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Controlled Evaluation of the Volume of Blood Cultured in Detection of Bacteremia and Fungemia

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To evaluate the role of the volume of blood cultured in the detection of clinically important bacteremia and fungemia in adults, we evaluated the yield and speed of detection of microorganisms from 5,317 paired 2- and 5-ml samples of blood. The same kind of medium (supplemented peptone broth with 0.03% sodium polyanetholsulfonate) and atmosphere of incubation (open venting units) were used for all blood cultures. Only adequately filled (≥80% of stated volume) sets (20-ml tube and 50-ml bottle) were compared statistically. Significantly more bacteria ($P < 0.01$), Pseudomonas spp. in particular ($P < 0.05$), were isolated from the 5-ml samples of blood. We conclude that the volume of blood cultured is a critical factor in the detection of septicemia. Consequently, valid evaluation of other factors influencing the detection of septicemia must be based on comparisons in which equal volumes of blood are cultured.

The isolation of bacteria or fungi from the blood of seriously ill patients has great diagnostic and prognostic importance (16). For the detection of bacteremia or fungemia, however, at least one viable microorganism must be present in the sample of blood cultured. Commercially available blood culture systems are designed for 2- to 10-ml samples of blood. The effect of the volume of blood cultured on the detection of septicemia has rarely been tested independently (7) without the confounding variables of medium and method of detection (8, 12). Therefore, we studied the role of volume of blood cultured in the detection of clinically important bacteremia and fungemia in adults. The medium, atmosphere of incubation, ratio of blood to broth, and processing methods were identical for all blood cultures; only the volume of blood sampled differed.

MATERIALS AND METHODS

Collection of samples. For a 12-month period, supplemented peptone broth (SPB) with 0.03% sodium polyanetholsulfonate (SPS) in 20-ml tubes and 50-ml bottles (Becton Dickinson VACUTAINER Systems, Rutherford, N.J.) was used for all blood cultures at the University of Colorado Hospital and the Denver Veterans Administration Medical Center. House staff and medical students obtained the cultures at the bedsides of the patients after preparation of the skin with a 10% povidone-iodine solution (1% available iodine) and then 70% isopropyl alcohol. Blood from each venipuncture was distributed by needle and syringe as follows: 2 ml of blood was added to a tube with 18 ml of SPB, and 5 ml of blood was added to a bottle with 45 ml of SPB. Thus, the 1:10 ratio of blood to broth was the same for both blood culture containers (1). Both tubes and bottles were under vacuum and had been evacuated and back flushed with 10% carbon dioxide in nitrogen at stoppering in the manufacturing process.

Volume standards. To ensure that the culture tubes and bottles actually received the specified amounts of blood, we measured the level of fluid in each container before and after it was filled with blood. Tubes or bottles inadequately filled with SPB were not released for use on the wards. Although all blood-containing tubes and bottles were incubated, those with fluid levels below the standards were computer coded as inadequate and were excluded from the subsequent analyses. Fluid level standards were set to ensure that at least 1.6 and 4 ml of blood were added to each 20-ml tube and 50-ml bottle, respectively.

Processing of samples. Identical methods were used for processing the blood cultures in the clinical microbiology laboratories at both hospitals. Each 20-ml tube and 50-ml bottle was incubated aerobically in a CO_2 incubator at 35°C with the same unplugged venting units.

Cultures were examined for macroscopic growth twice daily for 7 days and then reincubated for an additional 7 days. Routinely, all blood cultures were Gram stained and subcultured on chocolate agar in 5%

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TABLE 1. Comparison of yields of clinically important bacteria and fungi isolated from 2-ml (20-ml tube) and 5-ml (50-ml bottle) samples of blood cultured in SPB

Type of microorganism	No. of isolates from:			P value[a]
	Both 2- and 5-ml samples	2-ml sample only	5-ml sample only	
Aerobic and facultative bacteria	228	47	70	<0.05
Gram positive				
Staphylococci	51	6	7	NS
Streptococci	85	11	20	NS
Other	3	3	2	NS
Gram negative				
E. coli	52	11	15	NS
Other *Enterobacteriaceae*	20	14	12	NS
Pseudomonadaceae	14	2	11	<0.05
Other	3	0	3	NS
Anaerobic bacteria	21	10	19	NS
Gram positive	6	2	9	NS
Gram negative	15	8	10	NS
All bacteria	249	57	89	<0.01
Fungi	23	16	11	NS

[a] NS, Not significant ($P \geq 0.05$). P value for all bacteria, <0.01.

carbon dioxide and on 5% sheep blood agar enriched with vitamin K_1 and hemin in anaerobic GasPak jars (BBL Microbiology Systems, Cockeysville, Md.) at 1, 7, and 14 days and at the first sign of possible growth, such as turbidity, hemolysis, or gas production. Routine subcultures were done regardless of whether the blood culture was previously positive. Plates were incubated at 35°C for a total of 3 days (aerobic) or 5 days (anaerobic). All microorganisms isolated were identified by standard procedures (9).

Clinical assessment. Clinical records of all patients with positive blood cultures were reviewed by specialists in infectious diseases, and, after a thorough evaluation of all available data, each organism from a positive culture was designated as representing true bacteremia, true fungemia, or a contaminant (10). Criteria for the decision included pre- and posttreatment clinical course and findings, evidence for a source of sepsis, and multiple positive cultures which yielded the same microorganism(s).

Analysis of data. Paired comparisons of adequately filled bottles were made for all microorganisms causing septicemia. Significance testing was done with the modified chi-square test described by McNemar (11). Confidence limits for the binomial distribution (6) were used to determine the probability that the number of microorganisms isolated only in a 20-ml tube in relation to the number isolated only in a 50-ml bottle was consistent with the hypothesis that the volume of blood sampled was the only difference between the two culture containers.

RESULTS

A total of 6,782 blood culture sets were obtained during the study: 5,317 (78.4%) sets with both the 20-ml tubes and 50-ml bottles adequately filled; 571 (8.4%) sets, each having one or both containers inadequately filled; and 894 (13.2%) sets, each having one container missing. Of the 5,317 adequate blood culture sets, 498 (9.4%) were positive, including 358 (6.7%) that grew microorganisms causing illness, 125 (2.4%) that grew contaminants, and 15 (0.3%) that grew at least one pathogen and one contaminant.

A total of 445 microorganisms associated with sepsis were isolated from 5,317 adequately filled pairs of 20-ml tubes and 50-ml bottles (Table 1). Both the 2- and 5-ml samples of blood enabled the detection of 272 (61.1%) of the 445 clinically important microorganisms. Pseudomonads grew more frequently ($P < 0.05$) from the bottles with the larger volume of blood sampled (5 ml). Overall, more bacteria were isolated from bottles with 5 ml of blood than from tubes with 2 ml of blood ($P < 0.01$). The improvement in yield with the increasing volumes of blood cultured is shown graphically for all organisms in Fig. 1. The yield when 5 ml of blood was cultured was 8% greater than when 2 ml of blood was cultured (2.6% increase per ml of blood sampled). If the total blood sample was considered, the yield when 7 ml of blood was cultured was 29% greater than when 2 ml was cultured (5.8% increase per ml of blood sampled).

Although the 20-ml tubes of SPB did not

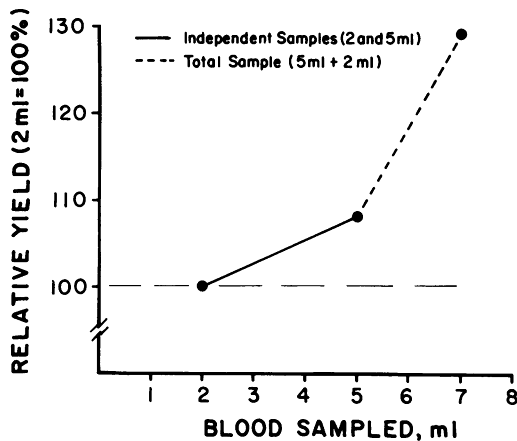

FIG. 1. Yield of organisms causing sepsis, as related to volume of blood cultured.

significantly favor the isolation of any category of microorganisms, the tubes outperformed the 50-ml bottles in two specific ways: the yield of microorganisms per milliliter of blood cultured and the speed of detection of *Escherichia coli*. When the number of organisms isolated only from the tubes was compared with the number of organisms isolated only from the bottles, it was found that the tubes yielded significantly more bacteria (28.5 versus 17.8; $P < 0.01$) and fungi (8 versus 2.2; $P < 0.01$) per milliliter of blood cultured. This finding is reflected in the nonlinear, concave-upward, schematic representation of yield (Fig. 1). Even when microorganisms were isolated from both culture units, more *E. coli* isolates ($P < 0.05$) grew at least 24 h earlier in the 20-ml tubes of SPB than in the 50-ml bottles (Table 2). Of the 10 *E. coli* isolates that grew faster in the 20-ml tubes, 8 were from two patients; of these 8, 6 were from a single patient with an intraabdominal abscess that seeded the bloodstream over a period of weeks.

DISCUSSION

We have shown that significantly more microorganisms were isolated when 5 ml of blood was cultured in SPB with SPS than when 2 ml was cultured. Hall and co-workers have reported similar findings for 5- and 10-ml samples of blood cultured in casein-soybean digest broth with SPS (7). Based on these studies, the value of sampling additional blood over the range of 2 to 15 ml is substantial. We found that the microorganism yield of 7-ml samples was 29% greater than that of 2-ml samples (Fig. 1), and the Mayo Clinic (Rochester, Minn.) data suggest that the yield of 15-ml samples is 25% greater than that of 5-ml samples (15). In fact, the volume of blood cultured appears to be relatively more important than the medium or atmosphere of incubation in the detection of sepsis (15).

Since we controlled variables other than the amount of blood sampled, we did not expect the

TABLE 2. Comparison of speed of detection of clinically important bacteria and fungi isolated from 2-ml (20-ml tube) and 5-ml (50-ml bottle) samples of blood cultured in SPB

Type of microorganism	No. of isolates from: 2- and 5-ml samples positive at same time	2-ml sample positive ≥1 day earlier	5-ml sample positive ≥1 day earlier	*P* value[a]
Aerobic and facultative bacteria	183	26	19	NS
Gram positive				
Staphylococci	39	7	5	NS
Streptococci	71	6	8	NS
Other	3	0	0	NS
Gram negative				
E. coli	40	10	2	<0.05
Other *Enterobacteriaceae*	19	1	0	NS
Pseudomonadaceae	10	1	3	NS
Other	1	1	1	NS
Anaerobic bacteria	9	4	8	NS
Gram positive	2	1	3	NS
Gram negative	7	3	5	NS
All bacteria	192	30	27	NS
Fungi	12	7	4	NS

[a] NS, Not significant ($P \geq 0.05$). P value for all bacteria, NS.

20-ml tubes to perform more favorably than could be explained by volume alone. The relative advantage of the tube was most marked for fungi, which are known to prefer an aerobic environment (13). It is possible that the location of the unplugged venting unit (closer to the broth surface in the tube than in the bottle) provided better aeration in the 20-ml tubes of SPB, which has a low Eh potential. This hypothesis, however, does not explain why the general yield of bacteria of the 20-ml tubes was relatively greater than that of the 50-ml bottles. Since 8 of the 10 *E. coli* isolates detected earlier in the tubes came from two patients, this result may be related to specific strains of bacteria.

What should be the volume of blood cultured, if more is generally better? The amount for children logically must be different from that for adults, but data are not available for definitive answers. There are practical constraints. Pediatricians, particularly neonatologists, are unable to sample large volumes of blood. Nosocomial anemia is also a reality in adults (4). Although cultures of less than 1 ml of blood will enable the detection of bacteremia when the concentrations of microorganisms are sufficiently high (2, 3, 5), Szymczak et al. showed that a more rapid detection of clinically significant organisms is achieved when ≥ 1 ml of blood from children is cultured (14). For adults, a 2-ml sample of blood will not enable the detection of some bacteremias (Table 1; Fig. 1); 10 ml of blood per culture appears to be a reasonable lower limit, based on data from the Mayo Clinic (7, 15). The value of culturing even larger volumes of blood from adults remains to be established in a controlled trial.

Three conclusions are possible from this study. First, the volume of blood cultured is a critical factor in the detection of bacteremia. Second, future studies of the effect of volume should include an analysis to determine if variables other than volume are involved. Third, conclusions about other factors, such as medium, atmosphere of incubation, and ratio of blood to broth, in the detection of bacteremia and fungemia are valid only if equal volumes of blood are cultured in controlled comparisons.

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