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Diagnostic value of *Candida* mannan antigen and anti-mannan IgG and IgM antibodies for *Candida* infection

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Summary

Objective: To assess the diagnostic value of serum *Candida* mannan antigen (MN) and anti-mannan IgG and IgM antibodies for candidiasis.

Methods: This study was a prospective cohort study. Clinical data and venous blood samples from 23 medical centres in Beijing, China were collected between 1 January 2017 and 31 December 2018. All collected specimens were tested within one week for serum *Candida* MN and IgG and IgM antibodies using an ELISA kit.

Results: A total of 452 patients were enrolled, including 188 patients in the *Candida* exposure groups (56 patients with *Candida* bloodstream infection, 69 patients with *Candida*-positive tracheal aspirate cultures and 63 patients with *Candida*-positive urine cultures) and 264 patients in the control groups (212 healthy controls and 52 patients with bacteraemia). The receiver operating characteristic (ROC) curve of the 56 patients with *Candida* bloodstream infection and 212 healthy controls showed that serum MN and IgG had good diagnostic value. The area under the ROC curve (AUC) values were 0.812 (95% CI, 0.750-0.873) and 0.866 (95% CI, 0.808-0.924), respectively, wherein the MN specificity and sensitivity were 86.79% and 60.71%, and the IgG were 84.43% and 80.36%, respectively. The AUC of the combination of serum MN and IgG was 0.871(95% CI, 0.813-0.929), and the specificity and sensitivity were 93.87% and 57.14%.

Conclusions: The serum levels of *Candida* MN and its IgG antibody have diagnostic value for *Candida* bloodstream infection, and combination of MN and IgG can improve diagnostic specificity and may provide a new approach for diagnosis of candidaemia.

KEYWORDS

anti-mannan IgG, anti-mannan IgG, *Candida* infection, *Candida* mannan, colonisation, rapid diagnosis

1 | INTRODUCTION

With the advancement of modern medical technology, fungal infections have gradually increased, which has attracted extensive attention in the medical community. The literature reports that fungal infections now affect 1 billion people, and 1.5 million die from these infections every year.[1] Candidiasis accounts for approximately 50% of invasive fungal infections and poses a threat to the widest range population; patients with invasive candidiasis have a mortality rate of 40% or higher.[2] Clinical studies have shown that the early diagnosis of invasive candidiasis and early drug treatment can reduce the mortality rate.[3-5] The current clinical diagnostic tests for candidiasis include *Candida* culture, smear microscopy and 1,3-β-D glucan assay.[6] Among these methods, *Candida* culture is time consuming,

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usually taking 3-7 days, with a sensitivity of approximately 50%.[7-9] The 1,3-β-D glucan assay is not specific, and in addition to *Candida*, it can also be positive for *Aspergillus* and *Pneumocystis*.[10,11] A specific and rapid diagnostic test for candidiasis would enable early treatment to be implemented, thereby increasing the survival rate of patients. In this paper, we investigated the diagnostic value of specific *Candida* mannan antigen (MN) and its IgG and IgM antibodies for candidiasis.

2 | MATERIALS AND METHODS

2.1 | Experimental design

This experiment was registered at clinical trials with the registration number gov: NCT03766932. The Ethics Committee of the Chinese People's Liberation Army (PLA) General Hospital (S2017-015-01) is responsible for ethical approval and supervision. The patients enrolled in this study were from 23 medical centres, including the Chinese PLA General Hospital, the Fourth Medical Center of PLA General Hospital, the Sixth Medical Center of PLA General Hospital, the PLA Rocket Force General Hospital and the PLA Air Force General Hospital. The research period was from 1 January 2017 to 31 December 2018. The inclusion criteria for the test group were as follows: (a) hospitalised patient ≥18 years old; (b) positive *Candida* culture in body fluid specimens, including blood, airway aspirate and urine, with two positive cultures in succession required for airway aspirate and urine specimens; and (c) patient agreement to participate in the study and provision of signed informed consent form. The inclusion criteria for the control group were as follows: (a) volunteer older than 18 years, with no clinical symptoms or signs of infection; and (b) patient agreement to participate in the study and provision of signed informed consent form. When people meeting the above criteria were identified, the researcher contacted the individual (or other authorised person) on the same day and fully informed them of the content of the study. After the relevant informed consent was signed, venous blood was taken within 24 hours.

2.2 | Data collection

(a) Clinical data of each enrolled patient were collected, including sex, age, specimen type, culture results, admitting diagnosis, white blood cell count, neutrophils, C-reactive protein (CRP) and interleukin-6 (IL-6). (b) The researchers from the Microbiology Department of each research centre were responsible for monitoring the body fluid culture results of all patients in each medical centre. If the culture result was positive for any *Candida* species, including *Candida albicans*, *Candida tropicalis*, *Candida parapsilosis*, *Candida krusei* or *Candida glabrata*, and the patient met the inclusion criteria, the researchers contacted the relevant clinical department and patient to discuss whether they would like to join the study. After the informed consent was signed, venous blood was taken within 24 hours. The control group consisted of healthy volunteers (including healthy examinees for a wellness visit and volunteers from medical professionals) and patients with bacteraemia. After receiving the informed consent, venous blood samples were collected within 24 hours. Vacutainer was used, and venous blood was collected by professional nurses. All blood samples were centrifuged at 1500 *g* for 15 minutes, and then 2 mL of the supernatant was removed and placed in an Eppendorf (EP) tube and stored in a −80°C freezer for subsequent testing.

2.3 | Test principle and steps

The samples were tested for Candida MN, IgG and IgM within one week using an ELISA kit. The testing work was completed by the laboratory staff of the PLA General Hospital using the Candida MN, IgG and IgM test kit provided by Dynamiker Biotechnology Co., Ltd., China. All the steps strictly followed the product operation manual.

Detection of *Candida* MN: Competitive ELISA was used for this detection. The pretreated human serum samples are first added to the ELISA microplate, which is precoated with *Candida* MN, and the enzyme-labelled antibody is then added. After mixing and incubation, the antigen in the sample competes with the coated antigen to bind to the enzyme-labelled antibody, forming an antigen-antibody immune complex. After washing, 3,3',5,5'-tetramethylbenzidine (TMB) substrate is added to produce a colour change reaction which dependent on the concentration of target, and a stop solution is applied to stabilise the colour development. The absorbance is then measured at a wavelength of 450 nm using a microplate reader. The absorbance value is inversely related to the MN content, thereby enabling the detection of *Candida* MN. The threshold for the assay is 1 AU/mL to 1500 AU/mL.

Candida IgG/IgM antibody detection: After the diluted patient serum sample is added to the ELISA microplate (precoated with a *Candida*-specific antigen), the *Candida* IgG/IgM antibody in the serum will bind the antigen. An anti-human IgG/IgM enzyme-labelled antibody and a TMB substrate are then added to produce the colour change reaction. The absorbance is measured at a wavelength of 450 nm using a microplate reader. The concentration of *Candida* IgG/IgM antibody in the sample is calculated from a standard curve. The threshold for the assay is 1 AU/mL to 1500 AU/mL.

2.4 | Statistical methods

Statistical analysis was performed using SPSS statistics 17.0. The numerical variables that were normally distributed are represented by the mean ± standard deviation, and the difference between the mean of two groups was compared using the *t* test. The median was used to describe the numerical variables that were not normally distributed. The Mann-Whitney *U* test was used for the non-parametric test of two independent samples, and the Kruskal-Wallis *H* test was used for the non-parametric test of multiple independent samples.

The unordered categorical variable was expressed as a rate, and Pearson's chi-squared test was used to compare the difference in the rate of two groups. The receiver operating characteristic (ROC) curve analysis was used to show the area under the ROC curve (AUC) value and its 95% confidence interval (95% CI). The ROC curve plot was constructed using SPSS statistics 17.0, and the other graphs were carried out using GraphPad prism 7. The optimal cut-off for the *Candida* diagnostic test was calculated using Youden's J statistic (sensitivity + specificity − 1). $P < .05$ was considered to be statistically significant.

3 | RESULTS

3.1 | Patient characteristics

From 1 January 2017 to 31 December 2018, a total of 452 patients were enrolled, including 188 patients in the *Candida* exposure groups and 264 patients in the control groups. According to the location from which the positive *Candida* culture specimens were taken, the exposure group was divided into three subgroups: 56 patients with one or more positive blood cultures, 69 patients with double positive tracheal aspirate cultures and 63 patients with double positive urine cultures. The control group was divided into two subgroups: 212 healthy volunteers and 52 patients with bacteraemia. Venous blood was collected from all patients and volunteers and was tested for *Candida* MN, IgG and IgM. The clinical characteristics of all the enrolled subjects are shown in Table 1. Infection indicators were not tested in the 212 healthy controls. In the *Candida* exposure group, the ratio of males was higher than that in the control group ($P < .001$), especially in patients with a positive lower respiratory tract culture, where the ratio of males was as high as 91.3%. The average age of the *Candida* exposure group was higher than that of the control group ($P < .001$), and the average age of patients with a positive lower respiratory tract culture was 80.94 years. The intensive care unit is an important source of *Candida* exposure, resulting in up to 76 cases. Since the lower respiratory tract specimens in this study required specimens obtained by electronic bronchoscopy, only the intensive care unit and respiratory department met this requirement. The mean of all infection indicators was higher in the bacteraemia group than that in the *Candida* exposure group, but the difference was not

TABLE 1 Baseline characteristics of the study population

	Positive patients				
Characteristic	Blood	Lower respiration secretion	Urine	Healthy control	Bacteraemia
All patients	56	69	63	212	52
Male gender	38 (67.9%)	63 (91.3%)	44 (69.8%)	108 (50.9%)	29 (55.7%)
Age (years) (mean ± SD)	60.5 ± 18.9	80.94 ± 9.77	73 ± 20.02	42 ± 14.22	58 ± 15.21
Underlying status					
Intensive care	15	29	32	—	11
Surgical postoperative	20	0	5	—	19
Respiratory disease	2	40	10	—	1
Digestive disease	5	0	6	—	4
Heart disease	4	0	2	—	5
Malignancy	3	0	2	—	4
Emergency	3	0	1	—	3
Other or unknown	3	0	5	—	5
Inflammation marker (mean ± SD)					
WBC ($\times 10^9$/L)	9.23 ± 5.09	8.44 ± 2.50	10.58 ± 4.51	—	11.49 ± 4.77
NE ($\times 10^9$/L)	7.91 ± 4.88	6.2 ± 2.59	8.35 ± 3.92	—	9.02 ± 4.45
CRP (mg/dL)	6.87 ± 6.85	1.46 ± 1.91	3.76 ± 4.56	—	7.51 ± 5.69
IL-6 (pg/mL)	109.67 ± 130.84	18.93 ± 16.98	46.96 ± 61.25	—	727 ± 1469
Assay [M (P25-P75)]					
MN	101.89 (7.85-948.86)	98.32 (23.59-628.16)	95.62 (22.20-496.99)	14.45 (7.41-31.45)	16.30 (8.63-47.66)
IgG	121.6 (25.55-410.44)	84.76 (42.65-174.94)	40.57 (21.29-54.63)	57.33 (39.86-81.67)	57.33 (39.86-81.67)
IgM	109.75 (4.26-1289.78)	106.74 (51.21-241.83)	74.24 (33.45-143.68)	25.93 (5.54-92.13)	25.93 (5.54-92.13)

TABLE 2 MN, IgG and IgM levels in 188 *Candida*-positive culture patients

Candida species	No. (%)	MN[M (P25-P75)] (AU/mL)	IgG[M (P25-P75)] (AU/mL)	IgM[M (P25-P75)] (AU/mL)
C. albicans	93 (49.5)	387.51 (67.74-656.15)	141.96 (83.78-222.21)	96.13 (29.21-234.04)
C. glabrata	28 (14.9)	73.09 (21.63-423.49)	96.22 (49.78-127.64)	83.43 (30.84-186.11)
C. parapsilosis	25 (13.3)	29.30 (18.21-205.67)	96.47 (61.37-150.88)	72.85 (49.92-143.76)
C. tropicalis	37 (19.7)	46.27 (15.70-457.398)	76.25 (36.13-169.93)	84.97 (13.17-136.81)
C. krusei	5 (2.7)	98.32 (20.32-457.40)	161.75 (72.84-332.23)	155.63 (130.24-163.84)
Total	188 (100)	108.83 (24.68-610.01)	116.02 (52.07-197.22)	88.27 (29.90-182.63)

Note: Abbreviations: IgG, Anti-mannan IgG; IgM, Anti-manana IgM; M, Median; MN, Mannan of *Candida*; P25-P75, interquartile range, represents the 25th percentile and the 75th percentile.

statistically significant. The levels of *Candida* MN and antibodies were higher in the *Candida* exposure group than in the control group ($P < .001$).

3.2 | *Candida* exposure group

The culture results of the 188 patients in the *Candida* exposure group showed five species of *Candida*, including *C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis* and *C. krusei*. *C. albicans* accounted for the most cases, up to 93, while only five cases of *C. krusei* were found, accounting for the least number of cases. There were significant differences in the levels of MN and IgG among the different *Candida* species ($P = .001$, $P = .008$), but no significant difference was observed in the level of IgM ($P = .368$). The lowest level of MN was 29.30 (P25-P75, 18.21-205.67 AU/mL), which was found in *C. parapsilosis* infections. The MN level of the healthy population was 14.45 (P25-P75, 7.41-31.45 AU/mL), which was significantly different from the lowest MN level, observed in *C. parapsilosis* infections ($P < .001$). The lowest IgG level, 76.25 (P25-P75, 36.13-169.93 AU/mL), was found in *C. tropicalis* infections, which was significantly different from the MN level of the healthy population

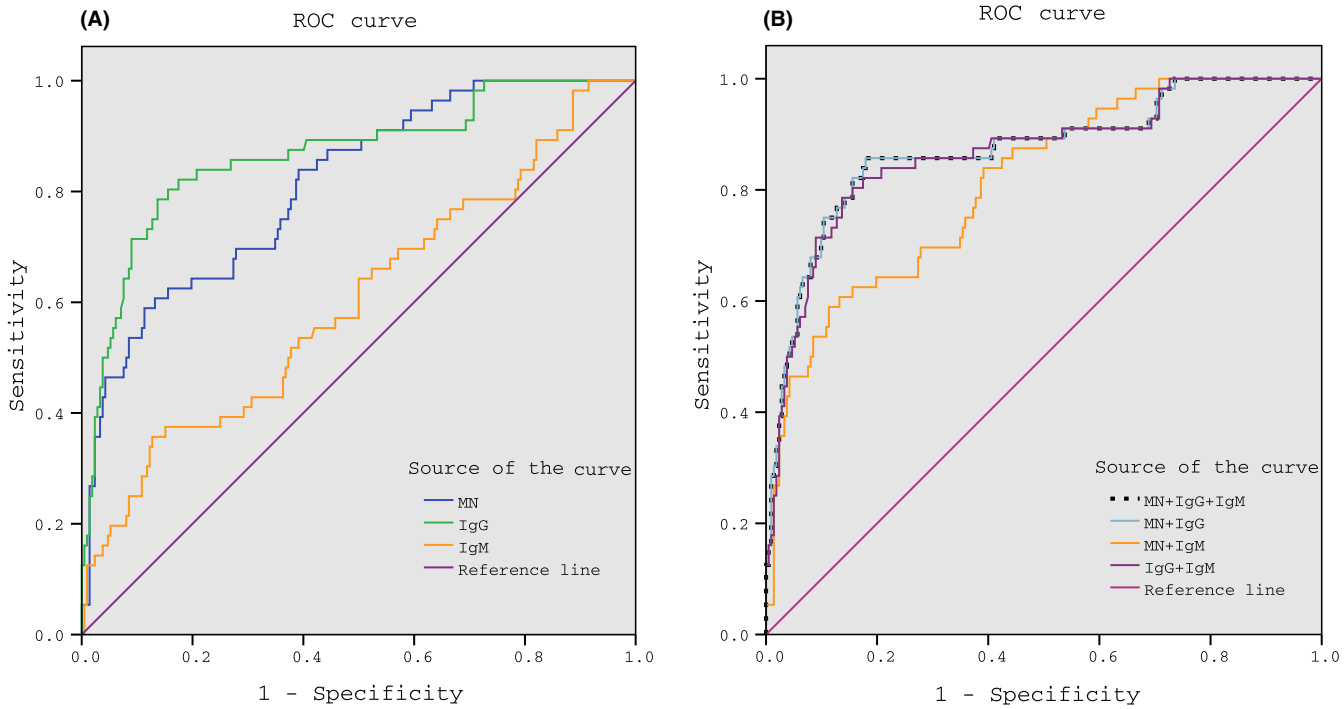

FIGURE 1 ROC curves indicates the diagnostic value of MN, IgG and IgM for *Candida* bloodstream infection vs healthy controls (A); ROC curves indicates the diagnostic value of combination of multiple assays (B). MN, Mannan of *Candida*; IgG, Anti-mannan IgG; IgM, Anti-manana IgM

40.57 (P25-P75, 21.29-54.63 AU/mL) ($P < .001$) (Table 2). While there were no significant differences with IgM between species, *C. krusei* levels were much higher, but numbers were limited.

3.3 | *Candida*-positive blood cultures group

The levels of MN, IgG and IgM of the 56 patients with a *Candida*-positive blood culture were compared with those of the 212 healthy controls, and ROC curve analysis was performed (Figure 1). The AUC values of MN, IgG and IgM were 0.812 (95% CI, 0.750-0.873), 0.866 (95% CI, 0.808-0.924) and 0.601 (95% CI, 0.514-0.689), respectively (Figure 1A). The AUC values of the combination of serum MN and IgG were 0.871 (95% CI, 0.813-0.929) (Figure 1B), and the specificity and sensitivity were 93.87% and 57.14%, respectively. The highest positive predictive value (PPV) and positive likelihood ratio (PLR) were obtained with the combination of MN, IgG and IgM (76.19%, 12.11), the highest negative predictive value (NPV) was IgG (94.21%), and the best diagnostic odds ratio (DOR) was IgG (22.19), followed by the combination of MN, IgG (20.41) Details of the specificity, sensitivity, Youden's J statistic, PPV, NPV, PLR, negative likelihood ratio (NLR) and DOR are presented in Table 3.

3.4 | *Candida*-positive blood cultures group vs pulmonary and urine exposure

Comparing the results of the *Candida* bloodstream infection group (56 cases) with the lung and urinary *Candida* exposure group (132 cases), statistical analysis revealed no significant differences in MN levels; however, IgG and IgM levels were significantly different between the two groups ($P = .002$, $P = .005$). The diagnostic value of single assay and combinations was statistically analysed. The results of ROC curve were shown in Figure 2. The detailed statistics were shown in Table 4. From the data in the table, the largest AUC was the three assays combined diagnosis with 0.649 (0.566-0.732). However in single assay, the largest AUC was IgG with 0.640 (0.558-0.722). In the subgroup analysis, MN levels did not show significant differences as well. Comparing the *Candida* bloodstream infection with the lung exposure group showed that only IgM levels revealed a significant difference ($P = .028$), and the AUC values of IgM were 0.615 (0.517-0.713). Comparison between *Candida* bloodstream infection and urine exposure group showed that there were statistical differences in IgG and IgM ($P < .001$; $P = .005$), and the AUC of IgG and IgM was 0.689 (0.595-0.783) and 0.649 (0.550-0.748), respectively.

4 | DISCUSSION

Candida is a yeast and is conditionally pathogenic. It can colonise the upper respiratory tract, oropharynx and lower digestive tract. When the host immune system is impaired, the skin mucosal barrier is destroyed, the flora is displaced, and the microenvironment can

TABLE 3 Performance of potential diagnostic statistical data from *Candida* bloodstream infection vs healthy controls

Assay	AUC (95%CI)	Cut-off (AU/mL)	Sensitivity	Specificity	Youden's J statistic	PPV	NPV	PLR	NLR	DOR
MN	0.812 (0.750-0.873)	59.16	0.6071	0.8679	0.475	0.5484	0.8932	4.597	0.453	10.16
IgG	0.866 (0.808-0.924)	66.42	0.8036	0.8443	0.648	0.5769	0.9421	5.162	0.233	22.19
IgM	0.601 (0.514-0.689)	200.39	0.3571	0.8726	0.23	0.4255	0.8371	2.804	0.737	3.81
MN + IgG	0.871 (0.813-0.929)	–	0.5714	0.9387	0.51	0.7111	0.8924	9.319	0.4566	20.41
MN + IgM	0.812 (0.750-0.873)	–	0.3036	0.967	0.2706	0.7083	0.8402	9.194	0.7202	12.77
IgG + IgM	0.866 (0.808-0.924)	–	0.3393	0.9575	0.2968	0.6786	0.8458	7.992	0.69	11.58
MN + IgG+IgM	0.871 (0.813-0.929)	–	0.2857	0.9764	0.2621	0.7619	0.8381	12.11	0.7315	16.56

Note: Abbreviations: 95%CI, Asymptotic 95% Confidence Interval; AUC, Area under the ROC curve; DOR, Diagnostic odds ratio; NLR, Negative likelihood ratio; NPV, Negative predictive value; PLR, Positive likelihood ratio; PPV, Positive predictive value.

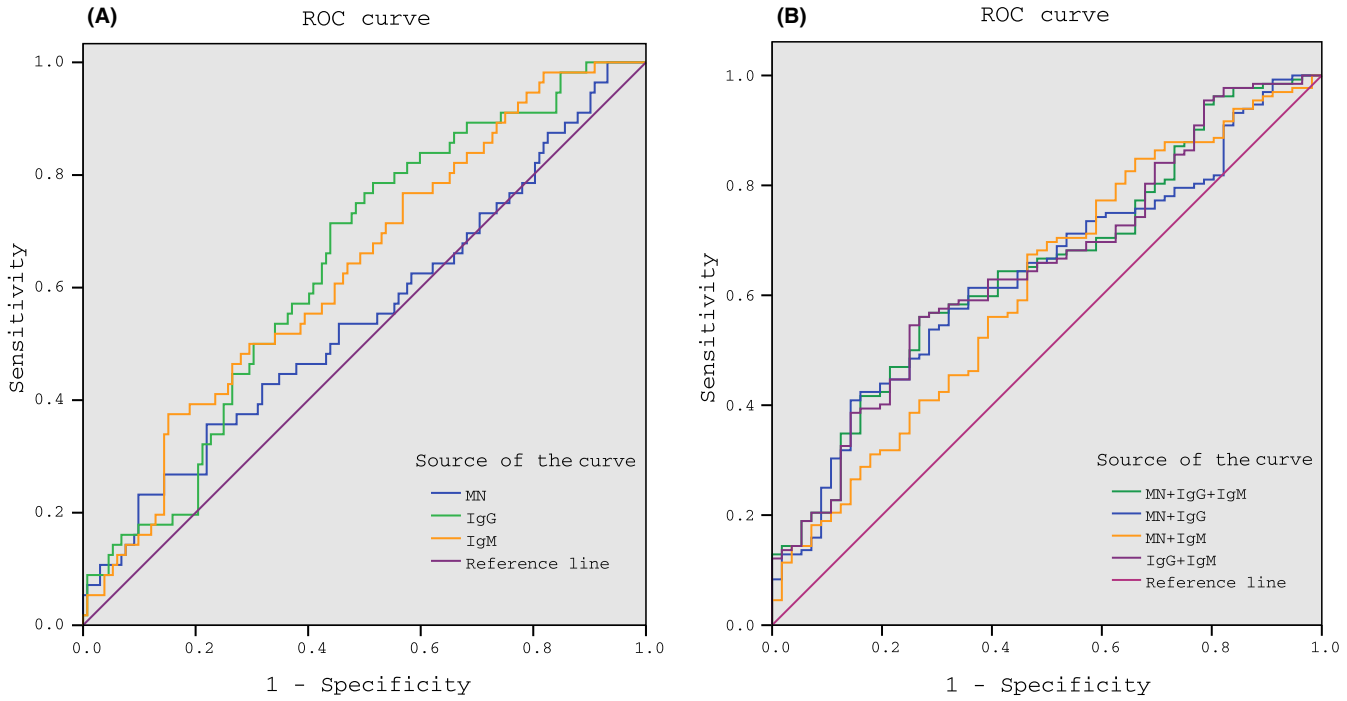

FIGURE 2 ROC curves indicates the diagnostic value of MN, IgG and IgM for *Candida* bloodstream infection vs pulmonary and urine exposure (A); ROC curves indicates the diagnostic value of combination of multiple assays (B)

become dysregulated. *Candida* can lead to the infection of multiple systems.[12-15] The diagnosis of invasive candidiasis is an important step before treatment. As a rapid diagnostic method for candidiasis, the detection of *Candida* MN and antibodies has been approved for drug marketing in Europe, but it has not been widely recognised in Asia and the Americas.[16] This study included a total of 452 cases, which expands the current state of clinical information regarding *Candida* MN and antibody detection. This study also extends the current state of the research for bloodstream, lower respiratory tract and urinary tract *Candida* infections.

Compared with infections of other locations, the diagnostic criteria for *Candida* bloodstream infection are objective and clear.[17] When the serum *Candida* MN and antibody levels in patients with bloodstream *Candida* infections were compared with those in the healthy population, the cut-off value for a diagnosis of candidiasis could be determined with a high accuracy and a high reference value. According to the ROC curve analysis, the AUC value of *Candida* MN was 0.812. When 59.16 AU/mL is used as the cut-off value for the MN level, the specificity for the positive diagnosis of a *Candida* bloodstream infection is 86.79%, and the sensitivity is 60.71%. The AUC value of the mannan IgG was 0.866. When 66.42 AU/mL is used as the cut-off value of the IgG level, the specificity is 84.43%, and the sensitivity is 80.36%. Compared with *Candida* MN, IgG parameter has slightly lower specificity but a higher sensitivity. When compared with blood culture, the diagnostic performance of both *Candida* MN and IgG is less specific but more sensitive,[9] but these values have a shorter time to diagnosis. The AUC value of mannan IgM was 0.601, which is not considered to have a high diagnostic value for candidiasis and may suggest that many patients mount rapid amnestic responses.[16] The AUC values of the combination of MN and IgG were 0.871 that would increase the PPV and PLR for candidaemia. However, the combination of three biomarkers may further improve these statistical indicators, but not obvious, and combination of MN and IgG in cases with clinically suspected candidemia may be preferred.

The *Candida* cell wall can be divided into two layers. The outer layer is primarily composed of O-linked and N-linked glycoproteins, of which 80%-90% are mannan proteins, and the inner layer is composed of β-1,3/1,6 glucan.[18] As an important component of the *Candida* cell wall, mannan is present in *C. albicans* and other *Candida* species, but the mannan in different species of *Candida* has different specific structures and is present in different amounts.[19] Studies have shown that the mannan has a higher specificity for invasive *Candida* infection than anti-mannan.[20,21] The mannan component enters the blood circulatory system during candidiasis; mannan has strong antigenicity and plays an important antigenic role in humoural immune response.[22] In this study, the serum *Candida* MN concentration of patients with *Candida*-positive venous blood, tracheal aspirate and urine cultures was significantly higher than that of the healthy controls and patients with bacteraemia ($P < .001$), suggesting the good specificity of this test. Considering the differences among *Candida* species, 188 patients with positive *Candida* cultures were used for a stratified analysis. The highest serum concentration of *Candida* MN was observed in patients with *C. albicans* infection, with a median level of 387.51 (P25-P75, 67.74-656.15 AU/mL), and the lowest was found in patients with *C. parapsilosis* infection, with a median level of 29.30 (P25-P75, 18.21-205.67 AU/mL). This is consistent with the difference in sensitivity for antigenic detection by EB-CA1 monoclonal antibody of the different *Candida* species Mannose epitopes

TABLE 4 Performance of potential diagnostic statistical data from *Candida* bloodstream infection vs pulmonary and urine exposure

Assay	AUC (95%CI)	Cut-off (AU/mL)	Sensitivity	Specificity	Youden's J statistic	PPV	NPV	PLR	NLR	DOR
MN	0.548 (0.455-0.641)	540.63	0.3571	0.7803	0.137	0.4081	0.7410	1.626	0.8239	1.97
IgG	0.640 (0.558-0.722)	86.23	0.7142	0.5606	0.275	0.4082	0.8222	1.626	0.5097	3.19
IgM	0.631 (0.546-0.716)	190.11	0.375	0.8485	0.223	0.5122	0.7619	2.475	0.7366	3.36
MN + IgG	0.636 (0.553-0.719)	–	0.3571	0.8409	0.198	0.4878	0.7551	2.245	0.7645	2.94
MN + IgM	0.614 (0.526-0.702)	–	0.1964	0.9470	0.143	0.6111	0.7353	3.704	0.8486	4.37
IgG + IgM	0.648 (0.565-0.732)	–	0.3392	0.8864	0.226	0.5588	0.7597	2.986	0.7454	4.01
MN + IgG+IgM	0.649 (0.566-0.732)	–	0.1964	0.9621	0.158	0.6875	0.7384	5.186	0.8352	6.21

and is less likely to detect *C. parapsilosis* mannan.[23] The difference in the *Candida* MN serum concentrations in different *Candida* species infections was statistically significant ($P = .001$), and the difference was also statistically significant ($P < .001$) when compared with the *Candida* MN level of healthy controls, which was 14.45 (P25-P75, 7.41-31.45 AU/mL). The results show that the *Candida* MN serum level was increased in the *Candida* exposure group patients, and there were also species differences. In tracheal aspirate specimens, the level of mannan in patients with *Candida*-positive cultures was significantly different from that in healthy controls ($P < .001$). In urine specimens, the level of mannan in patients with *Candida*-positive urine cultures was significantly different from that in healthy controls ($P < .001$). Therefore, high levels of mannan indicate Candida exposure in the respiratory and urinary systems.

Candida antibodies include IgG and IgM produced by the humoural immune response when stimulated by *Candida* mannan. Because the serum samples were collected on the same day as a *Candida*-positive culture was observed (or on the day after), there was a concern that the window for antibody production might be too short, leading to negative IgG and IgM detections; however, the results confirm that this was not a problem. According to the ROC curve analysis, as a diagnostic method for the detection of *Candida* bloodstream infection, the AUC value of mannan IgG was 0.866, with a specificity of 84.43% and a sensitivity of 80.36%. The use of mannan IgG for detection provides a similar specificity and higher sensitivity compared with *Candida* MN. The IgG levels found in patients with different Candida species infections show that *C. krusei* had the highest level at 161.75 (P25-P75, 72.84-332.23 AU/mL), while *C. tropicalis* had the lowest level at 76.25 (P25-P75, 36.13-169.93 AU/mL), and the difference was statistically significant ($P = .008$). The IgG levels from the different *Candida* species infections were significantly different from that of the healthy population, which was 40.57 (P25-P75, 21.29-54.63 AU/mL) ($P < .001$). In the tracheal aspirate and urine samples, the *Candida* IgG levels were significantly different from that in the healthy population ($P < .001$). Similar to the results of MN, high levels of IgG may serve as a biomarker for *Candida* exposure. Differently, the results suggest that it was difficult to use *Candida* antigen and antibody assays to identify bloodstream infections and other local exposures. The results showed no significant difference in antigen between the groups. Although there were significant differences in antibody assays, the diagnostic value was limited. It may be due to the intersection of exposure and *Candida* infection. However, 60%-70% of paediatric ICU patients with *Candida* bloodstream infection are colonised with the same *Candida* spp.,[24] indicating that some exposed groups will progress to candidaemia or have occurred but not been diagnosed.

According to the current clinical research reports on the detection of *Candida* MN and antibodies, at present this study included the largest number of enrolled subjects. However, for further stratification analysis based on the infection sites, *Candida* species, and immune status, more clinical and laboratory data are needed. Since the enrolment across 23 centres is still in progress, please look forward to our future research results.

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Not applicable.

CONFLICT OF INTEREST

The authors declare that they have no competing interest.

AUTHORS' CONTRIBUTIONS

All authors participated equally in the study in recruiting patients. KW carried out the conception and design of the review and drafted the manuscript. Yanping L carried out the design of the review and drafted the manuscript. SX and WZ carried out the data collection and revised the manuscript. YL, Yanqin L and XM performed the numerical experiments. KX and PY guided the data analysis and the use of medical statistics. HF and JC carried out the data cheque and helped to edit the manuscript. LX participated in conception and design of the review, performed the statistical cheque and helped to draft the manuscript. All authors read and approved the final manuscript.

ETHICAL APPROVAL

All experimental protocols were approved by the Ethics Committee of the Chinese People's Liberation Army (PLA) General Hospital.

EXPERIMENTAL STATEMENT

All methods were carried out in accordance with the guidelines and regulations.

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