RESEARCH NOTE

Experience with the Platelia *Candida* ELISA for the diagnosis of invasive candidosis in neonatal patients

S. Oliveri[1], L. Trovato[1], P. Betta[2], M. G. Romeo[2] and G. Nicoletti[1]

[1]Department of Microbiological Science, University of Catania Laboratory Analysis Unit and [2]Department of Paediatrics, University of Catania Neonatal Intensive Care Unit, Policlinico G. Rodolico, Catania, Italy

ABSTRACT

This preliminary study evaluated the use of the Platelia *Candida* antigen kit for the diagnosis of invasive candidosis in 70 of 184 pre-term infants admitted to a neonatal intensive care unit between March 2004 and March 2006. The frequency of confirmed candidaemia was 6.5%. The sensitivity and specificity of the assay were 94.4% and 94.2%, respectively, with a positive predictive value of 85% and a negative predictive value of 98%. These results suggest that the inclusion of regular serological surveillance for mannanaemia in some pre-term infants would complement blood cultures for the early detection of candidosis.

Keywords Antigen kit, candidaemia, detection, mannanaemia, neonates, Platelia *Candida* assay

Original Submission: 10 May 2007; **Revised Submission:** 15 October 2007; **Accepted:** 9 November 2007

Clin Microbiol Infect 2008; **14:** 391–393
10.1111/j.1469-0691.2007.01938.x

The incidence of candidaemia in neonatal intensive care units (NICUs) is steadily increasing [1–3]. Pre-term and low birth-weight infants are particularly susceptible to infection because of their immature immune system, disruptions in their cutaneous barrier and iatrogenic factors [4,5]. Clinical signs of invasive candidosis can be non-specific, and diagnosis is still based mostly on blood cultures; however, blood cultures are thought to be positive for *Candida* in only 24–60% of cases [6,7]. In view of this limitation, other techniques to facilitate a diagnosis of invasive fungal infection have been investigated. Mannan, a high molecular weight polysaccharide found in the cell wall of *Candida* spp., can be measured in blood [8], but serological data concerning the mannan antigen are very limited for patients in NICUs. Accordingly, this report presents preliminary data from an observational study concerning the use of Platelia *Candida* antigen kits (Bio-Rad, Segrate, Italy) for the diagnosis of invasive candidosis in pre-term infants.

In the present analysis, the mannan antigen test was considered positive when at least two serum samples with mannan levels of ≥0.5 ng/mL were obtained. All other cases, i.e., only one positive result or intermediate results, were considered to be negative. All positive results were interpreted in the context of the patient's clinical, radiological and laboratory data [9]. Proven candidosis, defined in the present study as clinical signs of sepsis and positive blood culture, was observed in 12 of 184 patients admitted to the NICU between March 2004 and March 2006, i.e., a frequency of 6.5%. Seventy patients were included in this preliminary analysis, based on the availability of at least three mannan antigen tests during their stay in the NICU. The mean number of serum samples/patient was 3.9. The method used was as described previously [10].

Of the 12 patients with proven candidosis, 11 were positive according to the mannan antigen test and one was negative. The latter patient had fungaemia caused by *Candida parapsilosis*. Of the remaining 58 patients, 49 were negative according to the mannan antigen test and blood cultures, while nine were negative according to fungal and bacterial blood cultures, but positive according to the mannan antigen test. Three of these patients were not considered to be suffering from invasive candidosis, because all colonisation cultures were negative and antibacterial therapy was effective in resolving the clinical conditions. Therefore, the mannan test results for these three patients should be considered to be false-positives. The remaining six patients were considered to be suffering from invasive

Corresponding author and reprint requests: S. Oliveri, Dipartimento di Scienze Microbiologiche, Via Androne 81, 95124 Catania, Italy
E-mail: oliveri@unict.it

Table 1. Characteristics and test results of infants with and without proven/probable candidosis

	n	M/F	Gestational age (weeks)[a]	Birth-weight (g)[a]	Mechanical ventilation		Parenteral nutrition		Positive blood culture	Positive antigen test[b]
					<15 days	>15 days	<15 days	>15 days		
Proven and probable candidosis	18	8/10	30.72 ± 3.31	1622 ± 556	13	5	7	11	12	17
Without candidosis	52	25/27	34.9 ± 3.1	2262 ± 692	12	0	39	1	0	3

M, male; F, female.
[a]Mean value ± SD.
[b]At least two positive serum samples from the patient.

candidosis. Indeed, alterations in the laboratory parameters (quantitative C-reactive protein, leukocytosis, neutrophilia), despite aggressive broad-spectrum antibiotic therapy for at least 2 days, an elevated level of *Candida* intestinal colonisation and, particularly, an improvement in clinical symptoms following administration of antifungal drugs, supported this hypothesis. Two of these patients also had a previous positive *Candida* culture from an intravascular catheter tip. The characteristics of infants with proven or presumed candidosis, or without candidosis, are summarised in Table 1.

Based on these results, the sensitivity and specificity of the Platelia *Candida* antigen test for cases of proven and probable candidosis were 94.4% and 94.2%, respectively. The positive predictive value and negative predictive value were 85% and 98%, respectively. For the 12 cases of proven candidosis, sensitivity was 91.7%, specificity was 84.5%, the positive predictive value was 55%, and the negative predictive value was 98%. For eight patients with proven candidosis, the antigen test was positive before blood culture sampling, for one patient on the day of blood culturing, and for three patients during the following days. Notably, for the initial eight patients, the test was positive between the fourth and 18th days before blood cultures became positive (median value 8.5 days). For six patients with proven candidosis, it was possible to obtain serological data at diagnosis and after 3 and 6 weeks (Fig. 1). These patients were treated with liposomal amphotericin B (1–5 mg/kg/day) for a period of 6–7 weeks following their diagnosis.

In conclusion, this preliminary study suggests that the inclusion of the mannan antigen determination test in a microbiological investigation of at-risk patients in NICUs may be a useful contribution to the diagnosis of invasive candidosis and to effective therapy. The Platelia *Candida* assay

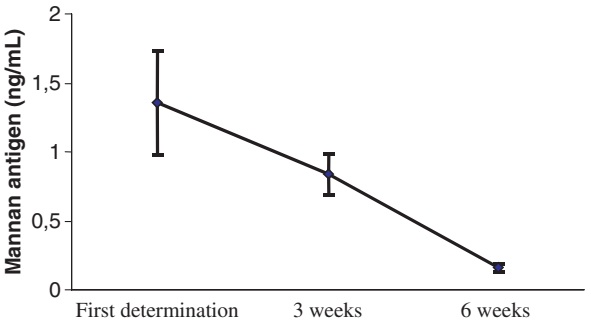

Fig. 1. Mean levels ± SE of mannan antigen observed in six patients with proven candidosis at diagnosis and after therapy with liposomal amphotericin B for 3 and 6 weeks.

showed a greater sensitivity in NICU patients than the global sensitivity of 51.5% reported by Sendid *et al.* [10] for intensive care unit and haematology patients with clinically suspected systemic candidosis. The greater sensitivity observed in the present study was probably associated with an absence of circulating anti-mannan antibodies, or a low concentration of circulating lectins (mannose-binding protein), or to the pathophysiology of candidosis in neonates. Nevertheless, it is important to note the low sensitivity for the detection of *C. parapsilosis* and *Candida krusei* infection. This difference in the sensitivity for antigenic detection of different species is consistent with the nature of the *Candida* mannose epitope recognised by the EBCA1 monoclonal antibody used in the test [10–12]. Nevertheless, since the frequency of *C. parapsilosis* and *C. krusei* recovery in the NICU was ≤20% in terms of blood culture and colonisation isolates, the Platelia *Candida* assay could be a good test for serological monitoring of patients with *Candida* colonisation, who show the highest frequency of invasive candidosis [13], and could allow the possibility of early diagnosis, as observed in this preliminary study, in *c.* 50% of patients with invasive *Candida* infection.

ACKNOWLEDGEMENTS

The authors declare that they have no conflicting interests in relation to this study.

REFERENCES

1. Kossoff E, Buescher S. Candidemia in a neonatal intensive care unit: trends during fifteen years and clinical features of 111 cases. *Pediatr Infect Dis J* 1998; **17**: 504–508.
2. Makhoul IR, Kassis I, Smolkin T *et al.* Review of 49 neonates with acquired fungal sepsis: further characterization. *Pediatrics* 2001; **107**: 61–66.
3. Stoll BJ, Hansen N, Fanaroff AA *et al.* Changes in pathogens causing early-onset sepsis in very low birth weight infants. *N Engl J Med* 2002; **347**: 240–247.
4. El-Masry F, Neal TJ, Subhedar NV. Risk factors for invasive fungal infection in neonates. *Acta Paediatr* 2002; **91**: 198–202.
5. Saiman L, Ludington E, Pfaller M *et al.* Risk factors for candidemia in neonatal intensive care unit patients. *Pediatr Infect Dis J* 2000; **19**: 319–324.
6. Rabalais GP, Samiec TD, Bryant KK, Lewis JJ. Invasive candidiasis in infants weighing more than 2500 grams at birth admitted to a neonatal intensive care unit. *Pediatr Infect Dis J* 1996; **15**: 348–352.
7. Smith H, Congdon P. Neonatal systemic candidiasis. *Arch Dis Child* 1985; **60**: 365–369.
8. Schreiber JR, Maynard E, Lew MA. *Candida* antigen detection in two premature neonates with disseminated candidiasis. *Pediatrics* 1984; **74**: 838–841.
9. Manzoni P, Pedicino R, Stolfi I *et al.* Criteria for the diagnosis of systemic fungal infections in newborns: a report from the Task Force on neonatal fungal infections of the GSIN. *Pediatr Med Chir* 2004; **26**: 89–95.
10. Sendid B, Poirot JL, Tabouret M *et al.* Combined detection of mannanaemia and antimannan antibodies as a strategy for the diagnosis of systemic infection caused by pathogenic *Candida* species. *J Med Microbiol* 2002; **51**: 433–442.
11. Trinel PA, Faille C, Jacquinot PM *et al.* Mapping of *Candida albicans* oligomannosidic epitopes by using monoclonal antibodies. *Infect Immun* 1992; **60**: 3845–3851.
12. Sendid B, Tabouret M, Poirot JL *et al.* 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* 1999; **37**: 1510–1517.
13. Farmaki E, Evdoridou J, Pouliou T *et al.* Fungal colonization in the neonatal intensive care unit: risk factors, drug susceptibility, and association with invasive fungal infections. *Am J Perinatol* 2007; **24**: 127–135.

RESEARCH NOTE

High-affinity iron permease (*FTR1*) gene sequence-based molecular identification of clinically important Zygomycetes

I. Nyilasi[1,2], T. Papp[2], Á. Csernetics[2], K. Krizsán[2], E. Nagy[1] and C. Vágvölgyi[2]

[1]Hungarian Academy of Sciences, University of Szeged Microbiology Research Group and [2]Department of Microbiology, Faculty of Sciences and Informatics, University of Szeged, Szeged, Hungary

ABSTRACT

The clinical importance of zygomycosis, an emerging and frequently fatal mycotic disease, has increased during recent years. This report describes an identification method based on PCR amplification and sequencing of the high-affinity iron permease 1 gene (*FTR1*). Primers and amplification protocols were established and tested for the identification of *Rhizopus oryzae*, *Rhizopus microsporus* var. *rhizopodiformis*, *R. microsporus* var. *oligosporus*, *Rhizopus schipperae*, *Rhizopus niveus* and *Rhizopus stolonifer*. *Rhizomucor* and *Syncephalastrum* could be identified at the genus level. PCR–restriction fragment length polymorphism analysis of the amplified gene fragment using *Alu*I digestion distinguished three subgroups among the *R. oryzae* isolates.

Keywords Identification, iron permease 1 gene, Mucorales, PCR–restriction fragment length polymorphism analysis, *Rhizopus*, zygomycosis

Original Submission: 27 April 2007; **Revised Submission:** 20 October 2007; **Accepted:** 12 November 2007

Clin Microbiol Infect 2008; **14:** 393–397
10.1111/j.1469-0691.2007.01932.x

Corresponding author and reprint requests: T. Papp, Department of Microbiology, University of Szeged, Közép fasor 52, Szeged H-6723, Hungary
E-mail: pappt@bio.u-szeged.hu