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Relevance of the Number of Positive Bottles in Determining Clinical Significance of Coagulase-Negative Staphylococci in Blood Cultures

STANLEY MIRRETT,[1,2]* MELVIN P. WEINSTEIN,[3,4,5] LARRY G. REIMER,[6] MICHAEL L. WILSON,[1,2]† AND L. BARTH RELLER[1,2,7]

Clinical Microbiology Laboratory, Duke University Medical Center,[1] and Departments of Pathology[2] and Medicine,[7] Duke University School of Medicine, Durham, North Carolina 27710; Microbiology Laboratory, Robert Wood Johnson University Hospital,[3] and Departments of Medicine[4] and Pathology,[5] University of Medicine and Dentistry of New Jersey—Robert Wood Johnson Medical School, New Brunswick, New Jersey 08901; and Department of Veterans Affairs Medical Center, Salt Lake City, Utah 84148[6]

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Coagulase-negative staphylococci (CNS) are the most commonly isolated contaminants from blood cultures, yet they frequently cause true infections. Determining the clinical significance of CNS is difficult, and clinicians often consider the number of positive bottles within a set of blood culture bottles in their assessment. Therefore, in three separate studies, we counted the number of positive bottles within blood culture sets comprising two, three, or four bottles in order to predict whether or not CNS were clinically significant isolates (CSI) in adult patients with suspected sepsis. Each culture was evaluated by independent, published clinical criteria to determine its clinical importance. Of 486 positive sets that included two adequately filled bottles, 127 (26%) CNS were CSI, 329 (67%) were contaminants, and 30 (6%) were indeterminate as a cause of sepsis. Among CSI, 39 and 61% were isolated from one and two bottles, respectively. The positive predictive value for sepsis was 18% when one bottle was positive and 37% when both bottles were positive. Of 235 positive sets that included three adequately filled bottles, 81 (34%) were CSI, 109 (46%) were contaminants, and 45 (19%) were indeterminate as a cause of sepsis. Of CSI, 43, 38, and 19% were found in one, two, and three bottles, respectively. The positive predictive value for sepsis was 28, 52, and 30% when one, two and three bottles were positive. Of 303 positive blood culture sets that included four adequately filled bottles, 64 (21%) were considered CSI, 197 (65%) were contaminants, and 42 (14%) were indeterminate as a cause of sepsis. Of CSI, 27, 28, 19, and 27% were found in one, two, three, and four bottles, respectively. The positive predictive value for sepsis was 11, 30, 34, and 37% when one, two, three, and four bottles were positive. We conclude that the number of culture bottles positive in a given culture set cannot reliably predict the clinical significance of the CNS isolated and, therefore, should not be used as a criterion for determining whether or not an isolate represents true infection or contamination.

Coagulase-negative staphylococci (CNS) are the most commonly isolated contaminants from blood cultures (12). Contaminated blood cultures result in increased lengths of stay and higher pharmacy, laboratory, and total costs (1). Determining the clinical significance of CNS is difficult, and some clinicians and microbiologists consider the number of positive bottles within a blood culture set in their assessment (4, 6–8). Our laboratories have accumulated data on CNS while participating in several multicenter, controlled clinical evaluations of blood culture media and systems (9, 10, 13). In this report, we review the data from these studies to determine whether the number of positive bottles within a blood culture set was useful for determining whether or not CNS were clinically significant isolates (CSI).

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* Corresponding author. Mailing address: Clinical Microbiology Laboratory, Duke University Medical Center, Box 2902, Durham, NC 27710. Phone: (919) 684-2562. Fax: (919) 684-8519. E-mail: stanley.mirrett@duke.edu.

† Present address: Department of Pathology and Laboratory Services, Denver Health Medical Center, Denver, CO 80204.

MATERIALS AND METHODS

Blood culture collection. All blood cultures were collected by the house staff as part of routine care of adult patients suspected of sepsis. Skin was prepared with 10% povidone-iodine and alcohol, and blood was withdrawn using sterile needles and syringes (i.e., collection devices were not used). There was no designation to differentiate blood samples obtained by peripheral venipuncture from those that were drawn through lines. Three different evaluations of blood culture media were included in the data analysis. Specimens of blood were inoculated into various blood culture media depending on which study was being performed. Study 1 (two bottles) consisted of a BacT/ALERT standard aerobic and anaerobic blood culture bottle, each inoculated with 5 ml of blood, and a BacT/ALERT standard aerobic bottle inoculated with 10 ml of blood Organon Teknika Corporation, Durham, N.C.) (10). This latter bottle was not used in the data analysis, since it was inoculated with a different volume of blood from the other bottles. Study 2 (three bottles) consisted of BACTEC aerobic (Plus 26) and anaerobic (Plus 27) resin bottles and a Septi-Chek (BD Biosciences, Sparks, Md.) aerobic bottle. In this study, the three bottles were each inoculated with 10 ml of blood (9). Study 3 (four bottles) consisted of BACTEC standard aerobic (NR 6A) and anaerobic (NR 7A) bottles (BD Biosciences) and BacT/ALERT aerobic and anaerobic standard bottles (Organon Teknika Corporation, Durham, N.C.). In this study, the four bottles were each inoculated with 5 ml of blood (13).

Data analysis. Only adequately filled (80 to 120% of stated blood volume) bottles in culture sets were included in the analysis. Each isolate was reviewed by an infectious disease consultant or fellow who used published criteria (11) to determine whether the isolate was clinically important or indeterminate as a

TABLE 1. CNS isolated from various blood culture studies

Type of blood culture set	Total no. of sets	Total no. of adequate sets	No. of positive cultures (%) for:			Total no. of cultures
			Sepsis	Contaminants	Indeterminate result	
2-bottle set (study 1) (BacT/ALERT Standard O_2/AnO_2)[a]	17,120	12,956	127 (26)	329 (67)	30 (6)	486
3-bottle set (study 2) (BACTEC Resin O_2/AnO_2 and Septi-Chek O_2)	11,393	5,043	81 (34)	109 (46)	45 (19)	235
4-bottle set (study 3) (BACTEC and BacT/ALERT Standard O_2/AnO_2)	7,925	5,389	64 (21)	197 (65)	42 (14)	303
Total for all sets	36,438	23,387	272 (27)	635 (62)	117 (11)	1,024

[a] O_2, aerobic bottle; AnO_2, anaerobic bottle.

cause of sepsis or was a contaminant. The study data were analyzed by comparing the proportion of bottles positive in two-, three-, and four-bottle sets for isolates judged to be contaminants versus those assessed to be causes of sepsis.

RESULTS

Of 36,438 blood culture sets received during the three studies, there were 23,387 adequately filled blood culture sets consisting of two, three, or four bottles. Of 1,024 blood cultures with CNS, 272 (27%) were clinically significant, 635 (62%) were judged contaminants, and 117 (11%) could not be categorized with confidence (Table 1). CNS that were judged to be the cause of sepsis represented 21 to 34% of the isolates depending on the specific study; 46 to 67% of CNS were contaminants. The studies that incorporated the BACTEC resin media had the highest percentage of isolates judged to represent sepsis.

Table 2 shows a summary of 486 two-bottle sets received during the study period. The 127 isolates of clinically significant CNS were found more often in both bottles (61%) of the set than in a single bottle (39%). The positive predictive value for sepsis, however, of both bottles being positive was only 37% and overlapped appreciably with the 18% positive predictive value of a single positive bottle. Conversely, the 76% probability of a single positive bottle containing a contaminant versus the 58% probability even with two positive bottles rendered the difference meaningless for clinical use.

Of 235 three-bottle sets positive with at least one bottle being positive, paradoxically more isolates of CNS were deemed to cause sepsis in sets with only a single positive bottle (43%) than in those with two (38%) or three (19%) positive bottles. Moreover, the positive predictive values of only 28, 52, and 30% for one, two, and three positive bottles, respectively, made this information useless for determination of sepsis in an individual patient (Table 3). As with the two-bottle study, the patterns of positivity (one versus two and three bottles) for contaminants showed extensive overlap (51, 33, and 50% positive, respectively).

Of 303 four-bottle sets, the number of positive bottles with CNS causing sepsis was 27, 28, 19, and 27% for one, two, three, and four positive bottles, respectively. The positive predictive values of 11, 30, 34, and 37% for one, two, three, and four positive bottles (Table 4) would not be helpful in making a judgment on the likelihood of sepsis. Of 46 isolates detected in all four bottles, 20 (43%) were judged to be contaminants versus 17 (49%) detected in three, 33 (55%) in two, and 127 (78%) in one of the bottles. As with the two- and three-bottle studies, the patterns of positivity (one versus two or three or four positive bottles) for contaminants showed extensive overlap (78, 55, 49, or 43% positive, respectively).

DISCUSSION

Clinicians and some microbiologists traditionally have assumed that growth of a microorganism in both blood culture bottles of a conventional culture set supports the clinical significance of the isolate. The basis for this assumption is that there are many microorganisms per milliliter of blood with true bacteremia, whereas only a few microorganisms per milliliter are present in contaminated blood cultures (5). Thus, the presence of many CFU per milliliter increases the likelihood that microorganisms will be isolated from more than one blood culture bottle. Conversely, the presence of fewer CFU per milliliter increases the likelihood of contamination being the cause of the positive blood culture (6). Because of this assumption, when reporting positive blood cultures to the clinician, the clinical microbiologist frequently is asked how many bottles are positive. Because they rarely are contaminants (11), isolation of common pathogens such as *Escherichia coli, Staphylococcus aureus*, and *Klebsiella pneumoniae* seldom elicits this question. This is not the case, however, with growth of CNS.

The issue is, therefore, how useful is this information for assessing an individual patient. Several studies have shown that in many adults with true bacteremia, the number of microorganisms present in blood is small, often less than 1 CFU/ml regardless of the pathogen (2, 3). If the number of bottles positive in a culture set could reliably predict clinical signifi-

TABLE 2. CNS isolated from two-bottle blood culture sets

Clinical significance	No. of cultures (%) positive in:		Total no. of cultures
	1 bottle	2 bottles	
Sepsis	49 (18)	78 (37)	127
Contaminant	207 (76)	122 (58)	329
Indeterminate	18 (7)	12 (6)	30

TABLE 3. CNS isolated from three-bottle blood culture sets

Clinical significance	No. of cultures (%) positive in:			Total no. of cultures
	1 bottle	2 bottles	3 bottles	
Sepsis	35 (28)	31 (52)	15 (30)	81
Contaminant	64 (51)	20 (33)	25 (50)	109
Indeterminate	26 (21)	9 (15)	10 (20)	45

TABLE 4. CNS isolated from four-bottle blood culture sets

Clinical significance	No. of cultures (%) positive in:				Total no. of cultures
	1 bottle	2 bottles	3 bottles	4 bottles	
Sepsis	17 (11)	18 (30)	12 (34)	17 (37)	64
Contaminant	127 (78)	33 (55)	17 (49)	20 (43)	197
Indeterminate	18 (11)	9 (15)	6 (17)	9 (20)	42

cance, the clinical laboratory could report this information routinely. Therefore, in this study we analyzed data from previous blood culture evaluations performed by our group to determine whether the number of bottles positive within a blood culture set could in fact predict whether the CNS isolate was clinically significant.

If an increasing number of positive bottles correlated well with the probability that the positive culture set denoted sepsis rather than contamination, one might expect that the most definitive answer would come from our analysis of four-bottle sets. Our data showed that all four bottles were positive in only 37% of sets judged to represent sepsis owing to CNS. Similar results were obtained in a smaller study of 147 blood culture sets reported by Peacock et al. (6), in which only 27% of the septic patients had four bottles positive with CNS. However, 10% of our four-bottle sets judged to be contaminants also had all four bottles positive, and 43% of all sets with four positive bottles were contaminants. Thus, the probability of contamination remains even if all four bottles filled from a single venipuncture grow CNS.

Not surprisingly, analysis of two- or three-bottle blood culture sets similarly failed to differentiate CSI from contaminants. For the most common situation, in which two blood culture bottles constitute a blood culture set, it is true that CSI are more commonly found in both bottles (61%) than in a single bottle (39%). However, the positive predictive value for CSI when both bottles grew CNS was only 37 versus 18% when one bottle grew CNS. These low positive predictive values for CSI are not sufficient for clinical application.

The data that we report occurred during studies of different formulations of blood culture media from two different commercial manufacturers. Moreover, other investigators have reported similar observations (6). Thus, we believe that our findings are not unique to one medium or system but rather constitute a fundamental truth regarding interpretation of positive blood cultures. The number of culture bottles positive in a given culture set cannot reliably predict the likelihood of clinical significance of the CNS isolated and, therefore, should not be used as a criterion for determining whether or not an isolate represents true infection versus contamination.

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