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Volume of Blood Submitted for Culture from Neonates

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We prospectively examined 298 sets (298 aerobic, 299 anaerobic, and 73 resin cultures) of blood cultures from 161 critically ill newborns. The attending physicians were unaware of the study. The mean blood volume per patient (aerobic and anaerobic) was 1.05 (range, 0.11 to 3.04) ml. The mean blood volume per aerobic bottle was 0.53 (range, 0.01 to 1.90) ml. Among aerobic samples 2.7% were ≤0.1 ml, 16% were ≤0.3 ml, 33% were ≤0.4 ml, and 55% were ≤0.5 ml. For anaerobic cultures the mean blood volume was 0.52 (range, 0.01 to 1.79) ml. Among anaerobic samples 2.7% were ≤0.1 ml, 15% were ≤0.3 ml, 35% were ≤0.4 ml, and 58% were ≤0.5 ml. Blood volume did not correlate with gestational age, chronologic age, or weight. The mean volume of blood submitted in positive cultures was not significantly greater than that in negative cultures. The blood volume used for culture from ill newborns may be inadequate for detecting sepsis, and the adequacy of currently available culture methods needs to be assessed for the small samples submitted from critically ill newborns.

Despite the uniform use of blood cultures for the diagnosis of sepsis, the optimal volume of blood required to detect bacteremia in newborns has not been established. We are aware of no systematic evaluation of the quantity of blood submitted for culture from newborns. Investigations using quantitative blood cultures for newborns with septicemia have stimulated questions concerning the sensitivity of cultures utilizing small blood volumes (3). Although dissimilar techniques of blood collection and methodology make it difficult to compare published reports, at least 1.0 ml of blood has been recommended for cultures of newborns (9, 10). However, in critically ill very low birth weight infants, volumes of 1 ml or more may be difficult to obtain. Furthermore, blood sampling for diagnostic studies may increase the need for blood transfusions. We prospectively and blindly evaluated the volume of blood submitted to the bacteriology laboratory for culture of critically ill newborns undergoing evaluation for sepsis. The purposes of the study were: (i) to examine the volume of blood routinely submitted for culture, (ii) to determine whether any clinical parameters affect the volume, and (iii) to investigate the relationship of volume to culture positivity.

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MATERIALS AND METHODS

BACTEC (Johnston Laboratories, Inc., Towson, Md.) 6B, 7D, and 16B blood culture bottles were weighed by one investigator (J.R.) using a Mettler top-loading tare balance. Weighed bottles were coded and exchanged for the existing supply in the neonatal intensive care units. The exchange was unrecognized by nursery staff, and all blood cultures obtained from March to June 1983 were submitted in weighed bottles. Blood was obtained by percutaneous venous or arterial puncture after skin preparation with 10% povidone–iodine followed by 70% isopropyl alcohol. Blood samples were also obtained from umbilical arterial catheters only at the time of insertion. Blood samples were divided among culture bottles by the nursery staff drawing the blood. Since the staff was unaware of the study, the order of filling and the volume distributed to individual bottles could not be evaluated. Bottles were reweighed when received by the laboratory, and the volume of blood inoculated in each bottle was determined by using the difference between pre- and postinoculation weights. Aerobic resin and regular aerobic bottles were evaluated by the BACTEC radiometric blood culture system. Isolation and identification of isolates were accomplished by using standard BACTEC radiometric methods (5). Aerobic resin and aerobic cultures were incubated on a rotator for 1 day at 35°C, examined twice a day on days 1 and 2 and once a day on days 3, 4, and 7. Anaerobic cultures were also incubated at 35°C and examined once a day on days 1, 2, 3, 4, and 7. Cultures were kept for 7 days. Blind subcultures were not done; however, if the radiometric growth index was positive, Gram stain, acridine orange stain, and subcultures were done.

Information recorded for each blood culture included the patient's chronologic and gestational ages, sex, weight, clinical findings leading to the evaluation, the presence of an umbilical arterial catheter, antibiotic therapy, and culture results. The blood sampling site was not always available. All bacterial isolates were considered significant except for *Staphylococcus epidermidis*, which was identified as a pathogen only if isolated from two or more blood cultures or if the attending physician elected to treat the patient with antibiotics.

Data were analyzed by using the *t* test for two independent variables or one-way analysis of variance for more than two groups. Computations were done with an SAS (SAS Institute Inc., Cary, N.C.) system.

RESULTS

Blood samples for culture were submitted from 105 patients once during their hospital course, twice from 24 patients, and three or more times from 32 patients. The mean weight of the patients was 2,052 (range, 580 to 5,500) g. The mean gestational age was 33 (range, 22 to 41) weeks, and the mean chronologic age was 23 (range, 0 to 142) days.

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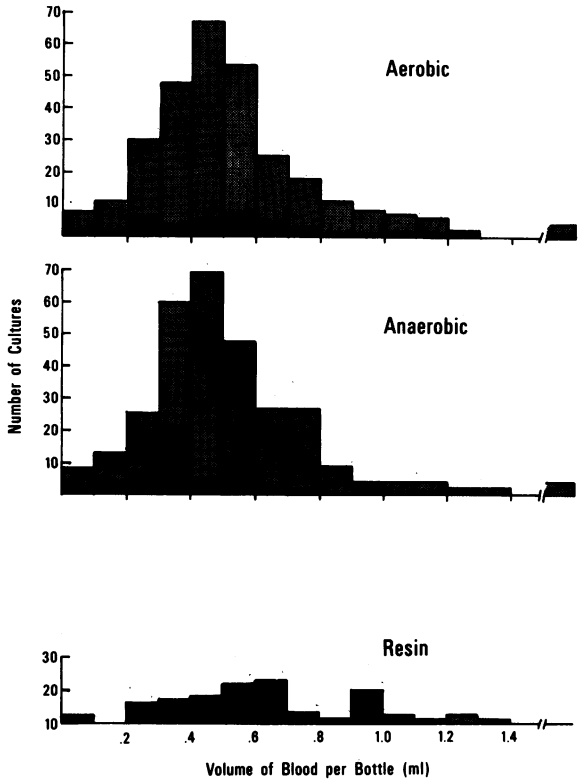

FIG. 1. Distribution of the volume of blood submitted in aerobic (n = 298), anaerobic (n = 299), and resin (n = 73) bottles for negative (shaded) and positive (solid) cultures.

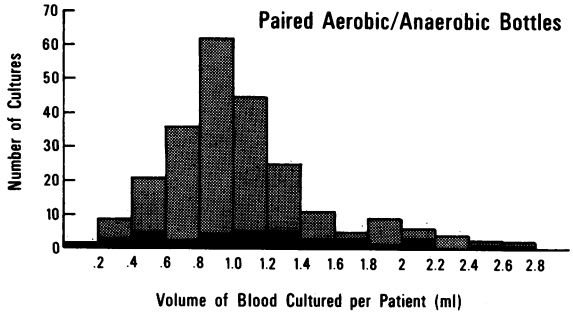

FIG. 2. Distribution of the total blood volume submitted for culture in 298 paired aerobic and anaerobic bottles. Cultures were considered positive if an isolate was recovered from one or both bottles of a pair (solid bars, positive cultures; shaded bars, negative cultures).

Overall, 298 aerobic, 299 anaerobic, and 73 resin bottle cultures were analyzed. The mean blood volume inoculated in aerobic bottles was 0.53 (standard deviation [S.D.], ± 0.27; range, 0.01 to 1.90) ml, and in anaerobic bottles it was 0.52 (S.D., ± 0.26; range, 0.01 to 1.79) ml. Among aerobic samples 2.7% were ≤0.1 ml, 16% were ≤0.3 ml, 33% were ≤0.4 ml, and 55% were ≤0.5 ml. Among anaerobic samples 2.7% were ≤0.1 ml, 15% were ≤0.3 ml, 35% were ≤0.4 ml, and 58% were ≤0.5 ml. The mean volume in aerobic resin bottles was 0.65 (S.D., ± 0.34; range, 0.03 to 2.29) ml. The mean volume obtained for paired aerobic and anaerobic cultures was 1.05 (S.D., ± 0.45; range, 0.11 to 3.04) ml (Fig. 1 and 2). In 65 cases, sets of aerobic, anaerobic, and resin bottles were submitted, and the mean total inoculum was 1.70 (S.D., ± 0.62; range, 0.38 to 4.03) ml divided among the three bottles. There was no statistical correlation between the volumes inoculated and patient weight or gestational or chronologic age (Table 1).

Organisms were isolated from 38 aerobic, 32 anaerobic, and 25 resin cultures obtained from 23 patients. Forty-eight percent of these patients were receiving antibiotics when blood was taken. Four of the patients died during acute illness. Twenty-five percent of all aerobic and anaerobic cultures and 63% of positive aerobic and anaerobic cultures were obtained from patients receiving antibiotics; blood samples for 25 resin cultures were taken from 11 patients. Blood samples for all 25 positive resin cultures were obtained while the patients were receiving antibiotics. Isolates included *S. epidermidis* (n = 16), *Candida albicans* (n = 8), *Escherichia coli* and *Staphylococcus aureus* (n = 7 each), *Pseudomonas aeruginosa* (n = 6), *Streptococcus faecalis* (n = 4), and *Serratia marcescens* (n = 3). No correlation was found between blood volume cultured and type of pathogen isolated except in the case of *C. albicans*. Cultures positive for *C. albicans* contained less blood than cultures containing other isolates ($P < 0.05$). We were unable to assess the significance of this finding. In patients for whom cultures were positive the mean weight was 1,584 g, the mean gestational age was 30 weeks, and the mean chronologic age was 43 days. Patients with positive cultures were significantly smaller ($P < 0.05$), gestationally less mature ($P < 0.05$), and chronologically older ($P < 0.05$) than patients with

TABLE 1. Correlation of gestational age, chronologic age, and body weight with volume of blood cultured

Parameter	Aerobic cultures				Anaerobic cultures				Resin cultures			
	No.	Mean ± S.D.	r[a]	P[b]	No.	Mean ± S.D.	r	P	No.	Mean ± S.D.	r	P
Gestational age (wk)												
Negative culture	258	33 ± 6	0.18	0.005	265	33 ± 6	0.20	<0.001	48	28 ± 29	0.05	NS
Positive culture	38	30 ± 5	0.03	NS	32	30 ± 5	0.02	NS	25	47 ± 35	0.20	NS
Chronologic age (days)												
Negative culture	260	19 ± 26	−0.12	0.04	267	21 ± 27	0.12	NS	48	33 ± 6	0.02	NS
Positive culture	38	45 ± 33	0.02	NS	32	33 ± 30	0.25	NS	25	29 ± 4	0.17	NS
Body wt (g)												
Negative culture	258	2,132 ± 1,165	0.20	<0.001	264	2,124 ± 1,158	0.20	<0.01	48	2,071 ± 1,208	0.12	NS
Positive culture	35	1,523 ± 846	0.07	NS	30	1,470 ± 841	0.01	NS	25	1,475 ± 740	0.08	NS

[a] r = Correlation coefficient.
[b] P = P value; $P > 0.05$ is not significant (NS).

TABLE 2. Comparison of positive and negative cultures: volume of blood cultured and clinical parameters

Culture type or parameter	Negative cultures		Positive cultures		P[a]
	No.	Mean ± S.D.	No.	Mean ± S.D.	
Aerobic (ml)[b]	260	0.52 ± 0.26	38	0.55 ± 0.34	NS
Anaerobic (ml)[c]	267	0.53 ± 0.26	32	0.41 ± 0.20	0.005
Resin (ml)[d]	48	0.68 ± 0.37	25	0.60 ± 0.27	NS
BV (ml)[e]	260	1.06 ± 0.44	37	0.96 ± 0.49	NS
TBV (ml)[f]	43	1.74 ± 0.63	22	1.63 ± 0.62	NS
Gestational age (wk)	264	33 ± 6	41	30 ± 5	0.002
Chronologic age (days)	264	20 ± 26	41	43 ± 32	<0.001
Body wt (g)	264	2,120 ± 1,162	38	1,584 ± 872	0.001

[a] $P > 0.05$ is not significant (NS).
[b] Volume of blood in aerobic culture bottle.
[c] Volume of blood in anaerobic culture bottle.
[d] Volume of blood in aerobic resin culture bottle.
[e] BV, Total blood volume in aerobic and anaerobic bottles.
[f] TBV, Total blood volume in aerobic, anaerobic, and aerobic resin bottles.

sterile blood cultures (Table 2). Among aerobic, anaerobic, and resin bottles evaluated individually, positive anaerobic cultures contained less blood than did negative anaerobic cultures ($P < 0.05$). There was no difference among the inoculated volumes of positive and negative aerobic, resin, and paired aerobic-anaerobic bottles (Table 2). Of 16 *S. epidermidis* isolates, 10 were considered pathogens by attending physicians and were treated with antibiotics. The frequency of isolation of *S. epidermidis* from the blood of newborns was similar to that observed in blood cultured from adults (4%). The volume of blood cultured and the clinical parameters of patients for whom cultures were negative, positive, or contaminated with *S. epidermidis* were not significantly different.

DISCUSSION

The sensitivity of blood culture methods is strongly influenced by the volume of blood cultured. Plorde et al. (8) found that the total isolate yield was predominantly a function of specimen volume in the BACTEC system. Knudson and Alden (6) and Mangurten and LeBeau (7) found comparable recovery rates in heelstick- and venipuncture-derived blood samples from neonates. Knudson and Alden (6) hypothesized, but presented no evidence, that peripheral pooling increased the concentration of bacteria in the 0.2-ml heelstick samples utilized in their study. Sixty-six percent of the patients studied by Mangurten and LeBeau died of sepsis. Since the mortality rate of septic newborns with greater than 1,000 CFU of *E. coli* per ml has been shown by others to be 73% (3), the high sensitivity of the 0.02-ml microheelstick method used by Mangurten and LeBeau may have been due to a high concentration of bacteria in the samples studied (7). Dietzman et al. (3) evaluated 35 quantitative cultures obtained from 30 patients with *E. coli* sepsis. Blood samples from infants suspected of having septicemia were evluated with agar pour plates and standard broth culture methods. Eleven blood samples contained more than 1,000 CFU/ml; five had 50 to 1,000 CFU/ml; 11 had 5 to 49 CFU/ml, and eight had 0 to 4 CFU/ml (3). Data from Dietzman et al. make it apparent that 23% of episodes may not have been detected by using a 0.2-ml sample; 54% may have been missed with a 0.02-ml sample. Szymczak et al. (10) evaluated 7,768 blood cultures from children; they performed quantitative cultures and inoculated two 50-ml aerobic and anaerobic bottles. Bottles inoculated with ≥1 ml of blood showed growth earlier than bottles inoculated with <1 ml. The concentration of organisms in blood was as low as 0.5 CFU/ml with a mean of 51 CFU/ml (10). Hall et al. (4) compared rates of recovery of bacteria from blood of adults by using 50- and 100-ml bottles of soybean-casein digest broth inoculated with 5- or 10-ml blood samples. It was concluded that optimally 20, but at least 10, ml of blood should be divided and inoculated into two 100-ml culture bottles. It was suggested that a smaller but unspecified volume be used for infants and children; however, no data were presented to support this recommendation. Newer culture techniques, such as the pediatric isolator, which requires a 0.5 to 1.5-ml blood sample, are undergoing evaluation for use in children. Several investigators have reported more rapid detection and isolation (1, 2, 11) as well as quantitation of pathogens with this method. However, a 2.5- to 5-fold increased rate of isolation of organisms considered to be contaminants was also reported (2, 11). We are not aware of any culture method which has been demonstrated to be superior to that used in our study for detecting bacteremia in neonates.

In the present blind study, the volume of blood obtained for culture in critically ill newborns was often considerably smaller than could be expected to detect all episodes of clinically significant bacteremia. Using the present methods, we could not demonstrate a correlation between volume cultured and culture positivity. We conclude that there is a need (i) to determine the concentration of organisms found in the blood of low-birth-weight newborns with clinically significant bacteremia, (ii) to examine present blood culture methods using the small volumes often submitted for critically ill newborns, and (iii) to establish the minimum volume of blood which should be cultured to ensure adequate sensitivity for detecting significant bacteremia in newborns.

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