
29	–	32	0.364	2	0.209	*Cryptococcus neoformans*	Positive

GXM galactoxylomannan, *GM* galactomannan, *BAL* bronchoalveolar lavage, *TBLB* transbronchial lung biopsy

GalXM-deficient NE365 (*Δuge1*) mutant and its parent strain NE241 were gifts from Dr. Guilhem Janbon, Pasteur institute, France [14].

Results

Serum cross-reactivity with GM

Between 2008 and 2014, 40 patients with pulmonary cryptococcosis were identified. Serum GM assay testing was performed on 29 of these patients. Clinical characteristics of these patients are summarized in Table 1. All patients were non-HIV infected and had negative fungal blood cultures. No patients had neutropenia. Two patients were diagnosed with concomitant *Cryptococcus* meningitis. No patient died due to *Cryptococcus* infection.

The mean serum GM index of the 29 tested patients was 1.06 (min; 0.15, max; 6.255) and was positive in 16/29 (55.2%) patients using a cutoff value of 0.5. No patients exhibited microbiological, histopathological, or clinical evidence of *Aspergillus* spp. co-infection, and none had a history of PIP/TAZ or AMP/CLAV use, or other known cause of false positive GM reactivity. No correlation between the serum GM index and GXM antigen titer at the time of diagnosis was observed (Fig. 1). Other clinical information including age, sex, radiological findings, comorbidities, and medications are summarized in Table 2. There was no correlation of GM false positivity with type or size of pulmonary lesion, use of corticosteroids, immunosuppressants, or biological agents. However, univariate analysis identified female sex and rheumatologic diseases (rheumatoid arthritis, 5; polymyalgia rheumatica, 2; and takayasu arteritis, 1) as significantly more common in the GM false positive group.

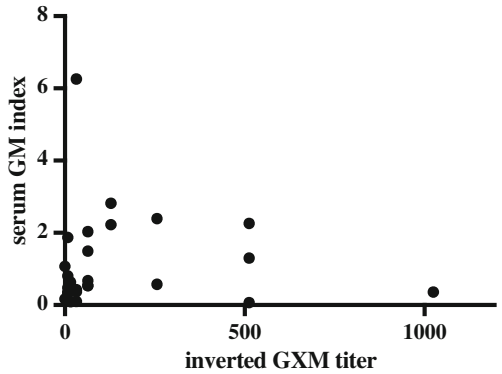

Fig. 1 Correlation between serum GM antigen and GXM antigen at the time of diagnosis of cryptococcal infection. No significant correlation was observed between serum galactomannan index and glucronoxylomannan antigen titers (Pearson $r = -0.013$, $P = 0.9459$)

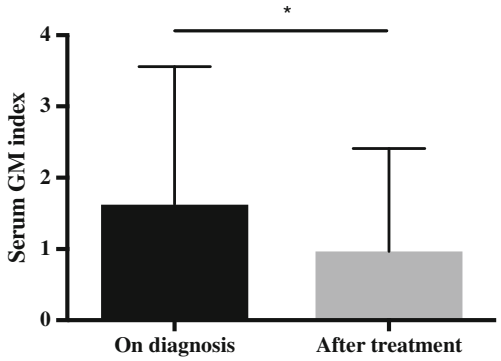

Fig. 2 Galactomannan (GM) antigen change following the treatment for cryptococcosis. GM index significantly decreased after fluconazole treatment for cryptococcosis ($n = 9$, $^*P = 0.0395$, paired t test). Average duration between tests was 15.6 months (range 7 to 43 months)

GM index change after treatment

Repeat GM assays were performed after the treatment completion in 9 of 29 patients with pulmonary cryptococcosis. Repeat testing was performed after 15.6 months on average (min; 7, max; 43 months) after diagnosis. All of these patients were treated with fluconazole, and in all cases, a significant reduction was observed after fluconazole treatment (Fig. 2), suggesting that false positive GM results were a consequence of a factor produced during cryptococcal infection.

GM index in BAL fluid

Bronchoscopy with bronchoalveolar lavage was performed in 17 of 29 patients in whom serum GM testing was performed. Surprisingly, the BAL GM index was significantly lower than the serum GM index in these 17 patients ($P = 0.0087$, paired t test).

Analysis of GM production in vitro by *Cryptococcus neoformans* clinical isolates and mutant strains

A total of 10 clinical strains were available for further study, five from patients with false positive serum GM (false positive strains), and five from patients with negative serum GM (negative strains). To determine if differences in capsule production could underlie the ability of these strains to produce substances that cross-react in the GM assay, strains were grown in Sabouraud dextrose broth and the capsule size compared. Although false positive strains exhibited a trend towards smaller capsular size, this difference was not statistically significant (Fig. 3a). None of the culture supernatants from strains grown in Sabouraud dextrose broth were reactive in the GM assay.

Transcriptional profiling studies have demonstrated that the in vivo expression of many cryptococcal genes is altered during growth in rich media in vitro. Most notably, growth in vivo is associated with the upregulation of genes associated with

Table 2 Clinical characteristics of pulmonary cryptococcosis patients

	GM-false positive patients ($n = 16$)	GM-negative patients ($n = 13$)	
Age	69.5 (± 12.5)	59.9 (± 17.2)	N.S.
Sex (M/F)	3/13	9/4	$P = 0.0061$
Consolidation/nodules	4/12	5/8	N.S.
Maximum size of pulmonary lesion (mm)	26.3 (± 19.3)	22.5 (± 16.7)	N.S.
White blood cell (/μl)	7143 (± 2143)	5838 (± 1625)	N.S.
C-reactive protein (mg/dl)	1.29 (± 2.02)	1.65 (± 3.73)	N.S.
Albumin/globulin ratio[a]	1.18 (± 0.22)	1.31 (± 0.33)	N.S.
Collagen diseases	8	1	$P = 0.02$
Diabetes mellitus	4	4	N.S.
Lymphoma	2	1	N.S.
Corticosteroid usage	8	4	N.S.
Immunosuppressant usage	3	1	N.S.
Biological agent usage	1	1	N.S.

Continuous numbers are presented as mean (± S.D.). Fisher's exact test and Mann-Whitney test are used for statistical analysis

[a] Data of albumin/globulin ratios were available in 11 and 9 cases, respectively

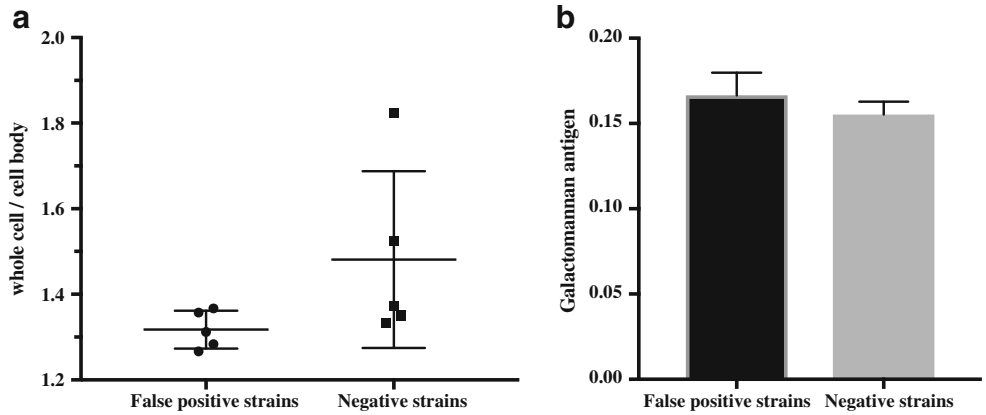

Fig. 3 Comparison of *Cryptococcus neoformans* strains associated with a positive serum GM test ($n = 5$) and those in which GM testing was negative ($n = 5$). **a** Capsule sizes of the indicated strains ($P = 0.15$, unpaired t test). **b** GM antigen testing from culture supernatants of the indicated after 48 h incubation in Sabouraud dextrose broth. No clinical strains produced significant amounts of GM antigen, and there was no significant difference observed between strains from patients with a GM-false positive or -negative test result

stress response [15]. We therefore tested the effects of osmotic stress on the production of compounds that cross-react with the GM assay by supplementing Sabouraud dextrose broth with NaCl. The addition of NaCl resulted in an increase in GM cross-reactivity in seven of ten clinical strains (Fig. 4a, $P = 0.0409$, paired t test). No difference was observed between false positive and negative strains.

To determine if the observed cross-reactivity in the GM assay was due to the production of GXM or GalXM, isogenic mutants deficient in production of each of these polysaccharides were compared with the H99 wild-type parent strain. As with clinical isolates, an increase in the production of GM cross-reacting substances was observed with the by wild-type *C. neoformans* H99 strain in the presence of NaCl (Fig. 4b). A similar increase in GM positivity was observed in response to NaCl when culture supernatants from the acapsular GXM-deficient Δ*cap59* mutant were tested. In contrast, culture supernatants from a GalXM-deficient *Δuge1* mutant of *C. neoformans* remained non-reactive in the GM assay even in the presence of NaCl.

Discussion

We report a high false positive rate of GM assay testing in patients with pulmonary cryptococcosis. Pre-existing rheumatologic disease was a risk factor of GM false positivity in this study, with a false positive GM index was observed in 18.2% of rheumatoid arthritis patients [16]. Although rheumatologic disease may result in the production of antibodies with the potential to interfere with the serologic testing, rheumatologic disease alone did not account for a significant proportion of false positive results. Eight of 20 (40%) patients with pulmonary cryptococcosis patients exhibited false positive GM tests in the absence of evidence of rheumatologic disease, suggesting another source for this cross-reaction. The lack of evidence for filamentous fungal infection and the decrease in GM index following fluconazole, which does not possess antifungal activity against filamentous fungi, suggests that this finding reflects the production of a cross-reacting antigen produced by *C. neoformans*. Further, our in vitro studies corroborate the ability of *C. neoformans* to produce antigens that

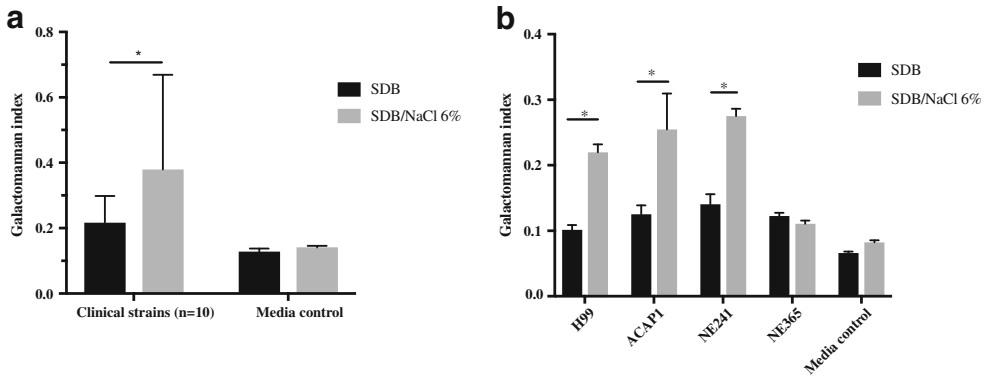

Fig. 4 Exposure of *C. neoformans* to NaCl induces the production of substances that cross-react in the galactomannan (GM). **a** Strains were grown in Sabouraud dextrose broth (SDB). Clinical isolates ($n = 10$) showed significantly higher galactomannan index in the presence of sodium chloride media. **b** Both wild-type strains (H99 and NE241) and ACAP1 (glucoronoxylomannan deficient) produced significantly higher GM index levels when grown in sodium chloride-containing media. NE365 (galactoxylomannan deficient) did not produce an increase in GM index when grown in in sodium chloride-containing media. *$P < 0.05$ by t test

cross-react in the GM assay under conditions of cell wall stress.

The EBA-2 antibody used by the GM assay reacts specifically with β(1,5)-galactofuranose oligosaccharides [17]. An early report suggested that small amounts of galactofuranose were present in GalXM and that GalXM was reactive in the GM assay [9]. However, a follow-up study failed to reproduce these findings, and a detailed compositional analysis of purified GalXM failed to identify β(1,5)-galactofuranose epitopes within this glycan. We found that culture supernatants from wild-type *C. neoformans*, but not a GalXM-deficient *Δuge1* mutant, cross-reacted in the GM assay. Although this observation is consistent with the hypothesis that GalXM is the source of GM-cross-, it is also possible that this mutant is also deficient in the production of other galactose-containing glycans. The *uge1* gene encodes a UDP-glucose/-galactose 4-epimerase which mediates the interconversion of glucose and galactose, and as a result, this strain is unable to synthesize galactose [14]. It is therefore possible that *C. neoformans* produces another galactan containing β(1,5)-galactofuranose epitopes that are recognized by the EBA-2 antibody. Co-purification of this galactan with GalXM could explain the original reports of EBA-2 recognition of GalXM. Detailed analysis of the secreted glycans produced by *C. neoformans* under conditions of cell wall stress should be performed to test this hypothesis.

This single-center retrospective study has several limitations. For 11 of 41 cases of pulmonary cryptococcosis, GM testing was not performed, and thus a selection bias cannot be excluded. In 2015, Bio-Rad Japan modified the sample preparation step for the Platelia *Aspergillus* assay, increasing the temperature of sample treatment from 100 to 120 °C in order to better dissociate immune complexes and to precipitate serum proteins that could interfere with the test. As clinical samples from patient were tested for GM prior to this protocol revision, some false positive GM results could have been a consequence of antibody complexes, particularly in patients with rheumatologic diseases. However, clinical isolates from these patients were still observed to produce GM cross-reacting substances in vitro using the new GM protocol, suggesting that differences in sample preparation do not account for the observations in this study.

Our findings suggest that clinicians should consider not only aspergillosis but the possibility of pulmonary cryptococcosis when interpreting positive serum GM assay results. Although the production of GM assay cross-reacting antigens by *C. neoformans* is dependent on growth conditions in vitro, the factors underlying the variability in the rates of serum GM index positivity among patients in this, and other studies, remain unclear. Further in vitro and clinical studies to elucidate the source of this variability are warranted.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The study was approved by the Nagasaki University School of Medicine Research Ethics Committee (Approval no. 16082210).

Informed consent As this study was a retrospective cohort study, written informed consent was not obtained. However, after approval from the ethics committee of our institute, we have shown the document of "Disclosure of information on clinical research" in our homepage to give the subjects opportunity to declare their will not to participate in this study.

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References

1. Maertens JA, Klont R, Masson C, Theunissen K, Meersseman W, Lagrou K, Heinen C, Crepin B, Van Eldere J, Tabouret M, Donnelly JP, Verweij PE (2007) Optimization of the cutoff value for the Aspergillus double-sandwich enzyme immunoassay. Clin Infect Dis 44(10):1329–1336
2. Zandijk E, Mewis A, Magerman K, Cartuyvels R (2008) False-positive results by the platelia Aspergillus galactomannan antigen test for patients treated with amoxicillin-clavulanate. Clin Vaccine Immunol 15(7):1132–1133
3. Mennink-Kersten MA, Ruegebrink D, Klont RR, Warris A, Gavini F, Op den Camp HJ, Verweij PE (2005) Bifidobacterial lipoglycan as a new cause for false-positive platelia Aspergillus enzyme-linked immunosorbent assay reactivity. J Clin Microbiol 43(8):3925–3931
4. Murashige N, Kami M, Kishi Y, Fujisaki G, Tanosaki R (2005) False-positive results of Aspergillus enzyme-linked immunosorbent assays for a patient with gastrointestinal graft-versus-host disease taking a nutrient containing soybean protein. Clin Infect Dis 40(2):333–334
5. Viscoli C, Machetti M, Cappellano P, Bucci B, Bruzzi P, Van Lint MT, Bacigalupo A (2004) False-positive galactomannan platelia Aspergillus test results for patients receiving piperacillin-tazobactam. Clin Infect Dis 38(6):913–916
6. Vergidis P, Razonable RR, Wheat LJ, Estes L, Caliendo AM, Baden LR, Wingard JR, Baddley J, Assi M, Norris S, Chandrasekar P, Shields R, Nguyen H, Freifeld A, Kohler R, Kleiman M, Walsh TJ, Hage CA (2014) Reduction in false-positive Aspergillus serum galactomannan enzyme immunoassay results associated with use of piperacillin-tazobactam in the United States. J Clin Microbiol 52(6):2199–2201
7. Tortorano AM, Esposto MC, Prigitano A, Grancini A, Ossi C, Cavanna C, Cascio GL (2012) Cross-reactivity of Fusarium spp. in the Aspergillus galactomannan enzyme-linked immunosorbent assay. J Clin Microbiol 50(3):1051–1053

8. Vergidis P, Walker RC, Kaul DR, Kauffman CA, Freifeld AG, Slagle DC, Kressel AB, Wheat LJ (2012) False-positive Aspergillus galactomannan assay in solid organ transplant recipients with histoplasmosis. Transpl Infect Dis 14(2):213–217
9. Dalle F, Charles PE, Blanc K, Caillot D, Chavanet P, Dromer F, Bonnin A (2005) Cryptococcus neoformans Galactoxylomannan contains an epitope(s) that is cross-reactive with Aspergillus galactomannan. J Clin Microbiol 43(6):2929–2931
10. De Jesus M, Hackett E, Durkin M, Connolly P, Casadevall A, Petraitiene R, Walsh TJ, Wheat LJ (2007) Galactoxylomannan does not exhibit cross-reactivity in the platelia Aspergillus enzyme immunoassay. Clin Vaccine Immunol 14(5):624–627
11. De Pauw B, Walsh TJ, Donnelly JP, Stevens DA, Edwards JE, Calandra T, Pappas PG, Maertens J, Lortholary O, Kauffman CA, Denning DW, Patterson TF, Maschmeyer G, Bille J, Dismukes WE, Herbrecht R, Hope WW, Kibbler CC, Kullberg BJ, Marr KA, Munoz P, Odds FC, Perfect JR, Restrepo A, Ruhnke M, Segal BH, Sobel JD, Sorrell TC, Viscoli C, Wingard JR, Zaoutis T, Bennett JE, European Organization for R, Treatment of Cancer/Invasive Fungal Infections Cooperative G, National Institute of A, Infectious Diseases Mycoses Study Group Consensus G (2008) Revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. Clin Infect Dis 46(12):1813–1821
12. Zaragoza O, Casadevall A (2004) Experimental modulation of capsule size in Cryptococcus neoformans. Biol Proced Online 6:10–15
13. Nelson RT, Hua J, Pryor B, Lodge JK (2001) Identification of virulence mutants of the fungal pathogen Cryptococcus neoformans using signature-tagged mutagenesis. Genetics 157(3):935–947
14. Moyrand F, Fontaine T, Janbon G (2007) Systematic capsule gene disruption reveals the central role of galactose metabolism on Cryptococcus neoformans virulence. Mol Microbiol 64(3):771–781
15. Chen Y, Toffaletti DL, Tenor JL, Litvintseva AP, Fang C, Mitchell TG, McDonald TR, Nielsen K, Boulware DR, Bicanic T, Perfect JR (2014) The Cryptococcus neoformans transcriptome at the site of human meningitis. MBio 5(1):e01087–e01013
16. Horie M, Tamiya H, Goto Y, Suzuki M, Matsuzaki H, Hasegawa WT, Noguchi S, Kawakami M, Matsuta K, Nagase T, Sakamoto Y (2016) Nonspecific elevation of serum Aspergillus galactomannan antigen levels in patients with rheumatoid arthritis. Respir Investig 54(1):44–49
17. Stynen D, Sarfati J, Goris A, Prevost MC, Lesourd M, Kamphuis H, Darras V, Latge JP (1992) Rat monoclonal antibodies against Aspergillus galactomannan. Infect Immun 60(6):2237–2245