

Improved Method for Bacteriological Diagnosis of Spontaneous Bacterial Peritonitis

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The definitive diagnosis of spontaneous bacterial peritonitis is made by a positive ascitic fluid culture. Causative organisms cannot be isolated in up to 65% of patients with well-defined spontaneous bacterial peritonitis, probably due to inadequate ascites culture techniques. We prospectively compared two ascites culture methods: conventional (on chocolate agar and thioglycolate broth) and modified (inoculation of 10 ml of ascites in a tryptic soy broth blood culture bottle at the patient's bedside). In a 10-month period, 31 cirrhotic patients met our diagnostic criteria for spontaneous bacterial peritonitis; both culture methods were performed on their ascitic fluid. The conventional method grew bacteria in only 16 of the 31 episodes (52%), whereas the modified method grew bacteria in 25 (81%), a significantly higher sensitivity ($P < 0.05$). The modified method also shortened significantly the time for detection of bacterial growth. We conclude that ascites inoculation into a blood culture bottle at the patient's bedside should be the routine method for ascites culture.

Spontaneous bacterial peritonitis (SBP) is a serious complication of cirrhosis with ascites. It consists of the infection of ascitic fluid without any apparent extra- or intra-abdominal foci of infection. Its prevalence has been reported to vary from 8 to 25% in cirrhotic patients with ascites, with a mortality ranging from 57 to 95% (1-3, 8). In our institution, SBP occurs in 24 to 30% of cirrhotic patients with ascites with an early mortality of 53% (G. Garcia-Tsao and R. Gomez-Arnau, *Rev. Gastroenterol. Mex.* 52:294, 1987). The definitive diagnosis of SBP is made by a positive ascitic fluid culture. However, a group of patients has been identified in whom clinical findings are those of SBP, including a high leukocyte count in ascites, but whose ascitic fluid cultures fail to yield bacteria (2, 9, 11). These false-negative cases have been reported in up to 65% of patients (13) and are probably due to inadequate ascites culture techniques.

In a study that used a historical cohort (13), Runyon et al. reported that direct inoculation of ascites into blood culture bottles was a more sensitive method for detecting SBP than the conventional method. Runyon et al. recently confirmed these findings in a study with concurrent controls (10).

The purpose of our study was to prospectively compare the routine method of ascites culture used in our hospital, in which a small volume of ascites is inoculated into thioglycolate broth and on chocolate agar (conventional method), with the inoculation of a larger volume of ascites into a blood culture bottle at the patient's bedside (modified method). We found a significantly higher sensitivity for the modified method (81%) than for the conventional method (52%).

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MATERIALS AND METHODS

Since December 1986, all cirrhotic patients with ascites admitted to the Instituto Nacional de la Nutricion Salvador Zubiran have undergone routine paracentesis. Patients who

develop signs or symptoms of SBP during hospitalization also undergo paracentesis.

During the period from March to December 1987, two ascites culture methods were performed on all ascitic fluid samples obtained under sterile conditions by paracentesis.

(i) In the conventional method (5, 14), a sample of ascitic fluid was sent to the Clinical Microbiology Laboratory; 5 ml was centrifuged at 29,400 × g for 10 min, and the sediment was inoculated on enriched chocolate agar (1 drop) and into 8 ml of thioglycolate broth (2 to 4 drops). The chocolate agar plates were incubated in a candle jar at 35°C for 48 h. The broth was incubated under anaerobic conditions at 35°C for 7 days. Blind subcultures on sheep blood agar and MacConkey agar (incubated in aerobic conditions) and in phenylethyl alcohol agar (incubated in anaerobic conditions) were performed at 48, 72, and 168 h. Both plates and broth were examined daily for visible growth. When turbidity was detected, additional subcultures were performed.

(ii) In the modified method, 10 ml of ascites fluid was inoculated in one 100-ml tryptic soy broth culture bottle (containing 0.05% sodium polyanethol sulfonate) at the patient's bedside and was transported to the Microbiology Laboratory, where it was incubated at 35°C for 7 days, with blind subcultures on sheep blood agar and MacConkey agar (incubated in aerobic conditions) and in phenylethyl alcohol agar (incubated in anaerobic conditions) at 24, 48, and 168 h. Bottles were examined daily for visible growth. When turbidity was detected additional subcultures were performed.

The times at which cultures became positive were recorded, and organisms were identified by standard methods (6, 7).

Ascitic fluid total leukocyte count was performed in all samples by diluting 0.5 µl of the sample with Turk solution in a Thoma pipette. After vortexing for 60 s, the fifth drop was placed in a Neubauer chamber, where leukocytes were counted under a microscope (×40). The total count was calculated according to the formula (counted leukocytes × 10 × 20)/4.

The diagnosis of SBP was established according to the following criteria: (i) clinical signs and symptoms compatible with SBP (abdominal pain and/or fever); (ii) ascitic fluid

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TABLE 1. Comparison of two ascites culture methods ($n = 31$)

Culture result	No (%) of results by:	
	Conventional method	Modified method
Positive	16 (52)	25 (81) ^a
Negative	15 (48)	6 (19)

^a $P < 0.05$ compared with the conventional method.

polymorphonuclear leukocyte count greater than $500/\text{mm}^3$; and (iii) absence of clinical, laboratory, and radiological data suggestive of secondary peritonitis. In cases with culture-negative SBP the existence of pancreatitis, cholecystitis, peritoneal carcinomatosis, peritoneal tuberculosis, and hemorrhage into ascites could be excluded.

Statistical analyses were performed by using the McNemar chi-square test.

RESULTS

During this 10-month study, 31 episodes of SBP were diagnosed with our criteria in 31 cirrhotic patients with ascites.

Of the 31 patients with SBP, ascites culture was positive by both methods in 14, in 11 cases culture was positive only by the modified method, 2 cultures were positive only by the conventional method, and 4 cases were culture negative by both methods.

Therefore, of the 31 cases of SBP a microorganism was isolated more frequently with the modified method (25 of 31, 81%) than with the conventional method (16 of 31, 52%). This difference in sensitivity was statistically significant ($P = 0.026$) (Table 1).

Of the four cases that were negative by both methods, the etiologic diagnosis was confirmed in two by positive blood cultures (*Klebsiella pneumoniae* and *Enterobacter cloacae*, respectively), and in the remaining two cases an etiologic diagnosis could not be established; however, their therapeutic response to broad-spectrum antibiotics was diagnostic.

The modified method of ascites culture also shortened the time for detection of bacterial growth (Table 2). Whereas only nine cultures by the conventional method were positive within 24 h, 19 cultures by the modified method demonstrated growth within 24 h. This difference was statistically significant ($P < 0.005$). None of the cultures that were negative at 48 h showed detectable bacterial growth after 7 days of observation.

Of 27 culture-positive SBP episodes by either method, *Escherichia coli* was the most frequently isolated microorganism (Table 3). Of 11 ascites cultures that were positive only with the modified method, 7 episodes corresponded to *E. coli*, 2 corresponded to coagulase-negative staphylococci, 1 corresponded to *Enterococcus faecalis*, and 1 corre-

TABLE 2. Time interval to detection of bacterial growth

Culture result	No. (%) of results by:	
	Conventional method	Modified method
Positive in <24 h	9 (29)	19 (61) ^a
Positive after >24 h	7 (23)	6 (19)
Negative	15 (48)	6 (19)

^a $P < 0.005$ compared with conventional method.

TABLE 3. Microorganisms isolated by two ascites culture methods

Microorganism	No. of isolates		
	Conventional and modified	Conventional alone	Modified alone
<i>Escherichia coli</i>	10	1	7
<i>Klebsiella pneumoniae</i>	2		
Coagulase-negative staphylococci		1	2
<i>Pseudomonas aeruginosa</i>	1		
<i>Streptococcus faecalis</i>			1
<i>Serratia marcescens</i>	1		
Gram-negative rod			1

sponded to a gram-negative rod. Of the two cases that were positive only with the conventional method, one episode was due to *E. coli* and the other was due to a coagulase-negative staphylococcus (Table 3).

Blood cultures were obtained in 13 patients and were positive in 9 (69%); in seven cases, the same microorganism was isolated in blood as in ascites (in four cases the organism was cultured with both methods, in two cases the organisms were only cultured with the modified method, and in one case the organisms had grown only with the conventional method), and in the two remaining cases the organism was only isolated in blood.

DISCUSSION

This study confirms that the modified method of ascites culture, consisting in the inoculation of 10 ml of ascitic fluid into a blood culture bottle at the patient's bedside, has a significantly higher sensitivity for the diagnosis of SBP than the method used routinely at our hospital (81 versus 52%, $P < 0.05$). Our routine method of ascites culture has a widespread use and has been recommended by several other institutions (5, 14).

Our results are in accordance with those of Runyon et al. (13), who found, in ascites samples obtained from different patients at different time periods, that the inoculation in blood culture bottles improved sensitivity from 42 to 91%. They are also in accordance with a more recent prospective and controlled study by the same authors (10) in which the conventional method (or a modification of it) had a sensitivity of 57% compared with 93% for the blood culture bottle method.

As proposed by these authors, the higher sensitivity obtained with this modified method may be due to the fact that SBP is an infection that involves a low concentration of bacteria (4, 12), and thus by culturing larger amounts of ascites the yield of the method is higher; in fact, they were able to demonstrate that the inoculation of 10 or 20 ml was more sensitive than inoculum sizes of 1, 2, or 5 ml (10).

The presence of antiphagocytic and anticomplementary activity of the anticoagulant sodium polyanethol sulfonate, in the blood culture bottles may also account for a higher bacterial yield, as has been shown to occur with blood cultures (14).

In addition to the higher sensitivity of the modified method, organisms grown in blood culture bottles were obtained earlier (<24 h) than with the conventional method. This is an important finding, because the prompt administration of specific antimicrobial therapy is the goal of SBP treatment, as it is for treatment of any other severe infection.

Contrary to what would be expected, we did not find an increase in sensitivity with longer periods of observation; however, this may be due to our relatively small number of cases, and we still consider that ascites cultures should be incubated for 7 days like blood cultures.

As for the culture medium contained in the bottles, Runyon et al. (10) found a higher sensitivity for the bottle containing Thiol (93%) than for the bottle containing tryptic soy broth (54%). In our study we only used the 100-ml tryptic soy broth bottle, with which we had an 81% sensitivity, comparable to that of Runyon with the Thiol bottle. We consider that this difference is not due to medium composition in itself but to differences in the microaerophilic environment, since their tryptic soy broth bottle was vented and neither our tryptic soy bottle nor their Thiol bottle was vented.

Overall, 4 of our 31 patients (13%) had false-negative ascites cultures, but 2 of them had positive blood cultures. When only the modified method is considered, 6 of the 31 patients had false-negative ascites cultures and 3 of them had positive blood cultures. These findings are consistent with reports in the literature in which simultaneously taken blood cultures are positive in 54% of patients with SBP (12) and in one-third of patients with culture-negative SBP (11).

We conclude that the modified culture method, which consists of inoculating 10 ml of ascitic fluid into a blood culture bottle at the patient's bedside, should be used routinely for ascites culture of cirrhotic patients and that, in cases in which SBP is suspected, blood cultures should be performed simultaneously.

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