ORIGINAL RESEARCH

Role of Candida Mannan Antibody Assay in the Diagnosis of Invasive Candidiasis (IC) in Patients with Malignant Conditions

Arunjyoti Sarmah[1], Dipak Kumar Das[2], Ajanta Sharma[3], Jagannath Deb Sharma[4]

ABSTRACT

Introduction: Mannan is a major component of the *Candida albicans* cell wall, composing up to 7% of the cell dry weight, and is one of the main Candida antigens that circulate during infection. It is a serum marker for the presence of Invasive Candidiasis is Candida Mannan antibody, which has been included in the relevant European Organization for Research and Treatment of Cancer/ Mycoses Study Group (EORTC/MSG) diagnostic criteria. Aims of the study were to study the prevalence of invasive candidiasis in patients with malignant conditions and to assess the role of Candida Mannan antibody assay in the diagnosis of invasive candidiasis.

Material and method: A total of 88 individuals attending Gauhati Medical College and Hospital (GMCH) and Dr. B. Barooah Cancer Institute (BBCI) with diagnosed malignancy who are at risk of developing invasive infection with *Candidaspp* according to Revised EORTC criteria were enrolled for the study

Results: Out of the 88 study cases, 38 (43.18%) patients were found to fulfil the criteria necessary for qualifying as cases of invasive candidiasis. Hence, the prevalence rate of invasive candidiasis in the study group was calculated to be 43.18%. The sensitivity, specificity, Positive predictive value (PPV) and Negative predictive value (NPV) of Candida Mannan Antibody assay wascalculated as 60.52%, 92.0%, 85.18% and 75.41% respectively.

Conclusions: The high Specificity, PPV and an at-par NPV suggest that the test may be particularly useful in excluding Invasive Candidiasis. It is thus feasible to explore the use of serial measurements of Mannan and Anti-Mannan as part of a broader diagnostic strategy for selecting patients to receive antifungal drug therapy.

Key-words: Candida, EORTC, Invasive, Mannan, Malignancy

How to cite this article: Arunjyoti Sarmah, Dipak Kumar Das, Ajanta Sharma, Jagannath Deb Sharma. Role of candida mannan antibody assay in the diagnosis of invasive candidiasis (IC) in patients with malignant conditions. International Journal of Contemporary Medical Research 2015;2(5):1219-1223.

[1]Research Scientist I- Medical, Department Of Microbiology, State Level VRDL, [2]Associate professor, Department of Microbiology, Gauhati Medical College, [4]Professor & Head, Department of Pathology, B. Barooah Cancer Institute, Guwahati, [3]Professor, Department of Microbiology, Assam Medical College, Dibrugarh, India

Corresponding author: Dr. Arunjyoti Sarmah, 5th Floor, Department of Microbiology, Gauhati Medical College, Narakasur Hilltop, P.O.- Indrapur, Guwahati, India.

Source of Support: Nil

Conflict of Interest: None

INTRODUCTION

Candida species are part of normal flora of human beings found commonly on the skin, throughout the gastrointestinal tract and female genital tract especially higher in the vagina during pregnancy. A number of factors are known to predispose candidiasis (both superficial and deep seated) either by lowering the host resistance or by altering the balance of normal microbial flora of the body.[1,2]

During the last century a number of predisposing factors have been surfaced, two of which seem to be of utmost importance. The first is the advent of antibacterial antibiotics and their indiscriminate use and the second being the emergence of pandemics like AIDS, Malignancy, Diabetes mellitus and other immunocompromising diseases. Despite the prevalence of *C. albicans* as the most common causative agent of superficial and deep fungal infections, a rising incidence of non-*albicans Candida* like *C. tropicalis, C. krusei, C. glabrata and C. Parapsilosis* has been documented in the last few years.[1,3]

Invasive candidiasis is a fungal infection that occurs when Candida enters the bloodstream and then spreads through the body. Invasive candidiasis (IC) includes disseminated candidiasis with deep organ involvement, candidemia,endocarditis and meningitis. IC has an attributable mortality of 40% to 50% and is increasingly reported in intensive care units (ICUs).[4]

Candida is the fourth most common cause of bloodstream infection among hospitalized patients in developed countries. A survey conducted at Oxford University found that candidemiaoccurs in 8 of every 100,000 persons per year. Persons at high risk for candidemia include low-birth-weight babies, surgical patients, and immunosuppressed individuals.[4]

Studies from European countries like Iceland, Norway and Denmark have shows a considerable rise in the incidence of candidemia over a period of a decade from 1990-1991 to 2002-2003.[4] A 10 year period study in Switzerland reported *Candida* species to be the seventh among the most common causes of BSI in hospitals.[5] Another study from Finland stated an increase in the yearly incidence rate of candidemia from 1.7 per 100,000 persons in 1995 to 2.2 in 1999.[6] A Norwegian study reported candidemia episodes from approxi-

mately 1-2 episodes in the early 1990s to around 3 episodes during 2001 to 2003.[7] Studies carried out in developing countries have also resonated on similar lines. An overall incidence of 2.49 cases of candidemia for every 1000 admissions was reported in yet another study of candidemia carried out in Brazil.[8]

Invasive candidiasis typically present with non specific symptomatology; chills and fever that do not improve after antibiotic therapy being the most common symptoms. Additional specific symptoms develop only when the infection spreads to deep organs such as liver, bones, kidneys, muscles, joints, spleen or eyes, which may vary depending on the site of infection. Occaisionally, it can also be introduced iatrogenically when medical devices or equipment are contaminated with Candida species. Invasive candidiasis (IC) is usually diagnosed either by culture of blood or tissue or by examining samples of infected tissue under the microscope.[1]

Invasive candidiasis is fast gaining clinical importance owing to an increase in the population at risk, which generally includes patients with immunosuppression (both disease-related and iatrogenic) and patients on various types of supportive care, especially in Intensive Care Units and Burn Wards.[1]

Technological advances in recent times have drastically curtailed most of the infectious diseases but the picture is opposite in the context of cancer. Increment in life span alongwith modern way of living and pollution resulted in a upward trend of this dreaded disease. In fact, there is gradual yet consistent increase in the incidence of cancer worldwide. The present cancer burden is alarming with approximately 25 millions globally and 2.5 millions for India. Research studies have shown the rise of invasive mycoses as the major mortality factor in malignancy as evidenced by approximately 20% rise in mortality rate due to delay in anti-fungal administration by a single day.[9,10] The rising trend of various malignant disorders in this part of the country associated with high mortality rate necessitates a study on invasive candidiasis among patients with malignancies to understand the actual burden of the disease and to study the scope of various modalities for the early diagnosis of this condition.

There is increasing interest in pre-emptive antifungal therapy, defined as treatment that is deferred until there is substantial evidence for the presence of an IFI.[6,7] Blood culture or Culture from any normally sterile site for the diagnosis of IC, although highly specific but are not without their disadvantages. The most important disadvantages being the long waiting period and low sensitivity. It has been found that Culture requires at least 8-10 days for reporting a positive result, which can be decisive when it comes to treatment of IC where delay by even a day results in increase of mortality rate. Early detection of diagnostic markers of a fungal infection, such as fungal antigens, fungal nucleic acids, antibodies, or cell wall components, is essential in this regard. A serum marker for the presence of Invasive Candidiasis is Candida Mannan antibody, which has been included in the EORTC/MSG diagnostic criteria.[11]

Hence, this study was taken up in order to assess the role of Candida Mannan antibody assay in early diagnosis of invasive candidiasis with respect to conventional methods to reduce the associated morbidity and mortality.

MATERIALS AND METHODS

This study is a hospital based prospective study carried out in Dr. B. Barooah Cancer Institute (BBCI), Guwahati and Department of Microbiology, Gauhati Medical College, Guwahati during a period of one year from June, 2013 to May,2014. A total of 88 individuals with diagnosed malignancy who fulfil the Revised EORTC criteriafor either possible, probable or proven case of Invasive Candidiasiswere taken up for the study.[11]

Subjects with diagnosed malignant conditions fulfilling the EORTC criteria foreither possible, probable or proven case of Invasive Candidiasis were selected for thestudy.

Inclusion criteria

(As per revised EORTC guidelines 2008):[11]

Criteria for Probable case of IFD: As per EORTC guidelines, Probableinvasive Fungal Disease is defined by at least.

a) One host criterion

AND

b) One clinical criterion

AND

c) One mycological criterion

Host Criteria

1. Recent history of neutropenia (< 0.5 x 109/L {<500 neutrophils/mm3} for >10days) temporally related to the onset of fungal disease or ongoing neutropenia
2. Receipt of an allogenic stem cell transplant
3. Prolonged use of corticosteroids (excluding patients with ABPA) at an averageminimum dose of 0.3 mg/kg/day prednisone equivalent for > 3 weeks
4. Treatment with other recognized T-cell immune suppressants such as cyclosporine, TNF- blockers, specific monoclonal antibodies alemtuzumab, nucleosideanalogues during the past 90 days
5. Inherited severe immunodeficiency (e.g., chronic granulomatous disease, severecombined immunodeficiency)

Clinical criteria

Lower respiratory tract fungal disease

Presence of one of the following "specific" imaging signs on CT:-

1) Non-specific focal infiltrate. 2) Pleural Effusion. 3) Cavity

CNS infection

At least one of the following:- Focal lesions on imaging or Meningeal enhancement on MRI or CT.

Chronic disseminated candidiasis

Small, peripheral, target like abscesses (new nodular filling defects, bull's-eye lesions) in
liver and/or spleen

Mycological Criteria

- Isolation of *Candida* species from a specimen obtained by biopsy or needle aspiration from a otherwise sterile site on histopathologic, cytopathologic, or direct microscopic examination.
- Isolation of *Candida* species by culture of a sample obtained by a sterile procedure from a otherwise sterile site showing a clinical or radiological abnormality consistent with an infectious disease process.
- Blood culture that yields yeast or yeast like fungi
- A single serum sample positive for beta-D-glucan
- A serum sample positive for *Candida* Mannan antigen assay

Criteria for Proven case of IFD

Above Criteria Plus isolation of Yeast from one or more normally sterile sites like Blood, CSF etc

Patients fulfilling at least one Host criterion and one Clinical criterion with orwithout one Mycological criterion of the probable/Proven invasive candidiasis wereincluded in the study.

Exclusion criteria

- Critically ill patients who are not deemed fit to undergo the study by the attendingclinician
- Patients already on prophylactic antifungal therapy

5 ml of venous blood was collected from each individual under study, following all aseptic precautions, in sterile tubes. Serum/Plasma was separated by centrifuging the sample at 3000 rpm for 5 minutes. Separated serum/plasma was transferred to screw capped sterile vials, labelled properly and stored at -20^0 C until tested for *Candida*Mannan Antigen and Antibody by Platelia™ Candida Mannan Antibody Plus ELISA assay (BIORAD).

Simultaneously, 10 ml (patients above 15 yrs of age) and 5 ml (patients below 15 yrs of age) of Venous blood was collected from each individual maintaining all aseptic measures and incubated at 37^0 C for 10 days in Sterile Brain Heart Infusion (BHI) broth. Serial subculture was done from each BHI bottle at intervals of 5, 7 and 10 days onto Sabouraud Dextrose Agar (SDA) with 0.005% Chloramphenicol. Furthermore, other clinical samples like tracheal aspirates, CSF, Sputum & non-catheterised clean catch mid stream urine were collected based on patient symptomatology and cultured on SDA with 0.005% Chloramphenicol.

The *Candidaspp*. were identified by Gram stain and Lactophenol Cotton blue (LPCB).

Sensitivity, Specificity, PPV and NPV were calculated by the following formulae:

$$\text{Sensitivity} = \frac{\text{True Positive}}{\text{True Positive+False Negative}} \times 100\%$$

$$\text{Specificity} = \frac{\text{True Negative}}{\text{True Negative +False Positive}} \times 100\%$$

$$\text{PPV} = \frac{\text{True Positive}}{\text{True Positive+False Negative}} \times 100\%$$

$$\text{NPV} = \frac{\text{True Negative}}{\text{True Negative +False Negative}} \times 100\%$$

Statistical Analysis of data was done with the help of analytical software EPI Info 7 and $P< 0.05$ was considered to be significant.

RESULTS

It was observed in the study that out of 88 cases, the highest numbers of 29 (32.95%) cases were in the 46-60 yearsage group, followed by 20 (22.73%) cases in the age group of 31-45 years and the lowest numbers of 6 (6.82%) cases were`in the age group of 0-15 years (Table 1, Fig 1).The study group comprised of 40 (45.45%) males and 48 (54.54%) females (Table 2, Fig 2).

On the basis of culture positivity of blood and other body fluids and Mannan antigen seropositivity, 38(43.18%) patients qualified as IC as per EORTC guidelines (Table 3). Out of the 38 IC cases, 23 patients were seropositive for Mannan antibody and 15 patients were seronegative. Further out of the rest 50 caseswho did not qualify for IC as per EORTC guidelines, 4 patients were seropositive for Mannan antibody. Considering the patients with mannanantigenemia and/or positive culture of *Candida* from a normally sterile site as cases of Invasive candidiasis and considering the anti-Mannanseroequivocal cases as negative result, we get the following values for the Platelia Candida anti-mannan antibody assay: Sensitivity 60.52%, Specificity 92.0%, Positive

Age Group in Years	No. of Cases (%)	No of Cases of IC (%)	No of Mannan Antibody Positive
0 – 15	6 (6.82%)	3 (7.89%)	1
16 – 30	16 (18.18%)	5 (13.16%)	3
31 – 45	20 (22.73%)	12 (31.58%)	7
46 – 60	29 (32.95%)	12 (31.58%)	8
Above 60	17 (19.32%)	6 (15.79%)	4
Total	88 (100%)	38 (100%)	23

Table-1: Age Distribution of the study cases and cases of ICs

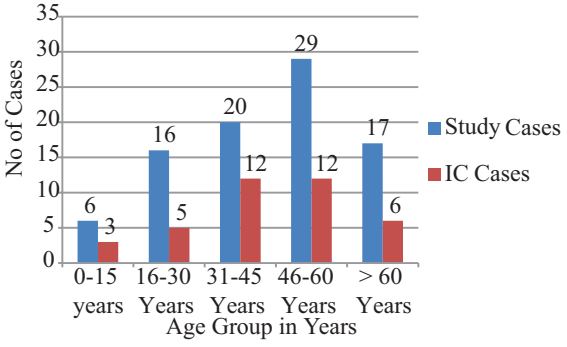

Figure-1: Age Distribution of the study cases and cases of ICs

predictive value (PPV) 85.18% & Negative predictive value (NPV) 75.41%.

DISCUSSION

At the outset, it has to be mentioned that although prior to this study, a large number of studies on the role of Candida Mannan antibody as well as Candida Mannan Antigen have been carried out worldwide, but not much data was available on the prevalence of IC or the role of Mannan Antibody in Malignant patients exclusively.

Out of the 88 study cases, 38(43.18%) patients were found to fulfil the criteria necessary for qualifying as cases of invasive candidiasis. Hence, in this study we have found a high prevalence rate of invasive candidiasis (43.18%)which is contradictory to the report published by Tritipwanit*et al* (2005)[12], Kumar *et al* (2005)[13], Sahni*et al* (2005)[14], Xess*et al* (2007)[15], Kothari *et al* (2008)[10] and Goel*et al* (2009)[16] They have reported the prevalence of invasive candidiasis to be within the range of 6-18% only. However, it is worth mentioning that none of the above studies have been done exclusively on malignant cases.

	No of Study cases (%)	No of cases of IC (%)	No of Mannan Antibody positive cases
Male	40 (45.45%)	15 (39.47%)	10
Female	48 (54.54%)	23 (60.53%)	13
Total	88 (100%)	38 (100%)	23

Table-2: Gender Distribution of the study cases and cases of IC

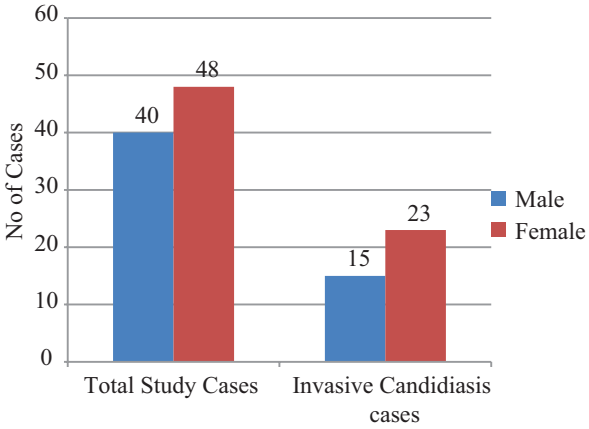

Figure-2: Gender Distribution of the study cases and cases of IC

Out of the 38 cases invasive candidiasis (Mannan antigen seropositive and/or culture positive), it was found that 23 patients were seropositive for Mannan antibody and of theremaining 50 cases, 4 patients were seropositive for Mannan antibody. On application of Chi square test to this data, we get an x^2 value of 28.01 and a $P = 0.00000012$, which signifies that mannan antibody detection in the antigen seronegative patients is statistically significant when compared to the antigen seropositive patients group. This observation is also supported by the data published in studies by Sendid*et al* (2003)[17] and Lunel*et al* (2009).[18]

The performance parameters of Platelia Candida MannanAntibody plus (i.e. Sensitivity 60.52%, Specificity 92.0%, Positive Predictive Value 85.18% & Negative Predictive Value 75.41%) obtained in our study resonates well with the studies by Tabouret*et al* (1999)[19], Alam*et al* (2007)[20] and Mikulska*et al* (2010).[21] Candida Mannan antigen is detectable in blood for 5-7 days only after initial infection after which it disappears from the systemic circulation. Candida Mannan Antibody starts appearing in blood by 7th day of infection and persists upto around 2 months. This means that a serum sample collected between 5-7 days after initial infection will show detectable levels of Mannan antigen but antibody levels in serum might not be in sufficient quantities so as to be detectable by ELISA. These cases are likely to be marked as False Negative cases. To overcome this drawback repeated sampling is needed. This can explain to some extent the high number of False Negative cases and hence the low sensitivity and Negative Predictive value of Candida mannan antibody ELISA.

CONCLUSION

From the study, it can be concluded that Candida Mannan Antibody can be used as an adjunct to the routinely used laboratory diagnostic tests for the diagnosis of invasive candidiasis owing to its reliable test parameters.

ACKNOWLEDGEMENT

The authors would like to thank Dr.AnupamSarma, Assistant Professor, Dept. Of Pathology, Bhubaneswar Barooah

Sample cultured	No. of Culture positives	Mannan antigen			Mannan Antibody Positive
		Positive (%)	Equivocal (%)	Negative (%)	
Blood c/s	2*	2 (100%)	0 (0%)	0 (0%)	2
Sputum c/s	7	1 (14.28%)	0 (0%)	6 (85.71%)	3
Body Fluid c/s	1	1 (100%)	0 (0%)	0 (0%)	1
Urine c/s	18	9 (50%)	5 (27.78%)	4 (22.22%)	11
Tracheal aspirate c/s	2	2 (100%)	0 (0%)	0 (0%)	2
Culture Negative = 60		23 (39.65%)	6 (10.34%)	29 (50%)	10

*One of the two cases of Positive blood Culture was also simultaneously positive for Urine culture and the other was positive for Body Fluid culture

Table-3: Mannan Antigen seropositive cases in Culture positive/negative cases

Cancer Institute (BBCI), Dr. Shiny Baruah&Dr.Anjanjyoti-Bhargab, Registrar(s), Dept. Of Medicine, Gauhati Medical College & Hospital, Mr.Dhrubajyoti, Technician, Dept. Of Pathology, BBCI,Miss JyotishmitaSaikia&Mr.BipulRabha, Data Entry Operator, Dept. Of Microbiology, Gauhati Medical College & Hospital for their help and assistance in carrying out the study. The authors would also like to thank DBT Nodal Centre, Napaam, Tezpur for the financial assistance in the form of MD/MS Thesis Grant of Rupees 1.10 Lakhs for carrying out the study.

REFERENCES

1. Chander J. A Textbook of Medical Mycology, 3rd edition. New Delhi: Mehta Publishers;2009.pp.266-90
2. Chauhan N, Li D, Singh P, Calderone R, Kruppa M. 2002. The cell wall of Candida spp., pp 159–75 Calderone RA, editor. (ed), Candida and candidiasis. ASM Press, Washington, DC
3. Chakraborty P. "A Textbook of Microbiology". 3rd ed. Kolkata: New Central Book Agency (P) Ltd. 2013;pp 686-88
4. Edwards, J. E., Jr., G. P. Bodey, R. A. Bowden, T. Büchner, B. E. de Pauw, S. G. Filler et al. 1997. International conference for the development of a consensus on the management and prevention of severe candidal infections/ cdc.gov/fungal. 25:43-59.
5. Marchetti O, Bille J, Fluckiger U, Eggimann P, Ruef C, Garbino J. Epidemiology of candidemia in swiss tertiary care hospitals: Secular Trends, 1991-2000. Clin Infect Dis 2004;38:311-20
6. Poikonen E, Lyytikainen O, Antilla VJ, Ruutu P. Candidemia in Finland, 1995-1999. Emerg Infect Dis 2003;9:985-90.
7. Sandven P, Bevanger L, Digranes A, Haukland HH, Mansaker T, Gaustad P. Candidemia in Norway (1991 to 2003): Results from a nationwide study. J ClinMicrobiol 2006;44:1977-81
8. Colombo AL, Nucci M, Park BJ, Nouer SA, Arthington-Skaggs B, daMatta DA. Epidemiology of candidemia in Brazil: A nationwide sentinel surveillance of candidemia in eleven medical centers. J ClinMicrobiol 2006;44:2816-23
9. Verma AK, Prasad KN, Singh M, Dixit AK, Ayyagari A. Candidaemia in patients of a tertiary health care hospital from north India. Indian J Med Res 2003;117:122-8.
10. Kothari A, Sagar V. Epidemiology of Candida Bloodstream Infections in a Tertiary Care Institute in India. Indian J Med Microbiol 2008;27:171-2.
11. Ben De Pauwa, Thomas J. Walsh, J. Peter Donnelly, David A. Stevens, John E. Edwards, Thierry Calandra, Peter G. Pappas, Johan Maertens, Olivier Lortholary, Carol A. Kauffman, David W. Denning, Thomas F. Patterson, Georg Maschmeyer, Jacques Bille, William E. Dismukes, Raoul Herbrecht, William W. Hope, Christopher C. Kibbler, Bart Jan Kullberg, Kieren A. Marr, Patricia Munoz, Frank C. Odds, John R. Perfect, Angela Restrepo, Markus Ruhnke, Brahm H. Segal, Jack D. Sobel, Tania C. Sorrell, Claudio Viscoli, John R. Wingard, TheoklisZaoutis, and John E. Bennett:Revised Definitions of Invasive Fungal Disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. Clinical Infectious Diseases 2008; 46:1813–21
12. Tritipwanit K, Chindamporn A, Suankratay C. Epidemiology of candidemia at King Chulalongkorn Memorial Hospital, Thailand. J Infect Dis Antimicrob Agents 2005;22:59-69.
13. Kumar CP, Sundararajan T, Menon T, Venkatadesikalu M. Candidiosis in children with onco-hematological studies in Chennai, India. Jpn J Infect Dis 2005;58:218-21.
14. Sahni V, Agarwal SK, Singh NP, Anuradha S, Sikdar S, Wadhwa A. Candidemia – An Under-recognized nosocomial infection in Indian Hospitals. J Assoc Physicians India 2005;53:607-11
15. Xess I, Jain N, Hasan F, Mandal P, Banerjee U. Epidemiology of candidemia in a tertiary care centre of North India: 5- Year Study. Infection 2007;35:256-9.
16. Goel N, Ranjan PK, Agarwal R, Chaudhary U, Sanjeev N. Emergence of nonalbicansCandida in neonatal septicemia and antifungal susceptibility: Experience from a tertiary care centre. J Lab Physicians 2009;1:53-5.
17. Sendid B, Caillot D, Baccouch-Humbert B, Klingspor L, Grandjean M, Bonnin A, Poulain D (2003) Contribution of the Platelia Candida-specific antibody and antigen tests to early diagnosis of systemic Candida tropicalisinfection in neutropenic adults. J ClinMicrobiol 41:4551–4558
18. Lunel FM, Mennink-Kersten MA, Ruegebrink D, van der Lee HA, Donnelly JP, Blijlevens NM, Verweij PE (2009) Value of Candida serum markers in patients with invasive candidiasis after myeloablative chemotherapy. DiagnMicrobiol Infect Dis 64:408–15
19. Tabouret M, BoualemSendid, Jean Louis Poirot, Daniel Mathieu, Jeanine Fruit, and Daniel Poulain. New Enzyme Immunoassays for Sensitive Detection of CirculatingCandidaalbicansMannan and Antimannan Antibodies: Useful Combined Test for Diagnosis of Systemic Candidiasis. J. Clin. Microbiol. May 1999;37:1510-1517
20. Alam FF, Mustafa AS, Khan ZU. Comparative evaluation of (1, 3)- b -D-glucan, mannan and anti-mannan antibodies, and Candida species-specific snPCR in patients with candidemia. BMC Infect Dis2007; 7:103–111
21. MikulskaM, Thierry Calandra, Maurizio Sanguinetti, Daniel Poulain, Claudio Viscoli and the Third European Conference on Infections in Leukemia Group. 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. Critical Care 2010, 14:R222