ISHAM INTERNATIONAL SOCIETY FOR HUMAN AND ANIMAL MYCOLOGY

Medical Mycology, 2019, 0, 1–11
doi: 10.1093/mmy/myy158
Advance Access Publication Date: 0 2018
Original Article

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Original Article

Clinical value of (1,3)-β-D-glucan, mannan, antimannan IgG and IgM antibodies in diagnosis of invasive candidiasis

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Received 23 June 2018; Revised 3 November 2018; Accepted 2 January 2019; Editorial Decision 26 November 2018

Abstract

Diagnosis of invasive candidiasis (IC) is still challenging due to absence of specific clinical signs and symptoms. In this study we investigate the clinical value of (1,3)-β-D-glucan (BDG), mannan (MN), antimannan immunoglobulin G (AM-IgG), and antimannan immunoglobulin M (AM-IgM) assay in diagnosis of IC. During 2016 to 2018 serum samples from 71 patients with IC and 185 patients without IC were collected. Serum samples from 41 patients with bacteremia were also enrolled as additional control. Significant differences in mean serum biomarkers levels between IC and control group were observed. At low cutoff threshold the sensitivity and specificity of BDG (70 pg/ml), MN (50 pg/ml), AM-IgG (80 AU/ml), and AM-IgM (80 AU/ml) assay were 64.8% and 90.8%, 64.8 and 89.2%,74.6% and 87.0%, 57.7% and 60.0%, respectively. Combined use of BDG/MN, BDG/AM-IgG and MN/AM-IgG improved the sensitivity and specificity to 85.9% and 81.1%, 85.9% and 80.0%, 81.7% and 81.6%, respectively. The combination of BDG/MN, BDG/AM-IgG, or MN/AM-IgG may provide an encouraging approach for diagnosis of IC.

Key words: invasive candidiasis, (1,3)-β-D-glucan (BDG), mannan, antimannan antibody, diagnosis.

Introduction

Invasive fungal disease (IFD) is a life-threatening disease that affects millions of people worldwide. *Candida* species are one of the major pathogens that cause IFD and the estimated number of patients with life-threatening *Candida* infection are over 400 thousand per year.[1] Candidemia and deep-seated invasive candidiasis (IC) are the most common forms of invasive *Candida* infection. These infections are usually associated with high mortality ranging from 30% to 70%.[2,3] Early diagnosis of IC is critical in disease management as delay in antifungal treatment can increase the mortality rate by up to 30%.[3,4] However, diagnosis of IC is still challenging due to absence of specific clinical signs and symptoms. Positive blood culture is considered as gold standard for diagnosis of candidemia, but the sensitivity and turnaround time are limited.[5] (1,3)-β-D-glucan (BDG) assay provide an encouraging approach for diagnosis of IFD, but BDG is not a *Candida*-specific biomarker. Mannan is a polysaccharide antigen and *Candida* cell wall component that can be used as the potential *Candida*-specific biomarker for diagnosis of IC. Some studies reported the clinical value of *Candida* Mannan (MN), antimannan immunoglobulin G (AM-IgG) and antimannan immunoglobulin M (AM-IgM) assay in diagnosis of IC with mixed sensitivity (40% to 70%) and specificity (50% to 80%) depending on different study design and population.[6–9]

Despite the recent research achievements, there is still not enough clinical evidence to fully support the use of MN, AM-IgG, and AM-IgM in diagnosis of IC. In this study, we aim to evaluate the clinical performance of single and combining use of BDG, MN, AM-IgG, and AM-IgM in diagnosis of IC.

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Table 1. Baseline characteristics of the study patients.

Characteristic	Candidemia $n = 49$	Deep-seated IC[a] $n = 22$	Total[b] $n = 71$	Bacteremia $n = 41$	Disease Control $n = 89$	Disease Control+ Healthy Control $n = 185$
Male Gender no, (%)	31 (63.3)	16 (72.7)	47 (66.2)	22 (53.7)	53 (59.6)	100 (54.1)
Mean Age±SD, years	67 ± 12	59 ± 15	63 ± 14	59 ± 14	54 ± 16	44 ± 16
Age range, years	23–92	29–87	23–92	29–88	20–84	18–84
Underlying disease (%)						
Diabetes	5 (10.2)	4 (13.6)	8 (11.3)	1 (2.4)	7 (7.9)	
Hypertension	8 (16.3)	3 (13.6)	11 (15.5)	1 (2.4)	10 (11.2)	
Surgical operation	5 (10.2)	9 (40.9)	14 (19.7)	2 (4.9)	13 (14.6)	
Pancreatitis	4 (8.2)	1 (4.5)	5 (7.0)	1 (2.4)	3 (3.4)	
Cancer	8 (16.3)	11 (50)	17 (26.7)	4 (9.8)	5 (5.6)	
Urinary tract infection	4 (8.2)	1 (4.5)	5 (7.0)	1 (2.4)	0 (0.0)	
Obstructive jaundice	8 (16.3)	2 (9.1)	10 (14.1)	3 (7.3)	1 (1.1)	
Respiratory symptoms	7 (14.3)	2 (9.1)	9 (12.7)	3 (7.3)	0 (0.0)	

[a]IC, invasive candidiasis.
[b] Patients with candidemia or deep-seated IC.

Table 2. Baseline characteristics of the deep-seated IC group patients.[a]

Characteristic	No. (%)
Abdominal conditions	
Pancreatitis	1 (4.5)
Gastroduodenal perforation	2 (9.1)
Peritonitis	3 (13.6)
Cholecystitis	2 (9.1)
Invasive treatment[b]	
ERCP	2 (9.1)
PTCD	5 (22.7)
Abdominal surgery	9 (40.9)
Solid tumor	
Liver/Gall cancer	5 (22.7)
Lung cancer	2 (9.1)
Pancreatic/ glandular cancer	2 (9.1)
Other solid tumor	2 (9.1)
Fever	13 (59.1)
Broad spectrum antibiotics	21 (95.5)
Corticosteroids	2 (9.1)
Antifungal treatment	19 (86.3)
Candida isolated sites/fluid type	
Pleural fluid	3 (13.6)
Ascites	11 (50)
Bile	9 (40.9)
joint	1 (4.5)

[a]IC, invasive candidiasis.
[b]ERCP, endoscopic retrograde cholangiopancreatography; PTCD, percutaneous transhepatic cholangial drainage.

Methods

Study design

This single site retrospective case-control study was performed at PLA General Hospital (Beijing, China) between June 2016 and February 2018. Residual serum samples from routine examination of 49 patients with candidemia and 22 patients with deep-seated IC were collected. Residual serum samples from 89 patients who attended our hospital in study period but had no evidence of IC during their hospital stay were collected as disease control, and 96 healthy persons were used as healthy control. Forty-one patients with bacteremia were included as additional disease control to investigate the potential cross-reaction with these biomarkers. The study was approved by local ethics committee.

Patient enrollment and sample collection

The diagnostic criteria for candidemia were at least one positive blood culture that yielded a *Candida* spp. with related clinical symptom (e.g., fever, chills). Diagnosis of deep-seated IC required clinical signs of infection and at least one positive *Candida* culture from normal sterile body fluid (e.g., pleural fluid) or drainage fluid within 24 hours. All patients enrolled in this study for deep-seated IC group had negative blood culture for *Candida* spp. Diagnosis of bacteremia required isolation of bacteria from patients' blood samples with related clinical symptom. All patients enrolled in bacteremia group had negative *Candida* blood culture through their hospital stay.

Residual serum samples from routine examination were collected and stored at –80°C to determine the biomarker levels. All samples were thawed once for this study. For IC group 65 patients had at least one serum samples available within ±2 days of positive blood or body fluid culture. Two patients had their serum samples within 10 days prior to blood culture (day 10 and day 9, respectively). Serum samples from four patients were within 6 days after isolation of *Candida* spp. (day 5, day 6, day 4, and day 3, respectively). To calculate the sensitivity, specificity

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Table 3. Distribution of isolated *Candida* species.

No. (% of patients)	*Candida* species					
	C. albicans	*C. parapsilosis*	*C. tropicalis*	*C. glabrata*	*C. krusei*	Other *Candida*
Candidemia[a]	13(26.5)	18(36.7)	7(14.3)	10(20.4)	1(2.0)	2(4.0)
Deep-seated IC[b,c]	14(63.6)	1(4.5)	7(31.8)	2(9.0)	1(4.5)	2(9.0)

[a]Two patients had two different *Candida* species isolated.
[b]IC, invasive candidiasis.
[c]Five patients had two different *Candida* species isolated.

Table 4. Number of patients with positive BDG (cutoff value 70 or 95 pg/ml), MN (cutoff value 50 or 100 pg/ml), AM-IgG and AM-IgM (cutoff value 80 or 120 AU/ml) used alone for Candidiasis diagnosis.

Biomarker[a]	Cutoff value	Candidemia $n = 49$	Deep-seated IC[b] $n = 22$	Total $n = 71$	Bacteremia $n = 41$	Disease control $n = 89$	Disease control+ Healthy control $n = 185$
BDG (%)	≥70	38 (77.6)	8 (36.4)	46 (64.8)	7 (17.1)	13 (14.6)	17 (9.2)
	≥95	31 (63.3)	5 (22.7)	36 (50.7)	4 (9.8)	8 (9.0)	10 (5.4)
MN (%)	≥50	31 (63.3)	15 (68.2)	46 (64.8)	9 (22.0)	12 (13.5)	20 (10.8)
	≥100	25 (51.0)	11 (50.0)	36 (50.7)	6 (14.6)	10 (11.2)	14 (7.6)
AM-IgG (%)	≥80	38 (77.6)	15 (68.2)	53 (74.6)	12 (29.3)	14 (15.7)	24 (13.0)
	≥120	27 (55.1)	10 (45.5)	37 (52.1)	7 (17.1)	7 (7.9)	10 (5.4)
AM-IgM (%)	≥80	29 (59.2)	12 (54.5)	41 (57.7)	11 (26.8)	26 (29.2)	74 (40.0)
	≥120	22 (44.9)	9 (40.9)	31 (43.7)	8 (19.5)	18 (20.2)	53 (28.6)

[a]BDG, 1,3-β-D-glucan; MN, Mannan; AM-IgG, Anti-Mannan IgG; AM-IgM, AM-IgM, anti-mannan IgM.
[b]IC, invasive candidiasis.

Table 5. Number of patients with positive results from combined BDG (cutoff value 70 pg/ml), MN (cutoff value 50 pg/ml), AM-IgG and AM-IgM (cutoff value 80 AU/ml) in candidiasis diagnosis.

Biomarker[a,b] no. (%)	Candidemia $n = 49$	Deep-seated IC[c] $n = 22$	Total $n = 71$	Bacteremia $n = 41$	Disease control $n = 89$	Disease control+ Healthy control $n = 185$
BDG/MN	43 (87.8)	18 (81.8)	61 (85.9)	14 (34.1)	23 (25.8)	35 (18.9)
BDG/AM-IgG	45 (91.8)	16 (72.7)	61 (85.9)	17 (41.5)	24 (27.0)	37 (20.0)
BDG/AM-IgM	45 (91.8)	16 (72.7)	61 (85.9)	16 (39.0)	35 (39.3)	85 (45.9)
MN/AM-IgG	39 (79.6)	19 (86.4)	58 (81.7)	17 (41.5)	18 (20.2)	34 (18.4)
MN/AM-IgM	37 (75.5)	18 (81.8)	55 (77.5)	14 (34.1)	28 (31.5)	78 (42.2)
BDG/MN/AM-IgG	46 (93.9)	20 (90.9)	66 (93.0)	20 (48.8)	28 (31.5)	47 (25.4)
MN/AM-IgG/AM-IgM	40 (81.6)	20 (90.9)	60 (84.5)	21 (51.2)	33 (37.1)	87 (47.0)
BDG/MN/AM-IgG/AM-IgM	47 (95.9)	21 (95.5)	68 (95.8)	24 (58.5)	41 (46.1)	96 (51.9)

[a]BDG, 1,3-β-D-glucan; MN, mannan; AM-IgG, anti-mannan IgG; AM-IgM, anti-Mannan IgM.
[b] Parallel combination, at least one of the combined biomarkers is positive, presented as biomarker/biomarker.
[c]IC, invasive candidiasis.

and mean biomarker levels we only use the data from serum samples that were closest to the day of positive blood or body fluid culture.

Biomarker measurement

All tests were performed according to the manufacturer's recommendation at the hospital clinical laboratory. Dynamiker Fungus (1,3)-β-D-glucan assay (Dynamiker Biotechnology Co., Ltd, China) was used to measure serum BDG level. This assay required 20 μl of serum per sample and the cutoff value was 95 pg/ml (high cutoff threshold). BDG level between 70 and 95 pg/ml were considered indeterminate and level below 70 pg/ml (low cutoff threshold) were negative.

Serum AM, AM-IgG, and AM-IgM levels were determined by using Plate ELISA kit (Dynamiker *Candida* Mannan assay,

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Table 6. Diagnostic performance of single and combined BDG (cutoff value 70 pg/ml), MN (cutoff value 50 pg/ml), AM-IgG and AM-IgM (cutoff value 80 AU/ml) for candidiasis diagnosis.

	Sensitivity % (95% CI)			Specificity % (95% CI)	
	Candidemia ($n = 49$)	Deep-Seated IC[d] ($n = 22$)	Total ($n = 71$)	Bacteremia ($n = 41$)	Disease control+ Healthy control ($n = 185$)
BDG[a]	77.6 (65.9–89.2)	36.4 (16.3–56.5)	64.8 (53.7–75.9)	82.9 (71.4–94.4)	90.8 (86.6–95.0)
MN[a]	63.3 (49.8–76.8)	68.2 (48.7–87.6)	64.8 (53.7–75.9)	78.0 (65.4–90.7)	89.2 (84.7–93.7)
AM-IgG[a]	77.6 (65.9–89.2)	68.2 (48.7–87.6)	74.6 (64.5–84.8)	70.7 (56.8–84.7)	87.0 (82.2–91.9)
AM-IgM[a]	59.2 (45.4–72.9)	54.5 (33.7–75.4)	57.7 (46.3–69.2)	73.2 (59.6–86.7)	60.0 (52.9–67.1)
BDG/MN[b]	87.8 (78.6–96.9)	81.8 (65.7–97.9)	85.9 (77.8–94.0)	65.9 (51.3–80.4)	81.1 (75.4–86.7)
BDG/AM-IgG[b]	91.8 (84.2–99.5)	72.7 (54.1–91.3)	85.9 (77.8–94.0)	58.5 (43.5–73.6)	80.0 (74.2–85.8)
BDG/AM-IgM[b]	91.8 (84.2–99.5)	72.7 (54.1–91.3)	85.9 (77.8–94.0)	61.0 (46.0–75.9)	54.1 (46.9–61.2)
MN/AM-IgG[b]	79.6 (68.3–90.9)	86.4 (72.0–100)	81.7 (72.7–90.7)	58.5 (43.5–73.6)	81.6 (76.0–87.2)
MN/AM-IgM[b]	75.5 (63.5–87.6)	81.8 (65.7–97.9)	77.5 (67.7–87.2)	65.9 (51.3–80.4)	57.8 (50.7–65.0)
BDG/MN/AM-IgG[b]	93.9 (87.2–100)	90.9 (78.9–100)	93.0 (87.0–98.9)	51.2 (35.9–66.5)	74.6 (68.3–80.9)
Mn/AM-IgG/AM-IgM[b]	81.6 (70.8–92.5)	90.9 (78.9–100)	84.5 (76.1–92.9)	48.8 (33.5–64.1)	53.0 (45.8–60.2)
BDG/MN/AM-IgG/AM-IgM[b]	95.9 (90.4–100)	95.5 (86.8–100)	95.8 (91. —100)	41.5 (26.4–56.5)	48.1 (40.9–55.3)
BDG+MN[c]	53.1 (39.1–67.0)	22.7 (5.2–40.2)	43.7 (32.1–55.2)	95.1 (88. —100)	98.9 (97.4–100)
BDG+AM-IgG[c]	63.3 (49.8–76.8)	31.8 (12.4–51.3)	53.5 (41.9–65.1)	95.1 (88.5–100)	97.8 (95.7–99.9)
BDG+AM-IgM[c]	44.9 (31.0–58.8)	18.2 (2.1–34.3)	36.6 (25.4–47.8)	95.1 (88.5–100)	96.8 (94.2–99.3)
MN+AM-IgG[c]	61.2 (47.6–74.9)	50.0 (29.1–70.9)	57.7 (46.3–69.2)	90.2 (81.2–99.3)	94.6 (91.3–97.9)
MN+AM-IgM[c]	46.9 (33.0–60.9)	40.9 (20.4–61.5)	45.1 (33.5–56.6)	85.4 (74.5–96.2)	91.4 (87.3–95.4)
BDG+MN+AM-IgG[c]	53.1 (39.1–67.0)	22.7 (5.2–40.2)	43.7 (32.1–55.2)	100.0 (99.7–100)	98.9 (97.4–100)
MN+AM-IgG+ AM-IgM[c]	44.9 (31.0–58.8)	27.3 (8.7–45.9)	39.4 (28.1–50.8)	95.1 (88.5–100)	95.1 (92.0–98.2)
BDG+MN+AM-IgG+AM-IgM[c]	38.8 (25.1–52.4)	9.1 (0–21.1)	29.6 (19.0–40.2)	100.0 (99.7–100)	98.9 (97.4–100)

[a]BDG, 1,3-β-D-glucan; MN, mannan; AM-IgG, anti-mannan IgG; AM-IgM, anti-mannan IgM.
[b]Parallel combination, at least one of the combined biomarkers is positive, presented as biomarker/biomarker.
[c]Consecutive combination, all combined biomarkers are positive, presented as biomarker+biomarker.
[d]IC, invasive candidiasis.

Dynamiker *Candida albicans* IgG assay and Dynamiker *Candida albicans* IgM assay, Dynamiker Biotechnology Co., Ltd, China). Briefly, 300 μl of serum were used for MN measurement. The high and low cutoff threshold of MN assay were 100 pg/ml and 50 pg/ml, respectively. For AM-IgG and AM-IgM measurement 1 μl and 1.5 μl of serum was used. The high and low cutoff threshold were 120 AU/ml and 80 AU/ml, respectively.

The optical density (OD) values were obtained by using Plate reader from TECAN. The serum concentration of biomarkers was calculated by using specific software provided by the manufacturer.

Statistical analysis

Statistical analysis was performed by using SPSS 18.0 (SPSS Inc., Chicago, IL, USA) and Graphpad Prism 5 (Graphpad Software, Sab Diego, CA, USA). For comparison of different values and percentage, Mann-Whitney *U* test, Kruskal-Wallis test, and Fisher exact test were used. A *P* value of $<.05$ was considered statistically significant. The optimal cutoff values and area under curve (AUC) were calculated by receiver operating characteristic (ROC) analysis.

Results

Study population and *Candida* isolation

Total of 75 *Candida* spp. were isolated from 71 patients with IC in this study. For patients with candidemia *C. parapsilosis* was the most isolated *Candida* spp. (36%), followed by *C. albicans* (26%), *C. glabrata* (20%), *C. tropicalis* (14%), *C. krusei,* and *C. guilliermondii* (both 2%). For patients with deep-seated candidiasis, 56% of isolated *Candida* spp. were *C. albicans*, followed by *C. tropicalis* (28%), *C. glabrata* (8%), *C. parapsilosis* (4%), and *C. krusei* (4%). The details of patient demographics and *Candida* isolation are shown in Tables 1, 2, and 3.

Diagnostic performance

The diagnostic performance of BDG, MN, AM-IgG, and AM-IgM assay were demonstrated in Tables 4, 5, and 6, and Fig. 2. The mean BDG, MN AM-IgG, and AM-IgM concentration in IC

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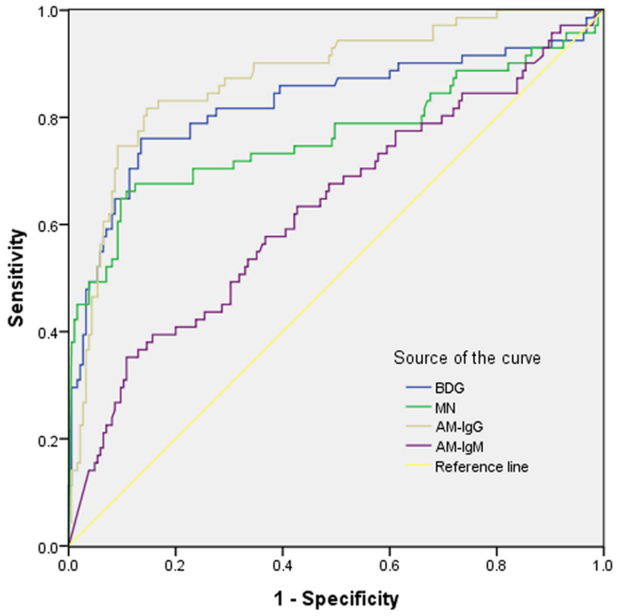

Figure 1. The ROC curves (total IC vs total control, $n = 185$) of the BDG, MN, AM-IgG, and AM-IgM assay for the diagnosis of Candidiasis. The receiver operating characteristic (ROC)-recommended cutoff value was 53.7 pg/ml for BDG. At this cutoff, sensitivity for BDG was 76.1% and specificity 86.5%, area under the curve (AUC) was 0.825 (95% CI, 0.757–0.893). The ROC-recommended cutoff value was 48.7 pg/ml for MN. The sensitivity for MN was 66.2% and specificity 89.2%, the AUC was 0.765 (95% CI, 0.687–0.844). The ROC-recommended cutoff value was 87.1 AU/ml for AM-IgG. At this cutoff, the sensitivity for AM-IgG was 74.6% and specificity 90.8%, the AUC was 0.874 (95% CI, 0.824–0.924). The ROC-recommended cut off value was 206.8 AU/ml for AM-IgM. At this cutoff, the sensitivity for AM-IgM was 12.7% and specificity 93.0%, the AUC was 0.454 (95% CI, 0.370–0.538). Abbreviations: AM-IgG, Anti-Mannan IgG; AM-IgM, Anti-Mannan IgM; BDG, 1,3-β-D-glucan; CI, confidence interval; IC, invasive candidiasis; MN, mannan.

group were 242 pg/ml, 244 pg/ml, 148 AU/ml, and 175 AU/ml, respectively. Compared with the total control group (disease control + health control), the concentration of all biomarkers significantly elevated in IC group. At high cutoff threshold (indeterminate results were considered as negative) the sensitivity and specificity of BDG, MN, AM-IgG, and AM-IgM were 50.7% and 94.6%, 50.7% and 92.4%, 52.1% and 94.6%, 43.7% and 71.4%, respectively. The sensitivity and specificity of BDG, MN AM-IgG, and AM-IgM at low cutoff were 64.8% and 90.8%, 64.8% and 89.2%, 74.6% and 87.0%, 57.7% and 60.0%, respectively. Significant improvements in sensitivity of all four biomarkers were shown at low cutoff threshold setting compared with that at high cutoff threshold, and the decrease in specificity were marginal (Table 4). Results from ROC analysis were demonstrated in Fig. 1. ROC analysis revealed that the AUC of IC group versus total control group for BDG, MN, AM-IgG, and AM-IgM were 0.825, 0.765, 0.874, and 0.454, respectively. The optimized cutoff for BDG, MN, AM-IgG and AM-IgM were 53.7 pg/ml, 48.7 pg/ml, 87.1 AU/ml, and 206.8 AU/ml, respectively. With the optimized cutoff the sensitivity and specificity of BDG, MN, AM-IgG, and AM-IgM were 76.1% and 86.5%, 66.2% and 89.2%, 74.6% and 90.8%, 12.7% and 93.0%, respectively.

Sensitivity of serological assay in candidemia and deep-seated IC group

The mean BDG value in candidemia group (313 pg/ml) was significantly higher than that in deep-seated IC group (83 pg/ml, $P = .0043$). There were no significant difference of mean MN, AM-IgG, and AM-IgM values between candidemia and deep-seated IC group, as demonstrated in Fig. 3. Noticeably, the sensitivity of BDG in candidemia group was also lower than that in deep-seated IC group (77.6% vs 36.4%, $P < .005$), while the sensitivity of MN, AM-IgG, and AM-IgM between candidemia group and deep-seated IC group were similar (P value of .79, .55, and .79, respectively).

Combination of BDG, MN, AM-IgG and AM-IgM in diagnosis of IC

Parallel combine use (at least one biomarker was positive) of BDG, MN, AM-IgG, and AM-IgM improved the sensitivity in diagnosis of IC (Tables 5 and 6). The combination of BDG and MN, BDG and AM-IgG increased the sensitivity to 85.9% with slightly decreased specificity of 81.1% and 80%, respectively. The sensitivity and specificity of combined MN and AM-IgG were 81.7% and 81.6%, respectively. When combining BDG, MN, and AM-IgG together the sensitivity increased to 93% but the specificity decreased to 74.6%. Adding AM-IgM to the combination also increased the sensitivity, but there were significant negative impacts on the specificity.

Consecutive combined use (all biomarkers were positive) of BDG and MN, BDG, and AM-IgG increased the specificity up to 98.9%, but the decreased in sensitivity was significant (29.6% to 57.7%).

False positive in bacteremia group

The mean values of BDG, MN, AM-IgG and AM-IgM were 44 pg/ml, 62 pg/ml, 90 AU/ml, and 63AU/ml, respectively (Fig. 2). At high cutoff value the false positive rate of BDG, MN, AM-IgG, and AM-IgM were 9.8%, 14.6%, 17.1%, and 19.5%, respectively, while at low cutoff value the false positive rate were 17.1%, 22.0%, 29.3%, and 26.8%, respectively (Table 4). There was no significant difference in false positive rate of biomarkers between Gram-positive and Gram-negative patients.

Diagnostic performance of serological assay for different *Candida* species

The sensitivity of BDG, MN, AM-IgG, and AM-IgM varied for different *Candida* species. At low cutoff value the highest sensitivity of all four biomarkers was for *C. albicans* (84.6%

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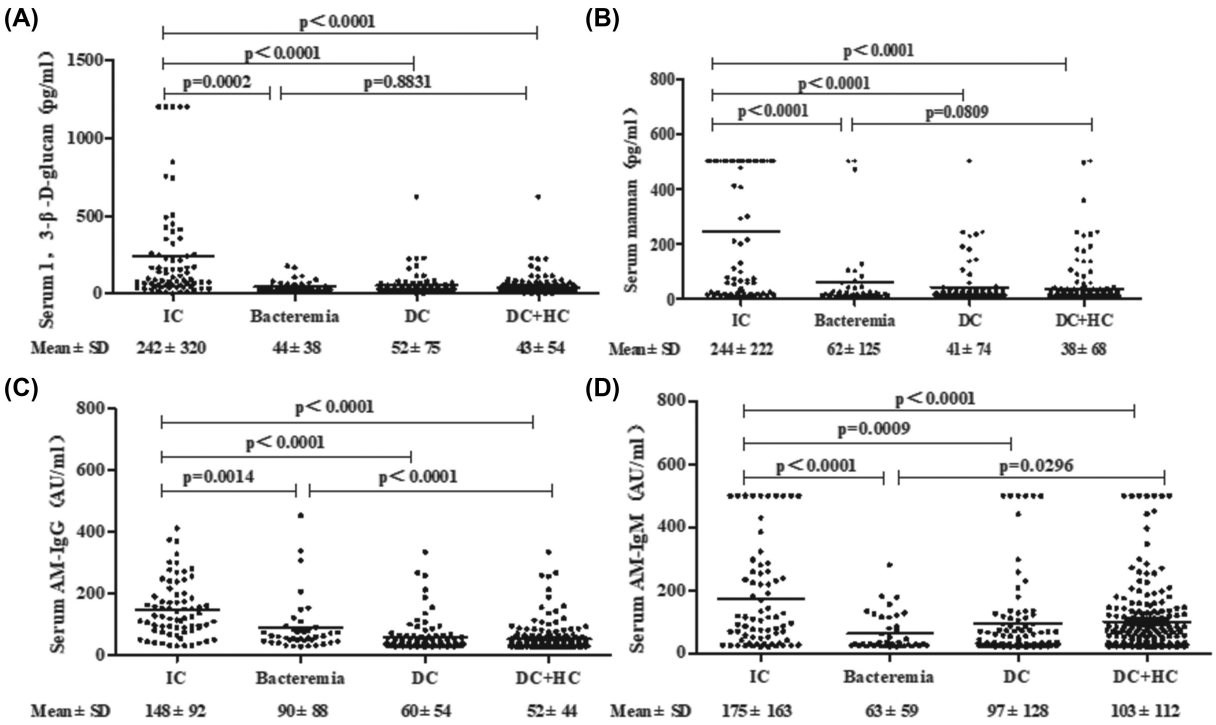

Figure 2. The mean concentration of biomarkers. The mean concentration of BDG (A), MN (B), AM-IgG (C) and AM- IgM (D) in IC ($n = 71$), bacteremia ($n = 41$), DC ($n = 89$), and DC+HC ($n = 185$) groups. Data were presented as mean values ± SD. $P < .05$ was considered as significant difference. Abbreviations: AM-IgG, Anti-Mannan IgG; AM-IgM, Anti-Manana IgM; BDG, 1,3-β-D-glucan; DC, disease control; HC, healthy control; IC, invasive candidiasis; MN, mannan; SD, standard deviation.

for BDG, MN, AM-IgG, and 92.3% for AM-IgM) and the lowest sensitivity was for *C. parapsilosis* (68.8%, 43.8%, 62.5%, and 37.5% for BDG, MN, AM-IgG, and AM-IgM, respectively) in candidemia patients, as demonstrated in Table 7. For patients with deep-seated IC at low cutoff value the sensitivity of BDG, MN, AM-IgG, and AM-IgM for *C. albicans* were 45.5%, 54.5%, 81.8%, and 45.5%, respectively, and 20%, 100%, 60%, and 40% for *C. tropicalis*. The remaining patients had more than one *Candida* species isolated, so we cannot compare the inter-species difference of biomarkers (Table 7). The mean value of MN and AM-IgM for *C. albicans* were significantly higher than that for *C. parasilosis*. The mean concentration of BDG, MN, AM-IgG, and AM-IgM for different *Candida* species in candidemia patients were shown in Fig. 4.

Serial monitoring of serum biomarker

Three patients in candidemia group had at least one serum sample available per week. The kinetics of BDG, MN, AM-IgG, and AM-IgM in the three patients were demonstrated in Fig. 5. Patient I (Fig. 5A) had positive *C. parapsilosis* culture from blood and received caspofungin as treatment. The infection was initially improved but became worse on July 25th. Antifungal treatment was changed to voriconazole and his conditions were significant better on August 8th. Patients II (Fig. 5B) had positive *C.parapsilosis* culture from blood and received fluconazole treatment. On July 20th the patient still had intermittent fever and the antifungal treatment continued. Patient III had positive culture of *C. albicans* and *C. parapsilosis*. Fluconazole was initially used then changed to voriconazole from July 11th. The infection was significantly improved on August 8th.

Discussion

We conducted a retrospective study to investigate the clinical value of BDG, MN, AM-IgG, and AM-IgM in diagnosis of IC. For the diagnostic performance of individual biomarker, the sensitivity of AM-IgM assay at low cutoff threshold was 57.7%, but the specificity is significantly lower than the other biomarkers (60%). Among the remaining biomarkers, AM-IgG showed the highest sensitivity of 74.6%, and a reasonable specificity of 87%. BDG and MN showed a sensitivity of 64.8% and the specificity were 90.8% and 89.2%, respectively. Meta-analysis suggested that the sensitivity and specificity of MN (Platelia *Candida* Ag, Bio-Rad) is 58% and 93%, respectively.[10] Our data revealed that the MN assay from Dynamiker produced a slightly higher sensitivity and lower specificity. In the same meta-analysis, the reported sensitivity and specificity of antimannan antibody are both lower than that of AM-IgG in our study. The decreased sensitivity and specificity of antibody assay from Mikulska et al. can be partly explained by different patient populations. Half of

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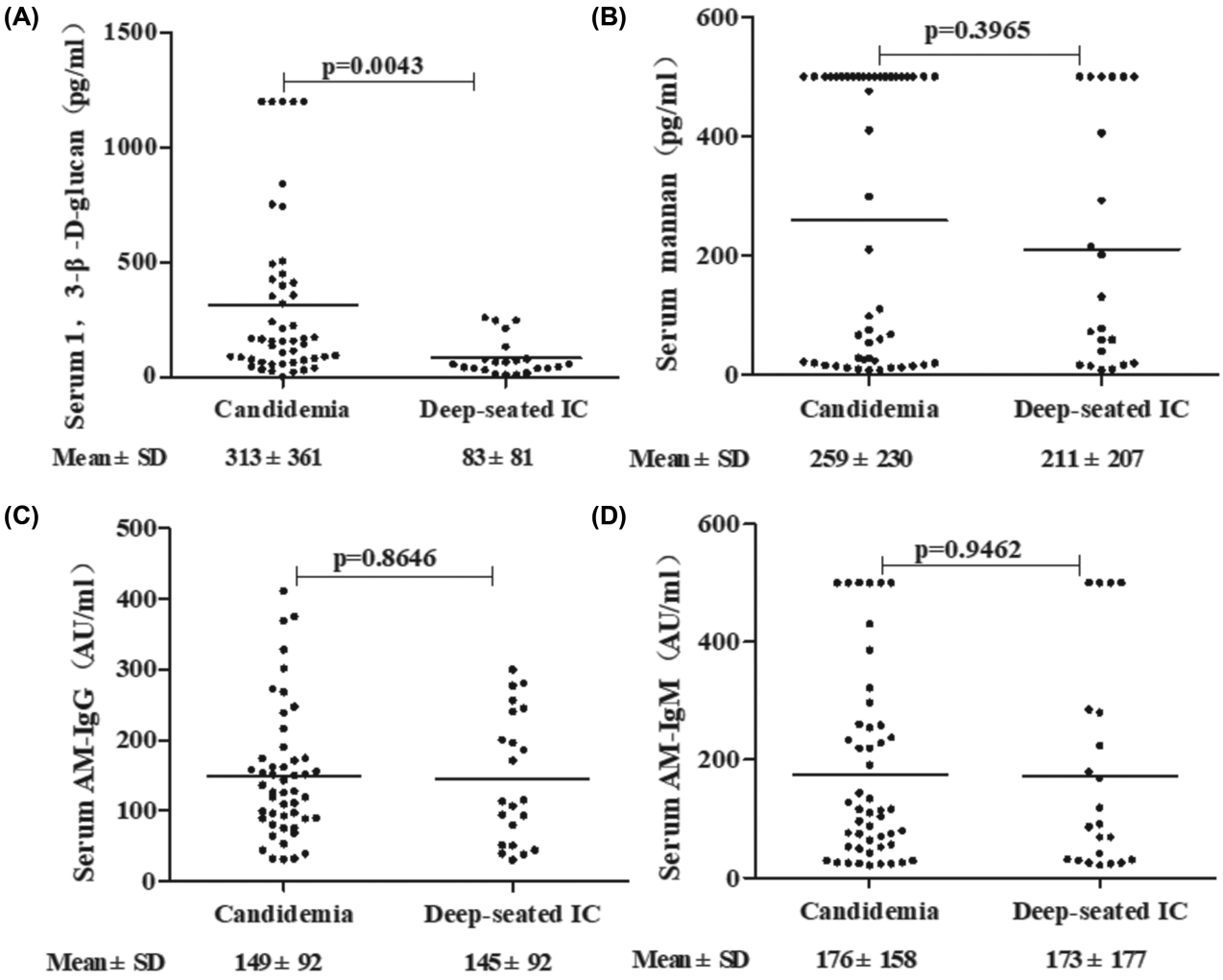

Figure 3. The mean concentration of each biomarkers in candidemia and deep-seated IC groups. The mean concentration of BDG (A), MN (B), AM-IgG (C) and AM-IgM (D) in candidemia and deep-seated IC groups. Data were presented as mean value ± SD. The mean concentration of BDG in candidemia group was significantly higher than that in deep-seated IC group. $P < .05$ was considered as significant difference. Abbreviations: AM-IgG, Anti-Mannan IgG; AM-IgM, Anti-Manana IgM; BDG, 1,3-β-D-glucan; IC, invasive candidiasis; MN, mannan; SD, standard deviation.

the studies analyzed by Mikulska et al. were patients with hematological and cancer cases, where in our study the number of immunosuppressed or immunocompromised patients were rare. Noticeably, Platelia *Candida* Ab assay detect total antimannan antibody, where the assay used in our study measured AM-IgG and AM-IgM separately. AM-IgM produced a relatively high false rate of 40% in our study, which is similar to the results from certain studies that using Platelia *Candida* Ab assay.[7,11] These data suggested that detection of AM-IgM may contribute to the false positive results from total anti-mannan antibody assay.

BDG is a pan fungi biomarker that can be used in diagnosis of invasive aspergillosis, invasive candidiasis, and *Pneumocystis* pneumonia. Meta-analysis showed that the sensitivity and specificity of BDG assay in diagnosis of IC are 81% (95% CI: 77%–85%) and 81% (95% CI: 80%–83%).[12] Another study reported that a sensitivity of 75% for BDG in diagnosis of IC.[13] White et al. showed the sensitivity and specificity of Dynamiker BDG assay are 93.3% and 78.1% for diagnosis of IC.[14] The overall sensitivity of BDG assay in our study is lower (64.8%) than that from other studies, but the specificity is higher (90.8%). Interestingly, the sensitivity of BDG in candidemia group is significantly higher than that in deep-seated IC group (77.6% vs 36.4%, cutoff of 70 pg/ml, Table 6). Recently some other studies also reported decreased sensitivity of BDG in patients with blood-culture negative IC: Leon et al. showed that the sensitivity of BDG is only 51.6% in a study that 77.4% of the IC patients enrolled were blood culture negative deep-seated IC.[15] Another study conducted by Leon et al. in 2016 showed a sensitivity of 65% in patients with blood-culture negative deep-seated IC,

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Table 7. Diagnostic performance of serological assay for different *Candida* species.

Biomarker[a]	Cutoff value	C.albicans	C. tropicalis	C. glabrata	C. parapsilosis
Candidemia					
		n = 13	n = 7	n = 9	n = 16
BDG (%)	≥70	11 (84.6)	5 (71.4)	7 (77.8)	11 (68.8)
	≥95	9 (69.2)	5 (71.4)	6 (66.7)	8 (50.0)
MN (%)	≥50	11 (84.6)	5 (71.4)	4 (44.4)	7 (43.8)
	≥100	9 (69.2)	5 (71.4)	4 (44.4)	5 (31.3)
AM-IgG (%)	≥80	11 (84.6)	7 (100.0)	6 (66.7)	10 (62.5)
	≥120	9 (69.2)	5 (71.4)	4 (44.4)	7 (43.8)
AM-IgM (%)	≥80	12 (92.3)	5 (71.4)	4 (44.4)	6 (37.5)
	≥120	9 (69.2)	2 (28.6)	3 (33.3)	6 (37.5)
Deep-seated IC					
		n = 11	n = 5		
BDG (%)	≥70	5 (45.5)	1 (20.0)		
	≥95	2 (18.2)	1 (20.0)		
MN (%)	≥50	6 (54.5)	5 (100.0)		
	≥100	4 (36.4)	3 (60.0)		
AM-IgG (%)	≥80	9 (81.8)	3 (60.0)		
	≥120	6 (54.5)	2 (40.0)		
AM-IgM (%)	≥80	5 (45.5)	2 (40.0)		
	≥120	4 (36.4)	2 (40.0)		

[a]BDG, 1,3-β-D-glucan; MN, mannan; AM-IgG, anti-mannan IgG; AM-IgM, anti-mannan IgM.

compared with 80% in candidemia group.[8] Tissot et al. reported a sensitivity of 65% in patients with blood culture negative intraabdominal candidiasis.[16] The lower sensitivity of BDG from our study than that from meta-analysis or study using same kit can be partly explained by these results. However, the decreased sensitivity of BDG in patients with blood culture negative IC worth drawing the attention as it may affect the clinical utility of BDG assay, although more studies are required to investigate this issue.

Combination of serological biomarkers were reported to improve the performance in diagnosis of IFD.[8,10,17,18] Study performed by Mikulska et al. analyzed 14 studies and suggested that the sensitivity and specificity of combined MN/Anti-MN antibody were 83% and 86%, respectively.[10] However, some recent studies showed lower sensitivity and/or specificity of combined MN/anti-MN antibody.[7,11] In this study we demonstrated that parallel combination of BDG/MN, BDG/AM-IgG, and MN/AM-IgM produced a sensitivity and specificity of over 80%, which is consistent with the findings from other study.[10] The sensitivity can be further increased to 93% while using BDG/MN/AM-IgG, but the negative impact on specificity cannot be neglected at this setting. Combining AM-IgM with other biomarkers increased the sensitivity but significantly decreased the specificity. Consecutive combined use of BDG, MN, and AM-IgG generated moderate sensitivity from 43.7% to 57.7% with very high specificity (94.6% to 98.9%). Based on these data, combination of BDG/MN, BDG/AM-IgG, and MN/AM-IgG are superior to single assay and provide useful tool in diagnosis of IC, while two or more positive biomarker at the same time strongly suggest deep *Candida* infection.

Bacteremia is believed to cause false positive in certain serological assays.[19] Sulahian et al. reported 37% false positive rate of BDG in patients with bacteremia.[20] The false positive rate of BDG, MN, AM-IgG, and AM-IgM were indeed higher in bacteremia group compared with that in control group (Table 4). At low cutoff threshold AM-IgG had the highest false positive rate of 29.3%. There was no significant difference in false positive rate of the biomarkers between Gram-positive patients and Gram-negative patients. The exact mechanism of the bacteremia-induced false positive is still not well understood, and it may be associated with endotoxin or other antigen produced by *Bacteria* that cross react with the ELISA kits. We also cannot exclude the possibility of underlying *Candida* infection among these patients. In fact, one patient initially enrolled in bacteremia group had positive blood *Candida* culture 10 days after positive blood *Bacteria* culture. However, none of these patients had positive *Candida* isolated during their hospital stay. Noticeably, bacteremia may be associated with the false positive of these biomarkers, but the specificity of patients with two or more positive results are still excellent, as demonstrated in Table 6.

The sensitivity of serological biomarkers varied for different *Candida* species and the highest sensitivity was for *C.albicans*, followed by *C. glabrata*, *C. tropicalis,* and *C. parapsilosis*, as demonstrated by some studies.[10] Our data suggested that at low cutoff value the highest sensitivity of serological biomarkers was

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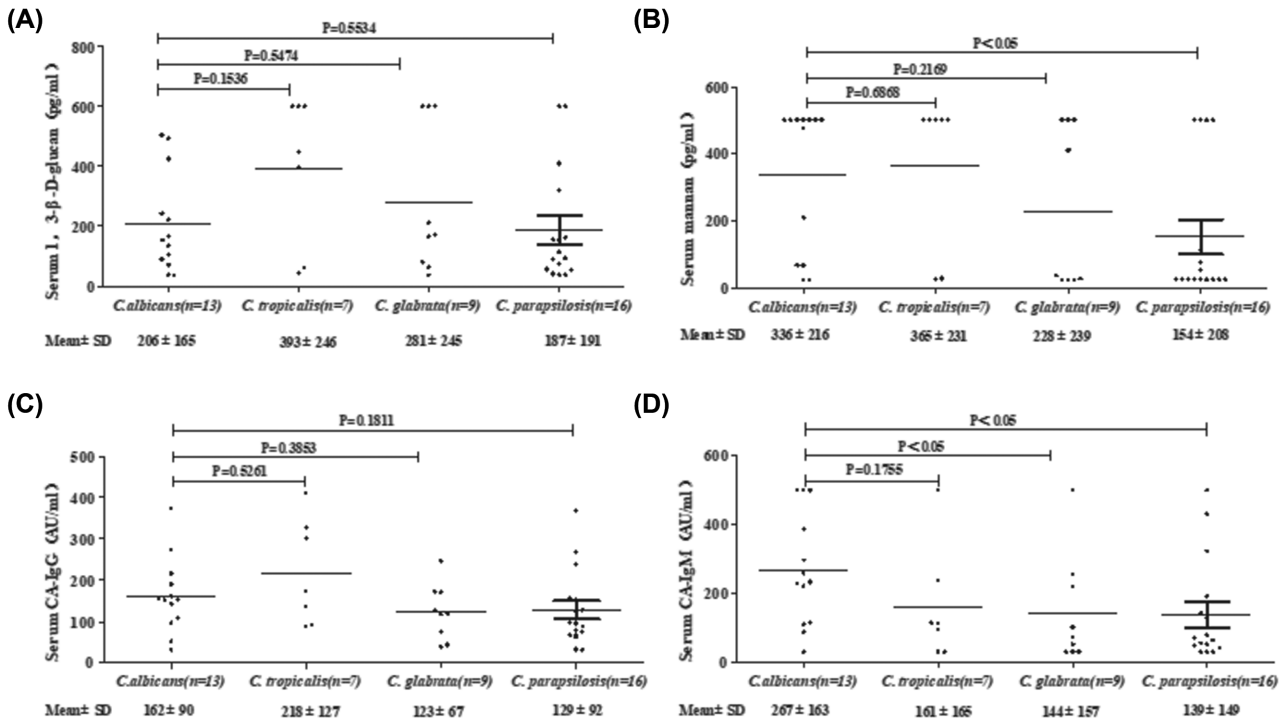

Figure 4. The mean concentration of each biomarkers for different *Candida* species in candidemia patients The mean concentration of BDG (A), MN (B), AM-IgG (C) and AM-IgM (D) in candidemia patients. Data were presented as mean value ± SD. The mean concentration of MN and AM-IgM for *C.albicans* was significantly higher than that for *C. parapsilosis*. $P < .05$ was considered as significant difference. Abbreviations: AM-IgG, Anti-Mannan IgG; AM-IgM, Anti-Manana IgM; BDG, 1,3-β-D-glucan; IC, invasive candidiasis; MN, mannan; SD, standard deviation.

for C. *albicans*, and it was the lowest for C. *parapsilosis* in Candidemia patients. There were also significantly differences in mean value of MN and AM-IgM between C. *albicans* and C. *parapsilosis* patients (Fig. 4). Interestingly, the sensitivity of MN, AM-IgG, and AM-IgM for C. *glabrata* was much lower than that for C. *tropicalis* (Table 7), while the sensitivity of BDG for C. *tropicalis* and C. *glabrata* was similar. There were also some differences in sensitivity of serological biomarkers for C. *albicans* and C. *tropicalis* in deep-seated IC group. Our study confirm the inter-species differences in the sensitivity of serological biomarkers, but more data are required to further analyze the results, especially for the deep-seated IC patients.

Few studies investigated the role of serial monitoring serological biomarkers in IC management. Ellis et al. showed kinetics of MN and AM antibody in seven selected patients with candidemia over 14 to 30 days, while Sendid et al. demonstrated that serial monitoring of MN and AM antibody can contribute to early diagnosis of IC.[17,21] In our study only four patients had at least one serum sample available per week in at least 2 weeks. One patient had negative serological results and the kinetics of the other three patients were showed in Fig. 4. Patients I and II received successful antifungal treatment, and most of the biomarker levels decreased compared with that from the day of positive blood culture. But there was no significant difference between serum AM-IgM levels on day 0 and end of monitoring period (Fig. 5A, C). The serum biomarker levels of BDG, AM-IgG, and AM-IgM significantly increased in patient II who had persistent *Candida* infection (Fig. 5B). Patient II had no severe immunosuppression during hospital stay. The reason of elevated levels of MN and AM-IgG at the same time was unclear, and it may be associated with increased fungi load. Noticeably the increase of MN level was more significant than that of AM-IgG. Another study conducted by Ellis et al. also showed a few patients may have positive correlation of MN and AM antibody levels during short period of time.[21] However, the individual case can only provide limited evidence, and further study is required to establish the role of serial monitoring in IC management.

MN and AM antibody can become positive several days prior to positive blood culture.[21] Due to the limitation of the retrospective design, we cannot evaluate the value of BDG, MN, AM-IgG, and AM-IgM in early IC diagnosis. Two patients had positive biomarkers 9 and 10 days prior to positive *Candida* blood culture. They were initially enrolled as non-IC group and developed candidemia a few days later. Prospective study is required to investigate the subject.

In this study we investigate the clinical value of BDG, MN, AM-IgG, and AM-IgM diagnosis of IC. Our data showed that BDG, MN, and AM-IgG provide reasonable sensitivity and specificity. Combined use of these biomarkers can significantly

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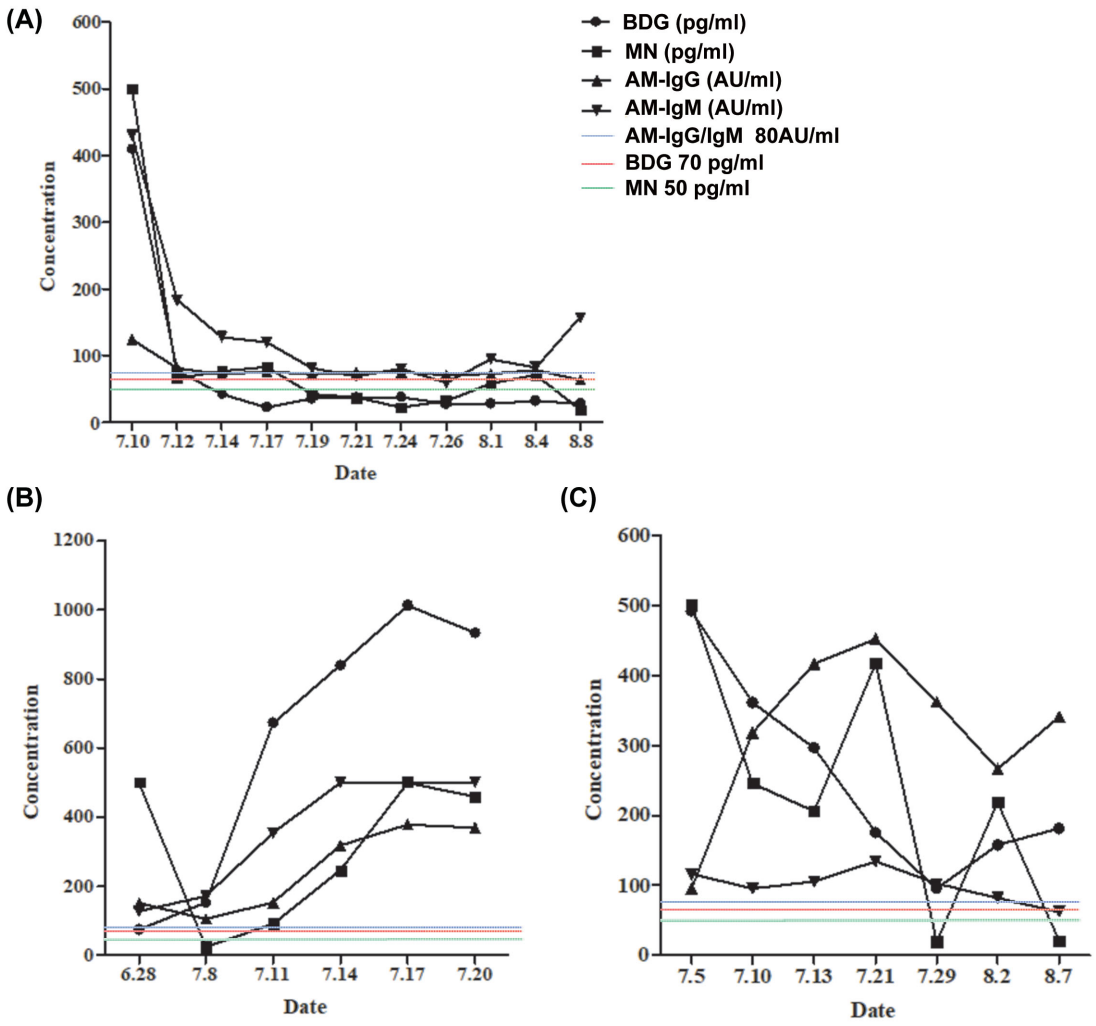

Figure 5. Diagnostic kinetics of one patient from candidiasis group. Diagnostic kinetics of BDG, MN, AM-IgG and AM-IgM of three selected patients: I (A), II (B) and III (C). Low cutoff thresholds of the biomarkers were plotted in the figures. Abbreviations: AM-IgG, Anti-Mannan IgG; AM-IgM, Anti-Manana IgM; BDG, 1,3-β-D-glucan; MN, Mannan.

increase the diagnostic performance. The combining of BDG and MN, BDG and AM-IgG, or MN and AM-IgG are useful tools in diagnosis of IC.

Supplementary material

Supplementary data are available at *MMYCOL* online.

Acknowledgments

This work was supported by PLA general hospital research funding (no. 2017MBD-024) and Natural Science Foundation of Tianjin (no.16JCYBJC43000). Dynamiker (Tianjin) provide the kits at no cost to perform this study.

Declaration of interest

X.Y. received an educational and post-doctoral funding from Dynamiker Limited. All other authors declared no conflicts of interest.

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