Laboratory diagnosis of late-onset sepsis in newborns by multiplex real-time PCR

Bloodstream infections (BSIs) are an important cause of neonatal morbidity and mortality, and often result in prolonged hospitalization of infants who are admitted to neonatal intensive care units (Verboon-Maciolek et al., 2006). Late-onset neonatal sepsis (occurring in newborns aged older than 3 days) occurs in approximately 0.1% of all newborns and in up to ~25% of very low birth weight infants (birth weight <1500 g) (Kaufman & Fairchild, 2004). Early diagnosis of sepsis and prompt treatment are critical in preventing severe and life-threatening complications in these patients (Harbarth et al., 2003; Kollef, 2003; Lodise et al., 2003). The clinical recognition of sepsis in neonates is difficult, however, because the signs and symptoms are often non-specific (Gerdes, 1991; Verboon-Maciolek et al., 2006) and blood cultures (BCs) are rarely positive.

In this study, we aimed to assess the clinical utility of a newly available, commercial real-time PCR test (LightCycler SeptiFast) in newborns aged older than 3 days who present with a clinical suspicion of sepsis. Peripheral venous blood was collected, and sepsis was evaluated by BC and the SeptiFast assay, using 1.0 and 1.5 ml blood for each method, respectively. BCs were performed according to the Clinical and Laboratory Standards Institute (CLSI) protocol (CLSI, 2007). The LightCycler SeptiFast MGRADE test was performed prospectively on blood samples, as reported in recent publications (Lehmann et al., 2007; Mancini et al., 2008).

A total of 34 newborns were enrolled in this study following the suspicion of late-onset sepsis, and the diagnosis of clinical sepsis was based on (1) the presence of at least one clinical sign (Philip & Mills, 2000) suggestive of clinical sepsis, and (2) elevated C reactive protein (CRP) values (>2.0 mg CRP dl⁻¹). Confirmation of BSI was determined by the presence of clinical sepsis and detection of pathogens in the blood by BC or LightCycler SeptiFast. Eight additional newborns, who showed no signs of sepsis and a normal CRP value (≤0.8 mg CRP dl⁻¹), were enrolled as negative controls.

A BSI was detected in 7/34 (20.6%) patients by BC or SeptiFast. In three cases, BC and SeptiFast results were in agreement (Table 1). In four cases, however, only SeptiFast identified the presence of pathogens in blood samples. In these four cases, BSI was confirmed by the presence of clinical signs of infection or additional microbiological data.

In one case, a mixed infection of Enterococcus faecium and Klebsiella pneumoniae was detected in the blood by PCR, and a concurrent urinary tract infection caused by K. pneumoniae was confirmed by standard urine culture. Additionally, this patient suffered from a congenital urinary tract malformation (bilateral renal pelvis dilatation). In another case, the bacterium that was detected by SeptiFast, Stenotrophomonas maltophilia, also was isolated from a pharyngeal swab culture that was performed on the same day.

One patient (infected with Streptococcus spp.) was admitted to the Neonatal Intensive Care Unit (St Orsola Hospital) for superficial cellulitis in the umbilical region, which were related to omphalitis. Streptococcus pyogenes infection is a prominent cause of omphalitis (Fraser et al., 2006); unfortunately, a culture from these lesions was not performed, due to the presence of the cellulitis.

The remaining patient (infected with Streptococcus pneumoniae) was affected by primary congenital immunodeficiency. No micro-organisms were detected by BC, but the patient was treated empirically with piperacillin and ceftazidime; the symptoms resolved within 48 h.

In one additional suspected case, BC identified Staphylococcus epidermidis, but results from the SeptiFast test were negative (Table 1). Analysis of SeptiFast results identified coagulase-negative staphylococci (CoNS) as a workflow contaminant, which was correctly confirmed and complemented by the lack of clinical relevance of the CoNS grown in culture.

In the remaining 26 cases (76.5%), both methods were negative and an analysis of the clinical records from the patients revealed that they were affected by other pathologies. All eight healthy infants tested negative by both BC and SeptiFast.

SeptiFast may prove to be a clinically useful laboratory diagnostic method for detecting late-onset sepsis in newborns. Consistent with recent findings in neutropenic patients (Mancini et al., 2008), the SeptiFast test was found to be a sensitive method that detected seven cases of BSI in newborns with suspected sepsis, while BC was positive in only three of these cases. Although the SeptiFast test does not provide information on antimicrobial susceptibility or micro-organism viability, its results are available sooner than BC results (~8 h after blood sampling versus 48–72 h for BC).

A more rapid availability of results could reduce the inappropriate use of antimicrobial therapy, the risk of developing antibiotic resistance and hospital stays. A prospective evaluation of a larger cohort of patients is required to assess the clinical benefit and the cost-effectiveness impact of SeptiFast in newborns who have a suspicion of sepsis.

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Table 1. Results of the SeptiFast and BC assays

<table>
<thead>
<tr>
<th>SeptiFast result/organism</th>
<th>BC result</th>
<th></th>
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<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
<td>Positive</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Enterobacter cloacae</td>
<td>Escherichia coli</td>
<td>CoNS</td>
</tr>
<tr>
<td>Negative</td>
<td>26</td>
<td>0</td>
<td>0</td>
<td>1*</td>
</tr>
<tr>
<td>Positive</td>
<td></td>
<td>0</td>
<td>1†</td>
<td>0</td>
</tr>
<tr>
<td>Enterobacter cloacae</td>
<td></td>
<td>0</td>
<td>0</td>
<td>1†</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CoNS</td>
<td></td>
<td>0</td>
<td>0</td>
<td>1†</td>
</tr>
<tr>
<td>Enterococcus faecium and Klebsiella pneumoniae</td>
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<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Streptococcus pneumoniae</td>
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<td>0</td>
</tr>
<tr>
<td>Stenotrophomonas maltophilia</td>
<td>1§</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Streptococcus spp.</td>
<td>1</td>
<td></td>
<td></td>
<td>0</td>
</tr>
</tbody>
</table>

*The BC was positive for Staphylococcus epidermidis, but the SeptiFast test identified CoNS contamination.
†Newborns infected with Enterobacter cloacae, Escherichia coli, and CoNS did not receive antibiotic therapy in the week prior to blood sampling.
‡This newborn was treated with ceftriaxone, amikacin and vancomycin in the 72 h prior to blood sampling.
§This baby was treated with ampicillin, amikacin and teicoplanin in the 72 h prior to blood sampling.
||This patient received ampicillin, amikacin, and subsequently ceftriaxone, in the 72 h prior to blood sampling.

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