GASTROENTEROLOGY 1988;95:1351–5

Optimization of Ascitic Fluid Culture Technique

BRUCE A. RUNYON, HANNA N. CANAWATI, and EVANGELOS A. AKRIVIADIS
Liver Unit and Department of Pathology, University of Southern California School of Medicine, Los Angeles, California

The conventional method of ascitic fluid culture detects bacteria in only 42%–65% of patients who have neutrocytic ascites and suspected spontaneous bacterial peritonitis. In this study ascitic fluid was cultured by the conventional method as well as by a new method consisting of bedside inoculation of blood culture bottles with ascites. The conventional cultures grew bacteria in only 13 (43%) of 30 episodes of neutrocytic ascites, whereas the blood culture bottles grew bacteria in 28 (93%); this difference was significant ($p < 0.0001$). The blood culture bottle method also resulted in more rapid detection of bacterial growth. The median concentration of bacteria in infected ascites was one organism per milliliter. Bedside inoculation of blood culture bottles with ascitic fluid is more sensitive than the conventional method in detecting bacterial peritonitis. The insensitivity of the conventional method is probably due to the low concentration of bacteria in infected ascites and the small volume of ascites cultured by this method.

Spontaneous bacterial peritonitis (SBP) has been defined as an ascitic fluid infection in which (a) the ascitic fluid culture grows bacteria, (b) the ascitic fluid neutrophil count is ≥250 cells/mm^3, and (c) there is no intraabdominal source of infection (1,2). Patients suspected of having SBP (based on signs, symptoms, and/or abnormal ascitic fluid neutrophil count) but whose ascitic fluid cultures grew no bacteria were excluded from the papers that originally described spontaneous peritonitis (3). However, subsequent reports have demonstrated that 35%–58% of these patients with suspected ascitic fluid infection have culture-negative ascites; this condition has been labeled culture-negative neutrocytic ascites (CNNA) (4,5). The signs, symptoms, and mortality of patients with CNNA are similar to those of culture-positive spontaneous peritonitis (4). Also, cultures of the blood of 33%–57% of patients with CNNA grow bacteria, confirming systemic bacterial infection (4,5). Finally, patients who survive SBP or CNNA frequently develop the other condition (4).

It has been suggested that the explanation of the culture-negativity of many episodes of suspected peritonitis may be simply the insensitivity of the "conventional" method of ascitic fluid culture (4). In fact, one study that used historical controls and another study that is published in French (and that used concurrent controls) both have demonstrated that inoculation of blood culture bottles with ascitic fluid at the bedside markedly improves the sensitivity of ascitic fluid culture to 91%–92.5% (5,6). However, because the reference source of the American Society for Microbiology recommends the conventional method, most microbiology laboratories continue to use this method, which detects bacterial growth in only approximately half of suspected peritonitis cases (7). In two large centers in the United States where spontaneous peritonitis research is being performed, the microbiology laboratories converted to the blood culture bottle method many years ago because of dissatisfaction with the routine method; however, no attention was drawn to the change in culture technique (1,8). Other hospitals have modified the routine method, e.g., by culturing the centrifuged pellet of a large aliquot of ascites, in an attempt to improve its sensitivity; whether this sort of modification is efficacious is unknown. In hospitals where infected ascites is uncommon, the insensitivity of the conventional method may be unrecognized.

The goals of this study were (a) to compare the sensitivity of the conventional method (and modifications of it) to the blood culture bottle method, (b)

Abbreviations used in this paper: CNNA, culture-negative neutrocytic ascites; SBP, spontaneous bacterial peritonitis.

0016-5085/88/$3.50

to determine the optimal volume of ascites that should be inoculated into blood culture bottles, and (c) to perform quantitative cultures of ascites.

Materials and Methods

Patients hospitalized with ascites at the University of Southern California Liver Unit at Rancho Los Amigos Medical Center between July 1986 and July 1987 were candidates for participation in this study. Paracentesis was performed if the patient developed signs or symptoms of peritonitis, i.e., fever, abdominal pain, or encephalopathy.

Conventional culture consisted of immediate transportation of the ascitic fluid specimen in a sealed syringe to the laboratory where (a) three drops were cultured on each of three plates (chocolate agar, blood agar, and MacConkey's agar) and (b) 1 ml of fluid was placed into a 10-ml tube containing 5 ml of brain-heart infusion broth. The chocolate and blood agar plates were incubated in a CO_2 atmosphere at 35°C; the broth and enteric plate were incubated in ambient air at 35°C. Two modifications of the conventional method were tested. One modification of the routine method consisted of culture of a larger aliquot of ascites (2 ml) into brain-heart infusion broth with incubation as above. The other modification involved (a) centrifugation (1000 g for 15 min) of 50 ml of ascites, (b) discarding the majority of the supernatant, (c) resuspending the pellet with enough residual supernatant to equal a final volume of 1 ml, and (d) culture of this sediment onto chocolate agar with incubation as above. All plates were inspected twice daily for 2 days and any colonies were Gram stained; if there was no growth after 48 h, the plates were discarded. The broth was inspected twice daily for 2 days, daily for 5 more days, and then discarded. Routine Gram stains and subcultures were performed on the broth at 24 and 48 h and after 7 days. Turbidity resulted in additional stains and subcultures.

The blood culture bottle method consisted of bedside inoculation of 10 ml of ascites into one 100-ml Difco (Difco Laboratories, Detroit, Mich.) tryptic soy broth bottle and one 100-ml Difco Thiol bottle (both with sodium polyanethol sulfonate and CO_2). Only the tryptic soy broth bottle was vented. These were inspected twice daily for 2 days and then daily for 5 more days. Routine Gram stains and subcultures were performed at 24 and 48 h, and after 7 days. Turbidity resulted in additional stains and subcultures. The blood culture bottles were incubated at 35°C.

To determine the optimal quantity of ascites that should be cultured in blood culture bottles, 1-, 2-, 5-, and 20-ml aliquots were also inoculated at the bedside into each bottle of pairs of tryptic soy and Thiol bottles as above. Therefore, five pairs of blood culture bottles were inoculated, including the 10-ml set.

Quantitative cultures were performed by culturing, on chocolate agar plates, 1 ml of vortexed uncentrifuged ascites as well as 1 ml of three serial log dilutions (in nonbacteriostatic normal saline) of ascites. Colonies were counted after 48 h of incubation so that a calculation of the concentration of bacteria in the undiluted ascites could be performed.

Diphtheroids, *Bacillus* species, and *Staphylococcus epidermidis* were considered contaminants. Identification of organisms and susceptibility testing were performed by routine methods (9). Twenty milliliters of blood (10 ml into each of the two bottles) was also cultured at the time of the paracentesis. In addition, Gram stains were performed on uncentrifuged fresh ascitic fluid and on the centrifuged sediment of 50 ml of ascites.

Spontaneous bacterial peritonitis was defined as above. Culture-negative neutrocytic ascites was diagnosed when (a) the ascitic fluid culture (of any type) grew no bacteria, (b) the ascitic fluid neutrophil count was $\geq$500 cells/mm^3, (c) there was no intraabdominal source of infection, (d) there was no antibiotic treatment within 7 days, and (e) there was no alternative explanation for an elevated ascitic fluid neutrophil count, e.g., pancreatitis, tuberculosis, peritoneal carcinomatosis, or hemorrhage into ascites (4,10). Secondary bacterial peritonitis was diagnosed when (a) the ascitic fluid culture grew bacteria, (b) the ascitic fluid neutrophil count was $\geq$250 cells/mm^3, and (c) there was an intraabdominal source of infection (1,11).

Patients were excluded from the study (a) if an inadequate quantity (<150 ml) of ascitic fluid was obtained or (b) if any antibiotic was given within 7 days of the paracentesis.

This study was approved by the Institutional Review Board of Rancho Los Amigos Medical Center. Patients gave written informed consent before entry into the study. The χ^2 test (with Yates' correction) and Fisher's exact test were used for statistical analysis. A p value <0.05 was considered significant.

Results

Thirty-one episodes of neutrocytic ascites in 26 patients met study inclusion criteria. One patient (4% of the group) was documented to have secondary bacterial peritonitis (perforated gastric ulcer) involving *Escherichia coli* and group D streptococcus; all of the ascites cultures, including all conventional culture methods and all blood culture bottles, grew bacteria. The remainder of the patients had no evident intraabdominal source for their infection; therefore, they were classified as having either SBP or CNNA. The remainder of the discussion involves only the 30 episodes of SBP and CNNA that occurred in 25 patients.

The blood culture bottles containing 10 ml of ascitic fluid grew bacteria in 28 (93%) of the 30 episodes of neutrocytic ascites, whereas the conventional cultures of ascites were positive in only 13 (43%) of 30 episodes (Table 1); this difference was statistically significant ($p < 0.0001$). In only two episodes were no cultures positive, i.e., there were only two episodes of CNNA. There were no instances in which the conventional cultures grew bacteria when the blood culture bottles (containing 10 ml of ascitic fluid) did not. The flora of the ascitic

Table 1. Comparison of the Conventional Method of Ascitic Fluid Culture (and Its Modifications) to the Blood Culture Bottle Method (Using 10 ml of Inoculum)

Culture method	Episodes of bacterial growth: Positive	Episodes of bacterial growth: Negative	Total episodes	Positive (%)	Difference (%)
Conventional[a]	13	17	30	43	50
Blood culture bottle[a]	28	2	30	93	
Conventional[b] (plus two modifications)	17	13	30	57	36
Blood culture bottle[b]	28	2	30	93	

[a] $\chi^2 = 17.33$, $p < 0.0001$, significant; Yates' correction = 17.04, $p < 0.0001$, significant; Fisher exact test (two-tail), $p < 0.0001$, significant. [b] $\chi^2 = 10.76$, $p < 0.001$, significant; Yates' correction = 8.89, $p < 0.01$, significant; Fisher exact test (two-tail), $p < 0.01$, significant.

fluid cultures are listed in Table 2. All cultures yielded pure growth of a single organism.

Of the 28 episodes in which the 10-ml "set" of bottles demonstrated bacterial growth, both bottles were positive in 13 (46%) of the episodes, the Thiol bottle alone was positive in 13 episodes, and the tryptic soy bottle alone was positive in only two (7%) episodes. Therefore, the sensitivity of the single 10-ml Thiol bottle (26 of 28, 93%) was greater than that of the single 10-ml tryptic soy bottle (15 of 28, 54%); this difference was significant ($p < 0.01$).

Cultures of the centrifuged sediment of 50 ml of ascites grew bacteria in only 10 (33%) of 30 episodes, including one episode in which the conventional method demonstrated no growth. Therefore, this modification of the conventional method appeared to be less sensitive than the conventional method in detecting bacterial growth; however, this difference, 33% vs. 43%, did not reach statistical significance. Culture of the sediment, however, was significantly less sensitive than the blood culture bottle method ($p < 0.0001$). The modification of the conventional method in which 2 ml of ascites was inoculated into brain-heart infusion broth grew bacteria in 16 (53%) of 30 episodes. Overall, in 17 (57%) of 30 episodes of neutrocytic ascites, the conventional method or a modification of it demonstrated bacterial growth. However, this degree of sensitivity (57%) remained significantly less than that of the blood culture bottle method (93%) ($p < 0.01$) (Table 1). The blood culture bottle method also resulted in more rapid detection of bacterial growth compared with the conventional method (Table 3).

The inoculation of increasing volumes of ascites into blood culture bottles demonstrated that the 10- and 20-ml inoculum sizes were equivalent in terms of overall sensitivity (both 93%) and were both statistically significantly more sensitive than the 1-, 2-, or 5-ml inoculum sizes (Table 4).

Table 2. Ascitic Fluid Flora of 28 Culture-Positive Episodes

Organism	No. of episodes (%)
Escherichia coli	12 (43)
Streptococcus pneumoniae	7 (25)
Klebsiella pneumoniae	3 (11)
Enterobacter cloacae	3 (11)
Streptococcus viridans group	1 (3)
Group A streptococcus	1 (3)
Citrobacter freundii	1 (3)

The concentration of bacteria in infected ascites ranged from 920,000 organisms per milliliter to one organism per 10 ml (as determined by culture-positivity of the 10-ml blood culture bottle with culture-negativity of smaller volume inocula). The median bacterial concentration was one organism per milliliter. Eighteen (64%) of the 28 culture-positive episodes involved a bacterial concentration of <10 organisms per milliliter, and 15 (53.5%) involved ≤1 organism per milliliter.

Of the 28 culture-positive episodes of ascitic fluid infection, 12 (43%) were associated with bacteremia involving the same organism. Gram stains of uncentrifuged and centrifuged sediment of ascites demonstrated bacteria in only 7% and 10%, respectively.

Discussion

This study demonstrates that inoculation at the bedside of 10 ml of ascites into each of two blood culture bottles is more sensitive than the conven-

Table 3. Rapidity of Detection of Bacterial Growth

Culture method	Episodes: Positive	Episodes: Negative	Total episodes	Positive (%)	Difference (%)
12 hours					
Conventional[a]	10	20	30	33	30
Blood culture bottle (10 ml)[a]	19	11	30	63	
24 hours					
Conventional[b]	13	17	30	43	44
Blood culture bottle (10 ml)[b]	26	4	30	87	

[a] $\chi^2 = 5.41$, $p < 0.05$, significant; Yates' correction = 4.27, $p < 0.05$, signficiant; Fisher exact test (two-tail), $p < 0.05$, significant. [b] $\chi^2 = 12.38$, $p < 0.0001$, significant; Yates' correction = 10.55, $p < 0.0001$, significant; Fisher exact test (two-tail), $p < 0.001$, significant.

Table 4. Comparison of Volume of Inoculum of Ascites Versus Detection of Bacterial Growth in Blood Culture Bottles

Volume of inoculum (per bottle)	Episodes: Positive	Episodes: Negative	Total episodes	Positive (%)	Difference (%)
1 ml[a]	16	14	30	53	40
10 ml[a]	28	2	30	93	
2 ml[b]	20	10	30	67	26
10 ml[b]	28	2	30	93	
5 ml[c]	21	9	30	70	23
10 ml[c]	28	2	30	93	
10 ml[d]	28	2	30	93	0
20 ml[d]	28	2	30	93	

[a] $\chi^2 = 12.27$, $p < 0.0001$, significant; Yates' correction = 10.31, $p < 0.001$, significant; Fisher exact test (two-tail), $p < 0.001$, significant. [b] $\chi^2 = 6.67$, $p < 0.01$, significant; Yates' correction = 5.10, $p < 0.05$, significant; Fisher exact test (two-tail), $p < 0.05$, significant. [c] $\chi^2 = 5.46$, $p < 0.05$, significant; Yates' correction = 4.01, $p < 0.05$, significant; Fisher exact test (two-tail), $p < 0.05$, significant. [d] $\chi^2 = 0$, $p < 1.0$, not significant; Yates' correction = 0, $p < 1.0$, not significant; Fisher exact test (two-tail), $p < 1.0$, not significant.

tional method in detecting bacterial peritonitis (93% vs. 43%, respectively; $p < 0.0001$). The blood culture bottle method was also found to more rapidly detect bacterial growth. Ten-milliliter and 20-ml inoculum volumes were superior to smaller volumes in detecting bacteria. Thiol media proved to be superior to tryptic soy broth in detecting bacterial peritonitis ($p < 0.01$). The median concentration of bacteria in "spontaneously" infected ascites was only one organism per milliliter. Fifty-seven percent of culture-positive ascites contained ≤2 organisms per milliliter. The insensitivity of the conventional method is, at least in part, explained by the low concentration of bacteria in infected ascites and the small volume of ascites (<2 ml) cultured by this method.

Two modifications of the conventional method could not be demonstrated to improve its sensitivity to a statistically significant degree. In fact, the modification that involved culture of the centrifuged sediment of 50 ml of ascites was slightly less sensitive than the conventional method in detecting bacterial growth (33% vs. 43%, respectively). Methods that would concentrate bacteria would be expected to improve the detection of bacterial growth in infections involving small numbers of organisms. However, centrifugation of the bacteria into the sediment, which also contains viable phagocytes (neutrophils), could result in a reduction in the numbers of viable bacteria because of engulfment and killing of microbes by phagocytes.

Two episodes (7%) of the 30 involved no positive cultures of any type. Both episodes occurred in 1 patient who also developed one episode of *E. coli* SBP between his two CNNA events. The explanation of the inability of the cultures to detect bacterial growth in two of three events in this 1 patient is not entirely clear. However, in one of these two episodes, the ascitic fluid neutrophil count was spontaneously decreasing (from 2123 to 1690 cells/mm^3) before antibiotic treatment was initiated. Perhaps episodes of CNNA that are diagnosed using sensitive culture methods are in fact episodes of SBP that are in the resolving phase, and the bacteria are no longer viable.

Body fluid infections that involve large concentrations of bacteria, e.g., infected urine or cerebrospinal fluid, can be cultured successfully using small quantities of fluid (12,13). Bacteremia involves small concentrations of bacteria (14). Blood culture bottles were devised to detect growth of bacteria when only one viable organism is present in the entire inoculum (15). By the inclusion of nutrients and sodium polyanethol sulfonate (an anticoagulant and opsonin inhibitor), blood culture media were formulated to support bacterial growth and prevent further killing of bacteria. It has been shown that the sensitivity of blood culture bottles in detecting bacteremia is largely dependent on the volume of blood cultured (16). Apparently those who recommend the conventional culture method of ascitic fluid (as well as pericardial, joint, and pleural fluid) have assumed that large numbers of bacteria are present in infected fluid and recommend culture of small volumes of fluid, similar to the methods used for culture of urine and cerebrospinal fluid (7). Also the assumption has been that ascitic fluid infection is often polymicrobial; therefore, selective agar media that suppress the growth of certain bacteria have been recommended (7). This study demonstrates (a) that ascitic fluid infection is usually spontaneous (30 of 31 episodes), (b) that SBP is nearly always monomicrobial (100% of the SBP episodes in this series), and (c) that SBP usually involves very low concentrations of bacteria. In this setting the blood culture bottle method would be expected to be superior to a method that cultures small volumes of fluid on media that can inhibit growth of bacteria.

In contrast to the conventional method, blood culture bottles provide a nutritious environment that also contains an anticoagulant and opsonin inhibitor that protect the bacteria from further complement or phagocyte-mediated killing, or both. Bacteria would be expected to grow rapidly in such an environment even if the cultures were delayed in reaching the incubators of the laboratory. Also, most commercially available blood culture bottles contain 50–100

ml of media and therefore permit culture of 5–20 ml of fluid—large enough volumes to result in detection of bacterial growth in most cases.

Bacterial peritonitis has become a major complication of continuous ambulatory peritoneal dialysis. Initially culture-negative peritonitis was a common problem, but more recently studies have demonstrated that (a) the concentration of bacteria in the infected fluid may be less than one organism per milliliter and (b) inoculation of blood culture bottles with fluid optimizes detection of bacterial growth (17–21). Infected peritoneal dialysate appears to be very comparable to spontaneous bacterial peritonitis. Both forms of peritonitis involve small numbers of bacteria. Cultures of large volumes of fluid are required to detect the offending organism.

Culture of ascitic fluid in blood culture bottles is superior to conventional techniques in detecting spontaneous bacterial peritonitis and should replace the conventional method as the preferred method of culture.

References

1. Hoefs JC, Canwati HN, Sapico FL, Hopkins R, Weiner J, Montgomerie JZ. Spontaneous bacterial peritonitis. Hepatology 1982;2:399–407.
2. Hoefs JC, Runyon BA. Spontaneous bacterial peritonitis. DM 1985;31:1–48.
3. Correia JP, Conn HO. Spontaneous bacterial peritonitis in cirrhosis: endemic or epidemic? Med Clin North Am 1975;59: 963–81.
4. Runyon BA, Hoefs JC. Culture-negative neutrocytic ascites: a variant of spontaneous bacterial peritonitis. Hepatology 1984; 4:1209–11.
5. Runyon BA, Umland ET, Merlin T. Inoculation of blood culture bottles with ascitic fluid: improved detection of spontaneous bacterial peritonitis. Arch Intern Med 1987;147:73–5.
6. Kammerer J, Dupeyron C, Vuillemin N, Leluan G, Fouet P. Apport des examens cytologiques et bacteriologiques du liquide d'ascite cirrhotique au diagnostic de peritonite bacterienne: a propos de 610 prelevements chez 156 malades. Med Chir Dig 1982;11:243–51.
7. Isenberg HD, Schoenknecht FD, Von Graevenitz A. In: Rubin SJ, coordinating ed. Cumitech 9: collection and processing of bacteriological specimens. Washington, D.C.: American Society of Microbiology, 1979:1–22.
8. Garcia-Tsao G, Conn HO, Lerner E. The diagnosis of bacterial peritonitis: comparison of pH, lactate concentration and leukocyte count. Hepatology 1985;5:91–6.
9. Lennette EH. Manual of clinical microbiology. 4th ed. Washington, D.C.: American Society for Microbiology, 1985.
10. Yang C-Y, Liaw Y-F, Chu C-M, Sheen I-S. White count, pH and lactate in ascites in the diagnosis of spontaneous bacterial peritonitis. Hepatology 1985;5:85–90.
11. Runyon BA, Hoefs JC. Ascitic fluid analysis in the differentiation of spontaneous bacterial peritonitis from gastrointestinal tract perforation into ascitic fluid. Hepatology 1984;4: 447–50.
12. Barry AL, Smith PB, Turck M. In: Gavan TL, coordinating ed. Cumitech 2: laboratory diagnosis of urinary tract infections. Washington, D.C.: American Society of Microbiology, 1975: 1–8.
13. Feldman WE. Concentrations of bacteria in cerebrospinal fluid of patients with bacterial miningitis. J Pediatr 1976;88: 549–52.
14. Weiss H, Ottenberg R. Relation between bacteria and temperature in subacute bacterial endocarditis. J Infect Dis 1932;50: 61–8.
15. Reller LB, Murray PR, Mac Lowry JD. In: Washington JA II, coordinating ed. Cumitech 1A: blood cultures II. Washington, D.C.: American Society of Microbiology, 1982:1–11.
16. Hall MM, Ilstrup DM, Washington JA II. Effect of volume of blood cultured on detection of bacteremia. J Clin Microbiol 1976;3:643–5.
17. Bint AJ, Finch RG, Gokal R, Goldsmith HJ, Junor B, Oliver D. Diagnosis and management of peritonitis in continuous ambulatory peritoneal dialysis: report of a working party of the British Society for Antimicrobial Chemotherapy. Lancet 1987;i:845–8.
18. Luce E, Nakagawa D, Lovell J, Davis J, Stinebaugh BJ, Suki WN. Improvement in the bacteriologic diagnosis of peritonitis with use of blood culture media. Trans Am Soc Artif Intern Organs 1982;28:259–61.
19. Hachler H, Vogt K, Binswanger V, von Graevenitz A. Centrifugation of 50 ml of peritoneal fluid is sufficient for microbiological examination in continuous ambulatory peritoneal dialysis patients with peritonitis. Infection 1986;14:102–4.
20. Cooper GL, White JA, D'Elia JA, et al. Lack of utility of routine screening tests for early detection of peritonitis in patients requiring intermittent peritoneal dialysis. Infect Control 1984;5:321–5.
21. Vas SI. Microbiological diagnostic approach to peritonitis in CAPD patients. In: Atkins RC, Thomson NP, Farrell PC, eds. Peritoneal dialysis. New York: Churchill Livingstone, 1981.

Received February 22, 1988. Accepted May 23, 1988.

Address requests for reprints to: B. A. Runyon, M.D., University of Southern California Liver Unit, Building 500, Room 114, Rancho Los Amigos Hospital, 7601 East Imperial Highway, Downey, California 90242.

This work was presented at the 88th Annual Meeting of the American Society for Microbiology, May 9, 1988, in Miami Beach, Florida.

This study was supported in part by a grant from the American College of Gastroenterology.