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PCR-Based Diagnosis of Neonatal Staphylococcal Bacteremias

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We compared PCR with blood cultures in the diagnosis of neonatal staphylococcal sepsis. Significant association was observed between PCR-based and culture-based diagnosis of staphylococcal bacteremia. Positive and negative predictive values for PCR were 100% and 98%, respectively. These data indicate that PCR may serve as a useful adjunct for the rapid diagnosis of staphylococcal sepsis.

Neonatal late-onset sepsis (LOS), a major problem in neonatal intensive care units (NICU), is defined as positive microbial growth in one or more bloodstream cultures obtained after 72 h of life with accompanying clinical signs of sepsis (7, 10). Late-onset sepsis can develop in any neonate but occurs mainly in very-low-birth-weight (VLBW) neonates (7, 10). In this group of patients, LOS due to coagulase-negative staphylococci (CoNS) and *Staphylococcus aureus* accounts for 47.9 to 55% and 3.9 to 7.8% of LOS episodes, respectively (7, 11). Nevertheless, it has been suggested that some cases of CoNS bacteremia, specifically, those with only one positive blood culture with CoNS, represent contaminated blood samples rather than true bacteremia cases (8, 11).

We prospectively compared during a 12-month period the results of staphylococcal infection diagnosis as assessed by blood cultures and by PCR amplification among all neonates with suspected LOS that were hospitalized at one tertiary NICU. We obtained written informed consent from the children's legal guardians according to a protocol approved by the local Helsinki Ethics Committee. We collected blood samples from all neonates who were hospitalized in the NICU during the study period and developed clinical signs suggesting sepsis after 3 days of life, as assessed by a senior neonatologist (signs included lethargy, irritability, apnea, cyanosis, respiratory distress, poor capillary refill, abdominal distension, vomiting, hypoglycemia, hyperglycemia, and excessive hyperbilirubinemia). During the study period, 124 neonates experienced 215 events of suspected sepsis and constituted the study population. In each case, two blood samples were obtained (when suspicion of sepsis was raised) from different sites (right and left saphenous veins). One sample (1.5 to 2.0 ml) was used for blood culture (1.0 to 1.5 ml) and complete blood count (0.5 ml), and the second (1.2 to 2.0 ml) was used for blood culture (1.0 to 1.5 ml) and PCR testing (0.2 to 0.5 ml) in EDTA-containing tubes. Blood cultures were analyzed by using an automated continuous-monitoring blood culture system (Bac-T-Alert; Biomerieux, Marcy l'Etoile, France). DNA was extracted by using the High Pure PCR template preparation kit (Roche Diagnostics GmbH, Mannheim, Germany) according to the manufacturer's instructions. Samples of DNA were PCR amplified by using a primer pair specific for staphylococcal (both CoNS and *S. aureus*) 16S rRNA genes as previously described (2). Inhibition controls (human globin genes) were included for each sample and confirmed the reliability of the PCR amplification. PCR detection threshold was assessed and determined to be 10 CFU/ml of blood (data not shown). The turnaround time from DNA extraction to detection of PCR product by agarose gel electrophoresis was less than 4 h. In comparison, cultures became positive after 24 to 72 h.

TABLE 1. Microbiological data of the study population at onset of suspected septic events

Parameter	No. (%) of suspected septic events	No. of cultures positive by *Staphylococcus*-specific PCR
Blood cultures[a]	215	
Two negative cultures	183 (85.1)	
One positive culture	23 (10.7)	
Two positive cultures	9 (4.2)	
No. of events with positive blood cultures		
Gram-positive bacteria		
Coagulase-negative *Staphylococcus*	9 (28.1)	5
Staphylococcus aureus	4 (12.5)	4
Alpha-hemolytic *Streptococcus* species	1 (3.1)	
Other (*Leuconostoc*, *Sarcina* species)	2 (6.2)	
Gram-negative bacteria		
Klebsiella species	2 (6.2)	
Escherichia species	3 (9.4)	
Other (*Enterobacter* species, *Proteus mirabilis*, *Providencia* species, *Citrobacter* species, unidentified bacillus)	6 (18.7)	
Candida species	5 (15.6)	

[a] Two aerobic blood cultures were drawn at the onset of every suspected septic event.

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TABLE 2. Staphylococcal bacteremia and staphylococcus-specific PCR[a]

Staphylococcus-specific PCR result	No. of cases in which staphylococcal bacteremia was present or absent		
	Present	Absent	Total
Positive	9	0	9
Negative	4	202	206
Total	13	202	215

[a] The sensitivity, specificity, positive predictive value, and negative predictive value of PCR in detecting staphylococcal bacteremia relative to blood cultures were 9/13 (69.2%), 202/202 (100%), 9/9 (100%), and 202/206 (98%), respectively ($P < 0.0001$).

Of the 360 neonates hospitalized in our NICU during the study period, 124 (34.4%) developed one or more events of presumed LOS. The mean gestational age was 33.5 ± 4.4 weeks (range, 24 to 42 weeks), the mean birth weight was 1,962 ± 874 g (range, 560 to 3,939 g), and the mean age at the onset of presumed sepsis was 15.4 ± 17.3 days (range, 4 to 96 days). The high value of the standard deviation (17.3) is due to skewed distribution of extreme range values (4 to 96). About 75% of enrolled infants were premature infants, 49% were males, and 40% had a birth weight of less than 1,500 g. We did not find statistically significant correlations between bacteremia and white blood cell count, immature neutrophil-to-total neutrophil ratio, or thrombocytopenia as previously shown (8).

Of the 215 events of clinically suspected LOS, there were 32 (14.9%; confidence interval [CI], 10.1% to 19.6%) with at least one positive blood culture for bacteria or fungi, of which 13 (6%; CI, 2.9% to 9.2%) were identified as staphylococci. Of these, nine grew CoNS (all with only one positive blood culture) and four grew *S. aureus* (Table 1).

Out of the 215 events of clinically suspected LOS, staphylococcal infections were diagnosed by PCR in nine instances; five were diagnosed as CoNS infections and four were diagnosed as *S. aureus* infections. All PCR-positive samples originated from the same blood draw as the corresponding positive blood culture. The association between PCR-based and blood culture-based diagnoses of staphylococcal bacteremia was statistically significant ($P < 0.0001$, Fisher's exact test). The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of PCR in diagnosing staphylococcal bacteremia relative to blood cultures were 69.2%, 100%, 100%, and 98%, respectively (Table 2).

Four previous studies established the usefulness of PCR in rapid diagnosis of neonatal LOS (Table 3), targeting universal bacterial (6, 13), fungal (12), and *Candida* (1) genes. Our study is the first to assess specifically staphylococcal infections. Compared with these previous studies, we achieved the shortest turnaround time and a slightly lower sensitivity but high specificity, PPV, and NPV. Previous reports have shown PCR to be a highly sensitive and specific method for the diagnosis of sepsis in neonates (1, 3, 9, 12). In contrast, PCR was 69.2% sensitive in our hands. The reason for this discrepancy is likely due to the fact that in previous studies, a 5-h preamplification culture was performed before PCR amplification, thereby increasing the sensitivity but at the same time decreasing the specificity of the technique. The low sensitivity for the diagnosis of CoNS infections by PCR could have been in part caused by a low bacterial concentration typically found in contaminated samples. Given the fact that our PCR assay detection threshold is about 10 CFU/ml, samples containing fewer bacteria would be diagnosed as PCR negative. This is in line with the fact that clinically relevant septicemia in children has been found to be associated with a bacterial blood concentration of more than 100 CFU/ml (14), although not all authors concur with this view (5). Contamination of blood samples during blood drawing has been suggested to have led to an overestimation of the incidence of CoNS-associated LOS and overuse of antistaphylococcal agents (4). This should be taken into consideration when analyzing previous data showing that LOS due to CoNS represents more than half of LOS cases among VLBW infants and affects more than 14% of all VLBW infants (10). Stoll et al. (11) have recently addressed this problem by proposing more strict criteria for the definition of CoNS-associated LOS, such as mandating the detection of a high C-reactive protein level when only one blood culture is positive for CoNS. This modified definition led to a decrease in the observed incidence of definite CoNS-associated LOS from 55% (10) to 29% (11).

Thus, the present study suggests that PCR-based diagnosis of staphylococcal infection in neonates is more rapid, as specific, and less sensitive than standard blood cultures but does not support the use of PCR-based diagnosis of staphylococcal infection as a substitute for standard blood cultures because of low sensitivity. Our results suggest that PCR-based diagnosis can serve as a useful adjunct when sepsis is suspected but only one positive blood CoNS culture is obtained; in this difficult but unfortunately common setting, PCR may be used to more rapidly establish a diagnosis of staphylococcal bacteremia.

TABLE 3. Reported studies of the use of PCR in early diagnosis of neonatal sepsis

Reference	Organism	Turnaround time	Sample size (no. of events)	% Sensitivity (no. detected/ total)	% Specificity (no. detected/ total)	% PPV (no. detected/ total)	% NPV (no. detected/ total)
Present study	*Staphylococcus*	<4 h	124 (215)	69.2 (9/13)	100 (202/202)	100 (9/9)	98 (202/206)
13	Bacterium	9 h	61	78	100	100	83
12	Fungus	5 h	70	77	77	43	93.6
1	*Candida*	9 h	61	95	95	90	97
6	Bacterium		33	100	93	67	100

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