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Time to Positivity for Detection of Bacteremia in Neonates

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The blood culture results of all samples obtained from newborns at Grady Memorial Hospital, Atlanta, Ga., during a 6-month period were analyzed to determine the time required for a blood culture to become positive, the time at which a culture could safely be considered negative, and the spectrum of isolated organisms. During the study period, 1,248 samples were submitted from all nurseries and processed by an automated detection instrument (BACTEC NR660). Of the 1,248 samples, 98 (7.8%) were positive by the end of a 7-day processing period; 29 of the 98 were classified as definite bacterial pathogens, 52 were classified as possible bacterial pathogens, 9 were classified as yeasts, and 8 were classified as contaminants. Virtually all organisms (28 of 29) categorized as definite pathogens were identified by day 2 of processing, and all were identified by day 4. All isolates of group B streptococcus, *Escherichia coli*, *Klebsiella* species, and *Staphylococcus aureus* were identified by day 2. Of all positive blood cultures, 79% were identified by day 2, 88% were identified by day 3, and 94% were identified by day 4. Of the 21 isolates identified after day 2, the only definite pathogen was from a sick baby in the intensive care unit. From among the 870 term low-risk newborns cultured because of maternal risk factors, only four possible pathogens were identified after day 2. The positive and negative predictive values of blood culture at days 2 and 4 were 92 and 99%, respectively. We conclude that, in our institution, (i) a 2-day processing period is sufficient to detect positive blood cultures in the asymptomatic term infant, (ii) a 4-day processing period will detect virtually all clinically important infections, and (iii) clinical yield from continuing blood culture processing beyond 4 days does not justify the time and cost involved.

The diagnosis of sepsis in the neonate is a difficult task for those involved in neonatal care. Sepsis is in the differential diagnosis of almost any sign of neonatal distress (e.g., apnea, bradycardia, respiratory problems, feeding intolerance, or temperature instability). Of all newborns, 3 to 5 per 1,000, and as many as 3 to 5% of newborns admitted to a newborn intensive care unit, have culture-proven sepsis (5, 13). Some investigators believe that these figures may underestimate the true bloodstream infection rate and that many cases remain undocumented. Because of this assumption, the length of treatment is often based as much on the physician's clinical assessment of the infant's status as on the microbiologic results. Sick newborns may be treated with antibiotics for 5 to 10 days if such therapy is warranted by clinical assessment, even if blood cultures remain negative.

Some asymptomatic newborns are started on antibiotics because of maternal risk factors, such as prolonged rupture of membranes, fever, or chorioamnionitis. Others receive antibiotics for minor symptoms that resolve quickly. Patients in these groups are often treated until it is clear that blood cultures are not positive. A critical issue, for these and other infants, is the time at which one can assume that blood cultures negative to date will not subsequently become positive.

The literature has not resolved the question of when a blood culture can safely be considered negative. Many laboratories continue to observe samples for 5 to 10 days. Yet, many nurseries discontinue antibiotic therapy after blood cultures have remained negative for 2 (8, 11; D. T. Crouse, J. S. Bhatia, and O. P. Mathew, Pediatr. Res. **19**:291A, 1985) or 3 days (4). The following unsettled questions for both the clinician and the laboratory were the subject of our study. (i) How many septic newborns would be inadequately treated if therapy were stopped at 2 days? (ii) How many newborns would be treated unnecessarily and have their hospital stay prolonged if a longer period of blood culture incubation were adopted? (iii) What is the optimal time for the laboratory to continue monitoring samples?

MATERIALS AND METHODS

Grady Memorial Hospital, Atlanta, Ga., is a large, urban teaching hospital which cares primarily for the indigent population of Atlanta. Neonates are hospitalized in several areas of the hospital on the basis of birth weight, gestational age, and health. Healthy newborns who weigh more than 2,200 g are admitted to one of three term nurseries (low risk). Healthy newborns who weigh between 1,900 and 2,200 g, as well as larger newborns who require an intravenous line for fluids or medications but are otherwise asymptomatic, are admitted to an intermediate care nursery. Newborns who weigh less than 1,900 g or are sick (i.e., experience respiratory distress, require oxygen, require mechanical ventilation, have seizures, have hypotension, etc.) or both are admitted to the newborn intensive care unit (NICU). These newborns are classified as very ill or ventilated infants (NICU) and more stable, nonventilated infants (intermediate care unit). Each year, about 5,500 babies are admitted to low-risk term nurseries, about 1,600 babies are admitted to one of the two intermediate care nurseries, and about 400 babies are admitted to the NICU.

The results of all blood cultures submitted to the Grady Memorial Hospital microbiology laboratory from all nurseries were collected from June through November of 1986. A previous report from our institution (7) reviewed the time to positivity of blood cultures from all patients, including

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neonates, in the period 26 June to 31 August 1986. The present study adds a review of epidemiologic and clinical data on these neonates and on others observed during a total period of 6 months. Blood from all neonates with maternal risk factors for infection or with signs and symptoms suggestive of sepsis was drawn and cultured. Most of these infants were treated with antibiotics while culture results and clinical course were pending. These included neonates from low-, intermediate-, and high-risk areas. One milliliter of blood was obtained from each neonate by puncture of a peripheral vein or artery after cleansing of the site with povidone-iodine solution and alcohol. The sample was transferred to BACTEC aerobic (NR6) and anaerobic (NR7) culture vials (Johnston Laboratories, Inc., Towson, Md.) and inoculated at the neonate's bedside (0.5 ml in each vial). The samples were collected and transported to the laboratory immediately by residents, medical students, and nurses assigned to the nursery areas. When the vials were brought to the laboratory, they were incubated at 35°C.

Aerobic culture bottles were agitated for the first day of incubation and examined by using an automated instrument used to detect microbial growth (BACTEC NR660; Johnston Laboratories) (7). Aerobic culture bottles received during the first morning test period were tested once on day 1 of incubation, twice on day 2, and once daily on days 3 to 7. Aerobic vials received after testing had been completed for the day were incubated for the remainder of that day (designated day 0) and tested on the BACTEC instrument twice each day on days 1 and 2 and once daily on days 3 to 7. Anaerobic vials were tested once each day for 7 days. The test protocol was followed daily, including weekends and holidays.

Bottles with an initial growth value of >30 or increasing by >15 between consecutive readings were examined by the Gram stain method and subcultured aerobically and anaerobically. If no organisms were seen by using the Gram stain, the bottles were returned to the test protocol, while subcultures were examined daily for 2 days. All isolates were identified by conventional methods (6, 9). We grouped all isolates according to the day of the test cycle on which growth was first detected in at least one bottle of the set.

Organism classification. To determine the clinical impact of the recovery of organisms from culture, we reviewed the medical records of all infants with positive cultures. We defined as definite pathogens those organisms which are known to cause disease in the newborn (4, 5). A possible pathogen was defined as one known to cause disease under special circumstances (e.g., immunosuppressed host, presence of an indwelling catheter, etc.) (2, 3, 12, 14). Since many of the patients had only one blood sample drawn, persistence of bacteremia could not be used to help determine the clinical importance of most of the possible pathogens (1, 2, 14). Finally, we defined as contaminants some organisms that rarely cause disease in the newborn and that were inconsistent with the patient's clinical picture.

RESULTS

During the 6-month study period, 1,248 blood cultures from neonates were submitted to the microbiology laboratory; 870 cultures were from infants in the low-risk term nurseries, 204 were from infants in the NICU, and 174 were from infants in the intermediate care areas. Of the 1,248 samples, 98 (7.8%) were positive by the end of the 7-day processing period, yielding 89 bacterial isolates and 9 yeasts (four cultures yielded two organisms each). One bacterial organism was recovered on the day the culture was submitted (defined as day 0), and 88% of all positive cultures were identified as positive by day 3 (Table 1). Of the 89 bacterial isolates recovered, 29 were classified as definite pathogens, 52 were classified as possible pathogens, and 8 were classified as contaminants (Table 1).

TABLE 1. Day on which organisms were recovered from blood cultures obtained from neonates

Organism category (no. of isolates)[a]	No. of blood cultures yielding organisms on day[b]:							
	0	1	2	3	4	5	6	7
Definite pathogens (29)								
Group B streptococcus	1	14	3					
S. aureus		1	2					
Group G streptococcus		1						
E. coli		3						
K. pneumoniae		1						
Enterobacter cloacae					1			
Citrobacter diversus		1	1					
Possible pathogens (52)								
Coagulase-negative staphylococci		8	10	5	2			2
Alpha-hemolytic streptococci		7	5	1				
Gamma-hemolytic streptococci		3						
Anaerobes		1	3		1			3
Neisseria species		1						
Yeasts (9)								
Candida albicans		2						
Candida parapsilosis			1	1				
Malassezia furfur			1	1				
Non-*Candida* species			1	1	1			
Contaminants (8)								
Corynebacterium species		2	1					
Diphtheroids		1			1			
Propionibacterium species						1		
Nonviable organisms			2					

[a] See Materials and Methods for definitions of the categories.
[b] Day of receipt of cultures in the lab is defined as day 0.

Of the 29 organisms identified as definite bacterial pathogens, 28 were from cultures identified as positive by day 2. All isolates of group B streptococcus, *Escherichia coli*, *Klebsiella* species, and *Staphylococcus aureus* were identified by day 2. If results for definite and possible bacterial pathogens are pooled together, 66 of 81 pathogens (81%) were in cultures detected as positive by day 2, 72 of 81 (89%) were in cultures detected as positive by day 3, and 76 of 81 (94%) were in cultures detected as positive by day 4. Of the cultures positive after day 4, one was a contaminant, three were anaerobes, and two were coagulase-negative staphylococci. No definite pathogens were isolated after day 4, on which one strain of *Enterobacter cloacae* was recovered.

Of 15 bacterial organisms identified after day 2, the sole definite pathogen and 7 of 14 possible pathogens were isolated from babies in the NICU; all of these babies were sick, with an episode of clinical deterioration compatible with sepsis (Table 2). The only definite pathogen in the group was an isolate of *Enterobacter cloacae* from a sick 43-day-old premature infant. Of the 14 possible pathogens, 9 were isolates of coagulase-negative staphylococci (three from infants in the NICU, five from infants in the intermediate care area, and one from an infant in the low-risk area). Two infants in the intermediate care area with blood cultures positive for coagulase-negative staphylococci were symptomatic from birth, were judged to have clinical sepsis, and

TABLE 2. Blood cultures positive after day 2 of processing by organism category and clinical risk group of newborn, Grady Memorial Hospital, June to November 1986

Organism category	No. of patients in clinical risk group		
	High risk (n = 204)	Intermediate risk (n = 174)	Low risk (n = 870)
Definite pathogens	1	0	0
Possible pathogens	3	7	4
Contaminants	0	1	1
Yeasts	4	0	0

would have been treated with antimicrobial agents for a full 7- to 10-day course regardless of blood culture results. One patient in the intermediate care area had a culture positive for an alpha-hemolytic streptococcus, and four infants (one from the intermediate care area and three term low-risk babies) had blood cultures from which anaerobes were recovered (*Clostridium* spp., two infants; *Clostridium* spp. plus *Propionibacterium* spp., one infant; mixed gram-positive rods and gram-negative cocci, one infant). Blood samples from four asymptomatic term babies, cultured solely because of maternal risk factors, yielded isolates identified after day 3; anaerobes were recovered in three cases and a coagulase-negative staphylococcus was recovered in one. These four infants had been discharged before the samples showed any growth and were not treated with antibiotics. All remained asymptomatic, and none required rehospitalization. Finally, cultures from three sick infants in the NICU yielded four yeast isolates that were recovered after day 2. In both the low- and intermediate-risk groups of patients, the microorganisms isolated after day 2 were either possible pathogens or contaminants.

Sensitivity, specificity, and predictive value of the blood culture in identifying pathogens were as follows. The sensitivities at days 2 and 4 were 81 and 94%, respectively. The specificity of the culture at both days 2 and 4 was 99%. The positive predictive value of the blood culture at both day 2 and day 4 was 92%. The negative predictive value of the culture at both days 2 and 4 was 99%. Thus, a blood culture negative for definite or possible pathogens at day 2 would have a 99% probability of being negative after 7 days of incubation. The numbers of true-positives (defined as definite and possible pathogens) at days 2 and 4 were 66 and 76, respectively.

DISCUSSION

Technological developments in the past 20 years have improved the survival of premature newborns and increased the diagnostic abilities of the clinician. However, in the field of neonatal infections, it remains difficult to make a quick and accurate diagnosis of sepsis.

Cultures are prepared from a large number of babies because of their perinatal history. Some of these babies are very sick and require hospitalization and treatment whether their cultures are positive or not. However, many more are healthy, asymptomatic full-term or near-term infants who will be treated with intravenous antimicrobial agents until their cultures can be safely considered negative. For this second group, a critical issue is how soon one can safely call a blood culture negative and discontinue therapy. In our study, 28 of 29 definite bacterial pathogens, but only 5 of 9 fungi, were identified by day 2 of culture processing. All isolates of group B streptococcus, *E. coli*, *Klebsiella* species, and *S. aureus* were identified by day 2. If results for definite and potential bacterial pathogens are considered together, 81% were identified by processing day 2 and 89% were positive by day 3.

The predictive value of blood cultures that were negative at day 2 was clinically similar to that of waiting for 4 days of processing before discontinuing therapy. No septic infant was missed in the low-risk nursery, since growth is not the only parameter taken into account for the treatment of newborns. If one chose to continue therapy for all 870 low-risk infants in this 6-month review until blood cultures were negative for 4 days of processing rather than for 2 days, approximately 1,740 newborns per year, already having been treated with antibiotics for 2 days, would have been treated and hospitalized for 2 extra days each to detect four microorganisms in the potential pathogen group and no definite pathogens. The extra stay involved represents 3,480 patient days per year. At an estimated cost of $200/day (an extremely conservative estimate), this would total almost $700,000. Moreover, earlier discontinuation of intravenous lines and antibiotic therapy and earlier discharge would decrease the risk of iatrogenic complications and nosocomial infections.

Laboratory processing for a total of 7 days also was not cost-effective. No definite pathogen, 5 of 52 possible pathogens, and 1 of 8 contaminants developed after day 4. Even if one considers all results through day 7 as clinically useful, 94% of definite and potential pathogens were recovered by day 4. These observations are not confined to the nursery; in all hospitalized patients, the value of 7 days of processing with the BACTEC NR660 was slight compared with that of 5 days of processing (7). With the BACTEC 460 system, only 4 of 268 isolates from previously healthy infants and children were detected as positive after day 2 of incubation (10).

Our study differed from earlier ones in the classification of the likely clinical impact of the organisms and in the use of a newer instrument for detection of bacteremia. In contrast to some prior studies (8, 11), we considered coagulase-negative staphylococci as possible pathogens rather than as contaminants. These organisms have been increasingly recognized as important nosocomial pathogens in neonates, especially in very-low-birth-weight infants (2, 3, 12), and our definition took this into account. In addition, our instrument, the BACTEC NR660, operates on a principle different from that of the BACTEC 460 used in earlier work (10, 11); it is not clear that results from our instrument, which uses infrared detection, would hold for the older instrument, which used radiolabeling for detection of positive cultures (7). Because of this, our results cannot be extrapolated to institutions with other patient populations or that use other methods for blood culture processing without confirmation of these findings in that setting. Despite these differences, our results were similar in clinical import to those of prior investigators.

From this study we conclude that, in our institution, a 2-day observation period is enough to rule out sepsis in the otherwise asymptomatic term infant, a 4-day observation period is sufficient to detect virtually all clinically important infections, and clinical yield from continuing blood culture processing beyond 4 days does not justify the time and costs involved.

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