

ORIGINAL ARTICLE

Medicine Science 2021;10(4):1368-72

The role of combined use of mannan antigen and anti-mannan antibodies in the diagnosis of invasive candidiasis in pediatric intensive care unit

Yasemin Ay Altintop¹, Ayse Betul Ergul², Ayse Nedret Koc³, Mustafa Altay Atalay³¹Kayseri Training and Research Hospital, Department of Microbiology, Kayseri, Turkey²Kayseri Training and Research Hospital, Department of Pediatrics, Kayseri, Turkey³Erciyes University, Faculty of Medicine, Department of Microbiology, Kayseri, Turkey

Received 15 June 2021; Accepted 14 September 2021

Available online 24.11.2021 with doi: 10.5455/medscience.2021.04.134

Copyright@Author(s) - Available online at www.medicinescience.org

Content of this journal is licensed under a Creative Commons Attribution-NonCommercial 4.0 International License.



Abstract

The combination of Mannan antigen and anti-mannan antibody (Ag / Ab) test is a specific diagnostic test for the diagnosis of invasive Candida (IC) infections. In this study, it was purposed to determine the roles of Ag and Ab in the diagnosis of IC in pediatric intensive care unit patients. Nineteen patients with candidiasis treated for various reasons in the pediatric intensive care unit, 25 patients colonized with Candida spp without candidemia, and 15 patients with neither candidemia nor colonized with a total number of 59, were included in the study. In colonized patients to consider colonization; cervical, nasal, throat, axillary, perineal and rectal swab cultures and urine culture were obtained every week throughout admission and Candida colonization index (CI) was calculated. Blood samples were stored at -80°C after separated to serum form till the tests performed. Candida mannan and anti-mannan (Ab) tests were performed in duplicate in a total of 59 serum samples. Serum was assayed with Candida mannan antigen (Ag) and anti-mannan antibody (Ab) with Platelia™ Candida Ag Plus and Platelia™ Candida Ab Plus (Bio-Rad, Marnes las Coquette, France). The Ag and Ab values of the candidemia and colonized group were higher than the non infected and non-colonized group ($p < 0.001$). The antigens were evaluated for threshold values of 125, 250 and 500 pg / ml while 10 IU /ml was the cut off value for antibody. Although the rate of Ag positive patients (48%) in the colonized group was significantly higher than the group with candidemia (21%) for threshold 125, this difference was not statistically significant. When the threshold value $Ag > 125$ was accepted, antigen positivity for *C. albicans* (12/22) was found to be higher than that of *C. parapsilosis* strains (1/4) ($p > 0.05$). The most compatible combination to detect IC was the combination of Ag 500 and Ab usage with a sensitivity of 57.8% and specificity of 80%. On the era of this combination, PPV was 42.8 and NPV was 80. The use of Candida mannan Ag and Ab together in children increases the diagnostic value in showing invasive candidiasis. Although concomitant use increases the diagnostic value, they need to provide higher NPV in order to exclude invasive candidiasis.

Keywords: Candida mannan antigen, Candida mannan antibody, pediatric intensive care unit

Introduction

There is increasing incidence of Candida infection in Intensive Care Units (ICU) [1]. It is the 3rd most common cause of blood circulation infections and the 2nd most common cause of death from sepsis in children [1-3]. Severe form of Candida infection is Invasive Candidiasis (IC) which is an infection with a mortal course often seen in ICUs and the most frequently seen form of

IC is Candidemia. Although candidemia is the most common IC finding, it is a little-diagnosed infection as sometimes production in blood cultures has been prevented by the initiation of empirical antifungals in ICUs; or in infections caused by some Candida species (eg, *C. glabrata*) growth is difficult to see so it can be overlooked because of the long incubation required [4].

As diagnosis is difficult and takes time, there is a need for tests to assist diagnosis so that appropriate treatment can be started without delay for ICU patients showing severe clinical findings. Although definitive diagnosis is made from culture, causes preventing growth in culture and the long processes have led to efforts to develop such new diagnostic methods like serological ones [5]. Leading to appropriate and rapid antifungal use; tests, applied to selected patients should be targeted to at least halting sequelae and overcoming antifungal resistance of invasive fungal diseases which can have severe outcomes. Biomarkers that can be

*Corresponding Author: Yasemin Ay Altintop, Kayseri Training and Research Hospital, Department of Microbiology, Kayseri, Turkey
E-mail: dryaltintop@hotmail.com

determined in blood and other clinical samples are used adjuvant to traditional pathological and microbiological methods [6]. There are ongoing studies of the fungal biomarkers of *Candida* mannan antigen (Ag) and anti-mannan antibodies (Ab) [7].

As the main component of the cell wall, mannan constitutes approximately 7% of the dry weight of the *Candida* cell, and is found in circulation during *Candida* infection [8]. The examination of mannan antigen and anti-mannan antibodies together is recommended because of the high sensitivity and specificity in IC diagnosis [9]. There are very few studies that have evaluated mannan and anti-mannan together [10]. Studies that have used these methods have generally been conducted on hematology cancer patients and usually in adult patient groups [11-13].

The purpose of this study was to determine the value of mannan antigen and anti-mannan antibody in IC diagnosis in pediatric patients with *Candida* colonization and candidemia in the Pediatric ICU.

Materials and Methods

The study is composed of three groups: I-Patients with no candidemia or colonization (n: 15), II- Patients with colonization but no growth in culture (n: 25), III- Patients with *Candida* spp growth in blood culture (n: 19). Blood samples were taken from the patients in exacerbation periods. Patients taken for colonization study were selected from those who had been in ICU for more than 7 days and had no previous *Candida* spp production. Throat, nose, neck, axillary, perineal and rectal smears and urine cultures were taken from the patients on first admittance to ICU and once a week throughout the stay, and were implanted in Sabaraud Dextrose Agar. Isolated yeasts were identified using standard microbiological procedures, which included identification based on germ-tube formation, colony morphology on SDA, and morphological characteristics on cornmeal agar, urea hydrolyses, and carbohydrate assimilation test using an API 208CAux yeast identification kit (BioMérieux, Marcy l'Etoile, France).

The *Candida* colonization index (CI) was calculated by identifying the *Candida* strains growth. The number of regions growth *Candida* spp in the culture as the proportion of the total number of cultures yielded the CI. A CI >0.2 was accepted as mild and CI >0.5 as intense colonization. The serums were separated from the blood samples taken from all the patients and were stored at -80°C until assay. Each serum sample from the total 59 patients was examined twice for *Candida* mannan antigen (Ag) and anti-mannan antibody (Ab). The *Candida* Ag and Ab in the serums were examined with the Platelia™ *Candida* Ag Plus and Platelia™ *Candida* AbPlus (Bio-Rad, Marnes las Coquette, France) tests.

In accordance with the manufacturer's recommendations, the results for Ag were evaluated according to 3 different threshold values of 125, 250 and 500 pg/ml. Values over 500 pg/ml for Ag were diluted and assayed again.

For Ab, the threshold value was accepted as antibody unit (AU) ≥10 per ml. The threshold values for Ag and Ab were determined with ROC analysis.

Approval for the study was granted by the Local Ethics Committee.

Informed consent was obtained from the parents of legal guardians of each patient.

Statistical analysis

Data obtained from patient records were transferred to a computer and a statistical analysis was performed using SPSS v22.0 software. Descriptive statistics for the measurement values were stated as mean±standard deviation, and for the numerical values, as number (n) and percentage (%). frequency table was created, and conformity of the data to normal distribution was assessed with the Kolmogorov Smirnov test; those conforming to the normal distribution were checked with the One Sample Kolmogorov Smirnov test. Specificity was calculated for the Ag and Ab analyses using the samples taken from the patients without IC. Continuous variables were reported as median and interquartile range (IQR) or mean values in a 95% confidence interval. In the comparative analyses, when parametric test assumptions were met, the Student's t test was applied to determine the difference between the measured variables of the two groups. When parametric test assumptions were not met, the Mann-Whitney U test was applied. To compare the difference between the two percentages, Chi-square analysis was applied. Spearman Correlation Analysis was applied to examine the relationships between variables. All statistical analyses were performed two-way at a 95% confidence interval. A value of $p < 0.05$ was accepted as statistically significant.

Results

The demographic data of patients, underlying diseases and *Candida* mannan values are shown in Table 1.

The 19 patients with candidemia comprised 11 males and 8 females with a mean age of 42 months. No statistically significant difference was found between the groups with colonization and without colonization in respect of age and gender. When the underlying diseases were evaluated, there was seen to be a significantly higher rate of hemato-oncological cancers in the candidemia group ($p < 0.001$). In the group with colonization, there were seen to be more neurological diseases but not of a statistically significant level. In both the colonised group and the candidemia group, the most growth *Candida* strain was *C.albicans* ($p < 0.001$).

The mean Ag value of all the groups was median 66.6 (0-1008) and an Ag value above the median was only obtained in the colonised group 98.9 (3-983) (200.86 ± 263.51). The Ag and Ab values in the candidemia group were determined to be statistically significantly higher than those of the colonised and non-infected groups ($p < 0.001$). In the 19 candidemia patients, Ab was positive in 4 patients with *C.albicans* growth, in 2 patients with *C.parapsilosis* and in 2 patients with *C.glabrata*. Antigen positivity was determined in 2 patients with *C.albicans* growth and in 1 patient with *C.parapsilosis* (Table 2).

Although not statistically significant, when the threshold value of $Ag > 125$ was accepted in the colonised group, Ag positivity was found to be proportionally greater for *C.albicans* than for *C.parapsilosis*. No statistically significant difference was determined between the groups in respect of *Candida* strain for *Candida* Ab. Antibody positivity was determined in 1 patient in the group with no colonization or candidemia. No Ag or Ab positivity

Table 1. Demographic data of the patients of study groups

	All patients (n = 59)	Study groups			P value
		Non colonize nor infected (n = 15)	Colonized by <i>Candida</i> spp (n = 25)	Candidemia (n = 19)	
Mean Age (month)(±SD)	36.01±47.31	27.93±42.47	36.04±57.57	42.36±35.97	NS
Gender M/F (%)	34/25 (57.6)	9/6 (60)	14/11 (56)	11/8 (57.8)	NS
Underlying Diseases (%)					
HEM	21 (35.6)	4 (26.7)	2 (8)	15 (78.9) *	<0.001
CARD	7 (11.9)	1 (6.7)	5 (20)	1 (5.3)	NS
MET	11 (18.6)	3 (20)	6 (24)	2 (10.5)	NS
NEURO	13 (22)	3 (20)	9 (36)	1 (5.3)	NS
RESP	7 (11.9)	4 (26.7)	3 (12)	-	NS
Antigen (mean)	150.15±235.11	95.85±56.19	200.86±263.51	126.28±276.50	NS
Antigen					
Cut off 125 positive/negative(%)	17/42 (40)	1/14 (0.6)	12/13 (48)	4/15 (21)	NS
Cut off 250 positive/negative(%)	10/49 (16.9)	0/15 (0)	7/18 (28)	3/16 (15.7)	NS
Cut off 500 positive/negative(%)	7/52 (11.8)	0/15(0)	4/21 (18.1)	3/16 (15.7)	NS
Antibody positive/negative(%)	12/47 (20.3)	1/14 (6.7)	3/22 (12)	8/11 (42.1) *	0.048

SD: Standard deviation, M: Male, F: Female, HEM:Hemato- oncological malignancy, CARD:Cardiovascular, MET: Methabolic, NEURO: Neurological, RESP. Respiratuar diseases NS. Non spesifik

was determined in patients colonised with *C.glabrata*, *C.keyfr* and *C.tropicalis*. In 2 of the 4 patients with *C.glabrata* candidemia, Ab positivity was determined.

In the group with colonization , Ab was positive in 4 patients with *C.albicans* and CI>0.2, and Ag positivity was determined in 4. In 1 patient with CI>0.5, Ab was positive, and in 1, Ag was found to be positive.

In the ROC analysis, the highest sensitivity (75%) and specificity (96%) was obtained at the value of >221 when the threshold value of >250 was accepted for Ag. Further analysis could not be made due to the low number of patients.

When the *Candida* mannan Ag and Ab findings were evaluated separately in the determination of IC because of candidemia, the highest specificity was 90% for Ab and 90% at the threshold value of 500 for Ag (Table 3). The sensitivity for these values was 42.1%

Table 2. Distribution of *Candida* species and Antigen and Antibody positivity in groups Colonized by *Candida* spp and Candidemia

Genus	Total	<i>Candida</i> spp colonization* n= 25				Candidemia n=19				
		Antigen 125** n (%)	Antigen 250**n (%)	Antigen 500**n (%)	Antibody n (%)	Total	Antigen 125 n (%)	Antigen250 n (%)	Antigen 500 n (%)	Antibody n (%)
<i>C. albicans</i>	22*	12 (54)	6 (27)	4 (18)	3(13.6)	7	2 (28)	2 (28)	2 (28)	4 (57)
<i>C.parapsilosis</i>	4	1 (25)	-	-	-	6	2 (33)	1 (16)	1 (16)	2 (33)
<i>C.krusei</i>	2	1 (50)	1 (50)	1 (50)	-	1	-	-	-	-
<i>C. glabrata</i>	1	-	-	-	-	4	-	-	-	2 (50)
<i>C. keyfr</i>	1	-	-	-	-	1	-	-	-	-
<i>C. tropicalis</i>	1	-	-	-	-	-	-	-	-	-

*some patients were colonized with >1 *Candida* species.: *C.albicans*+ *C.parapsilosis*, *C.albicans*+*C.krusei*, *C.albicans*+ *C.tropicalis*, *C albicans*+*C.keyfr*; *C.albicans*+*C.krusei*+*C.glabrata*; ** When the antigen threshold value is calculated separately as 125, 250, 500 pg / ml.

Table 3. Sensitivity and specifity of *Candida* mannan Ag ve Ab determined single or together

	Sensitivity	Specifity	PPV	NPV
Mn 125+ A. Mn	63	60	42.8	77.4
Mn 500+ A. Mn	57.8	80	42.8	80
Mn 125	21	70	25	65.1
Mn 500	15	90	42.8	69.2
A. Mn	42.1	90	66.6	76.5

Mn: *Candida* Mannan antigen, A.Mn: Anti Mannan antibody, PPV: Pozitif Predictive Value, NPV: Negatif Predictive Value

and 15% respectively. When the most compatible combination of Ag 500 and Ab was used in the determination of IC, sensitivity was found to be 57.8% and specificity 80%. The positive predictive value (PPV) for these values was 42.8 and negative predictive value (NPV) was 80.

Discussion

Invasive candidiasis (IC) can be a life-threatening disease if not diagnosed early and treated appropriately. It is seen in patients with suppressed immunity as a result of immune system treatments and a lengthy stay in ICU with various interventions applied. Pana et al reported that IC is widely seen in hematology patients [14]. In the current study, there were found to be statistically significantly more hemato-oncology patients in the candidemia group. The gold standard in diagnosis is culture, but it may not always be possible [15].

As growth in culture takes a long time, it is important that early warning systems are developed. *Candida* colonization is known to be a risk factor for the development of IC [16]. Chumbitazi et al suggested that the specificity of laboratory diagnosis of positive Ag was increased in patients colonised with *Candida* spp [8]. In the current study, Ag positivity was found to be greater in those with mild CI(>0.2) (4/25) compared to those with intense CI (>0.5) (1/25).

Mannan antigen found in the cell wall of *Candida* spp and the mannan antibody that forms against it are known to have a role in the determination of IC. However, there have been very few studies on this subject in pediatric populations [17].

Pana et al reported that there was no standard Ag threshold value for children [14]. In a study by Lunel et al, 21 IC patients were compared with 30 patients receiving chemotherapy and there was observed to be an increase in sensitivity at a low threshold value (>125) [18]. In the same study, it was also shown that lowering the threshold in candidemia patients did not make a difference in the determination of IC, and more positive results were found at a low threshold in patients who did not have candidemia, which they associated with the expression of mannan Ag into the blood of patients with superficial candidiasis. In the current study, high Ag positivity (13/40) was found at a low threshold (125 pg/ml) in the colonised group, with high specificity (70%) but low sensitivity (21%).

Similarly, Mokaddas et al found that when the Ag threshold was taken as 500, no significant increase was determined in diagnosis in the Ag levels of patients with mucosal *Candida* colonization [19]. In a study by Rao et al, in which children in ICU who could develop IC were examined, Ag was found to be 100% sensitive, whereas Ab had 60% sensitivity, and the 23% rate of false positivity was associated with IC not being able to be diagnosed because of antifungal treatment [20]. In the current study, while false positivity associated with colonization or non-diagnosed IC was seen at a low Ag threshold value (125 pg/ml), an Ag threshold value of 500 pg/ml could be an important limit value for potential IC.

In the ROC analysis applied with Ag to evaluate candidemia in the

current study, the highest sensitivity and specificity values were reached at the threshold of 221 pg/ml.

Previous studies have used threshold values of 5 and 10 AU/ml for Ab, and sensitivity and specificity have been reported as 47%-61% and 92%-94% respectively [21-23]. Similarly, in the current study when the threshold was taken as 10AU/ml, Ab sensitivity and specificity were found to be 42% and 90%, respectively. Using high threshold values for Ab (>20->50 AU/ml) with the algorithm developed by Rouze et al, sensitivity has been reported to be increased and it has provided less and shorter-term use of antifungals [24]. In the current study, the effect of Ab level on antifungal use could not be evaluated as there was no long-term follow-up of the patients. However, when the threshold value for Ab was taken as 5AU/ml, only 1 patient in the colonised group was Ab positive, suggesting that taking the threshold value at 10 AU/ml would be more sensitive.

Mikulska et al reported that with the use of Ag, Ab, and both together (Ag+Ab), sensitivity was 62%, 57% and 86% respectively and specificity was 93%, 83% and 86%, respectively and therefore concluded that whatever threshold value was used, using the Ag and Ab tests together was more advantageous than single use [25].

Prella et al demonstrated a large increase in sensitivity using Ag+Ab (from 29% to 79%), and a slight fall in specificity (from 92% to 84%) [22]. When evaluations in the current study were made at the Ag threshold value of 125 and 500 pg/ml, sensitivity in the Ag+Ab test was found to be 63% and 57.8%; and specificity was 60% and 80% respectively. Consistent with the findings of previous studies, the use of the tests together was observed to increase sensitivity with a slight drop in specificity.

When the sensitivity and specificity of the tests have been examined according to the *Candida* strain, more pleasing results have been obtained for *C.albicans*, *C.tropicalis* and *C.glabrata*, while lower sensitivity and specificity values have been shown for *C.parapsilosis* and *C.krusei* [7, 25]. Researchers have suggested that this difference could be attributed to the dominance in EB-CA1 monoclonal antibody of *C.albicans*, which is bound to the mannan epitope [26]. Similarly in the current study, Ag positivity was found to be proportionally greater in the colonised group. However, there can be considered to be a need for further studies of more extensive series to show the effect of the aforementioned epitope. Ellis et al stated that to obtain a reasonably PPV when the possibility of IC was low, the highest sensitivity should be obtained by lowering the threshold value, and at the threshold of 250 pg/ml, PPV was found to be 36% [27]. In the current study, the highest PPV (66%) was obtained when Ab used alone. With the combined use of Ag at a threshold of 500 pg/ml and Ab, a similar rate (42.8%) was obtained to that in the above-mentioned study. The highest NPV (80%) was also obtained with this combination. In a similar study, it was suggested that with a high NPV (>90%) in the study group, the probability of IC was low and thus there was high diagnostic benefit in discounting patients with a negative Platelia test [27]. In the current study to exclude invasive candidiasis higher NPV is essential.

Non-culture tests show the possibility of infection, not a definitive diagnosis. However, the use of *Candida* mannan Ag and Ab together

in children increases the diagnostic value in showing invasive candidiasis. Nevertheless, despite the increase in diagnostic value of taking the Ag threshold value of 221 pg/ml in the diagnosis of IC, there is a need for further studies of larger patient groups to be able to recommend threshold values for these tests in children.

Limitation of the study

The number of patients in groups is low but it could have not been increased due to the special patient group during study period. Larger patient and control groups will help to improve the specificity of the combined usage of *Candida* mannan Ag and Ab along with its sensitivity while distinguishing Candidemia and colonization.

Conflict of interests

The authors declare that they have no competing interests.

Financial Disclosure

All authors declare no financial support.

Ethical approval

Approval for the study was granted by the Local Ethics Committee (2015/177 03.04.2015).

References

1. León C, Ruiz-Santana S, Saavedra P, et al. 2006. A bedside scoring system ("Candida score") for early antifungal treatment in nonneutropenic critically ill patients with Candida colonization. *Crit Care Med.* 34: 730-7.
2. Brissaud O, Guichoux J, Harambat J, et al. 2012. Invasive fungal disease in PICU: epidemiology and risk factors. *Ann Intensive Care.* 2:6.
3. Pittet D, Monod M, Suter PM, et al. 1994. Candida colonization and subsequent infections in critically ill surgical patients. *Ann Surg.* 220: 751.
4. León C, Ruiz-Santana S, Saavedra P, et al. 2009. Usefulness of the "Candida score" for discriminating between Candida colonization and invasive candidiasis in non-neutropenic critically ill patients: a prospective multicenter study. *Crit Care Med.* 37:1624-33.
5. Yera H, Sendid B, Francois N, et al. 2001. Contribution of serological tests and blood culture to the early diagnosis of systemic candidiasis. *Eur J Clin Microbiol Infect Dis.* 20: 864-70.
6. Martínez JP, Gil ML, López-Ribot JL, et al. 1998. Serologic response to cell wall mannoproteins and proteins of *Candida albicans*. *Clin Microbiol Rev.* 11:121-41.
7. Sendid B, Poirot JL, Tabouret M, et al. 2002. Combined detection of mannanaemia and anti-mannan antibodies as a strategy for the diagnosis of systemic infection caused by pathogenic *Candida* species. *J Med Microbiol.* 51:433-42.
8. Chumpitazi BF, Lebeau B, Faure-Cognet O, et al. 2014. Characteristic and clinical relevance of *Candida* mannan test in the diagnosis of probable invasive candidiasis. *Med Mycol.* 52:462-71.
9. Mikulska M, Furfaro E, Viscoli C. 2015. Non-cultural methods for the diagnosis of invasive fungal disease. *Expert Rev Anti Infect Ther.* 13:103-17.
10. Oz Y, Kiraz N. 2011. Diagnostic methods for fungal infections in pediatric patients: microbiological, serological and molecular methods. *Expert Rev Anti Infect Ther.* 9:289-98.
11. Agvald-Öhman C, Klingspor L, Hjelmqvist H, et al. 2008. Invasive candidiasis in long-term patients at a multidisciplinary intensive care unit: Candida colonization index, risk factors, treatment and outcome. *Scand J Infect Dis.* 40:145-53.
12. Caggiano G, Puntillo F, Coretti C, et al. 2011. Candida colonization index in patients admitted to an ICU. *Int J Mol Sci.* 12:7038-47.
13. Leroy G, Lambiotte F, Thévenin D, et al. 2011. Evaluation of "Candida score" in critically ill patients: a prospective, multicenter, observational, cohort study. *Ann Intensive Care.* 1:50.
14. Pana Z-D, Vikelouda K, Roilides E. 2016. Diagnosis of invasive fungal diseases in pediatric patients. *Expert Rev Anti Infect Ther.* 14:1203-13.
15. Clancy CJ, Nguyen MH. 2018. Diagnosing invasive candidiasis. *J Clin Microbiol. JCM.* 01909-17.
16. Wey SB, Mori M, Pfaller MA, et al. 1989. Risk factors for hospital-acquired candidemia: a matched case-control study. *Arch Intern Med.* 149:2349-53.
17. Huppler AR, Fisher BT, Lehrnbecher T, et al. 2017. Role of Molecular Biomarkers in the Diagnosis of Invasive Fungal Diseases in Children. *J Pediatric Infect Dis Soc.* 6:S32-S44.
18. Verduyn Lunel F, Donnelly J, Van Der Lee H, et al. 2009. Circulating *Candida*-specific anti-mannan antibodies precede invasive candidiasis in patients undergoing myelo-ablative chemotherapy. *Clin Microbiol Infect.* 15:380-6.
19. Mokaddas E, Burhamah MH, Khan ZU, et al. 2010. Levels of (1→3)-β-D-glucan, *Candida* mannan and *Candida* DNA in serum samples of pediatric cancer patients colonized with *Candida* species. *BMC Infect Dis.* 10:292.
20. Rao DR, Ghosh A, Singhi S, et al. 2002. Mannan antigen detection in the diagnosis of patients with invasive candidiasis. *Indian J Med Res.* 116:13.
21. Alam FF, Mustafa AS, Khan ZU. 2007. Comparative evaluation of (1, 3)-β-D-glucan, mannan and anti-mannan antibodies, and *Candida* species-specific snPCR in patients with candidemia. *BMC Infect Dis.* 7:103.
22. Prella M, Bille J, Pugnale M, et al. 2005. Early diagnosis of invasive candidiasis with mannan antigenemia and antimannan antibodies. *Diagn Microbiol Infect Dis.* 51:95-101.
23. Sendid B, Tabouret M, Poirot JL, et al. 1999. New enzyme immunoassays for sensitive detection of circulating *Candida albicans* mannan and antimannan antibodies: useful combined test for diagnosis of systemic candidiasis. *J Clin Microbiol.* 37:1510-7.
24. Rouzé A, Loridant S, Poissy J, et al. 2017. Biomarker-based strategy for early discontinuation of empirical antifungal treatment in critically ill patients: a randomized controlled trial. *Intensive Care Med.* 43:1668-77.
25. Mikulska M, Calandra T, Sanguinetti M, et al. 2010. The use of mannan antigen and anti-mannan antibodies in the diagnosis of invasive candidiasis: recommendations from the Third European Conference on Infections in Leukemia. *Crit Care.* 14:R222.
26. Jacquinet PM, Plancke Y, Sendid B, et al. 1998. Nature of *Candida albicans*-derived carbohydrate antigen recognized by a monoclonal antibody in patient sera and distribution over *Candida* species. *FEMS Microbiol Lett.* 169:131-8.
27. Ellis M, Al-Ramadi B, Bernsen R, et al. 2009. Prospective evaluation of mannan and anti-mannan antibodies for diagnosis of invasive *Candida* infections in patients with neutropenic fever. *J Med Microbiol.* 58:606-15.