Molecular diagnosis of polymicrobial newborn sepsis by multiplex real-time PCR using a small volume of blood sample

Sepsis is an important cause of neonatal morbidity and mortality, especially in preterm, very low birth weight infants (birth weight <1500 g) (Kaufman & Fairchild, 2004). Early diagnosis of pathogens and prompt treatment are critical in preventing severe and life-threatening complications in these patients (Lott, 2003). However, the clinical recognition of sepsis in neonates is difficult, because the signs and symptoms are often non-specific and blood cultures are rarely positive (Peters et al., 2004). Recently available molecular assays aid rapid detection of micro-organisms and improve the diagnostic flow-chart (Peters et al., 2004; Gaibani et al., 2009). Among these, a commercial multiplex real-time PCR (LightCycler SeptiFast Test; Roche Molecular Systems) uses a novel technology that enables the direct detection from blood samples of a wide panel of bacterial and fungal micro-organisms commonly involved in systemic infections (Lehmann et al., 2008; von Lilienfeld-Toal et al., 2009). This technique was found to give promising results in the neonatal population with sepsis, particularly when antibiotic treatment is initiated (Mussap et al., 2007; Paolucci et al., 2009; Lucignano et al., 2011).

Under this perspective, the LightCycler SeptiFast assay was used in a male, preterm, low birth weight infant (birth weight 840 g). The neonate was delivered by lower Caesarean section at 27 weeks of gestation after a spontaneous rupture of membranes and had Apgar scores of four and eight at 1 and 5 min, respectively. In the delivery room, the infant was intubated and ventilated mechanically. Thereafter, he was transferred to the neonatal unit because of prematurity and respiratory distress syndrome. Umbilical vein and arterial catheters were inserted and empirical antibiotic therapy with ampicillin and gentamicin was initiated. On day 7 of life, the neonate developed symptoms of septicaemia and a standard volume of blood (4 ml) was drawn for blood culture (BacT/ALERT, PF Paediatric FAN; bioMérieux). The neonate was diagnosed with Stenotrophomonas maltophilia bloodstream infection and received antimicrobial treatment with teicoplanin (8 mg kg⁻¹ every 24 h), netilmicin (6 mg kg⁻¹ every 24 h) and ciprofloxacin (20 mg kg⁻¹ every 24 h). In addition, the umbilical vein catheter of the neonate was substituted after reporting the positive blood culture. S. maltophilia was also recovered from culture of the removed umbilical catheter.

Despite antimicrobial treatment, the condition of the neonate did not improve and a SeptiFast test was ordered on postnatal day 17. The molecular assay was performed in only 0.5 ml of whole blood, instead of the blood volume recommended in the manufacturer’s instructions (1.5 ml blood), due to difficulties in drawing blood from the small veins of his arm. In parallel, new cultures were requested, including peripheral blood and umbilical catheter tip cultures. Within 6 h, DNA of S. maltophilia, Escherichia coli and Candida albicans was amplified in the blood sample and reported to the neonatal unit. Antifungal treatment with amphotericin B and 5-flucytosine was then promptly added to his therapy. In the following days, culture-based approaches and clinical outcome strengthened the results of the molecular assay. Blood culture was positive for Gram-negative rods, which were identified as S. maltophilia in accordance with the initial blood culture. E. coli grew from the umbilical catheter tip culture after 24 h of incubation. C. albicans was not recovered from blood culture, after 5 days of incubation, or from the catheter tip, but rectal swab cultures detected heavy growth of the yeast. It should also be noted that the clinical condition of the newborn improved substantially after the addition of the antifungal treatment and the infant was discharged in good condition at 1 month of age.

Blood cultures are known to be negative for approximately 50 % of patients with candidaemia or disseminated candidiasis (Ostrosky-Zeichner & Pappas, 2006). Therefore, it is not surprising that C. albicans was not recovered from blood cultures from our neonate. The advantage of SeptiFast technology, especially in detecting fungal pathogens, has been previously documented (Lucignano et al., 2011; von Lilienfeld-Toal et al., 2009). Mixed blood infections have been sporadically reported in adults and newborns using LightCycler SeptiFast (Mancini et al., 2009; Paolucci et al., 2009). The present report indicates that even in a very small volume of blood sample polymicrobial septicemia can be rapidly detected with SeptiFast. This is of particular interest for neonates when drawing of the recommended blood volume is not always obtainable. This fact could widen the possible use of the assay, especially in the neonatal setting.

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