Blood culture volume and detection of coagulase negative staphylococcal septicaemia in neonates

G Jawaheer, T J Neal, N J Shaw

Abstract

A prospective, blind study was carried out to determine: the amount of blood submitted for culture from neonates; whether small blood volumes resulted in false negative results; and whether there was a temporal relation between volume of blood cultured and time to positivity.

Seventy three bottles were evaluated. They contained a median of 0.63 ml of blood. Twenty nine bottles (39.7%) contained less than 0.5 ml of blood; 21 bottles (28.8%) were positive. There were three false negative cultures, only one of which contained a blood volume below 0.5 ml. The median time to positivity was 22.4 hours. There was no correlation between blood volume cultured and time to positivity.

Neonatal cultures frequently contain less than 0.5 ml of blood. False negative cultures are rare. Neonatal blood culture bottles need to be validated for blood volumes below 0.5 ml.

(*Arch Dis Child* 1997;76:F57-F58)

Keywords: blood cultures; septicaemia; coagulase negative staphylococci

Blood cultures have an important role in the diagnosis of coagulase negative staphylococcal septicaemia in neonates. The bottles are validated only for a specific range of blood volumes. During the past two decades, coagulase negative staphylococci (CNS) have emerged as the principal pathogen associated with late onset disease in preterm infants.¹ As infection with this organism gives rise to a varied clinical picture, the results of blood cultures form the basis of accurate diagnosis and effective treatment. According to the data sheet, our blood culture bottles (Pedi-BacT, Organon Technika) were validated using blood volumes ranging from 0.5 ml to 4.0 ml. At least 1.0 ml of blood has been recommended for neonatal blood cultures.² However, it is often difficult to take a peripheral blood culture in a neonate. The occasions when the smallest quantity of blood is obtained are often those where there are the greatest difficulties obtaining the sample and where the risk of contamination is highest. Samples smaller than 0.5 ml may be submitted for culture. For such low blood volumes, the sensitivity of the bottles is unknown and, therefore, the reliability of the cultures is questionable. This problem has not received much attention and is not widely recognised by clinicians.

The aims of this study were first, to determine the volume of blood submitted for culture in neonates, second, to assess the reliability of such cultures, and third, to examine the relation between volume of blood cultured and time to positivity.

Methods

All the blood culture bottles on our neonatal intensive care unit were replaced by bottles which had been numbered and weighed on an electronic scale (Denver Instruments, UK) by one investigator (TJN). Blood cultures were drawn by physicians who were blind to the purpose of the study and unaware that the bottles were being weighed. In all cases blood was obtained by peripheral venepuncture after cleansing the skin with 70% isopropyl alcohol. After inoculation the bottles were reweighed in the public health laboratory. The difference between weight (g) before and after inoculation was considered to be equivalent to the volume of blood inoculated in millilitres. The bottles were incubated at 37°C and monitored using the BacT/Alert continuous monitoring blood culture system (Organon, Teknika Corporation, Durham, North Carolina, USA). The time taken for the bottles to signal positive was determined by the computer software in the blood culture machine. The cultures were kept for seven days. Empirical broad spectrum antibiotics, consisting of co-amoxycylav and netilmicin, were started after cultures were taken. If the cultures remained negative after an incubation period of 48 hours, antibiotics were stopped. When the cultures were positive with a pure growth of coagulase negative staphylococci, antibiotic treatment was adjusted according to the results of sensitivity tests and administered for five days. A blood culture result was considered to be false negative when clinical symptoms persisted and a subsequent blood culture drawn within 72 hours of the original was positive for the organism.

Data are expressed as median and range. The positive and the negative groups were compared using the Mann-Whitney U test. The correlation between the volume of blood cultured and time to positivity was evaluated using the Pearson product moment correlation coefficient. Differences were considered significant at $P < 0.05$.

Results

Eighty blood culture bottles were submitted for culture over a period of five months. Seven were rejected: organisms other than coagulase negative staphylococci were cultured in three

Neonatal Intensive
Care Unit, Fazakerley
Hospital, Liverpool
G Jawaheer
N J Shaw

Regional Public Health
Laboratory, Fazakerley
Hospital, Liverpool
T J Neal

Correspondence to:
Mr G Jawaheer,
Department of Paediatric
Surgery,
Royal Hospital for Sick
Children,
Edinburgh
EH9 1LF.

Accepted 5 November 1996

and the time of blood collection was not recorded in four. The 73 bottles evaluated were from 43 neonates. The weight (kg) of the neonates was 2.07 (0.7-4.24) and their gestational age (weeks) was 32 (24-41). The volume of blood inoculated in the 73 bottles was 0.63 ml (0.05- 2.57). Twenty one bottles (28.8%) were positive, yielding a pure growth of coagulase negative staphylococci. The positive bottles contained significantly smaller volumes of blood than the negative bottles: 0.41 ml (0.13-1.81) and 0.67 ml (0.05-2.57), respectively ($P = 0.03$). Twenty nine bottles (39.7%) contained less than 0.5 ml of blood; 14 were positive for coagulase negative staphylococci and 15 were negative. The median time to positivity was 22.4 hours (13.2-40.6). There was no significant correlation between the volume of blood cultured and time to positivity ($r = -0.13$; $P = 0.56$). Three cultures (4.1%) were considered false negative because subsequent positive cultures, with a pure growth of coagulase negative staphylococci, were obtained within 72 hours of the original blood culture. Only one of the three original cultures contained a blood volume below 0.5 ml.

Discussion

The management of neonates with suspected infection hinges on the results of blood cultures. In clinical practice a negative blood culture signifies the absence of bacteraemia and antibiotics are subsequently stopped. The reliability of the results is therefore of crucial importance. The single most important factor governing the sensitivity of blood cultures is blood volume.³ The yield of blood cultures in adults increases by 3% per millilitre of blood cultured.⁴ Our finding was that in 40% of cases the amount of blood inoculated in the bottles was less than 0.5 ml and therefore below the range for which the bottles had been validated. The volumes recorded in this study should be a true reflection of the volume of blood inoculated into neonatal blood culture bottles in clinical practice because the clinicians performing the cultures were unaware of the bottles being monitored. Similar findings were reported in a study of 298 sets of blood cultures from 161 neonates; 55% of the aerobic and 58% of the anaerobic bottles contained less than 0.5 ml of blood.⁵

Given the high prevalence of low volume blood cultures, a high incidence of false negative results is to be expected. We found the reverse to be true. The positive bottles contained a significantly smaller volume of blood than the negative bottles, with 14 of the 21 positive bottles (66.7%) containing less than 0.5 ml. Of the three false negative results,

only one had an initial blood culture volume of less than 0.5 ml. One possible explanation for our observation is the fact that neonates have higher levels of bacteraemia than adults. It has been shown that the concentration of micro-organisms in 50% of adult bloodstream infections is less than 1 CFU/ml.⁶ In contrast, a study of 787 neonatal blood culture specimens showed that there were no cases of coagulase negative septicaemia associated with counts of less than 5 CFU/ml.⁷ Therefore, a smaller amount of neonatal blood would be required to provide the same bacterial load as in an adult blood culture. Reducing the blood volume would decrease the blood:broth ratio, but this would not be expected to have a significant influence on the culture result as blood: broth ratios of up to 1 in 100 have been shown to have no deleterious effect on organism recovery.⁸

One drawback of our study was that coagulase negative staphylococcal pathogens could not be reliably distinguished from contaminants. Blood cultures are indicative of sepsis in only 26% of infants.⁹ On our unit, all babies with positive blood cultures receive antibiotics for five days. However, we only included blood cultures yielding a pure growth of coagulase negative staphylococci in our study to minimise the influence of contaminants.

The volume of blood inoculated into neonatal blood culture bottles is often less than 0.5 ml. It is clear from this study that organisms can be recovered from such low volumes, which in turn, do not affect the time taken for blood cultures to become positive. Paediatric blood culture bottles therefore need to be evaluated for blood volumes of less than 0.5 ml.

- 1 Hall SL. Coagulase-negative staphylococcal infections in neonates. *Pediatr Infect Dis J* 1991;10:57-67.
- 2 Szymczak EG, Barr JT, Durbin WA, Goldmann DA. Evaluation of blood culture procedures in a pediatric hospital. *J Clin Microbiol* 1979;9:88-92.
- 3 Hall MM, Ilstrup DM, Washington JA 2nd. Effect of volume of blood cultured on detection of bacteremia. *J Clin Microbiol* 1976;3:643-5.
- 4 Tenney JH, Reller B, Mirrett S, Wang W, Weinstein MP. Controlled evaluation of the volume of blood cultured in detection of bacteremia and fungemia. *J Clin Microbiol* 1982; 15:558-61.
- 5 Neal PR, Kleiman MB, Reynolds JK, Allen SD, Lemons JA, Yu PL. Volume of blood submitted for culture from neonates. *J Clin Microbiol* 1986;24:353-6.
- 6 Kellogg JA, Manzella JP, McConville JH. Clinical laboratory comparison of the 10-ml isolator blood culture system with BACTEC radiometric blood culture media. *J Clin Microbiol* 1984;20:618-23.
- 7 Phillips SE, Bradley JS. Bacteremia detected by lysis direct plating in a neonatal intensive care unit. *J Clin Microbiol* 1990;28:1-4.
- 8 Kennaugh JK, Gregory WW, Powell KR, Hendley JO. The effect of dilution during culture on detection of low concentrations of bacteria in blood. *Pediatr Infect Dis* 1984;3:317-8.
- 9 Baumgart S, Hall SE, Campos JM, Polin RA. Sepsis with coagulase-negative staphylococci in critically ill newborns. *Am J Dis Child* 1983;137:461-3.