TIME TO POSITIVITY OF BLOOD CULTURE IN NEWBORN INFANTS

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Abstract. The purpose of this study was to evaluate the minimum incubation time required to detect positive blood cultures from newborn infants with sepsis. Data were collected retrospectively on seventy-five positive blood cultures from newborn infants in the neonatal intensive care unit of Songklanagarind Hospital. The BacT/Alert Microbial Detection System had been used to culture the samples. Data were obtained retrospectively from the patients' medical records for positive blood cultures. A computer algorithm in the automated blood culture system determined the time to positivity, which was then evaluated for clinically important definite bacterial pathogens, possible bacterial pathogens, fungi and contaminants. Definite bacterial pathogens accounted for 46% (34/74) of the positive blood culture results, possible bacterial pathogens accounted for 39% (29/74), fungi for 7% (5/74) and contaminants for 8% (6/74). The cultures were positive at 24, 36 and 48 hours of incubation in 70.2%, 91.8% and 95.9% respectively. At 36 hours of incubation, the sensitivity, specificity and negative predictive value were 70.3%, 100% and 93.3%, respectively. All cultures growing clinically significant definite bacterial pathogens were positive by 36 hours of incubations, 88% by 24 hours. The cultures had 100% sensitivity, specificity and negative predictive value at 36 hours of incubation. If definite and possible bacterial pathogens were considered, the time to positivity was 71% at 24 hours, 95% at 36 hours and 97% at 48 hours, respectively. The sensitivity, specificity and negative predictive values were 70.3%, 100%, and 93.3%, respectively. Of cultures growing fungi, 80% were positive by 36 hours and all by 48 hours.

INTRODUCTION

Neonatal sepsis is an important cause of mortality and morbidity in neonatal intensive care units, with incidences of 1 and 4 cases per 1,000 live births for full-term and premature infants, respectively, in western countries. In Songklanagarind Hospital, the major tertiary referral institution in southern Thailand, a recent study found the incidence of neonatal sepsis to be 4.3% (Janjindamai *et al*, 1998). The susceptibility of a neonate to sepsis is multifactorial, and can be related to immaturity in humoral, phagocytic and cellular immunity, hypoxia, acidosis and metabolic derangements. The early identification of sepsis in infants is difficult because early symptoms of sepsis are non-specific. Delayed treatment raises the risk of mortality, however, and for this reason, when maternal risk factors for neonatal sepsis are present or the infant manifests symptoms suggestive of infection, cultures are obtained and antibiotic therapy is normally initiated (Gerdes, 1991). This procedure is long-established; for instance, the New England Journal of Medicine featured a study in 1977 in which between 11 and 23 non-infected newborns were treated with antibiotics for every one with proven sepsis. (Hammerschlag *et al*, 1977). However, in recent years we have begun to understand better that inappropriate use of antibiotics can be implicated in the development of multiresistant bacteria in the hospital setting. To reduce antibiotic overuse, it is common practice to discontinue antibiotic treatment if the blood culture is negative at 48-72 hours and the baby does not have any clinical or laboratory indicators of infection. This practice is based on retrospectively acquired data regarding time to positivity of blood cultures in the newborn in-

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fant. (Rowley and Wald, 1986; Kurlat *et al*, 1989; Hurst and Yoder, 1995). These data were generated by using non-instrument-based manual methods which assess blood culture turbidity (Pichicheo and Todd, 1979) to determine positivity as well as methods which use carbon dioxide detection. (Rowley and Wald, 1986; Kurlat *et al*, 1989). Blood culture technology has changed from tube or bottles of liquid culture medium requiring frequent inspection, microscopic or blind plating on to solid culture medium to see if growth has occurred to modern, closed, computer based systems, which assess changes in CO_2 indicating growth every 10-15 minutes (the BacT/ALERT microbial detection system, Organon Teknika Corporation, Durham, North Carolina, USA) (Butterly, 2002). The BacT/ALERT method is reported to more rapidly detect positive blood culture results in pediatric and newborn patients than other systems (Kumer *et al,* 2001). Our hospital has been using the BacT/ALERT microbial detection system since 1996. The aim of this study was to measure the time required for bacteria to be detected in blood cultures taken from suspected sepsis newborns by the BacT/ALERT microbial detection system.

MATERIALS AND METHODS

All positive blood cultures in both term and preterm newborn infants were retrospectively reviewed for the 1 year period from September 1, 2000 through August 31, 2001. All the babies had been admitted to the newborn intensive care unit (NICU), at Songklanagarind Hospital. A total of 75 positive blood cultures were identified from the microbiology database maintained at the hospital, which provides the microbiology service. Using a standardized form, data concerning microorganisms identified, number of hours from inoculation to positive culture, patient age at time of culture, patients' history and clinical presentations, and diagnosis were recorded. Cultures were excluded from analysis if they were obtained from newborns with an indwelling central venous catheter.

Blood (0.5 ml or more) was collected by venepuncture, after the skin had been cleaned with 70% alcohol, and inoculated into a BacT/ALERT aerobic blood culture bottle, which contained 20 ml of brain/heart infusion broth, with amino acid and carbohydrate substances, and 0.02% sodium polyanetholesulphonate as an anticoagulant (Wilson *et al*, 1994). In the BacT/ALERT system, the substrates in the aerobic culture bottle are in an atmosphere of carbon dioxide at subatmospheric pressure and are designed for the growth of common aerobic, microaerophilic and fastidious bacteria and common yeasts. The temperature is maintained at 35-37ºC. Each culture bottle contains a colorimetric carbon dioxide sensor to measure microbial growth. Because growth of microorganisms produces carbon dioxide, and with the increase in CO_2 the color of the gas permeable sensor changes from green to yellow. The BacT/ALERT system tests for carbon dioxide production every 10 minutes, and data points are plotted. Also as the amount of carbon dioxide dissolved in the culture medium raises, a light emitting diode reflects more light, indicating to the computer that the blood culture is positive. The time required for the blood culture to become positive is recorded in the data management system. In the current study, the time to positivity of a blood culture was taken as the time from inoculation of the blood into the culture bottle to the time at which the BacT/ALERT machine signaled a positive result.

Bottles identified as positive were immediately removed from the instrument, at any time during the day or night, and an aliquot was taken for Gram stain and subculture. Bottles were incubated for a total of 6 days before being declared negative.

The positive blood cultures were classified on the basis of the organism, isolated as bacterial or fungal. Bacteria were further subdivided into definite pathogens, possible pathogens, and contaminants. Definite pathogens were defined as organisms known to cause disease in newborns, for example *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Enterococcus* spp, or *Pseudomonas aeruginosa.* Possible pathogens were defined as organisms known to cause disease under special circumstances, for example, coagulase-negative *Staphylococci*, or α-hemolytic *Streptococci*. Blood cultures con-

taining two or more organisms were considered to be possibly pathogenic if one of the organisms was a definite or possible pathogen (Faix and Rovarik *et al*, 1989). Contaminants were defined as organisms that rarely cause disease in the newborn, for example, *Bacillus* spp or *Propionibacterium* spp.

Data analysis

The data was entered into Epidata, and diagnostic tests were used to analyze the time to positivity of the blood cultures at 24, 36 and 48 hours. The study was approved by the Ethics Committee Board of Prince of Songkla University.

RESULTS

During the study period, 379 blood cultures from 44 sick newborns were analyzed at the microbiology laboratory at Songklanagarind Hospital. Seventy-five (19.8%) were reported as positive. Complete information was available on 74 of the 75 (98.7%) positive blood cultures.

Sixty-four percent of the positive blood cultures were from male infants. The mean ± SD gestational age of the infants with positive blood cultures was 34 ± 4.2 weeks (range 24-41 weeks). The mean ± SD birth weight was 2,052±843 grams (range 650-4,045 grams). The median age at blood culture collection was 21 days. The median time to positivity of all blood cultures was 17 hours. Table 1 shows all organisms isolated in the blood cultures. Coagulase-negative *Staphylococci* accounted for 39% (29/74) of the total isolates.

Table 2 gives the time to positivity for all the blood cultures. Three of the 74 (4.1%) blood cultures that became positive after 72 hours were not considered by the attending clinicians to be indicative of true infection, and as all 3 of the babies were asymptomatic, no antibiotic treatment was given. No blood culture became positive between 48 and 72 hours. Of the 3 blood cultures that became positive between 36 and 48 hours, one was a possible pathogen (*S. epidermidis*), one was a fungus (*C. parpsilosis*) and one was a contaminant (*Bacillus* spp). All of the definite bacterial pathogens became positive in 36 hours. Ninety percent of the possible bacterial pathogens became positive within 36 hours and 93.1% became positive within 48 hours. All of the fungi became positive within 48 hours. Considering all positive cultures, the sensitivities for detection of bacteremia or fungemia after 24, 36 and 48 hours incubation were 70.3, 91.9 and 95.9%, respectively. The negative pre-

Table 1
Classification of organisms isolated in 74 positive blood cultures.

Definite pathogens		Possible pathogens		Fungi		Contaminants	
S. aureus	12	CoNS/*S. epidermidis*	29	*C. parapsilosis*	5	*Bacillus* spp	6
S. saprophyticus	1						
Propionibacterium spp	1						
P. aeruginosa	1						
K. pneumoniae	3						
E. coli	3						
Moraxella spp	3						
A. baumanni	9						
Enterococcus	1						
Total	34	Total	29	Total	5	Total	6

S. aureus (Staphylococcus aureus), S. saprophyticus (Staphylococcus saprophyticus), P. aeruginosa (Pseudomonas aeruginosa), K. pneumoniae (Klebsiella pneumoniae), E. coli (Escherichia coli), A. baumannei (Acinetobacter baumannii), CoNS/S. epidemidis (Coagulase negative staphylococci/*Staphylococcus epidermidis*), *C. parapsilosis (Candida parapsilosis).*

Table 2
Duration of time for blood cultures to become positive.

	0-12 hrs	12-24 hrs	24-36 hrs	36-48 hrs	48-60 hrs	60-72 hrs	> 72 hrs
Total positives, No. (%)	20 (27)	32 (43.2)	16 (21.6)	3 (4.1)	0	0	3 (4.1)
Definite bacterial pathogens, No. (%)	14 (44.1)	16 (47.1)	4 (11.8)	0	0	0	0
Possible bacterial pathogens, No. (%)	2 (6.9)	13 (44.8)	11 (37.9)	1 (3.5)	0	0	2 (6.9)
Fungi, No. (%)	1 (20)	2 (40)	1 (20)	1 (20)	0	0	0
Contaminants, No. (%)	3 (50)	1 (16.7)	0	1 (16.7)	0	0	1 (16.7)

Values in parentheses are percentages.

Table 3
Comparison of all positive cultures at 24, 36, and 48 hours incubation.

	Sensitivity	Specificity	PPV	NPV
All positive cultures				
Within 24 hours	70.3	100	100	93.3
Within 36 hours	91.9	100	100	98.1
Within 48 hours	95.9	100	100	99

PPV: positive predictive value, NPV: negative predictive value.

Table 4
Comparison of definite and possible bacterial pathogens at 24, 36, and 48 hours incubation.

	Sensitivity	Specificity	PPV	NPV
Definite and possible bacterial pathogens				
Within 24 hours	71.4	100	100	94.5
Within 36 hours	95.2	100	100	99
Within 48 hours	96.8	100	100	99.4

PPV: positive predictive value, NPV: negative predictive value.

Table 5
Comparison of definite bacterial pathogens at 24, 36, and 48 hours incubation.

	Sensitivity	Specificity	PPV	NPV
Definite bacterial pathogens				
Within 24 hours	88.2	100	100	98.7
Within 36 hours	100	100	100	100
Within 48 hours	100	100	100	100

PPV: positive predictive value, NPV: negative predictive value.

dictive values were 93.3, 98.1 and 99%, respectively. The specificities of the positive predictive values were 100% for all incubation groups (Table 3).

Considering only definite and possible bacterial pathogens, the sensitivities for significant bacteremia at 24, 36 and 48 hours incubation were 71.4, 95.2 and 96.8%, respectively. The

negative predictive values were 94.5, 99 and 99.4%, respectively. The specificities and positive predictive values were 100% for all incubation groups (Table 4). Considering only isolates of definite bacterial pathogens, the sensitivities at 24, 36 and 48 hours were 88.2, 100 and 100%, respectively, and the negative predictive values were 98.7, 100 and 100%, respectively. The specificities and the positive predictive values were 100% for all incubation groups (Table 5).

DISCUSSION

The management of neonates with suspected infection hinges on the result of blood cultures. It is standard clinical practice to discontinue antibiotic treatment in an asymptomatic infant if the blood cultures are negative at 48-72 hours (Pichicheo and Todd, 1979). The purpose of this study was to investigate the time to positivity of infant blood cultures using the single pediatrics BacT/ALERT aerobic bottle in newborn infants admitted to the NICU, Songklanagarind Hospital. Overall, the proportion of the total number of positive blood cultures was about 20%, which is about the same as an earlier study by Kumer *et al* (2001), although other known previous studies arrived at a figure of less than 10% (Rowley and Wald, 1986; Kurlat *et al*, 1989). Possible explanations for our higher findings include the possibility that we have a higher threshold before initiating a septic screen in our NICU, a newer and more sensitive blood culture system (new version of the BacT/ALERT microbial detection system), and more extremely low birth weight infants surviving for longer periods. The spectrum of organisms isolated from positive blood cultures in our study was also different from previous studies (Pichicheo and Todd, 1979; Rowley and Wald, 1986; Kurlat *et al*, 1989; Kumer *et al*, 2001). Forty-six percent of our positive blood cultures contained definite bacterial pathogens, whereas only 10% were definite bacterial pathogens in a similarly analyzed study (Kumer *et al*, 2001). Thirty-nine percent of positive blood cultures were classified as possible bacterial pathogens in this study, similar to the findings of Kurlat *et al*, (1989) whereas 77% were possible bacterial pathogens in the study of Kumer *et al* (2001). This difference is probably due to an increased proportion of coagulase negative staphylococci isolates in the study of Kumer *et al* (2001), and also reflects a difference in the study populations; in our study, the mean gestational age was 34, *vs* 28 weeks in the study of Kumer *et al* (2001). The more extremely low birth weight infants that survive for a long period, the more *S. epidermidis* infections are found. Fungi constituted about 7% of the total organisms, as in various other studies (Pichicheo and Todd, 1979; Rowley and Wald, 1986; Kurlat *et al,* 1989; Kumer *et al*, 2001). The median time to positivity of blood cultures in this study was 17 hours, which was shorter than in some earlier studies (Chichicheo and Todd, 1979; Kurlat *et al*, 1986; Rowley and Wald, 1986; Hurst and Yoder, 1995), but similar to the findings of recent studies that used modern blood culture incubation techniques (Garcia-Prats *et al*, 2000; Kumer *et al*, 2001). This may be explained by advances in laboratory methodology, such as an automated microbial detection systems. Another factor may be that neonates with bacteremia have been reported to have much higher concentrations of organisms per ml of blood than adults due to their immature immune defense mechanism (Philip and Bradley, 1990). All blood cultures with definite bacterial pathogens became positive within 36 hours. The cultures of the 2 babies which became positive for possible pathogens after 72 hours were considered by the attending clinician not be true infections, and normally if a blood culture is negative at 36 hours, it is 99% probable to remain negative for definite or possible bacterial pathogens. The diagnostic yield was not significantly improved by prolonging the incubation to 48 hours, and it can be concluded that a 36-hour observation period is enough to rule out sepsis in the asymptomatic baby. This shortened antibiotic course is especially practical when good follow-up is assured. Blood culture results should not be considered as a gold standard for sepsis and it may of course be necessary for some symptomatic babies to be treated as infected even though blood cultures remain negative, as many factors can affect blood culture yield, such as the

amount of the microorganisms present in the blood stream, the volume of the blood sample drawn for culture, the ratio of the blood to broth media and the type of broth media.

This study was unable to show any effect from prior specific antimicrobial treatment on the time to positivity of various groups of bacterial pathogens and fungi, however, the administration of antibiotics to the babies before blood collection had no effect on the time to positivity in the studies of Kumer *et al* (2001) or Gracia-Prats *et al* (2000).

We conclude that in asymptomatic babies, a 36-hour observation period is enough to rule out sepsis and stop antibiotic treatment. This should lead to a reduction in total antibiotic use in the neonatal unit, and the decreased use of antibiotics should help to reduce the emergence of antibiotic-resistant strains of bacteria in the long term (Lacey, 1994). There should also be cost savings through decreased length of stay of babies in the hospital, and decreased use of materials, such as intravenous cannulae. Other benefits include: a reduced workload for the neonatal staff, babies should suffer less pain overall through the reduced use of invasive procedures, and the nosocomial infection rate should be reduced resulting in earlier hospital discharges overall, which in turn should help to improve bonding between parents and babies.

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