Research Article

Antigen detection in comparison to conventional blood culture in fungal detection

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Abstract

Background and objectives: The aim of the present study is to compare the conventional blood culture vs ELISA for the diagnosis of invasive fungal infections in Pediatric Intensive Care Unit (PICU). **Subjects and methods:** The study was carried out on 80 critically ill patients who were admitted to Pediatric Intensive Care Unit (PICU) with one or more risk factors for fungal infection were included in the study. Blood Culture was done using Sabaroud dextrose agar (SDA). Also, ELISA using mannan antigen was done. **Results:** ten cases were positive for fungi by blood cultures out of 80 patients, 8(10%) cases were candida spp., 1 case (1.3%) was aspergillus fumigatus and 1case (1.3%) was kodamaea ohmeri . Among the 8 candida positive cases by blood culture, 8 cases had positive mannan antigen with sensitivity of 80% and specificity of 91.43 %. **Conclusion**: Candida species are the most frequent cause of IFI in PICU and the blood culture still the gold standard method yet has long turnaround time. Antigen detection lack sensitivity.

Keywords: IFI, invasive candidiasis, PICU.

Introduction

The methods available for detection of IFIs include direct or indirect methods of detection. No test is perfect and it is necessary to perform several diagnostic tests to achieve maximum accuracy. The diagnosis of invasive fungal infection is based upon results of combination of clinical and laboratory studies [(11)].

Invasive candidiasis comprises both candidemia and deep-seated tissue candidiasis. Candidemia is generally considered as the most common type of the disease. Deep-seated candidiasis arises from either hematogenous dissemination or direct inoculation of candida species to a sterile site, such as the peritoneal cavity [(9)].

Antigen testing is recommended by the European Organisation for Research and Treatment of Cancer (EORTC) and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (MSG) and may be considered part of the expected standard of care for haematology patients at risk of fungal infection. Antigen detection usually detects active infection. Antigen is generally detected in serum. However, it can also be detected in other body fluids, such as urine. [(2)]

Mannan is a major component of the Candida cell wall that induces a strong antibody response. [(10)]

The combined detection of mannan (Platelia Candida Ag, Bio-Rad Laboratories) and anti-mannan antibodies (Platelia Candida Ab, Bio-Rad Laboratories) considerably improved the diagnosis of candidemia. While individual sensitivities of these tests is low (<50%), the combined detection increases the sensitivity (60-89%).[(5)]

Subjects and Methods

Study design:

The current study was carried out at Obstetric, Gynecological and Pediatric Hospital, Minia University in the period from June 2016 to September 2017. The

study was performed on 80 critically ill patients who were admitted to Pediatric Intensive Care Unit (PICU).They were 40 males and 40 females. Their ages ranged from 1.2 to 70 months with a mean age of 14.7 months.

Patients clinically suspected to have invasive fungal infections such as fever, cough or retrosternal pain; oral mucositis or perianal pain, drug history for antibiotics or corticosteroids, history of chemotherapy, duration of the disease and hospital stay.
In addition to the laboratory investigations; five milliliters venous blood were withdrawn, one ml of venous blood was collected on Egyptian blood culture media (EDM bottles), one ml of venous blood was mixed with EDTA for CBC, three ml of venous blood was left to be clotted in the incubator and centrifuged at 2500 rpm for 10 minutes. Separated serum divided into aliquots and used for assay of urea, creatinine, random blood sugar and Candida antigen (in a sterile tube stored at -20ºC until the time of assay).

Routine laboratory investigations:
CBC was determined by automated cell counter Sysmex KX-21N (TAO Medical Incorporation, Japan), Renal function tests (urea and creatinine), and Random blood glucose were assayed using fully automated clinical chemistry auto-analyzer system Konelab 60i (Thermo Electron Incorporation, Finland)**.**

Special investigations:

- Blood Culture (EDM) bottles were incubated aerobically at 37°C.then, subculture was done every week for 4 weeks onto 2 plates of SDA , for primary isolation and propagation of molds and yeast. One plate of SDA was incubated at 37°C and the other was incubated at 25°C. Plates were inspected every other day for fungal growth for the 1st 2 weeks then twice weekly for the next 2 weeks. Also, the blood culture bottle was subcultured on blood agar and Mac Conkey agar (aerobically at 37°C) every 2 days for the 1st week for identification of bacterial growth. Any growth was observed and identified macroscopically according to organism morphology, microscopically according to shape of hypheal element, conidia production, scotch tape preparation (for molds) and budding oval bodies by gram stain (for yeast).
- ELISA assay for mannan detection; PLATELIA™ Candida Ag plus (Bio-Rad, France). According to the manufacturer's recommendations.

Statistical analysis

Data were coded and entered using the statistical package SPSS version 21. Data was summarized using mean and standard deviation for quantitative variables and frequencies (number of cases) and relative frequencies (percentages) for categorical variables. Frequencies were compared between the different methods of diagnosis and the risk factors using binary logistic regression.Odds ratio (OR) with 95% confidence intervals was calculated. Comparison of non-parametric quantitative variables was done using Mann Whitney test. Fisher exact test was used for qualitative data between two groups when the expected frequency is less than 5. P value <0.05 was taken as statistically significant.

Results

Among the studied 80 patients, 44 cases did not show microbial growth on blood culture, 26 patients (32%) had bacterial growth with CONs with the highest bacterial frequency (10%), 4 cases (5.1%) had fungal growth with Candida albicans represented 2(2.5%), Aspergillus fumigatus represented (1.3%) and Kodamaea ohmeri was isolated from one case (1.3%). Six cases of the studied patients had mixed bacterial and fungal infections 6/80 (7.8%) where Candida tropicalis 3/80 (3.8%), Candida parapsillosis 1/80 (1.3%) and Candida albicans represent 2/80 (2.5%) AS shown in Table (1). See figures (1&2).

The diagnostic performance of ELISA was calculated considering blood culture for the detection of fungal infections as the gold standard. The sensitivity of ELISA was found to be 80 %, the specificity was 91.43%, the positive predictive value (PPV)

was 57.1% and the negative predictive value (NPV) was 97% with overall accuracy was 90%. Cohen's kappa was 0.610. AS shown in Tables (2&3). See figure (3).

Table 1: Blood culture results of all patients:

Blood culture results [β]	Type	N (%)
-Ve	No growth	44 (55%)
Bacterial 26(32.1 %)	CoNS	8(10%)
	Klebseilla	6(7.5%)
	E.coli	3(3.8%)
	Acinetobacter	4(5%)
	Pseudomonas	2(2.5%)
	Enterobacter aerogenes	2(2.5%)
	Enterococci	1(1.3%)
Fungal 4(5.1%)	Candida albicans	2(2.5%)
	Aspergillus fumigatus	1(1.3%)
	Kodamaea ohmeri	1(1.3%)
Mixed infection 6(7.8%)	Candida albicans + CoNS	1(1.3%)
	Candida albicans + Klebseilla	1(1.3%)
	Candida tropicalis + E. coli	1(1.3%)
	Candida tropicalis + S.aureus	1(1.3%)
	Candida tropicalis + Pseudomonas	1(1.3%)
	Candida parapsillosis + E. coli	1(1.3%)

Table 2: ELISA using mannan antigen versus blood culture fungal results:

		Blood culture		P value	Kappa test	
		-Ve (n=70)	+Ve (n=10)		Kappa	P value
ELISA	-Ve	64(91.4%)	2(20%)			
	+Ve	6(8.6%)	8(80%)	**<0.001***	**0.610**	**<0.001***

Table 3: ROC curve analysis of ELISA for predicton of the presence of fungal infection:

Method	AUC	95% CI	P value	Sensitivity	Specificity	PPV	NPV	Accuracy
ELISA	0.86	0.76 - 0.93	**<0.001***	80	91.43	57.1	97	90

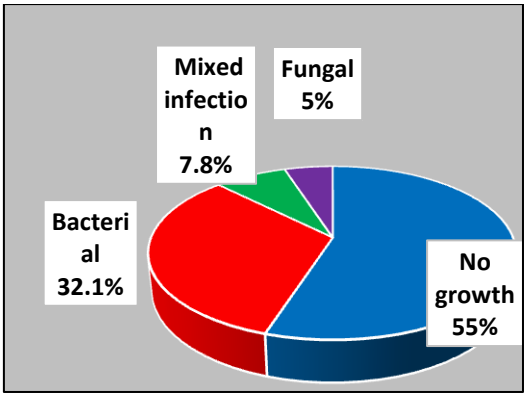

Figure 1: blood culture results for patients with suspected fungal infection
N.B: mixed infection=bacterial and fungal

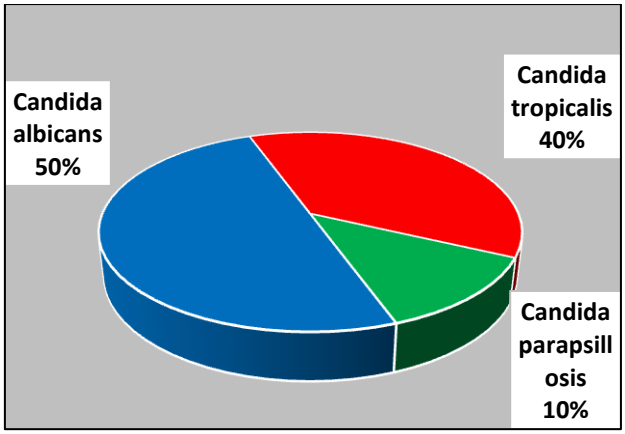

Figure 2: percentages of different candida species isolated from blood culture for patients with suspected fungal infection

Discussion

Invasive candidiasis is the most common fungal disease among hospitalized patients in the developed world. Invasive candidiasis comprises both candidemia and deep-seated tissue candidiasis[9].

Our results showed that 10 cases were positive for fungi by blood cultures out of 80 patients, 8(10%) cases were candida spp., 1 case (1.3%) was aspergillus fumigatus and 1case (1.3%) was kodamaea ohmeri .

In our study C.tropicalis was the most prevalent NCAC species 3/8(37.5%), followed by C. parapsilosis was 1/8 (12.5%). These results were in broad accordance with Awasthi et al., 2011[3] who reported higher percent of C.tropicalis that represented (71.5%) of candidaemia cases, also concomitant with Almeida et al., 2013[1] having C. tropicalis as the most common (42%) .

In contrast to, Dutta and Palazzi 201[6] who reported that among the NCAC group, C. parapsilosis was the most common (23.9%), followed by C.tropicalis (14.8%), also, Bruder-Nascimento et al., 2010[4] where C. parapsillosis had the highest percentage (48%) and Yapar, 2014[16] showing C. parapsillosis and C. tropicalis (23.9%) and (16.8%) respectively in a study made in Argentina .

The variable frequencies of different Candida species explained by Pfaller and Diekema 2004[12] who reported that, this may be affected by the geographic area, underlying diseases, antifungal treatment, differences in patient populations and infection control policies.

As regard ELISA results of mannan Ag of candida in this study, the diagnostic performance of ELISA was calculated considering blood culture for the detection of fungal infections as the gold standard. The sensitivity of ELISA was found to be 80 %, the specificity was 91.43, kappa was 0.610, indicating substantial agreement between both methods in detection of fungal infections.

Also, this is in agreement with Held et al., 2013[8], where mannan Ag detection showed an excellent specificity of 97.5%, but of low sensitivity of 58.9%. The ROC analysis in this study revealed that by lowering the Platelia Candida Ag Plus cutoff from 125pg/ml to 50pg/ml, the sensitivity would increase to 69.6% without severely affecting the specificity (93.0%).

However, Lunel et al., 2011[15], observed a modest increase in sensitivity 61.9%, while the specificity 43.3% was reduced by half due to a high number of false-positive results in patients with superficial candidiasis.

Mannan antigen tests gave false-negative results .This might be due to the fact that the antibodies used only have weak reactivity for the antigens from C. parapsilosis and C. krusei as was shown by Sendid et al., 2002[14] who observed poor detection of these species, and Yera at al., 2001[17] reported detection rates 44% for C. krusei and C. parapsilosis with the Platelia assay.

The impact of fungal colonization on false-positive results by Mannan antigen and the low specificity remains unclear.[13]

The major benefits of mannan antigen testing are the short time needed and its cost-effectiveness. However, inconsistent observations regarding the test accuracies were reported in previous studies. This might be due to the heterogeneity of the available studies and the low prevalence of IC, since small numbers of positive samples make it difficult to draw conclusions of statistical significance. Among intensive care patients, for example, the prevalence was reported to only lie between 0.5 and 1%.[7]

References

1. Almeida, Adriana Araujo de, Cristiane Suemi Shinobu Mesquita, Terezinha Inez Estivalet Svidzinski, and Kelly Mari Pires de Oliveira. "Antifungal susceptibility and distribution of Candida spp. isolates from the University Hospital in the municipality of Dourados, State of Mato Grosso do Sul, Brazil." Revista da Sociedade Brasileira de Medicina Tropical46, no. 3 (2013): 335-339.
2. Ascioglu, S. (2002). Invasive Fungal Infections Cooperative Group of the European Organization for Research and Treatment of Cancer; Mycoses Study Group of the National Institute of Allergy and Infectious Diseases. Defining opportunistic invasive fungal infections in immunocompromised patients with cancer and hematopoietic stem cell transplants: an international consensus. Clin Infect Dis., 34, 7-14.
3. Awasthi AK, Jain A, Awasthi S, Ambast A, Singh K and Mishra V (2011): Epidemiology and microbiology of nosocomial pediatric candidemia at a northern Indian tertiary care hospital. Mycopathologia; 172(4):269-277.
4. Bruder-Nascimento, Ariane, Carlos Henrique Camargo, Maria Fátima Sugizaki, Terue Sadatsune, Augusto Cezar Montelli, Alessandro Lia Mondelli, and Eduardo Bagagli. "Species distribution and susceptibility profile of Candida species in a Brazilian public tertiary hospital. " BMC research notes 3, no.1(2010): 1.
5. Cuenca-Estrella, M., P. E. Verweij, M. C. Arendrup, S. Arikan-Akdagli, J. Bille, J. P. Donnelly, Henrik Elvang Jensen et al., "ESCMID guideline for the diagnosis and management of Candida diseases 2012: diagnostic procedures. " Clinical Microbiology and Infection 18, no. s7 (2012): 9-18.
6. Dutta, A., & Palazzi, D. L. (2011). Candida non-albicans versus Candida albicans fungemia in the non-neonatal pediatric population. The Pediatric infectious disease journal, 30(8), 664-668.
7. Eggimann, Philippe, Jacques Bille, and Oscar Marchetti. "Diagnosis of invasive candidiasis in the ICU. " Annals of intensive care 1, no. 1 (2011): 37.
8. Held, Jürgen, Isabelle Kohlberger, Elfriede Rappold, Andrea Busse Grawitz, and Georg Häcker. "Comparison of (1→3)-β-D-Glucan, Mannan /anti-Mannan-antibodies and Cand-Tec Candida-Antigen as Serum Biomarkers for Candidemia."Journal of clinical microbiology (2013): JCM-02473.
9. Kullberg, B. J., & Arendrup, M. C. (2015). Invasive candidiasis. New England Journal of Medicine, 373(15), 1445-1456.
10. Lamoth, Frédéric, Mario Cruciani, Carlo Mengoli, Elio Castagnola, Olivier Lortholary, Malcolm Richardson, and Oscar Marchetti. "β-Glucan antigenemia assay for the diagnosis of invasive fungal infections in patients with hematological malignancies: a systematic review and meta-analysis of cohort studies from the Third European Conference on Infections in Leukemia (ECIL-3). " Clinical infectious diseases 54, no.5(2011): 633-643.

11. Miceli MH, Maertens J (2015) Role of non-culture-based tests, with an emphasis on galactomannan testing for the diagnosis of invasive Aspergillosis. Semin Respir Crit Care Med 36(5): 650–661.
12. Pfaller, M. A., and D. J. Diekema. "Rare and emerging opportunistic fungal pathogens: concern for resistance beyond Candida albicans and Aspergillus fumigatus." Journal of clinical microbiology 42, no.10 (2004): 4419-4431.
13. Poissy, Julien, Boualem Sendid, Sébastien Damiens, Ken Ichi Ishibashi, Nadine François, Marie Kauv, Raphaël Favory, Daniel Mathieu, and Daniel Poulain. "Presence of Candida cell wall derived polysaccharides in the sera of intensive care unit patients: relation with candidaemia and Candida colonisation."Critical Care 18, no. 3 (2014): R135.
14. Sendid, Boualem, Jean Louis Poirot, Marc Tabouret, Alain Bonnin, Denis Caillot, Daniel Camus, and Daniel Poulain. "Combined detection of mannanaemia and anti-mannan antibodies as a strategy for the diagnosis of systemic infection caused by pathogenic Candida species. " Journal of medical microbiology 51, no. 5 (2002): 433-442.
15. Verduyn Lunel, Frans M., J. Peter Donnelly, Henrich AL van der Lee, Nicole MA Blijlevens, and Paul E. Verweij. "Performance of the new Platelia Candida Plus assays for the diagnosis of invasive Candida infection in patients undergoing myeloablative therapy. " Medical mycology 49, no. 8 (2011): 848-855.
16. Yapar, Nur. "Epidemiology and risk factors for invasive candidiasis. " Therapeutics and clinical risk management 10 (2014): 95.
17. Yera H, Sendid B, Francois N, Camus D, Poulain D: Contribution of serological tests and blood culture to the early diagnosis of systemic candidiasis. Eur J Clin Microbiol Infect Dis. 2001, 20: 864-870.