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Performance of a new *Candida* anti-mannan IgM and IgG assays in the diagnosis of candidemia

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ABSTRACT

Candida is one of the most frequent pathogens of bloodstream infections, which is associated with high morbidity and mortality rates. Rapid immunological detection methods are essential in the early diagnosis of candidemia. Anti-mannan is one of host-derived biomarkers against cell wall components of *Candida*. We conducted this study to evaluate the diagnostic performance of two anti-mannan assays (IgM, IgG) for candidemia through the analysis of 40 candidemia patients, 48 participants with *Candida* colonization and 213 participants with neither *Candida* colonization nor *Candida* infections (13 patients with other bloodstream infections, 145 hospitalized patients and 55 healthy controls). The performance of the two assays were evaluated by calculating their sensitivity and specificity. The sensitivity ranged from 0.78 to 0.80 for the IgM assay and 0.68 to 0.75 for the IgG assay. The specificity ranged from 0.97 to 0.98 for the IgM assay and 0.91 to 0.94 for the IgG assay. The diagnostic performance of the anti-mannan IgM assay was better than that of IgG, with higher sensitivity and specificity. Combining the two assays (positive results of single or both assays are both considered as positive) could improve the sensitivity up to 0.93 (0.79-0.98) and only slightly reduce the specificity (0.93(0.89-0.95)). The anti-mannan IgM, IgG assays are rapid and cost-effective assays that may be probably useful in the diagnosis of candidemia.

KEYWORDS: Candidemia. Anti-mannan. Diagnosis.

INTRODUCTION

Candida is one of the leading causes of healthcare-associated bloodstream infections. Owing to the advances in medical technology, the application of invasive procedures is becoming more and more extensive, increasing significantly the burden of *Candida* infections, especially in critically ill patients[1,2]. Early initiation of effective antifungal therapy was essential to improve the outcome of patients, while misdiagnosis or delay in diagnosis of candidemia could result in substantial morbidity and mortality (as high as 46–75%)[3], so the early diagnosis of candidemia is of great value.

Blood cultures are the gold standard of candidemia's diagnosis. The current guidelines provided by the European Society for Clinical Microbiology and Infectious Diseases (ESCMID) and the Infectious Diseases Society of America (IDSA) have shown that it is essential to draw at least two pairs of blood cultures for the diagnosis of candidemia. The frequency recommended was daily and the incubation period was at least 5 days. When all these recommendations are followed, sensitivity of blood cultures to detect *Candida* will be between 50–75%[4,5]. The sensitivity will further decrease when detecting patients who have neutropenia or

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previous antifungal treatment[4,5]. In addition, its turnaround time was limited so it cannot be considered as an early diagnostic technique. Regarding the clinical use of some biomarkers, B-D-glucan, mannan and anti-mannan are recommended with levels of evidence being II (second) according to ESCMID guidelines for Candida diseases[6].

The Dynamiker *Candida* anti-mannan IgM and IgG assays are new enzyme-linked immunosorbent assays (ELISA) designed to rapidly identify antigens from *Candida*'s cell wall in serum samples. This study evaluated the performance of two different antibody isotypes for diagnosis of candidemia.

MATERIALS AND METHODS

Study population

This study was approved by the Ethics Committee of the West China Hospital, (The Ethics Committee of West China Hospital, Nº 75, 76). To assess the diagnostic performance of the new anti-mannan IgM, IgG antibody assays, three groups of cases were enrolled: (1) Culture-confirmed candidemia, (2) *Candida* colonization without infection, (3) Neither *Candida* colonization nor infections.

Candidemia was defined as the presence of one or more *Candida* species in the bloodstream. Its diagnostic criteria were at least one positive blood culture that yielded at least one of the *Candida* species in patients with consistent clinical manifestations. The exclusion criteria were as follow: neutropenia (total leukocyte count ≤ 500/mm^3), pregnancy, autoimmune diseases, recent or ongoing immunosuppressive or antifungal therapy, significantly abnormal immune function. The blood cultures were processed by the BacTAlert® Virtuo® (bioMérieux, Marcy l'Etoile, France) system. All the specimens with positive results of blood cultures were identified on microscopy and subcultured on blood agar and chocolate agar for 48 h at 35 °C under aerobic conditions. *Candida spp*. isolated from blood samples underwent identification by MALDI-TOF mass spectrometry.

Candida colonization was defined as the recovery of *Candida spp.* from nonsterile and noncontiguous sites including mouth, throat, bronchus, skin and urethra. In the present study, we only enrolled cases who have *Candida* colonization in the upper respiratory tract because it is the most common colonization site. The exclusion criteria were: positive blood cultures for *Candida*; neutropenia; antifungal/immunosuppressive therapy; positive (1-3)-β-D-Glucan.

In total, there were 213 cases with neither colonization nor infections, 48 with *Candida* colonization and 40 with candidemia in our study. Among the cases with neither *Candida* colonization nor infections, 55 of them were healthy controls randomly selected from the health examination center of the West China hospital, 158 were hospitalized patients, of whom 13 had other bloodstream infections. The 13 patients included 4 with *Staphylococcus spp.*, 2 with *Enterobacteria spp.*, 1 with *Enterococcus spp.*, 1 with *Talaromyces* (*Penicillium*) *marneffei*, and 5 with *Cryptococcus spp.* infection. None of them had neutropenia, recent or ongoing immunosuppressive therapy or antifungal therapy.

The baseline characteristics of the 40 candidemia patients were presented in Table 2, while characteristics of control group 1 (neither *Candida* colonization nor infections group) and control group 2 (*Candida* colonization group) were presented in Table 3.

ELISA assay

The new anti-mannan IgM and IgG assays (Dynamiker Biotechnology, Tianjin, China) were performed strictly according to the manufacturer's instructions. The

Table 1 - Accuracy indices of the *Candida* anti-mannan IgM, IgG assays for candidemia.

Diagnostic parameter	Value of different anti-mannan antibody isotypes		
	anti-mannan IgM	anti-mannan IgG	anti-mannan IgM+IgG
Sensitivity (% [95% CI])	0.78 (0.61-0.89)[a] 0.80 (0.64-0·90)b	0.68 (0.51-0.81)[a] 0.75 (0.58-0·87)b	0.93 (0.79-0.98)
Specificity (% [95% CI])			
Candida colonization	1 (0.91-1)[a] 1 (0.91-1)[b]	0.88 (0.74-0.95)[a] 0.77 (0.77-0.87)[b]	0.88 (0.74-0.95)
neither colonized nor infected	0.97 (0.94-0.99)[a] 0.96 (0.93-0.99)[b]	0.96 (0.92-0.98)[a] 0.94 (0.90-0.97)[b]	0.94 (0.90-0.97)
Overall	0.98 (0.95-0.99)[a] 0.97 (0.94-0·99)b	0.94 (0.90-0.97)[a] 0.91 (0.87-0.^{9}4)b	0.93 (0.89-0.95)

[a]Undetermined results (80-120 AU/mL) were considered as negative results; [b]Undetermined results were considered as positive results. CI- confidence interval.

Table 2 - Characteristics of the 40 blood culture-positive candidemia patients and detection of anti-mannan IgG and IgM antibodies results.

Case Nº	Age	Sex	Underlying condition	Time of incubation[a]	Anti-mannan IgG		Anti-mannan IgM	
					concentration (AU/mL)	results	concentration (AU/mL)	results
1	30	F	Heart failure	1.58	319.21	P	189.91	P
2	30	M	Acute pancreatitis	2	51.56	N	>500	P
3	29	M	Hematological system disease	0.95	217.52	P	147.56	P
4	71	M	Postoperative infection	1.5	150.88	P	>500	P
5	22	M	Aplastic anemia	0.7	190.33	P	28.16	N
6	45	F	Chronic hepatitis B	0.81	293.45	P	336.08	P
7	0	F	Postoperative infection	1.16	39.80	N	>500	P
8	48	M	Acute pancreatitis	2	156.90	P	425.57	P
9	66	F	Chronic renal failure	0.38	135.45	P	485.47	P
10	0	F	Intestinal fistula	2	27.38	N	>500	P
11	66	F	Heart disease	1.12	173.98	P	133.81	P
12	78	M	Intracranial infection	1.34	284.13	P	212.53	P
13	46	M	Heart disease	2	132.60	P	198.82	P
14	31	M	Acute pancreatitis	1.12	350.07	P	131.89	P
15	50	M	Cholecystolithiasis	3.29	30.30	N	199.61	P
16	40	M	Trauma	1.7	54.87	N	>500	P
17	68	M	Intestinal obstruction	1.5	>500	P	123.23	P
18	63	M	Chronic obstructive pulmonary diseases	1.29	22.67	N	>500	P
19	76	M	Esophageal stricture	1.75	257.64	P	382.81	P
20	61	F	Arthritis	1.18	191.80	P	208.57	P
21	64	F	Trauma	1.17	186.36	P	128.76	P
22	75	F	Abnormal liver function	0.25	>500	P	23.94	N
23	17	M	Trauma	0.45	>500	P	23.21	N
24	42	F	Acute pancreatitis	1.63	83.02	I	176.67	P
25	79	M	Intestinal obstruction	1.88	>500	P	29.20	N
26	44	M	Pulmonary infection	2.33	>500	P	61.04	N
27	44	F	Hepatic abscess	0.71	58.41	N	92.99	I
28	48	M	Trauma	0.8	96.98	I	264.82	P
29	35	M	Abnormal liver function	2.2	>500	P	46.76	N
30	20	M	Trauma	1.38	359.06	P	199.61	P
31	51	M	Cerebral hemorrhage	0.7	84.81	I	>500	P
32	33	F	Acute pancreatitis	0.87	54.20	N	181.47	P
33	49	F	Intestinal obstruction	1.58	490.59	P	345.08	P
34	63	M	Heart disease	2.29	28.68	N	20.58	N
35	60	M	Trauma	0.46	288.87	P	>500	P
36	39	M	Anemia	0.88	380.88	P	244.82	P
37	62	M	None	1.17	244.42	P	>500	P
38	56	M	Gastrointestinal perforation	1.12	141.06	P	>500	P
39	37	M	Acute pancreatitis	2.33	337.44	P	143.19	P
40	59	F	Arterial embolism of lower extremities	1.75	42.64	N	28.28	N

[a]Total incubation time to get a positive result of blood cultures; F = female; M = male; P = positive; N = negative.

recommended cut-off values are 120 AU/mL for both assays; 80 to 120 AU/mL is considered as undetermined results. All the data were analyzed anonymously and only routine diagnostic samples were used in this study, having no influence in therapeutic decisions.

Table 3 - Characteristics of control group 1 (without colonization) and control group 2 (with colonization).

Patient/parameter	control group 1	control group 2
Median age, y (range)	44(31-52)	69 (63-75)
Gender (men/women)	109/104	30/18
Cardiovascular disease	31/213	6/48
Renal disease	20/213	4/48
Lung disease	9/213	19/48
Brain disease	29/213	8/48
Neurological disease	3/213	-
Hypertension	4/213	3/48
Biliary tract disease	9/213	2/48
Trauma	7/213	2/48
Fever	1/213	1/48
Digestive tract disease	19/213	1/48
Endocrine disease	4/213	-
Eye diseases	3/213	-
Bone disease	6/213	-
others	13/213	2/48
Healthy control	55/213	-

Statistical analysis

To illustrate the diagnostic performance of the assays, sensitivity (percentage of correctly identified candidemia) and specificity (percentage of correctly eliminated candidemia) were calculated. Specificity is calculated separately for control group 1, control group 2, and overall (control group 1 plus control group 2). GraphPad Prism (version 8.0.1; GraphPad Software, La Jolla, California, USA) was used to illustrate the antibody concentration data.

RESULTS

Diagnostic performance of the anti-mannan IgG ELISA assay

Serum samples of 40 candidemia patients and 261 controls were tested. Among the 40 candidemia patients, anti-mannan IgG were positive in 13/19 of candidemia patients with *C. albicans* infection, 6/8 with *C. tropicalis*, 4/6 with *C. parapsilosis*, 2/5 with *C. glabrata*, 1/1 with *C. krusei* and 1/1 with *C. lusitaniae*. As for the undetermined results, they were considered to be positive and negative, respectively. If the undetermined results were considered as positive results, the IgG ELISA assay had a sensitivity of 0.75 (0.58-0.87) and an overall specificity of 0.91 (0.87-0.94). If the undetermined results were considered negative, the IgG ELISA assay had a sensitivity of 0.68 (0.51-0.81) and an overall specificity of 0.94 (0.90-0.97) (Table 1). The specific concentrations of anti-mannan IgG were shown in Table 2 and Figure 1.

Diagnostic performance of anti-mannan IgM ELISA assay

Anti-mannan IgM were positive in 17/19 of candidemia patients with *C. albicans*, 5/8 with *C. tropicalis,* 4/6 with *C. parapsilosis*, 3/5 with *C. glabrata* 1/1 with *C. krusei*,

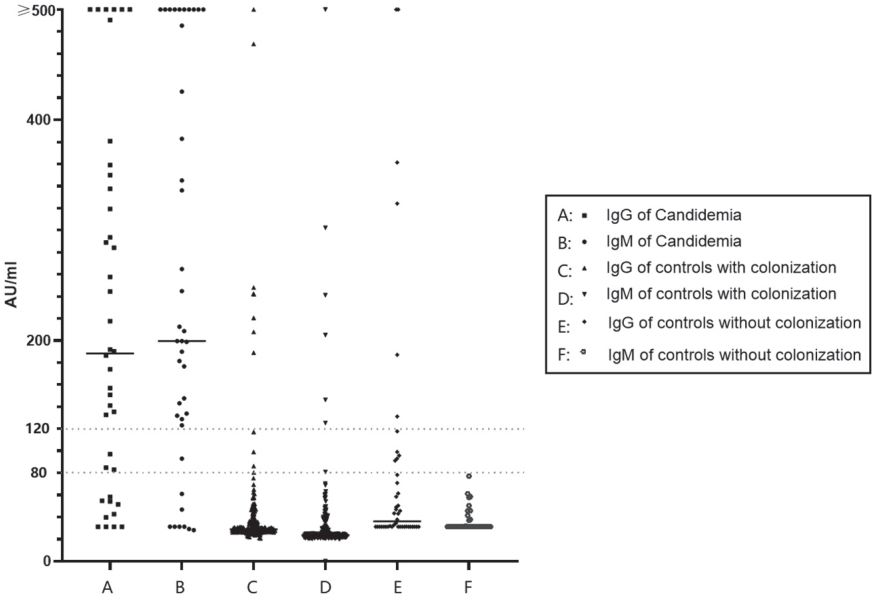

Figure 1 - The concentration of anti-mannan IgG and IgM antibodies in candidemia patients and controls. The dotted lines are the cut-off values of the assays (80AU/mL and 129AU/mL); the continuous lines are the median of the concentrations of the antibodies in each group

1/1 with *C. lusitaniae*. If the undetermined results were considered as positive results, the IgM ELISA assay had a sensitivity of 0.80 (0.64-0.90) and a specificity of 0.97 (0.94-0.99). If the undetermined results were considered as negative results, the IgM ELISA assay had a sensitivity of 0.78 (0.61-0.89) and a specificity of 0.98 (0.95-0.99) (Table 1). The specific concentrations of anti-mannan IgM were shown in Table 2 and Figure 1.

Diagnostic performance of the combined detection of the two assays

In the combined detection of IgG and IgM assays, serum samples with positive result (>120 AU/mL) of single or both assays were regarded as positive. Anti-mannan IgG and/or IgM were positive in 19/19 of candidemia patients with *C. albicans* infection, 7/8 with *C. tropicalis*, 5/6 with *C. parapsilosis*, 4/5 with *C. glabrata*, 1/1 with *C. krusei,* 1/1 with *C. lusitaniae*. The sensitivity of the combined detection of two assays for diagnosing candidemia was 0.93 (0.79-0.98) and the specificity was 0.93 (0.89-0.95) (Table 1).

DISCUSSION

Candidemia is one of the most common bloodstream fungal infections whose diagnosis is based mainly on blood cultures[7]. However, blood cultures lack sensitivity (ranging from 21% to 71% in different studies)[8] and its turnaround time is limited. As is known, even a mere 12 hours of diagnostic delay may expose infected individuals to a higher risk of disability and mortality[9], so the timely diagnosis is of cardinal importance.

Parra-Sánchez *et al*.[10] indicated that the sensitivity of the *C. albicans* germ-tube specific IgG antibody assay ranged from 61.1% to 85.7% and the specificity ranged from 75.8% to 80.3%. Mattsby-Baltzer *et al*.[11] study reported that IgG_2 anti-phosphopeptidomannan antibody is another biomarker with 88% sensitivity and 94% specificity in invasive candidiasis, but no IgG2 antibody response was observed in *C. parapsilosis* and *C. albicans* infections. White *et al*.[12] described that the sensitivity and specificity of a (1-3)-β-D-Glucan (D-BDG) assay were 90.7% and 73.4% in the diagnosis of invasive fungal disease, when optimal positivity threshold was 70 pg/mL. Alam *et al*.[13] reinforced the diagnostic value of semi-nested PCR (snPCR), which can detect DNA of different *Candida* species in serum samples of over 50% of the clinically suspected patients without positive blood cultures, while the combination of mannan and D-BDG is necessary to eliminate false positive reactions.

Antibodies against constituents of *Candida* are also sensitive biomarkers. As is known, antibodies to *Candida* class IgA, IgM, IgG and IgE[14-16], and different antibody isotypes differ in diagnostic performance. A study has shsown that *Candida albicans* could significantly decrease the secretion of IgG in a dose-dependent manner while enhancing IgM[16]. In addition, the difference in antibody levels between patients and healthy controls is more significant for anti-*Candida* receptor 3-related protein IgM than for IgG[17], and atopic dermatitis patients were found to have abnormalities in the production of IgG antibodies against *Candida albicans*[18]. To sum up, IgM is probably more useful than IgG for the diagnosis of candidemia.

In studies with different designs and populations, the clinical value of anti-mannan assays in the diagnosis of *Candida* infection had variable sensitivity (40% to 70%) and specificity (50% to 80%)[19-21]. Differences in diagnostic performance may be attributed to inclusion and exclusion criteria, different sample structure including number of cases enrolled, grouping mode, baseline characteristics and distribution of isolated *Candida spp.* In Mikulska *et al.*[22] meta-analysis, the sensitivity and specificity of the anti-mannan assays for invasive candidiasis were 59% and 86%, and the sensitivity and specificity for a combined mannan/anti-mannan assay were 83% and 86%. Meta-analysis can solve the problem of small sample size, yet it may suffer from publication bias. Many of these studies did not mention which isotype of antibody was used.

In the present study, we evaluated the diagnostic performance of a new anti-mannan IgM and IgG assays for candidemia. All the enrolled cases were candidemia patients without significantly abnormal immune function and this is the most likely explanation for the better diagnostic performance (higher sensitivity) than in other studies. Regarding the discordant results, only three confirmed that candidemia patients had negative results in both assays, including one with *C. tropicalis*, one with *C. parapsilosis* and one with *C. glabrata*. Anti-mannan IgM assay had a better diagnostic performance than the IgG assay, with higher sensitivity and specificity.

The combined assays in our study had relatively high sensitivity and there was a high concordance with the infections status. Among all the 40 candidemia patients, the serum sample collection of 23 candidemia patients took place before getting the positive results of blood cultures. We obtained positive detection of IgM antibodies in 21 of them, while IgG was detected in 15 of them. In the combined detection of IgG and IgM, only one of these 23 patients (infected with *C. parapsilosis*) had both, IgG and IgM negative antibodies. The combined assays can help to detect infections earlier because they can be positive as long as 4 days prior to blood cultures. Chumpitazi *et al*.[23] found that mannan is also a biomarker appearing early in

the development of candidemia. However, its specificity was 86% and its sensitivity was 77% in the meta-analysis. Although both, the sensitivity and specificity of the assays in our study showed better diagnostic performances, there were still several nonspecific results in the control groups, some of which had high antibody titers. According to the manufacturer's instructions, these assays are qualitative rather than quantitative with cut-off values of 80 AU/mL and 120 AU/mL, so that positive or negative results are more valuable for clinical practice. In Clancy *et al*.[24] review, invasive candidiasis patients with negative blood cultures were called the "missing 50%", which is one of the possible reasons for the nonspecific results with high titers especially in patients with *Candida* colonization. However, whether some of the patients without any evidence of infection had positive result is still uncertain. From the results, we suggested that candidemia cannot be judged only according to high titers of anti-mannan IgG or IgM. A correct diagnosis must be made on the basis of suggestive clinical symptoms, presence of risk factors for candidemia (such as abdominal surgery, central venous catheter, mechanical ventilation, etc). Unselected screening of anti-mannan for *Candida* infections in similar settings is inappropriate, while using anti-mannan as a complementary test to blood culture results may be preferred in clinically suspected candidemia patients and cases with high risk based on our study results.

CONCLUSION

Our study has shown that anti-mannan IgM and IgG assays are useful to assist clinicians in making an earlier diagnosis of candidemia and differentiating *Candida* colonization from candidemia in patients without significantly abnormal immune function. The tests have shown a high negative predictive value (>85%) and can be used to rule out infections, as part of diagnostic strategies to establish the absence of candidemia, reducing the unwarrant use of antifungal agents in patients.

AUTHORS' CONTRIBUTIONS

Concept, design: Ying Ma; literature search, clinical studies, experimental studies: Mei Kang; data acquisition, data analysis, statistical analysis: Ziwei Kuang, Dongdong Li; manuscript preparation, manuscript editing, and manuscript review: Yanming Meng, Tingting Wang.

CONFLICT OF INTERESTS

No potential conflict of interests relevant to this article were reported.

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