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ORIGINAL ARTICLE

A study of the role of multiple site blood cultures in the evaluation of neonatal sepsis

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Background: The optimal number of blood cultures needed to document sepsis in an ill neonate has undergone little critical evaluation. Multiple site cultures may improve pathogen detection if intermittent bacteremia occurs, or if a low density of bacteria is present in the blood. We hypothesized, however, that bacterial clearance is slower and bacteremia more continuous in septic neonates, so that a single site blood culture should be sufficient to accurately document true septicemia.

Objective: To determine the need for multiple site blood cultures in the evaluation of neonates for sepsis.

Design/Methods: Clinical data were prospectively collected for 216 neonates who had 269 pairs of blood cultures taken from two different peripheral sites for the evaluation of possible sepsis. A minimum of 1 ml of blood was obtained from the two peripheral sites within 15–30 min of each other. Based on prior retrospective data, we determined that 203 infants would need to have two site blood cultures to demonstrate a significant improvement in pathogen detection at an alpha of 0.05 and a beta of 0.20 (80%) power.

Results: A total of 186 culture pairs were taken for evaluation of early-onset sepsis in 186 neonates, while 83 pairs were drawn for evaluation of late-onset sepsis in 43 neonates. In all, 21 neonates from the late-onset group were evaluated more than once, and 12 neonates were evaluated for both early- and late-onset sepsis. In all, 20 (9.2%) of 216 neonates had 22 episodes of culture-proven sepsis at a median age of 18 days. All neonates with positive cultures had the same organism with a similar sensitivity pattern obtained from the two different peripheral sites. The other 196 study neonates had negative blood cultures from both sites. The single episode of early-onset sepsis was caused by *Listeria monocytogenes*, while all remaining episodes were late-onset with the following organisms: *Staphylococcus epidermidis* (7), methicillin-resistant *Staphylococcus aureus* (MRSA) (3), combined MRSA and *Candida albicans* (2), *Candida albicans* alone (2), late-onset Group B β-hemolytic *Streptococcus* (GBS) (2), *Klebsiella pneumoniae* (2), *Enterococcus fecalis* (1), *Escherichia coli* (1), and *Serratia marcescens* (1). Since no infant grew organisms from only one of the two sites, the data indicate that the diagnosis of sepsis would have been made correctly in all infants with a single site culture.

Conclusions: Two site blood cultures for the initial evaluation of neonatal sepsis do not have a better yield in pathogen detection. Sepsis in neonates can be detected with no loss of accuracy with a single site blood culture with blood volume of $\geqslant 1$ ml.
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Introduction

The isolation of microorganisms from blood is the standard method used to diagnose sepsis in the newborn infant.[1] There is limited information to guide the practitioner, however, on the optimal number of blood cultures that should be obtained when evaluating an infant for suspected neonatal sepsis.[1–4]

Some data have suggested that in the neonatal period, multiple site blood cultures may improve pathogen detection if bacteremia is intermittent, if there is a low density of bacteria present in the circulation, if there is an overdilution of the small volume of blood obtained during a culture with the blood culture broth, or if there is an inhibitory effect of the intrapartum antibiotic therapy administered to mothers.[4] Indeed, several authors have suggested that multiple site blood cultures may be more efficacious in diagnosing neonatal sepsis.[2,4–7] In addition, the recommendations for older children and adults are also to obtain blood cultures from at least two sites.[4,8–10]

To date, however, there is no prospective trial that has been performed which has evaluated the role of multiple site blood cultures in the detection of early- and late-onset sepsis in the neonates.

We hypothesized that a single site blood culture should be sufficient to document sepsis because the bacterial clearance is

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slower and the bacteremia more continuous in neonates with sepsis than in older patients.[11] We therefore conducted this prospective, observational study to determine the usefulness of two site blood cultures in the initial evaluation of neonates for sepsis.

Methods

Clinical data were prospectively collected on 216 neonates who had blood cultures taken from two different peripheral sites for the evaluation of possible early- or late-onset sepsis in the neonatal intensive care unit at the State University of New York at Stony Brook. The study was initiated as a QA process, and during the study period, between December 2001 and November 2002, the standard of care in the Neonatal Intensive Care Unit had been to obtain blood samples for culture from two different peripheral sites in neonates. Use of the QA data for research purposes was subsequently approved by the Committee on Research Involving Human Subjects of the State University of New York at Stony Brook.

Blood cultures were obtained from two different peripheral sites within 15–30 min of each other in each study neonate. A minimum of 1 ml of blood was obtained from each site for inoculation in the BD Bactec Pediatrics Plus/F aerobic bottle (Becton Dickinson Co., Sparks, MD 21152) after skin cleansing using the standard policy of the unit, comprising three consecutive at least 10 s cleanings with premoistened 10% povidone iodine swab with at least 30 s drying time before blood sampling. The use of two or more consecutive cleanings, and/or a longer duration of cleansing has been recommended for more effective skin sterilization in previous studies.[12] No central line blood samples were drawn for the study purposes.

The procedure guidelines for blood cultures were developed and the medical and nursing staff was observed to ensure proper technique during the study.

Statistical analysis

In a prior retrospective study from our group,[4] 18 of 460 infants (3.9%) from whom multiple site blood cultures were obtained, the second set of cultures helped to either confirm bacteremia ($n = 8$, 1.7%) or to confirm contamination ($n = 10$, 2.2%). Assuming 3.9% of multiple site blood cultures would add information additional to a single culture (0%), we estimated that 203 infants would require two site blood cultures to demonstrate a similar improvement in pathogen detection, at an alpha of 0.05 and a beta of 0.20 (80%) power.

Data were analyzed using a multivariate analysis using a logistic regression model to assess if specific neonatal characteristics and factors could predict positive result of blood cultures from both sites in neonates with suspected late-onset sepsis.

Results

During the study period, 216 consecutive infants with suspected early- and late-onset sepsis were studied. In all, 173 neonates were evaluated for possible early-onset sepsis alone, 30 neonates were studied only for possible late-onset sepsis, while 13 neonates were evaluated for both early- and late-onset sepsis. A total of 186 neonates in the early-onset group and 43 neonates in the late-onset group comprised the final totals. In all, 21 of the 43 neonates from the late-onset group were evaluated more than once for suspected late-onset sepsis.

A total of 269 pairs of blood cultures were drawn from two sites for evaluation of possible sepsis in the 216 neonates. In all, 186 culture pairs were obtained for evaluation of possible early-onset sepsis in 186 neonates, while 83 culture pairs were drawn during 83 episodes of suspected late-onset (>7 days of life) sepsis in 43 neonates.

Mean gestational age and mean birth weight for 186 neonates evaluated for early-onset sepsis were 34.8 weeks ±s.d. 4.5 and 2507.9 g ±s.d. 988.6 respectively; and gestation at delivery and birth weight in 43 neonates evaluated for late-onset sepsis were 28.7 weeks ±s.d. 4 and 1272.6 g ±s.d. 711, respectively. Figure 1 shows the distribution of gestational ages at birth in neonates who were evaluated for sepsis. The greatest number of infants from the early-onset group were born beyond 37 weeks of gestation, while the majority of the infants from late-onset group were born prior to 28 weeks of gestation and remained in the neonatal intensive care unit for a sufficient period of time to develop multiple episodes of suspected late-onset sepsis.

The maternal risk factors which prompted evaluation for early-onset sepsis in 186 infants were clinical chorioamnionitis in 127 infants (68%), preterm labor and premature rupture of membranes (PROM) for longer than 24 h in 54 infants (29%), and a positive GBS screening culture obtained during pregnancy, with inadequate intrapartum antibiotic prophylaxis in 29 cases (16%). Subsequent histopathological examination of the placenta was consistent with chorioamnionitis in 59 of the 127 (46.5%) women with clinical chorioamnionitis. All 127 women with clinical chorioamnionitis were treated with intrapartum antibiotics.

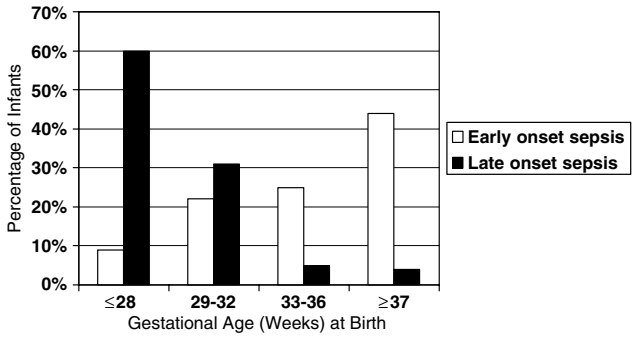

Figure 1 Distribution of gestational ages in neonates evaluated for early-onset ($N = 186$), and late-onset sepsis ($N = 43$).

In all, 126 of the infants (68%) from early-onset group were symptomatic soon after birth and 112 (89%) of those neonates had respiratory distress at the time of initial clinical examination. The causes of respiratory distress were subsequently noted to be due to transient tachypnea of the neonate (TTN) in 65 infants, RDS in 35 infants and other diagnoses (e.g., MAS, PPHN, pneumonia) were made in 12 infants. In all, 55 of the 112 (49%) infants with respiratory distress were being treated with nasal continuous positive airway pressure (NCPAP) or mechanical ventilation at the time of evaluation for early-onset sepsis. In all, 14 infants (8%) were febrile soon after birth. The secondary diagnosis in all 186 neonates from the early-onset group, whether symptomatic or asymptomatic, was suspected neonatal septicemia because of presence of maternal predisposing factors for sepsis.

In the late onset group, the indications for performing blood cultures during 83 episodes of possible sepsis in 43 infants were as follows: a septic appearance as evidenced by presence of lethargy, hypotonia and mottled appearance during 35 of 83 episodes (42%), hyperglycemia and temperature instability during 17 episodes (20.5%), feeding difficulties with suspected necrotizing enterocolitis in 14 instances (17%), recurring severe apnea and bradycardia during five episodes (6%), an abscess at the site of an intravenous catheter insertion or skin incision site for PDA ligation during two (2.4%) episodes, and combination of abovementioned symptoms during 10 other episodes.

Neonates who were evaluated for late onset sepsis were receiving treatment with CPAP or mechanical ventilation during 70 of the 83 episodes of possible sepsis. The indications for CPAP or mechanical ventilation were resolving RDS during 38 episodes, evolving BPD during 28 episodes, resolving MAS pneumonia, and recurrent apnea in two instances each. During 33 (41%) of those 70 possible sepsis episodes, infants had an increase in ventilatory requirement with onset of sepsis. Neonates from the late onset group had central venous catheters (peripherally inserted central catheter (PICC), umbilical venous catheter, or Broviac catheter) in place during 32 episodes and were receiving total parenteral nutrition and intralipid during 72 episodes of possible sepsis. Neonates with possible late-onset sepsis, compared with neonates with early-onset sepsis, had a significantly higher white blood cell count (mean 20 300/mm^3 ± s.d. 9500 vs mean 14 700/mm^3 ± s.d. 7900) and higher immature: total neutrophil ratio (mean 0.28 ± s.d. 0.2 vs mean 0.14 ± s.d. 0.1).

Blood culture results revealed that 20 (9.2%) of 216 neonates had 22 episodes of culture proven sepsis at a median age of 18 days. Only one of the 20 neonates with a positive blood culture had an episode of early-onset sepsis. This infant's septicemia was caused by *Listeria monocytogenes* while all other remaining 21 episodes of blood culture proven sepsis were late onset.

The organisms grown in addition to *L. monocytogenes* during remaining episodes were: coagulase negative *Staphylococci* in seven; methicillin-resistant *Staphylococcus aureus* (MRSA), late onset Group B *Streptococcus*, Gram-negative organisms and *Candida* in others (Table 1).

Table 1 Pathogens isolated from blood cultures during 22 episodes of Sepsis in 20 neonates

Pathogens	*Number of episodes*
Coagulase Negative Staph	7
MRSA	3
MRSA and *Candida*	2
Candida	2
Group B Strep (late onset)	2
Klebsiella pneumoniae	2
Escherichia coli	1
Enterococcus fecalis	1
Listeria monocytogenes	1
Serratia marcescens	1

All 20 neonates with positive blood cultures had the same organisms with similar sensitivity patterns obtained from the two different peripheral sites during 22 episodes of sepsis. The remaining 196 of 216 study neonates had negative blood cultures from both sites.

We performed a multivariate analysis using a logistic regression model to assess if specific neonatal characteristics and factors can predict positive result of blood cultures from both sites in neonates with suspected late-onset sepsis. The factors which showed a positive correlation on univariate analysis were I:T neutrophil ratio $\geqslant 0.2$ ($P = 0.011$), presence of thrombocytopenia ($P = 0.006$), fever ($P = 0.004$), presence of central line ($P = 0.012$), TPN/Lipid infusion ($P = 0.015$), hypo or hyperglycemia ($P = 0.004$), increase in ventilatory requirements ($P = 0.005$), and septic appearance ($P = 0.00$) but on multivariate analysis, the septic appearance (OR 35.53, $P = 0.000$) and thrombocytopenia (OR 8.97, $P = 0.007$) were the only significant predictors for positive blood culture in infants with suspected late-onset sepsis.

Discussion

The isolation of microorganisms from blood remains the most valid method of diagnosing bacterial sepsis in the newborn.[1,4] But the number of blood cultures required to document sepsis in an ill neonate, however, has had minimal evaluation to date.[3] Studies comparing two blood cultures vs a single site blood culture in the diagnosis of neonatal sepsis have been surprisingly small and have focused primarily on early-onset Group B *Streptococcus* or late onset coagulase-negative *Staphylococcal sepsis*.[4,13] The current prospective study strongly indicates that a single site blood culture with blood volume of $\geqslant 1$ ml should be sufficient to document 'true' Gram positive, Gram negative, or fungal sepsis in neonates.

The purpose of our study was not to evaluate if multiple blood cultures can pick up more cases of bacteremia in neonates with

presumed sepsis but to prove our hypothesis that because of higher bacterial load with slow clearance of bacteremia, if the blood culture is positive from one site it should be positive from other site as well, precluding the need for two-site cultures. All neonates with positive cultures in our study had the same organism with a similar sensitivity pattern obtained from the two different peripheral sites. Since no infant grew organisms from only one of the two sites, these data suggest that the correct diagnosis would have been made in all instances with a single site culture.

There was only one positive culture in the early-onset cohort and the agreement in two-site negative cultures in this cohort merely indicates that for infants who do not have septicemia one culture may be sufficient to document 'no bacterial disease'. The interpretation of this observation is limited by the fact that in the setting of early-onset sepsis, the use of a majority of study infants whose two-site cultures were sterile to provide the power to substantiate conclusions regarding positive cultures may not be completely accurate.

An earlier study, for the first time, investigated the usefulness of multiple site blood cultures in the initial evaluation for neonatal sepsis during the first week of life and contrary to the results of our current study, found two site blood cultures to be useful.[4] That study is of limited value, however, because of its retrospective nature. Most significantly, all neonates studied were evaluated for presumed sepsis during first week of life and no infants with late onset sepsis were included. With different patient populations in which intrapartum antibiotic prophylaxis for prevention of GBS sepsis is common, leading to a changed prevalence and incidence of early-onset neonatal sepsis,[14] as well as changing patterns of organisms that may be present in both early- and late-onset sepsis,[15] the earlier study may no longer be entirely applicable.

Another study by Struthers *et al*.[13] noted that in 5% of babies, cultures from a second site did not substantiate the diagnosis of coagulase-negative *Staphylococcus* sepsis when compared to the result from a single culture. This study was prospective, but evaluated neonates who were at least 48 h old. Also, 6% of those neonates had blood cultures taken for documentation of clearance of previous infection and not for the initial evaluation of sepsis. Finally, the study focused mainly on coagulase-negative *Staphylococcus* sepsis and was subject to selection bias, since not all the infants who had sepsis screens had blood cultures taken from two sites.[13] Moreover, in both of the previous studies,[4,13] approximately 0.5 ml of blood was inoculated into the blood culture bottle. Although a minimum of 0.5 ml of blood per culture bottle has been verified as adequate for Coagulase-negative *Staphylococcal* sepsis,[16] the reliability of such a small volume has not been validated for other organisms. This low volume may explain the negative culture results from the second site in those studies and prompted us to obtain at least 1.0 ml of blood for culture in the study neonates to avoid false negative results.

In our study, the blood cultures were obtained from two different peripheral sites within 15–30 min of each other in every neonate once sepsis was suspected and no anaerobic cultures were sent. Anaerobic sepsis in the neonatal population is exceedingly rare, with many centers preferring to use all the blood for aerobic cultures unless specific clinical indications exist.[17–20] Although there are no neonatal or pediatric data on timing of blood cultures, it is generally recommended that the optimal time to culture for bacteremia is 'as early as possible' in the course of a sepsis episode.[20] The interval between repeat blood cultures does not appear to be important[20,21] and the studies in adults demonstrate that high-volume blood cultures drawn serially or simultaneously return the best yields.

A single site blood culture should be sufficient to document sepsis, and is biologically plausible possibly because young children have high-colony-count bacteremia,[22] the bacterial clearance is slower, and the bacteremia more continuous in neonates with sepsis than in older patients.[11] High-colony-count bacteremia with slower bacterial clearance seems to be true for neonates with both early- and late-onset sepsis even though they represent two widely different groups, each with a totally different epidemiologic and pathogenetic background in so far as the acquisition of bacterial infection is concerned. On the basis of quantitative blood culture data from the 1970's, it has been shown that 80% of infected newborns with early-onset sepsis had high loads of *Escherichia coli*.[20,23] Similar observations were made with reference to late onset coagulase-negative *Staphylococcal* sepsis as well in a study of 787 neonatal blood culture specimens which showed that no cases of coagulase-negative septicemia were associated with counts of <5 CFU/ml,[16,24] in contrast to the concentration of microorganisms of <1 CFU/ml in 50% of adult bloodstream infections.[16,25]

Results of our current prospective study indicate that two site blood cultures for the initial evaluation of neonatal sepsis do not improve pathogen detection. Philip has also suggested that 'under most circumstances a single blood culture is satisfactory', because it was not prudent to withhold antibiotic therapy in a sick infant.[26] Sidebottom *et al*.,[27] commented that the use of a single blood culture may decrease the need for blood transfusions as compensation for repeated phlebotomy.[27] Finally, taking blood cultures from multiple sites require valuable physician and nurse time, appears to inflict unnecessary discomfort on the neonate, and costs money.[13] Our study proves that sepsis in neonates can be detected with no loss of accuracy with a single site blood culture with blood volume of $\geqslant 1$ ml.

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