SPONTANEOUS BACTERIAL PERITONITIS

pathogenesis, diagnosis and management

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PATHOGENESIS, DIAGNOSIS AND MANAGEMENT

SPONTANE BACTERIELE PERITONITIS

PATHOGENESE, DIAGNOSTIEK EN BELEID

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	Prof. Dr. S.W. Schalm
	Prof. Dr. H.A. Verbrugh

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'Het hart heeft zijn redenen die de rede niet kent'

Blaise Pascal, natuurkundige, (1623-1662).

Voor Drieka

Johanna en Jacob

Marien en Simon

Table of contents

CHAPTER 1

1.1 Introduction

On the 26 of March AD 1827 Ludwig van Beethoven died. The cause of Beethoven's death, liver failure due to cirrhosis, was confirmed by autopsy. From 1821 Beethoven had suffered regular attacks of jaundice and slowly progressive hepatic failure. In November 1826 he suffered from pneumonia. This infection probably triggered the terminal phase of Beethoven's illness. He was treated with bed rest and his health gradually improved. However on the seventh day of his illness he developed vomiting and diarrhoea. The jaundice deepened and his abdomen became distended due to the accumulation of fluid. He became oliguric and developed paroxysmal nocturnal dyspnoea. In the third week a paracentesis was carried out, draining 11 litres of straw coloured fluid. Three subsequent paracentesis were performed, the second yielding 22 litres of fluid. However the paracentesis wound did not close, and continuously leaked ascitic fluid. Over the next three months, Beethoven slowly deteriorated. He became increasingly anorectic and emaciated and died in coma on the 26 of March 1827 at the age of 57 years. At the autopsy it was noted that the abdominal cavity contained 8 litres of greyish-brown turbid fluid, representing infected ascites (1).

Although medical science made revolutionary progress during the last 150 years, a medical history similar to the above mentioned is familiar to many physicians even today. Although diuretics and antibiotics relieve some of the symptoms described above, end-stage liver disease remains one of the most difficult entities to treat. Spontaneous bacterial peritonitis was recognized as a specific entity in the early 1960s and clusters of cases were published from Paris (2), London (3) and West Haven, Connecticut (4). The first patients published by Harold O. Conn presented a discrete homogeneous syndrome. Each patient had been admitted to hospital with decompensated alcoholic cirrhosis and ascites. After one week in hospital they developed abdominal pain, fever and encephalopathy. Paracentesis showed cloudy ascitic fluid that contained large numbers of polymorphonuclear leukocytes and coliform bacteria were cultured from the ascitic fluid. All patients died (4).

Since then the spectrum of disease has broadened considerably. Initially it was almost exclusively described in alcoholic cirrhosis, but now it has been recognized in all types of

cirrhosis, but very rarely in non-cirrhotic ascites. Clinical signs and symptoms may be absent and deterioration of laboratory values may be the only sign that an underlying bacterial infection of ascitic fluid is present. At first it was thought to be a nosocomial infection with a prevalence of 10-25% of all patients with cirrhosis and ascites admitted to a hospital (5,6). However prospective studies learned that the infection is often community-acquired, with a 12% prevalence in patients with cirrhosis and ascites seeking admission for various reasons (7). Due to increased awareness of the disease, earlier diagnosis and better treatment, in-hospital mortality rates have improved during the last 20 years from an initial mortality rate of 95% to about 40% in more recent series (8-13). A high recurrence rate after a first episode of spontaneous bacterial peritonitis and progression of the underlying liver disease results in a poor 1-year survival of about 20-30% (9,11).

1.2 Definitions

Spontaneous bacterial peritonitis (SBP) is defined as the bacterial infection of the ascitic fluid without any identifiable intra-abdominal source of the infection (14-16). The most widely accepted diagnostic criteria of SBP include:

1) Increased polymorphonuclear leucocyte (PMN) count in the ascitic fluid. A cut-off level above 0.25×10^9 cells/l is most frequently used.

2) A positive ascitic fluid culture.

"Spontaneous" infections are distinquished from secondary bacterial peritonitis in that spontaneous infections do not have a surgically-treatable source. Although many patients with SBP have a focus of infection, e.g. urinary tract infection or pneumonia, they are diagnosed as having SBP.

There are two variants of SBP that require further clarification:

Culture-negative neutrocytic ascites, in which the the PMN count of ascitic fluid is elevated, but cultures remain negative. Obviously the culture technique determines which percentage of suspected episiodes of SBP will remain culture-negative. Cut-off levels for the PMN counts in the ascites are above 0.25 or 0.50×10^9 cells/l. Although there have been some discussions in the literature suggesting that culture-negative neutrocytic ascites is a less severe variant of SBP, usually they are regarded as similar diseases with identical prognosis (17-19).

Monobacter ascites, or monomicrobial nonneutrocytic bacterascites, is a controversial

entity and defined as a positive ascitic fluid culture with a single organism (excluding *Staph. epidermidis*) with an ascitic fluid PMN count $<0.25 \times 10^9$ cells/l (20,21). This transient residence of bacteria in the ascitic fluid without neutrocytic response may progress to real SBP, sometimes in a few hours time, or may resolve without treatment (7,20,21). Antibiotics are not routinely recommended for this subgroup of patients, but close monitoring of symptoms and early retapping of ascites is advisable.

1.3 Pathogenesis

H.O. Conn, who first used the term SBP (4), thought that bacteremia-induced infection of preexistent ascites was the most likely way of developing SBP. Another possibility he raised was the direct transmural migration from the gut to the peritoneal cavity, and he also suggested that paracentesis by itself could lead to SBP. Over the ensuing years the pathogenesis of spontaneous ascitic fluid infections has become a bit clearer.

It is possible that enteric bacteria regularly will enter the ascitic fluid, but that in the majority of cases the bacteria are cleared by local host defense mechanisms (7,20,21). SBP will occur if there is an increase of invading bacteria or impaired host defenses.

Assuming that the normally present equilibrium between host-environment-microorganism becomes disturbed, it seems worthwhile to have a look at the pathogenesis of SBP using this model.

Host factors; Most patients who develop SBP have severe liver disease with diminished function of the hepatic reticuloendothelial system (RES) and increased porto-systemic shunting. These changes increase the chance that viable bacteria will not be cleared from the blood stream. Rimola et al. (22) using an elimination constant for ^{99m}technetium-sulfur colloid as a marker of RES phagocytic activity in the liver demonstrated a consistently lower elimination rate in patients with cirrhosis and bacteremia occurred more frequently in the patients with the lowest elimination rates. Other deficits in cirrhosis, not limited to the portal circulation, include decreased intracellular killing properties, impairment of neutrophil chemotaxis, reduced serum complement levels and reduced opsonization unrelated to hypocomplementemia (23,24).

Factors present in ascites; Recent studies (25-28) have shown that patients who develop SBP have low chemoattractant and opsonic activities of the ascitic fluid, thus indicating that the ascitic fluid itself plays an important role in preventing colonization by pathogenic bacteria. Mal et al. (25) studied prospectively a number of patients with ascites due to

different etiologies and found that diminished complement C3 levels in ascites had an independent predictive value for the occurrence of SBP, which correlated with impaired chemoattractant and opsonic activity. This was confirmed in a number of other studies (26,27). By establishing a correlation between the total protein content and C3 concentration in the ascitic fluid, a number of studies found the total protein content of ascitic fluid to be a good parameter for predicting which patients are most at risk to develop SBP (28,29).

In summary, ascitic fluid opsonic activity correlates with ascitic fluid C3 concentration and total protein concentration. Patients whose ascitic fluid is dilute and deficient in these complement factors are predisposed to ascitic fluid infection. Runyon (28) found that patients with an ascitic fluid protein concentration < 10g/l. were ten times more likely to develop SBP during hospitalization than patients with higher protein ascitic fluid levels. This does not however completely explain why some patients are more susceptible than others. A very interesting French study (30) described an improved PMN function within cirrhotic ascitic fluid after serial dilutions or when adding ascitic fluid from malignant peritonitis. This suggests the presence of a suppressive factor in cirrhotic fluid which suppresses PMN function.

The bacteriological flora, responsible for the spontaneous infections of the ascitic fluid is mainly gut derived (5,8,31-35). Gram-negative aerobic rods such as *E. coli* and *Klebsiella pneumoniae* are responsible for about two-third of all cases of SBP. If organisms could easily traverse the gut wall and directly enter the ascitic fluid, polymicrobial infections would be the rule and not the exception. Additionally, the aerobic Gram-negative microorganisms in the gut are outnumbered by 2-4 orders of magnitude by anaerobes and enterococci (14), which seldom cause SBP. Apparently there is some kind of a "filter" between the gut lumen and the fluid.

Studies in rodents have demonstrated that under certain circumstances bacteria can "translocate" from the gut lumen across the mucosa into submucosal lymphatics and be detected in mesenteric lymph nodes (36). From the mesenteric lymph nodes bacteria may spread to the spleen, liver, or bloodstream. Interestingly, it has been confirmed that Gram-negative rods translocate in greatest numbers through the bowel wall, Gram-positive cocci moderately and anaerobes seldom (37). Circumstances which promote translocation include (1) bacterial overgrowth in the gut, (2) disruption of the gut mucosal barrier and (3) abnormal host defenses. It still a matter of debate if patients with cirrhosis have altered gut flora, but there is some evidence supporting this (38-40). Some authors describe a

contamination of the jejunum and duodenum in up to 75% of patients with cirrhosis (38). Biopsies of the upper small bowel in patients with cirrhosis often demonstrates venous stasis, edema of villi and a degeneration of epithelium and basement membrane. It might well be that the gut mucosa is abnormally permeable in patients with cirrhosis, promoting translocation of bacteria from the gut to mesenteric lymph nodes and on to the peripheral blood.

A report of Runyon et al. (41) is the first to describe a rodent model of SBP with experimental cirrhosis induced by the oral administration of phenobarbital and the intragastric installation of carbon tetrachloride. More than 50% of the rats who survived the cirrhosis-induction program developed spontaneous infections of the ascitic fluid caused by Gram-negative bacilli of enteric origin. However, the effect of the carbon tetrachloride on the mucosal gut permeability is unknown, which makes these results difficult to interpret. The ideal animal model to study the mechanisms of pathogenesis of SBP has not yet been developed.

In summary, general host defenses, factors present in the ascites, and the microorganisms in the gut flora, act together to facilitate the development of spontaneous ascitic fluid infections (figure 1). Most bacteria causing SBP are gut-derived and translocate through the mucosal wall to mesenteric lymphnodes, on to the thoracic duct and bloodstream and leak across the sinusoids of the liver to the ascites.

In some patients bacteria colonizing the urinary tract or the respiratory tract may cause bacteremia which subsequently leads to ascitic fluid infection. Invasive procedures such as paracentesis, endoscopy or sclerotherapy do not seem to play a major role in most cases, and SBP is not necessarily a nosocomial infection.

1.4 Prevalence, recurrence and survival

There is some variance in data as to how often SBP occurs in patients with cirrhosis and ascites, most articles quoting a figure between 10% and 25% (5-8). A great deal depends on the index of suspicion and the threshold to perform a paracentesis in hospitalized patients. Some studies prospectively looked at the prevalence of SBP when paracentesis was routinely performed at admission (7,42-47). Seven prospective studies are summarized in Table 1. SBP and culture-negative neutrocytic ascites are grouped together. Monobacter ascites has been excluded from these data. There is no great difference in the

prevalence of SBP between alcoholic and nonalcoholic cirrhosis. In 5,7% -21% of patients with cirrhosis and ascites, SBP was diagnosed when paracentesis was routinely performed at admission. The reason for admission and presenting symptoms varied in the different studies. Also the Child-Pugh classification, which is an important predictive factor for the development of SBP, differs in the different studies. Venturelli et al. (42) found a low prevalence of 5,7%, but most patients had a relatively mild cirrhosis (Child-Pugh class B) and this was a multicentre study involving general medical departments, and not referral centres. Three studies (45-47) did a follow-up of their patients with cirrhosis and ascites and calculated an annual cumulative probability of developing SBP varying between 11% and 29% within their patient groups. The latter figure probably being higher because more of these patients had Child-Pugh category C cirrhosis, compared to the other two studies. When in a multivariate analysis clinical and laboratory variables were analyzed to establish predictive factors, the total protein content of ascitic fluid and the bilirubin serum level were the most important predictors for the develoment of a first episode of SBP (9,12,46). Llach et al. (46) calculated a probability for a first episode of SBP within 3 years of follow-up of 24% when the total protein content in ascitic fluid was lower than 10 g/l and of 4% when the total protein content was greater than 10 g/l.

If a patient had an episode of SBP the recurrence rate is much higher, between 35% and 69% at 1 year (9,43,48). Wang et al. (45) found an 11% annual occurrence rate for a first episode of SBP and a 47% annual recurrence rate in patients with a previous episode of SBP. The only predictive factor for this high recurrence rate was an ascitic total protein level below 7.5 g/l, other clinical and laboratory data were not statistically different between the two groups, although Tito et al. (9) showed that the serum bilirubin and a delayed prothrombin time were also associated with a higher risk of recurrence.

SBP is a serious complication with a high in-hospital mortality and a poor 1-year survival. Most studies report a direct mortality related to the SBP of about 40-50%, older series have a higher mortality (70%-95%) than more recent published studies. When a more aggressive approach is followed and every patient undergoes a paracentesis on admission, irrespective of symptoms, the mortality rate may be as low as 17% (44). This again emphasizes the need to perform a paracentesis routinely in every patient with cirrhosis and ascites who is admitted in hospital, as early diagnosis results in a much better prognosis. However, long-term survival after a first episode of SBP is possible and has been reported by Hoefs (4 out of 13 patients survived more than 3 years,(49) and Tito (8 out of 59 patients survived more than 3 years (9).

1.5 Prevention

Because enteric bacteria are the most common causative agents of SBP, it has been suggested that selective intestinal decontamination, which largely eliminates the aerobic Gram-negative intestinal flora and preserves the remaining aerobic and anaerobic flora could be useful in its prophylaxis. Two recent placebo-controlled studies have demonstrated the efficacy of oral norfloxacin in the prevention of SBP episodes without attaining a reduction in mortality (50,51). The first study (50) used antibiotic prophylaxis in a group of patients recovering from a first episode of SBP and found a decreased risk of recurrence at 1-year of follow-up of 20% compared to 68% in the placebo group. The SBP recurrences in the norfloxacin treated group were mainly due to Gram-positive cocci, which are not sensitive to norfloxacin. There was no significant difference between both groups with respect to the probability of SBP recurrence caused by Gram-positive cocci or culture-negative SBP (17% in the norfloxacin group and 20% in the placebo group at 1-year of follow-up). In the second study (51) 63 patients with cirrhosis and with a total protein content in the ascitic fluid < 10g/l were randomized either to receive placebo or norfloxacin during hospitalization. Also in this study there was a lower incidence of SBP, but also in total number of infections, during hospitalization in the patients receiving selective intestinal decontamination, without effect on the overall mortality.

Norfloxacin was chosen in both studies because it is incompletely absorbed by the intestine, is highly effective against Gram-negative bacilli, has low effectivity against anaerobic bacteria and has a low incidence of side effects when administered chronically (52). At least two mechanisms may contribute to the efficacy of long-term norfloxacin administration in preventing SBP. The first and most attractive mechanism is the selective intestinal decontamination caused by this drug. Its effect on the fecal flora confirms that there is a marked reduction of the aerobic Gram-negative bacilli with no significant effects on Gram-positive cocci and anaerobic bacteria (50). On the other hand, in patients with cirrhosis and ascites, bactericidal levels are obtained in serum, urine and ascites, which could be an additional mechanism explaining the efficacy in the prevention of SBP. A possible third mechanism was pointed out by a study (53) which described increasing serum and ascitic fluid C3 levels during selective intestinal decontamination by norfloxacin. It is not clear from this study if the increase in serum C3 and ascitic fluid C3 is due to decreased consumption of complement or improved hepatic complement synthesis. In this way norfloxacin may indirectly increase the bactericidal and opsonic activity of ascitic

fluid.

Long-term prophylaxis with norfloxacin remains a controversial matter (54,55). What are the indications for prophylaxis? How long is long-term prophylaxis? What are the side effects, especially concerning infections with norfloxacin-resistant strains and yeasts.

Finally the issue of cost and cost-effectiveness should be considered, comparing long-term prophylaxis (which did not show a decrease in hospital admissions) compared to no prophylaxis with a "diagnose and treat" strategy.

Yet another group of patients who might benefit from antibiotic prophylaxis should be discussed in this setting. It has been noted that cirrhotics with a gastrointestinal hemorrhage are at an increased risk to develop bacterial infections, especially caused by enteric bacteria (56,57). In the most recent study (56) norfloxacin 400mg twice daily was given during 7 days, immediately starting after emergency gastroscopy. There was a lower incidence of infections in the treated group, mainly attributable to a decrease of bacteremia's and urinary tract infections, which did not result in a significantly decreased mortality in the treated group.

In conclusion, norfloxacin has been shown to be an effective agent in the prevention of SBP in patients with quiescent chronic liver disease, but the number of hospitalisations and the survival is similar to prospective detection and treatment of SBP. More studies are needed to define subgroups of patients with liver disease to determine which patients benefit most from proper antibiotic prophylaxis.

1.6 Antibiotic treatment of Spontaneous Bacterial Peritonitis

Survival of the patient with SBP can be improved by the early institution of antibiotic therapy. Empiric intravenous antibiotic treatment (before culture results are avaliable) should be given to patients with an elevated PMN cell count in the ascitic fluid. A PMN count $>0.5x10^9$ cells/l., independent of the presence or absence of symptoms, or a PMN count $>0.25x10^9$cells/l in the presence of a compatible clinical picture should be sufficient reason to start antibiotic therapy immediately. Because more than 90% of cases of SBP are caused by enteric Gram-negative aerobes and Gram-positive cocci, the combination of ampicillin with an aminoglycoside provides good coverage and has been shown to be effective in the treatment of SBP (58). However the nephrotoxicity of aminoglycosides in patients with cirrhosis limits its usefulness.

Aztreonam, a monobactam, has been compared with cefotaxim in the treatment of SBP

(59); its lack of activity against Gram-positive organisms and a higher frequency of Gram-positive superinfections makes it suboptimal as a first choice antibiotic.

Cefotaxim, a third generation cephalosporin, is most widely used as a first-choice antibiotic in the empiric treatment of SBP. A cure rate of 85% was described in a recent study (12). At least 10% of the infections did not respond satisfactorily to this first-choice treatment and the antibiotic regimen had to be changed. Interestingly, the *in vitro* resistance of the isolated bacteria to cefotaxim did not always correspond to infection resolution *in vivo*. The efficient and rapid ascitic fluid penetration may account for this finding. The combination of amoxicillin and clavulanic acid has recently been shown to be effective in 85% of acute episodes of SBP in a French study (60) with minimal side effects and low frequency of resistant organisms. Also for these drugs a good distribution in the ascitic fluid and prolonged serum half-lives due to slow return from the ascitic compartment was proven (61). Unfortunately, this combination has never been compared to cefotaxim, but one of these drugs or drug combinations are suitable as a first-choice treatment for acute SBP. Once culture results are available, optimal antibiotic treatment obviously depends on the organism identified. Monitoring the ascitic fluid PMN count during the first days of treatment is useful to detect at an early stage resistance to the antibiotic regimen or to discover secondary peritonitis, which does not respond as well as SBP to antibiotic treatment (62).

Because SBP is a severe infection with a high mortality, it is common practice to treat for 10 to 14 days with antibiotics. Two studies compared long and short-term antibiotic regimens. Fong et al. (63) stopped the antibiotic treatment when the PMN count in the ascites was below 0.25×10^9cells/l and they did not find a difference in survival or infection recurrence between patients who received conventional treatment and the patients whose antibiotic therapy was discontinued when the ascitic fluid PMN count was $< 0.25 \times 10^9$-cells/l. Runyon et al (64) compared 10 days cefotaxim with 5 days treatment and found similar results in both groups concerning hospital mortality and infection recurrence. Both studies are not very large, thus a type II error can not be excluded.

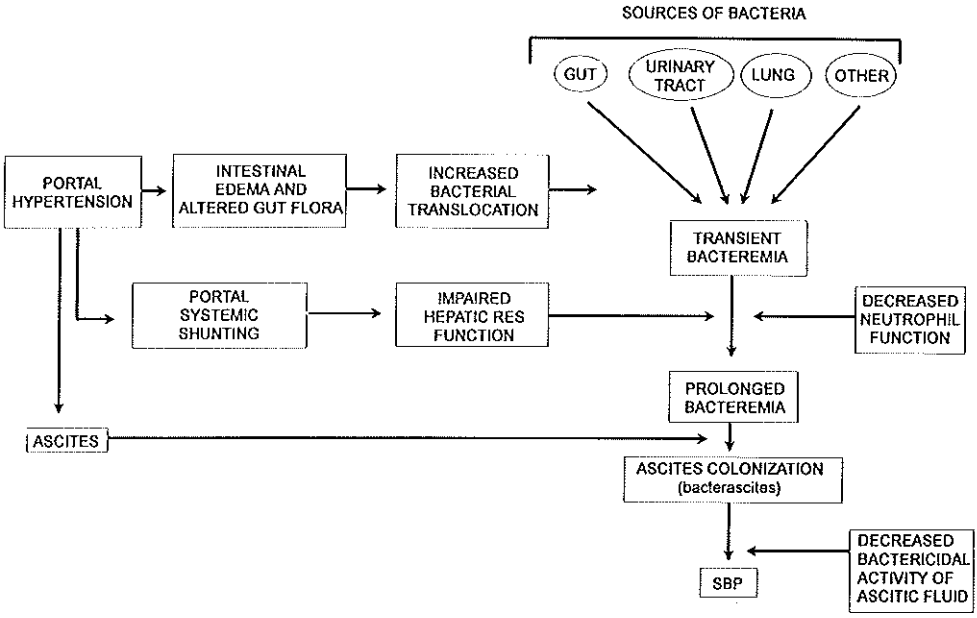

Figure 1. Mechanisms involved in the pathogenesis of SBP. RES = reticuloendothelial system.

Table 1; A summary of four studies (7,42-44) investigating the occurrence of SBP by routinely performing a paracentesis on all patients admitted to hospital, irrespective of their symptoms, and three prospective studies (45-47) calculating the annual cumulative probability of SBP.

Author/ref	Number of patients	Total percentage of patients with SBP	In-hospital mortality rate	Annual cumulative probability of SBP	Follow-up time months
Pinzello[7]	224	15	48		
Venturelli[42]	142	5,7	38		
Conte[43]	265	21	42		
Llovet[44]	278	16	17		
Wang[45]	153	22	36	11	30
Llach[46]	127	10		11	21 ± 22
Andreu[47]	110	25	41	29	11 ± 1

1.7 Aims of this thesis

1. To investigate and evaluate the occurrence of spontaneous bacterial peritonitis in a tertiary referral centre in the Netherlands.

2. To improve the diagnostic yield of ascitic fluid investigations and to develop standardization in diagnostic tests with respect to the bacteriological as well as to the haematological and biochemical analysis.

3. To clarify the pathogenesis of spontaneous bacterial peritonitis, especially concerning the presence of bacterial overgrowth and small bowel wall permeability in patients with portal hypertension.

4. To determine which subgroups of patients with decompensated cirrhosis might benefit from selective intestinal decontamination.

5. To augment knowledge and awareness of the existense of spontaneous bacterial peritonitis in order to improve early diagnosis and to lower the mortality due to this serious complication.

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CHAPTER 2

PARACENTESIS: THE IMPORTANCE OF OPTIMAL ASCITIC FLUID ANALYSIS.

This chapter is a modified and updated version of a review article published under the same title in Neth J Med 1993; 43: 147-155 by the following authors D.J. Bac, P.D. Siersema and J.H.P.Wilson.

Summary

An accumulation of peritoneal fluid can result from a variety of conditions, cirrhosis of the liver being responsible for about 75% of all patients with ascites. Malignancy accounts for 10-12% and cardiac failure for about 5%. The remaining 8-10% of ascites cases have a variety of causes, including tuberculosis, pancreatic disease and kidney disease. An early and accurate diagnosis often depends on an appropriate ascitic fluid analysis. Patients with known liver cirrhosis and clinical deterioration also need to have a paracentesis, with a determination of the ascitic fluid leucocyte and neutrophil count and adequate bacteriological cultures of their ascitic fluid.

The diagnostic value of different ascitic fluid parameters and their ability to separate between the several etiologies and their complications, is discussed.

Introduction

Diagnostic paracentesis has become increasingly important as the key initial test in the assessment of a patient with ascites. During the last two decades the value of paracentesis has been demonstrated for the rapid diagnosis of bacterial peritonitis [1-4], and for the differentiation of spontaneous from secondary peritonitis using chemical parameters [5] and bacteriological analysis [6,7]. The albumin gradient between serum and ascites has been used as an index of portal hypertension. This gradient is helpful to distinguish between different causes of ascites [2,4,8,9]. In addition, parameters such as cholesterol [10-13], fibronectin [11,13,14], ferritin [15,16], pH [17,18], and lactate [17] and the various tumor markers [15] in ascites have been evaluated in recent years. In every patient with new-onset ascites a paracentesis should be performed. This applies especially to patients with known pre-existent cirrhosis as this may be complicated by superimposed spontaneous bacterial peritonitis (SBP), hepatocellular carcinoma or peritoneal carcinomatosis. Even in cirrhotic patients with severe coagulopathy a lower abdominal paracentesis is unlikely to be harmful. On the other hand, the risk of undetected peritonitis is significant being a potential reversible cause of clinical deterioration, especially in patients with advanced liver disease.

Pathophysiology of ascites

The different causes of accumulation of fluid within the peritoneal cavity are summarized in Table 1. The pathogenesis of ascites formation in patients with hepatic disorders has not been fully elucidated. Traditionally, two theories have explained the excessive sodium retention in cirrhosis. The "underfill" theory proposes that the combination of an hepatic venous block in the liver and portal hypertension lead to fluid transudation into the abdominal cavity [19]. This results in a decreased effective plasma flow ("underfill"), leading to secondary sodium and water retention as a compensatory mechanism. The "overflow" theory proposes that ascites formation is a secondary phenomenon that results from primary renal sodium and water retention, probably due to a hepatorenal reflex [20], which predominates over the normal volume regulatory mechanism.
A third hypothesis, integrating the above mentioned pathogenetic mechanisms has been suggested by Schrier et al [21]. This theory is that cirrhosis leads to periferal arterial

vasodilatation as the initial event, resulting in a decreased "effective" plasma volume and the activation of compensating hormonal systems causing renal sodium retention [22]. This is supported by findings that demonstrate systemic haemodynamic changes which are characterized by primary peripheral vasodilatation and a secondary increase in cardiac output prior to ascites formation [23,24]. Patients with higher plasma renin activities tend to have more advanced liver disease, as evidenced by more ascites, a lower glomerular filtration rate and diminished sodium excretion and a shorter survival [25]. Further evidence for impaired renal sodium excretion is found by a blunted response to Atrial Natriuretic Factor (ANF) in patients with cirrhosis [26] and increased circulating plasma ANF levels in patients with cirrhosis and ascites [27].

Primary peritoneal processes, most importantly infection and malignancy causes usually high protein ascites due to increased fluid and lymph production from the peritoneum and increased permeability of the peritoneal capillaries, at the site of the inflamed peritoneum. Extravasation of pancreatic fluid , bile or lipid rich lymphe into the peritoneal cavity may also lead to chronic peritoneal fluid accumulation. Finally ascites may occur in patients with myxoedema or benign ovarian tumours (Meig's syndrome) in which the mechanism is unknown.

Initial evaluation of a patient with ascites

In many cases the history and physical examination will yield valuable clues to the likely cause of the ascites. For instance liver cirrhosis is suggested by stigmata such as spider angiomata and splenomegaly. Distended jugular veins may accompany congestive heart failure or constrictive pericarditis. The peritoneal cavity normally contains less than 50 ml of fluid. Accumulation of over 1 liter of ascitic fluid is required for detection during routine physical examination, using the signs of shifting dullness and positive fluid wave [28]. The reliability of these physical findings has been questioned and several studies have shown that the clinical diagnosis of ascites is incorrect in ± 50% of cases [29]. A plain abdominal X ray may suggest the presence of fluid through blurring of the psoas shadow, however, this requires over 2.5 liters of fluid to be accumulated in the abdominal cavity [30]. Ultrasonography is the most sensitive technique to detect ascites, since as little as 150 ml of fluid can be detected in the right lateral decubitus position [31]. Abdominal ultrasound can also detect potential causes of ascites, such as liver masses, pelvic and abdominal masses, or the presence of retroperitoneal lymphadenopathy.

The paracentesis

Diagnostic paracentesis can be performed with low morbidity if the ascitic fluid is accurately localized, an aseptic technique is followed, and a small needle (21-22 gauge) is used [32]. The patient is placed in a semi-recumbent position and a site caudal to the umbilicus is selected, either the avascular linea alba (midline) or the iliac fossae. The area selected should be away from visible vessels and surgical scars. If multiple scars are present, or obesity precludes reliable percussion of ascites an ultrasound guided paracentesis should be performed. Approximately 50 ml of fluid is withdrawn for diagnostic purposes. Paracentesis is not contraindicated in the presence of coagulopathy. Prophylactic transfusion of fresh frozen plasma should not be administered as the risk of hepatitis exceeds the risk of bleeding from the paracentesis, which has been estimated to be 1% [33].

Ascitic fluid analysis

The ascitic fluid sample should be analyzed using the tests listed in Table 2. The gross appearance of the fluid may help in the differential diagnosis. Bloody ascitic fluid is most often found in patients with malignant ascites, primary liver cancer, or as a result of a ruptured mesenteric varix [34]. Milky fluid suggests chylous ascites. This can be confirmed by determining its triglyceride content [35]. Turbid or cloudy ascites may indicate infection, though infected ascites is usually clear.

White blood cell count and differential

The white blood cell count (WBC) and the percentage of polymorphonuclear (PMN) cells is the most important determinator in ascitic fluid. As half of the patients with sterile ascites may have slightly increased WBC counts (usually < 300 cells/mm^3) in the ascites, mostly consisting of lymphocytes and only about 25% of PMN cells [36], the use of the PMN count in ascites has a higher specificty than the WBC count [2,4]. An ascitic fluid PMN count of > 250 cells/mm^3 (0.25×10^9 cells/l) suggests bacterial infection [1,3,36,37]. If the ascites is blood-tinged due to a traumatic tap one should correct for the contamination of ascitic fluid with blood by subtracting 1 PMN per 250 red cells from the absolute PMN count [1]. Eight studies have been published comparing the PMN count in ascites with the ascitic fluid pH and/or lactate level, as a means of making a rapid presumptive

diagnosis of SBP, using bacterial culture as the gold standard [37]. The PMN count (with a discriminant level above 500 cells/mm^3) remains the single best test with a sensitivity of 90%, a specificity of 98% and a positive predictive value of 86% [4]. The only exception to this rule is patients with malignancy-related ascites with a high WBC count with a low PMN percentage (<75%) in the ascites which may be due to tumor cell infiltration in peritoneal carcinomatosis, and not related to infection [38].

In our hospital we use an EDTA hematology tube to collect ascites for the determination of the white cell count, which is counted in a Sysmex NE 8000^R analyser (TOA Medical Electronics Co, Kobe Japan). If leucocytes are above 4000 cells/mm^3 a differential count by hand can be performed for the determination of the percentage of PMN cells. If there are between 300 and 4000 leucocytes/mm^3 the ascites is centrifuged at 2000 rpm for 5 min. and the pellet stained by Giemsa stain. Below 300 leucocytes/mm^3 a differential cell count is not reliable. This is described in more detail in chapter 3 of this thesis. A lymphocyte predominance in ascitic fluid is found in tuberculosis, fungal infections and peritoneal lymphoma [2,4].

pH and lactate

The ascitic fluid pH is decreased only if the ascitic fluid PMN count is > 250 cells/mm^3 ; therefore this test has little impact on clinical decision making regarding the use of antibiotics [2]. Lactate levels in ascites correlate inversely with ascitic fluid pH in most studies [17,18]. Ascitic fluid lactate is higher and pH lower in secondary peritonitis [5,7], than in SBP but add little to direct patient management.

Glucose and lactate dehydrogenase (LDH)

Ascitic fluid glucose levels do not differentiate between SBP, malignant ascites or uncomplicated ascites [16], although decreased glucose levels (<3.2 mmol/l; <50 mg/dl) are more frequent found in secondary peritonitis and in peritoneal carcinomatosis [7,11]. LDH in ascites is derived from disintegration of the PMNs or malignant cells. Thus the severest forms of infectious or malignant peritonitis can lead to low glucose and high LDH levels. The LDH ratio (ascites LDH/serum LDH) is usually above 0.6 in malignant ascites [16].

Total protein

The traditional concept of exudate (total protein >25 g/l) and transudate (<25g/l) is of

questionable value in the differential diagnosis of ascites. The normal peritoneal fluid protein concentration is >40g/l [2], the ascitic fluid protein concentration increases in cirrhotic patients during diuresis and albumin infusions [39], and some "transudative" etiologies such as ascites due to cardiac [40] and renal disease [41] have high protein concentrations, while some "exudative" etiologies including malignancy may have low concentrations [42]. The ascitic fluid protein concentration is the result of (a) the serum oncotic pressure, which is closely related to the serum albumin concentration, and (b) the hydrostatic pressure gradient, which is determined primarily by the portal pressure gradient [43]. In other words, the total protein concentration in ascitic fluid is influenced by serum protein concentration as well as by portal pressure. The total protein concentration does not directly influence clinical decision making, although in patients with liver cirrhosis a low ascitic fluid total protein concentration (<10g/l) might be predictive of the development of SBP [44]. A low protein concentration is associated with decreased opsonic activity and diminished complement concentrations, especially complement C3 concentration [45]. Three mechanisms are responsible for the low C3 levels in ascitic fluid; low hepatic synthesis, dilution with greater ascitic fluid volumes and high consumption due to classical pathway activation [46].

Albumin gradient (serum albumin minus ascites albumin concentration)

A close relationship between the portal pressure gradient and the albumin gradient has been established [43]. The albumin gradient correlates with only one physiologic factor, the portal pressure. This raised the possibility that the albumin gradient could be used clinically to separate etiologies of ascites based on the presence or absence of portal hypertension (Table 3). A wide gradient (>11g/l) could be described as portal hypertensive, and a narrow gradient (<11g/l) as non-portal hypertensive [2,8,47,50,51]. The diagnostic discrimination in distinquishing chronic liver disease from peritoneal carcinomatosis by the albumin gradient was established to be 95% and superior to the exudate-transudate concept in the differential diagnosis of ascites [48,51]. Moreover the albumin gradient can differentiate between ascites due to peritoneal carcinomatosis (non-portal hypertensive) and massive liver metastases (portal hypertensive) [49-51].

Fibronectin, cholesterol and triglycerides

These parameters have been used to distinquish malignant from non-malignant ascites [11-14]. Fibronectin is a glycoprotein with a molecular weight of 440.kD and is composed of

two identical subunits. Its insoluble form is associated with the extracellular matrix of many cells, and increased concentrations in body fluids are presumed to be due to shedding of the matrix of malignant cells [14]. Fibronectin, with cut-off levels above 50 or 75 μg/ml in ascitic fluid had a diagnostic accuracy of respectively 95 and 100% [11,14] in differentiating between malignant and non-malignant ascites.

Elevations of ascitic cholesterol levels have been described in peritoneal carcinomatosis . This is mainly caused by the increased movement of plasma HDL and LDL into the peritoneal cavity [10]. Cut-off levels of 46 and 48 mg/dl (respectively 1.20 and 1.25 mmol/l) had a diagnostic accuracy of 97 and 92 % in differentiating between malignant and non-malignant causes of ascites [11,12].

Triglycerides are only useful when chylous ascites is suspected. In cases of chylous leakage, ascitic concentrations are above serum values [35,52]. Chylous ascites is present in about 1% of patients with liver cirrhosis and ascites, [35] but it is usually found in patients with malignancies especially lymphomas [52].

Amylase

The determination of ascitic fluid amylase is performed when ascites due to pancreatitis is suspected. Ascitic levels of amylase are often above the serum values [15,54], and indicate leakage of pancreatic secretions to the peritoneal cavity. In these situations, endoscopic retrograde cholangio pancreatography (ERCP) will demonstrate passage of contrast material from a pancreatic duct or a pseudocyst into the peritoneal cavity.

Ferritin and tumor markers

The determination of ascitic fluid ferritin levels has not been widely investigated but some authors describe a 97% sensitivity in patients with malignant ascites. A cut-off level between 170 and 200 ng/ml has been suggested [16]. The usefulness of other tumor markers such as carcinoembryonic antigen, CA 125, CA 19-9, α-foetoprotein and prostate specific antigen has been disappointing [15,16,55-57].

Cytology

Cytological investigation, despite its high specificity, has been found unreliable in many cases of malignant ascites due to the high percentage (40-70%) of false-negative results [49,58]. Cytology is more likely to be positive with advanced ovarian carcinoma and with

peritoneal carcinomatosis than with gastro-intestinal tumors [58]. Examination of centrifuged larger volumes (> 200 ml) may increase the diagnostic yield to 60-90%. Up to now, the use of flow cytometry does not seem to add to the diagnostic accuracy [59,60].

Bacteriological examination

Spontaneous or primary bacterial peritonitis (SBP) occurs in 10-25% of hospitalized patients with cirrhosis [2,7,61,62]. SBP is rarely reported in patients with a non-cirrhotic cause of ascites [63,64, chapter 9 of this thesis]. This reflects probably the deficient opsonic activity of ascitic fluid in patients with liver cirrhosis [44,45]. Conventional culture results are negative in 40 to 60% of episodes with suspected SBP due to the low number of microorganisms (usually 1 microorganism/ml) in ascitic fluid [65]. Several prospective studies have documented the superiority of the use of blood culture bottles as medium for the ascitic fluid [65-69]. Moreover, the inoculation of ascites at the bedside is superior to delayed inoculation in the laboratory [70]. A sensitivity of about 80% in isolating microorganisms causing SBP can be expected, when 5-10 ml of ascites is inoculated at the bedside in blood culture bottles [65,68,69]. Aerobic Gram-negative bacilli from the intestinal flora are responsible for more than two thirds of the episodes of SBP in most series [6,65-68]. The remaining episodes are caused by Gram-positive cocci and rarely by anaerobes [6,65-69]. When an intra-abdominal source for the ascitic fluid infection is present it is called secondary peritonitis. In this situation usually several microorganisms can be isolated [5,7].

Infected versus sterile ascites

Several types of infected ascites can be distinquished, depending on the culture results, the PMN cell count and the biochemistry of ascites.

Spontaneous bacterial peritonitis: This is defined as a positive ascitic fluid culture and a PMN count of >250 cells/mm^3 in the absence of an intra-abdominal source of the infection. The total protein, albumin, and glucose levels of ascitic fluid do not add to the diagnosis. Lactate is usually elevated (47-89%) above 32 mg/dl and the pH is usually <7.32 however, only if the PMN count is above 250 cells/mm^3 [5,7].

Culture Negative Neutrocytic Ascites: This is identical to SBP except that culture results

remain negative, but the PMN cell count is elevated. (cutoff levels 250 or 500 PMN cells/mm^3)[70,71]. Obviously the culture technique determines which percentage of suspected episodes of SBP will remain culture negative. With bedside inoculation in blood culture bottles this will be between 15 to 20%. The patient should be treated as having an episode of SBP [70,71].

Monobacter ascites: This is a controversial entity and defined as a positive ascitic fluid culture with a single organism (excluding *Staph. epidermidis*) and an ascitic fluid PMN count of <250 cells/mm^3. The spontaneous evolution of asymptomatic monobacter ascites has been evaluated in several studies and progression to SBP has been noted in 14% to 38% of cases, usually in a short time, sometimes within a few hours [62]. In other cases however, the ascitic fluid spontaneously became sterile [62,72,73]. Early retapping of patients with asymptomatic monobacter ascites is recommended to assess the necessity of a specific treatment.

Secondary bacterial peritonitis: In ± 15% of infected patients an intra-abdominal source is responsible for the ascitic fluid infection [5,7]. Peritonitis associated with perforation may be identifiable by the following criteria: ascitic fluid total protein >10g/l, glucose <3.2 mmol/l and LDH greater than the upper limit of normal for serum [5,7]. It has been described that the ascitic fluid PMN count frequently increases despite therapy [75].

Tuberculous peritonitis: This is characterized by a lymphocyte predominance, a high total protein content and a narrow albumin gradient. The Ziehl-Neelsen staining is nearly always negative and positive culture results are reported to vary between 0 and 69% [76]. A laparoscopy with peritoneal biopsies is advised for establishing the diagnosis during the early clinical course [77].

Future developments

Concentrations in ascitic fluid of the cytokines Interleukin-1, Interleukin-6 and Tumor Necrosis Factor-α have been studied in patients with and without SBP [78-81], and a tremendous increase in concentrations of especially IL-6 and to a lesser extent of TNF-α within the peritoneal cavity was noted during infection, compared to only a slight increase of plasma values [78-81, chapter 5]. The high ratio of ascites to plasma values suggests that IL-6 is continuously produced within the peritoneal cavity. Following antibiotic treatment the cytokines levels returned to normal within a few days [79]. This suggests that IL-6 might become a usefull marker both for the diagnosis of SBP and the monitoring

of treatment. In Chapter 5 absolute levels of ascitic fluid IL-6 levels above 60.000 pg/ml. and a ratio of ascites/plasma IL-6 concentration above 40 were found in patients with spontaneous bacterial peritonitis.

Table 1. Causes of ascites

Portal hypertension
Cirrhosis
Hepatic congestion
Congestive heart failure
Constrictive pericarditis
Budd-Chiari syndrome
Veno-occlusive disease
Portal vein thrombosis
Infections
Spontaneous bacterial peritonitis
Secondary bacterial peritonitis
Tuberculous peritonitis
Fungal/parasitic peritonitis
Malignancy
Peritoneal carcinomatosis
Mesothelioma
Metastatic liver disease
Hepatocellular carcinoma
Other intra-abdominal malignancies
Other
Hypoalbuminaemia
Leakage of lymphatic tissue
pancreas
biliary tract
Renal failure
Myxedema

Table 2. Evaluation of ascitic fluid

Routine tests
White cell count and differential
Total protein
Albumin[1]
Lactate dehydrogenase [1]
Bacteriological examination
Bedside inoculation of 10 ml of ascites into blood culture bottles.
Gram and Ziehl-Neelsen stains
Mycobacterial cultures [2]
Cytology [2]
Optional tests
Amylase
Glucose
Triglycerides
Cholesterol

[1] Simultaneous plasma levels should be obtained.
[2] Optional, depending on index of suspicion

Table 3. Wide and narrow albumin gradient ascites [1]

Wide gradient (>11 g/l)	Narrow gradient (<11g/l)
Chronic liver disease	Peritoneal carcinomatosis
Massive hepatic metastases	Peritoneal inflammation
Veno-occlusive disease	Infection
Budd-Chiari syndrome	Serositis
Portal vein thrombosis	Hollow organ leak
Cardiac	Pancreas
	Lymphatic tissue
	Oncotic
	Nephrotic syndrome
	Chronic disease
	Idiopathic

[1] The albumin gradient reflects portal hypertension and is effective in distinquishing between etiologies based on its presence or absence. If peritoneal carcinomatosis or other typically narrow gradient etiologies occur in patients with portal hypertension, the gradient will be wide.

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CHAPTER 3

OPTIMAL ANALYSIS OF TOTAL PROTEIN, ALBUMIN, WHITE CELL COUNT AND DIFFERENTIAL IN ASCITIC FLUID

The contents of this chapter have been submitted for publication to Ann Clin Biochem under the same title with the following authors: H. Engel, D.J. Bac, R. Brouwer, B. G. Blijenberg, and J. Lindemans.

Summary

Accurate and precise procedures are described for the determination of total protein, albumin, absolute white cell count and differential count in ascitic fluid. The total protein method (biuret) on our routine serum chemistry analyzer, the Bayer-Technicon Chem-1™, was calibrated in the measuring ranges covering serum as well as ascitic fluid values (1 - 100 g/l) against the biuret reference method. The albumin method (BCG: bromcresol green) on the Chem-1™ was calibrated for these measurement ranges (1 - 50 g/l) against new human plasma protein international reference preparations and compared to the nephelometric method on the Beckman Array™, which was also calibrated against these reference preparations. A good correlation was obtained for total protein between the Chem-1™ (y) and the biuret reference method (x) in 58 ascitic fluids ($y = 1.02x - 0.3$; $r = 1.00$) and can be measured down to 1 g/l. Also, a good correlation between the Chem-1™ (y) and the Array™ (x) was obtained for albumin ($r = 0.99$), however the BCG method, which is not fully specific, results in significantly higher results ($y = 1.32x - 1.3$). Therefore, the immunochemical procedure will be more accurate for the determination of albumin in ascitic fluid.

The white cell count is carried out on a haematology analyzer. Depending on the absolute count, a reliable and convenient procedure was established for the manual differential. The ascitic fluid leucocytes in smears are equally distributed among artificial red and white blood cells from a quality control sample, the artificial white cells are clearly distinguisable from ascitic fluid white cells. The ascitic fluid is either directly mixed with this blood when the absolute count is above 4000 cells/mm^3, or the pellet is mixed with this blood after centrifugation of the ascitic fluid when the count is between 300 and 4000 cells/mm^3. This type of analysis is preferred above the cytospin procedure, and is currently also applied for other biological fluids, i.e. pleural fluid, and synovial fluid.

Introduction

A variety of conditions have been described to be responsible for the accumulation of peritoneal fluid. The major causes are cirrhosis of the liver (75%), malignancy (12%), and cardiac failure (5%). A more comprehensive list of causes is given in Table 1 (page 35). The diagnostic value of differences in ascitic fluid composition for the differentiation between the various aetiologies have been reported earlier (1,2). Traditionally, ascitic fluid was analyzed for absolute white cell count, total protein concentration, and for bacterial growth. The ascitic fluid total protein concentration was used to classify the fluids in categories like exudate (> 25 g/l) or transudate (< 25 g/l) in order to distinguish aetiologies like bacterial peritonitis, tuberculous peritonitis, pancreatitis, and peritoneal carcinomatosis from cirrhosis, heart failure, and nephrotic syndrome respectively. This exudate-transudate concept has resulted in many problems and exceptions (3,4). Since the serum oncotic pressure, which is closely related to the serum albumin concentration, and the hydrostatic pressure gradient, which is determined primarily by the portal pressure gradient, are both responsible for ascitic fluid accumulation, the serum-ascitic fluid albumin gradient is superior to the total protein concentration when used to distinguish between different causes of ascites (5). An albumin gradient wider than 11 g/l is indicative of the presence of portal hypertension, and a narrow gradient (< 11 g/l) of the absence of portal hypertension (6).

It might be clear in this respect that an accurate and precise determination of albumin in ascitic fluid is of great importance. Procedures are available for the determination of total protein and albumin in urine and cerebrospinal fluid in mg amounts per liter, however these methods are not directly applicable to ascitic fluid because the values range for total protein from 1 - 45 g/l and for albumin from 1-30 g/l. Also, the methods on the most common routine serum chemistry analyzers for total protein and albumin can not be directly applied for ascitic fluid analysis, the lower limits, as far as linearities are concerned, being approximately 25 g/l and 15 g/l respectively.

The ascitic fluid white cell count, especially the percentage of polymorphonuclear (PMN) cells, is the most important determinator for bacterial infection (7,8). The diagnosis of spontaneous bacterial peritonitis (SBP) in a patient with advanced cirrhosis is often difficult to make and easily missed, as clinical signs and symptoms are often lacking. A low threshold to perform a paracentesis followed by an accurate white cell count and differential of the ascitic fluid is essential to exclude the presence of peritonitis in any

patient with a deteriorating condition. A PMN count greater than 250 cells/mm^3 with signs or symptoms of SBP, or a PMN count > 500 cells/mm^3 without signs or symptoms of SBP are an indication to start broad-spectrum antibiotics, even before culture results are available. An early diagnosis of SBP is important because of several reasons. First of all, awareness and early diagnosis of SBP improves the mortality and secondly the presence of SBP precludes the possiblity of liver transplantation until the infection has been properly treated. A lymphocyte predominance in ascitic fluid is found in tuberculosis, fungal infections and peritoneal lymphoma. These considerations emphasize the importance of a reliable white cell count and differential but, again, established reliable techniques are lacking.

Therefore, we describe methods for the accurate and precise assessment of on the one hand total protein and albumin and on the other for the performance of an accurate white cell count and cell differentiation in ascitic fluid.

Materials and Methods

Materials

Haematology Quality Control blood was purchased from J.T. Baker (Philipsburg, PA, USA). Human plasma protein international reference preparations (OSAU 07 and ORDT 07), also containing albumin, were obtained from Behringwerke AG (Marburg, Germany).

Procedure for the accurate determination of total protein and albumin

Total protein and albumin were determined on a Chem-1™ routine serum chemistry analyzer (Bayer-Technicon, Tarrytown, NY, USA). The analytical ranges were verified using serum pools with high analyte concentrations and dilutions with saline according to NCCLS EP6-P recommendations (9). The total protein method on the analyzer was calibrated against the biuret reference method in a measuring range covering serum as well as ascitic fluid (1 - 100 g/l) with neat and saline-diluted patient sera (10). The albumin method (BCG: bromcresol green) on the Chem-1™ was calibrated for the combined measuring range for serum and ascitic fluid (1 - 50 g/l) using two human plasma protein international reference preparations and their dilutions with saline. The reference preparations had assigned values for albumin established by radial immunodiffusion, nephelometry, and turbidimetry,

performed by Behringwerke AG (Marburg, Germany) and were calibrated against the new International Reference Material CRM No. 470 (Bureau Communitaire de Réference of the European Economic Community, Brussels, Belgium). The Chem-1™ BCG method was compared with nephelometry using a Beckman Array™ Protein System nephelometer (Brea, CA, USA), also calibrated with these two reference preparations.

Procedure for assessment of haematology parameters

Ascitic fluid was collected in an EDTA Vacutainer tube for the determination of the absolute white cell count on a Sysmex NE-8000™ analyzer (TOA Medical Electronics Co., Kobe, Japan). If leucocytes were above 4000 cells/mm^3, a 5-part manual 100-cell microscopic differential count was performed. For that purpose, 1 volume ascitic fluid was mixed with 1 volume Haematology Quality Control blood and a wedge smear was prepared. With this procedure, the ascitic fluid white cells are equally distributed among red cells which results in a better assessment of the differential count. Direct manual analysis of ascitic fluid white cell differential results often in clumped leucocytes near the edge of the smear which, consequently, leads to an unreliable differential count. The artificial white cells in the control blood appear as filled blue spheres and can be clearly distinguished from ascitic fluid white cells.
If there are between 300 and 4000 leucocytes/mm^3, the ascitic fluid is centrifuged for 3 minutes at 2000 rpm, the supernatant is discarded and 50 μl of the pellet is mixed with 50 μl Baker blood. A manual 100-cell microscopic 5-part differential is carried out as described above. Below 300 leucocytes/mm^3, no differential is carried out.

Imprecision

Imprecision of the total protein and albumin methods was evaluated following the NCCLS EP5-T2 guidelines (11). The within-run and between-run imprecisions were determined using serum pools and dilutions thereof with low, normal and high concentration of total protein and albumin with respect to the ascitic fluid measuring ranges and were analyzed in single assays or on 10 different days, respectively.

Patient samples

A total number of 58 patient ascitic fluids were collected and analyzed for total protein on the Chem-1™ and with the reference biuret method, and for albumin on the Chem-1™ and the Array™.

Method comparison

The manual biuret reference procedure for the determination of total protein was performed on a Philips spectrophotometer (Eindhoven, The Netherlands). Regression analysis was performed according to the procedure described by Passing & Bablok (12).

Results and Discussion

The determination of total protein and albumin in ascitic fluid on a routine serum chemistry analyzer is described. The linearity ranges were expanded to lower regions for both parameters considering the ascitic fluid measuring ranges. The total protein and albumin methods, on our currently in use routine serum chemistry analyzer, were standardized over the complete ascitic fluid and serum measuring ranges against either a reference method for total protein or human plasma protein international reference preparation for albumin (13,14). These new international reference preparations for albumin have been prepared recently under auspicies of the International Federation of Clinical Chemistry (IFCC) in collaboration with the Bureau Communitaire de Réference of the European Economic Community (Brussels, Belgium), and the College of American Pathologists (CAP, Northfield, USA).

A total number of 58 ascitic fluids were analyzed for total protein and albumin on the Chem-1™ as well as total protein with the biuret reference method and albumin on the Array™ nephelometer (Table 2). Total protein could be measured down to 1 g/l on the Chem-1™ with respect to the lower limit of the linearities and a good correlation was obtained between the Chem-1™ and the biuret reference method (y (Chem-1) = 1.02x (biuret) - 0.3; r = 1.00; fig. 1A). In addition, a good correlation was found for albumin between the Chem-1™ and the Array™ (r = 0.99), however, above 7 g/l the Chem-1™ gives significantly higher results (y (Chem-1) = 1.32x (Array) - 1.3; fig. 1B). Since the BCG method is not fully specific for albumin and since albumin values of the reference

preparations were assigned by radial immunodiffusion, turbidimetry, and nephelometry, we conclude that albumin in ascitic fluid should be determined nephelometrically. This type of analysis might be difficult to perform when ascitic fluid, concerning total protein and albumin analysis, must be analyzed immediately 24 h a day. However, this is not the case in our hospital. When necessary, only total protein is analyzed immediately and albumin afterwards.

Total protein is accurately and precisely analyzed in the ascitic fluid measuring ranges. The between-run coefficients of variation are smaller than 3 % over the complete measuring range (Table 3).

The absolute white cell count in ascitic fluid with our haematology analyzer, a Sysmex NE-8000™, showed a good correlation with the manual count (Table 2). However, the result of white cell differential count by the analyzer is unreliable due to the abundance of degenerated leucocytes. Traditionally, a smear is prepared and manual white cell differential count is carried out, this leads however either to almost no leucocytes in the smear when the absolute count is low, or the leucocytes are clustered near the edge of the smear when the absolute count is high. In both cases, these manual differential counts are not reliable. Our current procedure, as described in the Materials and Methods section, results in smears with the ascitic fluid leucocytes equally distributed among artificial red and white blood cells from the haematology quality control material (fig. 2). This procedure is preferred above the cytospin procedure which is performed in order to differentiate cells present in cerebrospinal fluid. The cytospin procedure, applied to ascitic fluids with high absolute white cell counts, results in smears of highly concentrated leucocytes which are difficult to differentiate.

Depending on the absolute count, the ascitic fluid is mixed either directly with an equal volume of Baker blood (when the white cell count is above 4000 cells/mm^3) or the pellet is mixed after centrifugation of the ascitic fluid (when the white cell count is between 300 and 4000 cells/mm^3). This procedure is very convenient, gives reproducible results and is currently also applied for pleural and synovial fluids.

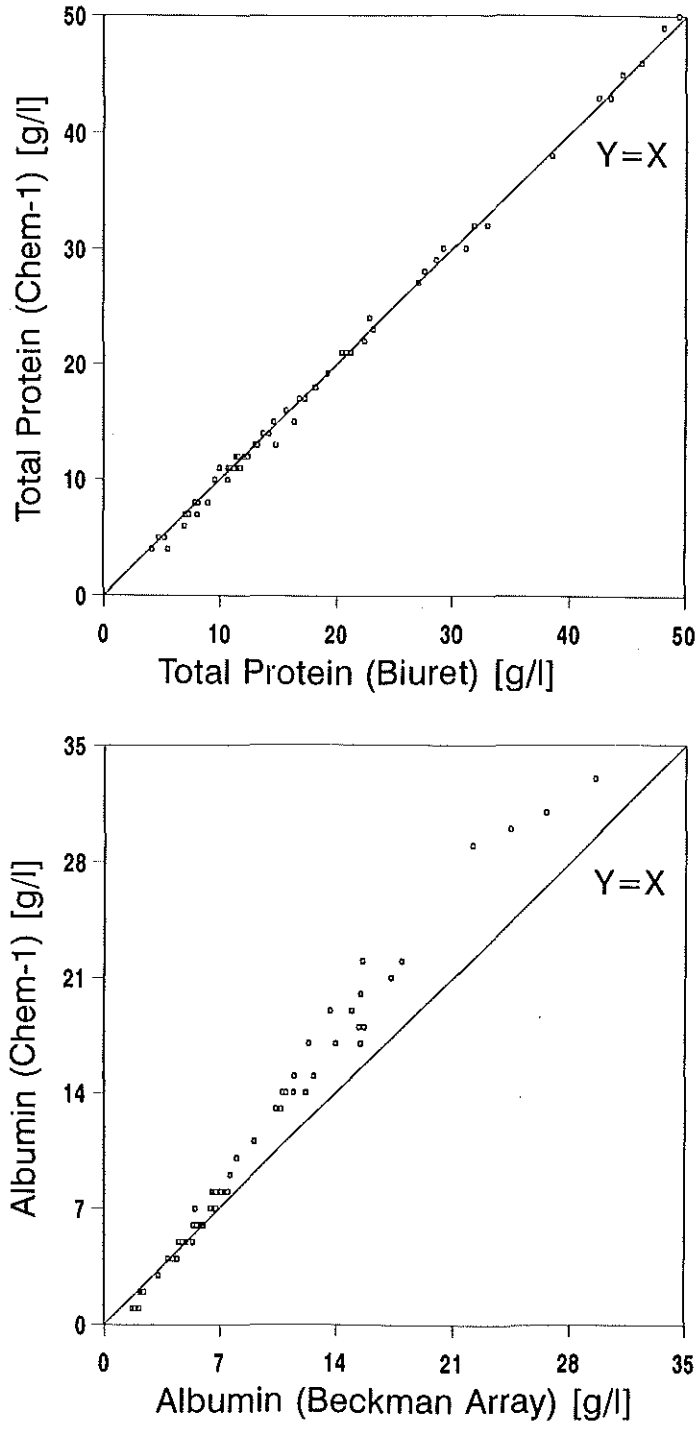

Figure 1. Comparison of measurements of total protein (A) and albumin (B) in ascitic fluid.

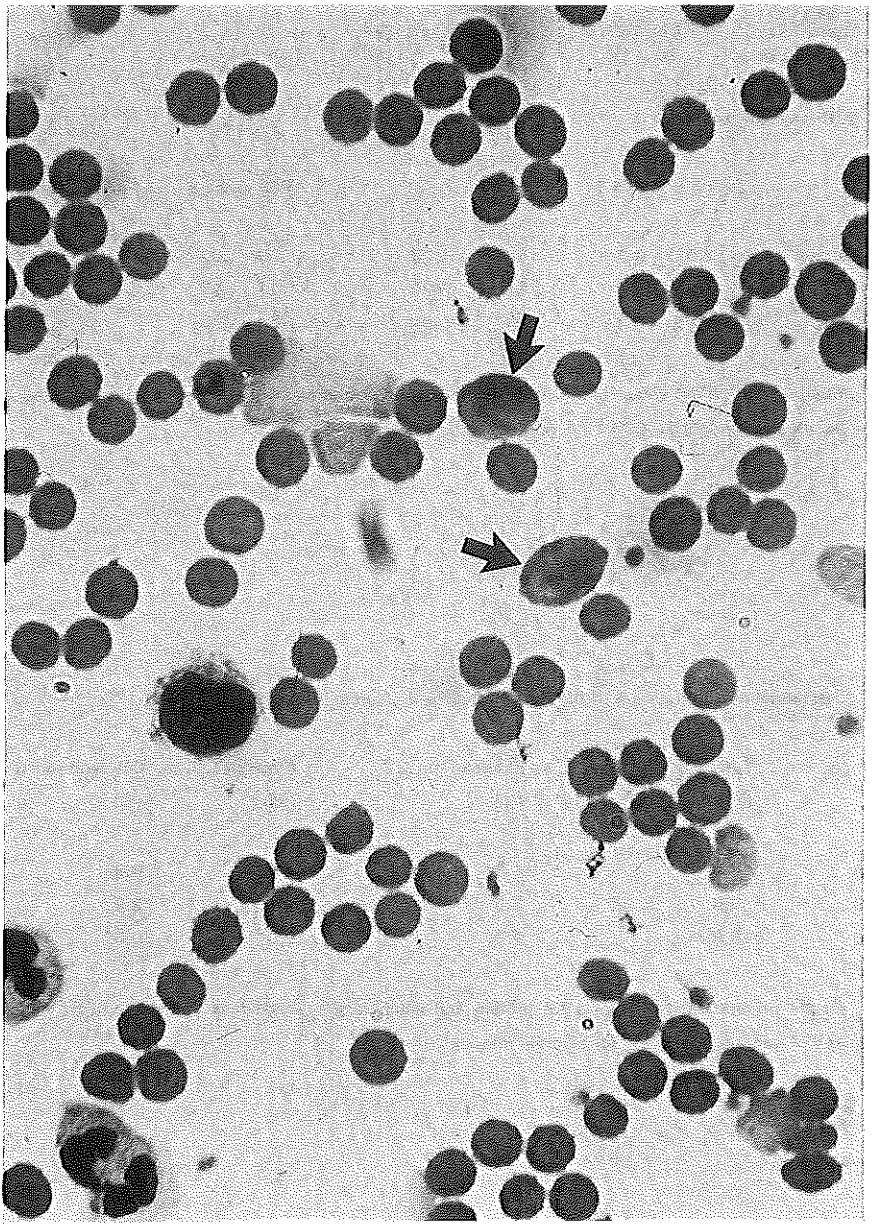

Figure 2. The equal distribution of ascitic fluid leucocytes among artificial red and white cells from a heamatology quality control blood. The artificial white cells appear as blue spheres and are indicated by an arrow.

Table 2. Regression analysis

	Passing & Bablok	n	r	95% range a	95% range b
Total Protein	y = 1.02x - 0.3	58	1.00	1.00 - 1.03	-0.6 - -0.1
Albumin	y = 1.32x - 1.3	58	0.99	1.27 - 1.37	-1.7 - -1.0
Leucocytes	y = 1.02x - 0.0	31	0.98	1.00 - 1.04	-0.1 - 0.0

Total protein: Chem-1™ (y) versus biuret reference method (x); Albumin: Chem-1™ (y) versus Beckman Array™ (x); Leucocytes: Sysmex NE-8000™ (y) versus manual count (x).

Table 3. Between-run imprecision of the total protein and albumin method on the Chem-1™.

	Total Protein (g/l)	Between-run CV (%)	Albumin (g/l)	Between-run CV (%)
pool A	68.8	1.8	46.2	0.9
pool B	30.8	2.6	23.9	2.9
pool C	15.8	2.7	9.0	0.0[a]

[a] The results on the Chem-1™ for total protein and albumin are reported as integers, which explains the coefficient of variation of 0.0 % for albumin in pool C.

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CHAPTER 4

BLOOD CULTURE BOTTLES ARE SUPERIOR TO LYSIS-CENTRIFUGATION TUBES FOR BACTERIOLOGICAL DIAGNOSIS OF SPONTANEOUS BACTERIAL PERITONITIS

The contents of this chapter have been published under the same title in J Clin Microbiol 1992; 30, 667-669 by the following authors: P.D. Siersema, S.de Marie, J.H. van Zeijl, D.J. Bac and J.H.P. Wilson.

Summary

The conventional method of ascitic fluid culture was compared with the bedside inoculation of ascites into blood culture bottles and into lysis-centrifugation tubes. The conventional culture method was compared with the blood culture bottle method in 31 episodes of spontaneous bacterial peritonitis (SBP). Cultures were positive with the conventional method in 11 (35%) and with the blood culture bottle method in 26 (84%) episodes ($p < 0.001$). The lysis-centrifugation tube method could be compared with the blood culture bottle method in 24 episodes of SBP. Cultures were positive with the lysis-centrifugation tube method in 11 (46%) and with the blood culture bottle method in 19 (79%) episodes ($p < 0.05$). Moreover, the blood culture bottle method also shortened the time for the detection of bacterial growth. In conclusion, bedside inoculation of ascites into blood culture bottles should be used routinely in patients with suspected SBP. Culturing of ascites in lysis-centrifugation tubes is more laborious than and inferior to that in blood culture bottles.

Introduction

Spontaneous bacterial peritonitis (SBP) is a frequent and severe complication of cirrhosis with ascites (4). With conventional ascitic fluid culture methods, consisting of inoculation of ascites into thioglycolate broth and onto agar plates, cultures are positive in only 42 to 57% of episodes of suspected SBP (1,10-12). In 1987, Runyon et al. reported that sensitivity of ascites cultures could be improved by inoculation of ascites into blood culture bottles (11). Recently this finding was confirmed in three prospective studies (1,10,12). When we started our study in 1987, the purpose was to prospectively compare the conventional method with the blood culture bottle method of ascitic fluid culturing. Meanwhile, it was demonstrated that even with blood culture bottles, ascites cultures were negative in up to 19% of cases of suspected SBP (1,10-12). Therefore, in an effort to further improve sensitivity of ascites cultures, starting in 1988, we also inoculated ascites into lysis-centrifugation tubes (8). Lysis-centrifugation tubes have been reported to be as good or even more effective than blood culture bottles for the detection of Gram-negative bacilli (5), which account for 69% of isolates in SBP (14). On the other hand, the detection rate of Gram-positive bacteria has been demonstrated to be lower with lysis-centrifugation tubes (5). Since streptococci account for 26% of isolates of SBP (14), this could cause a lower detection rate of these microorganisms in cases of suspected SBP.

Materials and methods

Between July 1987 and April 1991 all patients with liver cirrhosis and ascites, who were suspected of having SBP, were included in the study. A diagnosis of SBP was suspected if: a) patients had clinical signs and symptoms compatible with a diagnosis of SBP (abdominal pain and/or fever and/or worsening of hepatic encephalopathy without another explanation), b) the ascitic fluid polymorphonuclear (PMN) count was $\geq 500/mm^3$ (3) and c) there was no intra-abdominal source of infection. In all episodes of suspected SBP an alternative explanation for an elevated PMN count, e.g. pancreatitis, cholecystitis, peritoneal carcinomatosis, hemorrhage into ascites, or tuberculosis was exluded. Patients were also excluded if antibiotic therapy had been given within 14 days of presentation. During the period from June 1987 to April 1991, both the conventional culture method and the blood culture bottle method were simultaneously used to test ascitic fluid samples. In addition, from November 1988 to April 1991, the lysis-centrifugation tube method was

used to test all ascitic fluid samples. Ascites was obtained under sterile conditions by paracentesis with or without ultrasound-guided control.

For the conventional method, 10 ml of ascites was transported immediately to the Microbiology Laboratory. The specimen was centrifuged at 3000g for 5 min. Gram staining was performed on all centrifuged sediments. The sediments were inoculated on blood agar, MacConkey agar, chocolate agar and GC agar plates for aerobic cultures and incubated for 48 h in a 5% CO_2 atmosphere at 37°C. In addition, ascites was inoculated on Brucella blood agar, Brucella blood agar with 100 μg/ml neomycin, and Brucella blood agar plates with 100 μg/ml kanamycin and 7.5 μg/ml vancomycin, and incubated under anaerobic conditions for 5 days at 37°C. Thioglycolate medium was inoculated and subcultured onto Brucella blood agar plates after 2 days for aerobic culture and after 5 days for anaerobic culturing; these agar plates were subsequently incubated for 2 days at 37°C.

For the blood culture bottle method, 5 ml of ascitic fluid was inoculated at the bedside in 45 ml of Columbia broth (with sodium polyanetholesulfonate and increased cysteine) (Becton Dickinson vacutainer systems, Becton Dickinson and Company, Rutherford, New Jersey, USA) for aerobic incubation and 5 ml was inoculated in 45 ml of Columbia broth for anaerobic incubation at 37°C. Subculturing was performed as soon as growth was visible or blindly after 2 and 7 days from the aerobic cultures, and after 7 days for the anaerobic cultures.

For the lysis-centrifugation tube method, 10 ml of ascites was inoculated at the bedside into a lysis-centrifugation tube (du Pont Isolator 10-tube, E.I. du Pont de Nemours & Co., Inc., Wilmington, Delaware, USA). After centifugation at 3000g for 30 min, the pellet was inoculated onto the culture media of the conventional culture method.

The times at which cultures became positive were recorded and microorganisms were identified by standard laboratory methods as described in the *Manual of Clinical Microbiology* (7).

The ascitic fluid PMN count was calculated as the percentage of PMN's per total amount of leucocytes in ascites samples. The total amount of leucocytes was counted in a Sysmex NE 8000^R (TOA Medical Electronics, Kobe Japan). For the calculation of the percentage of PMN's, 10-20 ml of ascites was centrifuged at 2000 rpm for 10 minutes. The pellet was stained with Giemsa-stain. At least 50 cells were counted to determine the percentage of PMN's.

For the statistical analysis of the difference between the results of the three culture methods, the McNemar chi-square test (3) was used. For the statistical analysis of the

difference in the median time interval for detection of bacterial growth between the three culture methods, the SAS lifetest procedure was used (6). A p value of <0.05 was considered significant.

Results

During the study, 31 episodes of suspected SBP were diagnosed in 28 cirrhotic patients with ascites. In the 31 episodes of suspected SBP, the mean ± SD PMN count in ascites was 4,900 ± 2,700/mm^3 (range, 600 to 9,400/mm^3). In 2 episodes of SBP, the Gram stain of ascitic fluid revealed Gram-negative rods, whereas in the other 29 episodes the Gram stain did not detect bacteria. The conventional method was compared with the blood culture bottle method in 31 episodes of suspected SBP. Ascites cultures were positive by the conventional method in 11 of 31 (35%) episodes and by the blood culture bottle method in 26 of 31 (84%) episodes ($p<0.001$). Conventional cultures did not grow bacteria that were not also detected by the blood culture bottle method. The lysis-centrifugation tube method was compared with the conventional method and with the blood culture bottle method in 24 episodes of suspected SBP. Ascitic fluid samples were positive by the conventional method in 8 of 24 (33%) episodes, by the lysis-centrifugation tube method in 11 of 24 (46%) episodes (p, not significant) and by the blood culture bottle method in 19 of 24 (79%) episodes (blood culture bottle method vs. lysis-centrifugation tube method: $p<0.05$; blood culture bottle method vs. conventional method: $p<0.001$). All isolates detected by the lysis-centrifugation tube method were also detected by the blood culture bottle method. In Table 1 the microorganisms isolated by the conventional culture and the blood culture bottle methods were compared. For the 26 culture-positive episodes, Gram-negative bacilli were detected in 17 episodes (65%) and Gram-positive cocci in 9 episodes (35%). Multiple microorganisms in a single ascites specimen or anaerobes were not isolated. The median time intervals for detection of bacterial growth was 48 h (range, 24 h to 120 h) for the blood culture bottle method (26 episodes) and 72 h (range, 24 h to 120 h) for the conventional method (11 episodes) ($p<0.001$).

In Table 2 the microorganisms isolated by the lysis-centrifugation tube, the conventional culture and the blood culture bottle methods were compared. The median time intervals for detection of bacterial growth was 48 h (range, 24 h to 120 h) with the blood culture bottle method (19 episodes), 48 h (range, 24 h to 120 h) with the lysis-centrifugation tube method (11 episodes) ($p<0.01$), and 72 h (range, 48 to 120 h) for the conventional

culture method (8 episodes). (blood culture bottle method versus lysis-centrifugation tube method, $p<0.01$; blood culture method versus conventional culture method, $p<0.001$). Blood cultures were done for 24 patients and were positive in 10 (42%) episodes (in 9 episodes, the same microorganism was isolated from blood as from ascites; in 1 episode, *Klebsiella pneumoniae* was isolated only from blood).

Discussion

In accordance with the findings of others (1,10-12), our study demonstrates that the bedside inoculation of ascites into blood culture bottles is superior to conventional methods of ascitic fluid culturing in the detection of bacterial growth in SBP (84% versus 35%; $p<0.001$).

Among the isolated pathogens, staphylococci were detected in 2 episodes (1 with *Staphylococcus aureus* and 1 with *Staph. epidermidis*). Strikingly, both these patients were treated with esophageal injection sclerotherapy for variceal hemorrhage, 24 hrs. and 36 hrs. respectively before these microorganisms were isolated. Three cases of SBP, following injection sclerotherapy, have also been reported by Tam et al. (13). In one of these cases *S.aureus* was also isolated.

In cases of SBP very low concentrations of bacteria are detected in ascites (10). This fact is demonstrated by the finding that only 2 (6%) of the 31 Gram stains of centrifuged ascites, revealed microorganisms, in accordance with findings of Sainz et al. (12), who also found Gram stains of ascites to be positive in only 6% of cases of SBP.

In our study, ascitic fluid cultures remained negative in 5 (16%) of the 31 episodes of suspected SBP, comparable to the results of other studies (7 to 19% negative cultures) (1,10,11). There are two possible explanations for the failure rate of blood culture bottles in our study. In the blood culture bottle system that we used, only 5 ml of ascites is inoculated. Runyon et al. reported that inoculation of larger amounts of ascites (10 or 20 ml) resulted in more culture-positive episodes than the inoculation of smaller amounts of ascites (10). Moreover, blood culture bottles containing Thiol have been found to be more sensitive than bottles containing tryptic soy broth (10). Since we used Columbia broth bottles, it remains to be established whether these bottles are less sensitive than blood culture culture bottles containing Thiol for the detection of microorganisms from ascites. Blood cultures with lysis-centrifugation tubes have been described to increase the isolation rate of both Gram-negative bacilli, and fastidious and intracellularly growing micro-

organisms (5). We demonstrated that the sensitivity of ascites cultures could not be increased with lysis-centrifugation tubes. In 8 episodes of SBP (5 with Gram-negative bacilli and 3 with Gram-positive cocci) the lysis-centrifugation tube method failed to detect pathogens, that had been isolated by the blood culture bottle method (Table 2). Although the rate of detection of Gram-positive cocci may be decreased with lysis-centrifugation tubes (5), failures in isolating Gram-negative bacilli have not been reported. In contrast to our findings, Elston et al. reported lysis-centrifugation tube method to be a sensitive method for culturing body fluids (2). They tested 155 body fluid samples (including 45 peritoneal fluid samples); however, from these samples only 62 isolates were evaluated. Moreover, they did not mention the source of these 62 isolates. There are three possible explanations for the decreased sensitivity of the lysis-centrifugation tube method in detecting SBP. First, ascites in lysis-centrifugation tubes must be transported to the laboratory, causing a delay of about 30 to 60 minutes before inoculation onto culture media in our laboratory. The sensitivity of ascitic fluid cultures in blood culture bottles has been described to be increased if blood culture bottles are inoculated directly at the bedside and not in the laboratory after a delay (9). It is possible that in lysis-centrifugation tubes the few viable microorganisms present in ascites are exposed to unfavorable conditions and are likely to die before being cultured in the laboratory. Secondly, the constituents of lysis-centrifugation tubes, containing sodium polyethanol-sulfonate, saponin and EDTA, might be suboptimal for the preservation of microorganisms in ascites. Third, only thioglycolate medium was used as a broth, and this medium has been described to be less suitable for aerobic and facultative anaerobic microorganisms (8).

In conclusion, the inoculation of ascites at the bedside in blood culture bottles, should be used routinely in cirrhotic patients with suspected SBP. Culturing of ascites in lysis-centrifugation tubes is more laborious than and inferior to that in blood culture bottles.

Table 1: Microorganisms isolated from ascites by the conventional culture (CC) method and/or the blood culture bottle (BCB) method in 26 of 31 culture-positive episodes of suspected SBP.

Microorganism	CC and BCB positive	Only BCB positive
Gram-negative bacilli:		
Escherichia coli	6	4
Klebsiella pneumoniae		2
Enterobacter cloacae	1	
Acinetobacter sp.	1	
Pseudomonas aeruginosa		2
Not specified[1]		1
Gram-positive cocci:		
Alpha-hemolytic *Streptococcus*		2
Enterococcus faecalis	1	1
Streptococcus pneumoniae	1	2
Staphylococcus aureus	1	
Staphylococcus epidermidis		1
Total	11	15

[1] Gram-negative rod that died during the identification procedure

Table 2: Microorganisms isolated from ascites by the conventional culture (CC) method, the lysis-centrifugation tube (LCT) method and/or the blood culture bottle (BCB) method in 19 of 24 culture-positive episodes of suspected SBP.

Microorganism	CC, LCT and BCB positive	LCT and BCB positive	Only BCB positive
Gram-negative bacilli:			
Escherichia coli	4		3
Klebsiella pneumoniae			2
Enterobacter cloacae	1		
Acinetobacter sp.	1		
Pseudomonas aeruginosa		1	
Gram-positive cocci:			
Alpha-hemolytic *Streptococcus*		1	1
Enterococcus faecalis	1	1	
Streptococcus pneumoniae	1		1
Staphylococcus epidermidis			1
Total	8	3	8

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CHAPTER 5

HIGH INTERLEUKIN-6 PRODUCTION WITHIN THE PERITONEAL CAVITY IN DECOMPENSATED CIRRHOSIS AND MALIGNANCY-RELATED ASCITES.

The contents of this chapter have been submitted for publication in Liver under the same title with the following authors: D.J. Bac, W.M. Pruimboom, P.G.H. Mulder, F.J. Zijlstra and J.H.P. Wilson.

Summary

To assess the diagnostic and prognostic value of interleukin-6 (IL-6), interleukin 1β (IL-1β), and tumor necrosis factor-α (TNF-α) assays in plasma and ascites, we measured these cytokines in 8 patients with malignancy-related ascites and in 32 patients with decompensated cirrhosis. Five patients had an episode of bacterial peritonitis (BP) during which period one or more ascitic fluid samples were analyzed. IL-6 and TNF-α were not significantly different between the cirrhotic and malignant two groups: ascitic IL-6 13816 ± 15314 vs 28138 ± 23403 pg/ml, plasma IL-6 542 ± 719 vs 559 ± 604 pg/ml; ascitic TNF-α 19 ± 50 vs 12 ± 31 pg/ml, plasma TNF-α 3,4 ± 8,2 vs 6,1 ± 13,8 pg/ml. During an episode of BP there was a significant increase only in ascitic IL-6, (133268 ± 99743 pg/ml) which declined after antibiotic treatment. None of the parameters was associated with the 6 months survival (11 of the 40 patients died within 6 months). There was a correlation ($r = 0.675$; $p = 0.002$) between plasma IL-6 levels and the Child-Pugh score in patients with liver cirrhosis, but not with the etiology of the liver disorder. Plasma IL-6 levels correlated with Ig A levels ($r = 0.649$; $p = 0.004$) but not with CRP, ESR, fibrinogen, Ig M or Ig G.

Il-6 is produced within the peritoneal cavity in hepatic and malignant ascites. There is a sharp increase in the local production of IL-6 during an episode of bacterial peritonitis. This increase was not detectable for TNF-α. It remains to be assessed what the physiological meaning is for this over-production of locally generated cytokines and if this relates to the poor prognosis of patients with cirrhosis and infectious disorders.

Introduction

Interleukin-6 (IL-6), also called interferon β_2, or hepatocyte-stimulating growth factor, is produced by several cell types such as monocytes, fibroblasts and endothelial cells. Monocytes seem to be the most important in this process (1,2). IL-6 acts in concert with other cytokines such as IL-1 and TNF-α and leads to a variety of different actions including maturation of B cells for immunoglobulin synthesis, and induction of the synthesis of acute-phase proteins by hepatocytes (3). IL-6 appears to be a key member of the IL family, however many of the interactions with other lymphokines are poorly understood. Elevated serum levels of different cytokines in chronic liver disease have been reported (4,5). The pattern of cytokine elevation is stage dependant and is only moderately affected by the type of liver disease. There are some preliminary reports, suggesting high IL-6 production in ascitic fluid during spontaneous bacterial peritonitis (6-8), and without peritonitis (9). A correlation between survival and elevated TNF-α (10) and high serum IL-6 levels (11,12) has been established for patients with alcoholic hepatitis. A profound increase of IL-6 and TNF-α after the onset of septicaemia which lasted much longer in patients with cirrhosis compared to controls may be related to the high mortality of infectious complications in cirrhotic patients (13,14).

In order to assess the diagnostic and prognostic value of these cytokines, we measured plasma as well as ascitic fluid IL-6, TNF-α and IL-1β in patients with ascites of different etiology, including carcinomatous peritonitis and different causes of decompensated cirrhosis and portal hypertension. In some patients with an episode of SBP cytokines were measured serially in ascitic fluid. Plasma and ascitic fluid IL-6, TNF-α and IL-1β levels were compared to clinical data, biochemical measurements, diagnosis and prognosis.

Material and methods.

Patients

Thirty-two patients with hepatic ascites and 8 patients with malignancy-related ascites were prospectively studied from June to December 1993. Sixteen patients had alcoholic cirrhosis, 7 patients had hepatitis B or C virus induced cirrhosis, 5 patients had primary biliary cirrhosis or sclerosing cholangitis and 4 patients had other another liver disease (auto-immune disease 2x, cryptogenic 1x and M Wilson 1x). Of the malignancy-related ascites group all patients had metastasized disease (2 patients had pancreatic adenocar-

cinoma, 2 peritoneal adenocarcinomatosis with unknown primary tumor, 1 gastric carcinoma, 1 peritoneal mesothelioma, 1 coloncarcinoma and 1 leiomyosarcoma). Five patients developed SBP during the course of the disease, four patients with cirrhosis and one patient with a peritoneal adenocarcinomatosis. For further analysis patients are divided in three groups;cirrhosis (n = 28), malignancy-related ascites (n = 7) and bacterial peritonitis (n = 5). In 23 out of the 40 patients, plasma samples were available, taken within 24 hours after paracentesis. In those 40 patients a total of 56 ascitic fluid samples were available for biochemical analysis, cultures and cytokines assays. If more than one sample was analyzed in a patient, the patient's average was used in the statistical analysis. Patient characteristics are given in Table 1. Survival was assessed during a period of six months.

Cytokine assay

Ascites and blood were obtained under sterile conditions and immediately cooled to 0°C and centrifuged. Aliquots of cell-free samples were stored at -70 °C till assay. Il-6 was determined in ascites and plasma by an ELISA (High cult, Uden, The Netherlands) and TNF-α (Eurogenetics, Tessenderlo, Belgium) and IL-1β (Eurogenetics, Tessenderlo, Belgium). The determination threshold for IL-6 was 2 pg/ml., for TNF-α 10 pg/ml. and for IL-1β 2 pg/ml.

Blood and ascitic fluid sampling

Blood samples were taken within 24 h after the paracentesis was performed. In addition to routine biochemical assessment, plasma C reactive protein, IgG, IgM, IgA, and fibrinogen levels were measured. All ascitic fluid samples were cultured with bedside inoculation in blood culture bottles. Total protein, lactate dehydrogenase, cholesterol, triglycerides and leucocyte count with differential counting was measured in each ascitic fluid sample. In all patients with cirrhosis the severity was graded by the Child-Pugh score. Spontaneous bacterial peritonitis was defined as a positive ascitic fluid culture with more than 0.25×10^9 polymorphonuclear cells/l.

Statistical analysis

Data are summarized as the mean ± standard deviation. The Mann-Whitney U-test was used to compare data between two groups. The Kruskal-Wallis test was used to compare data between more than 2 groups. Correlations were estimated by the Spearman rank correlation coëfficient. p-Values (two-tailed) of less than 0.05 were considered statistically significant.

Results

Ascitic fluid IL-6 levels were higher in patients with malignancy-related ascites compared to hepatic ascites (28138 ± 23403 pg/ml vs 13816 ± 15314 pg/ml), but due to the wide scatter of values this did not reach statistical significancy ($p = 0.21$). However during an episode of SBP there was a marked increase in ascitic fluid IL-6 levels (133268 ± 99743 pg/ml, $p = 0.002$ when compared to hepatic and malignant ascites) while plasma values (1637 ± 907 pg/ml vs 542 ± 719 pg/ml, $p = 0.07$) were barely influenced by this sharp rise in ascitic fluid levels (fig 1). In 2 patients ascitic fluid levels were assessed after antibiotic treatment and declining values were obtained, finally similar to other patients with hepatic ascites without infection. The type of microorganism causing the ascitic fluid infection (*Escherichia coli* 2x, *Klebsiella pneumoniae* 1x, *Staph. Epidermidis* 1x, and culture-negative 1x) did not seem to influence the IL-6 levels. TNF-α levels were not statisticaly different between the patients with hepatic ascites and those with malignant ascites. Ascitic TNF-α 19 ± 50 pg/ml vs 12 ± 31 pg/ml and plasma TNF-α 3.4 ± 8.2 vs 6.1 ± 13.8 pg/ml. As TNF-α was only detectable in plasma in 7 patients and in ascites in 8 patients, no reliable statistical analysis was possible. Even in the patients with bacterial peritonitis and in some terminally ill patients with malignant ascites TNF-α could not be detected in ascites and plasma. IL-1β was not detected in any ascites or plasma samples. None of the parameters could differentiate between patients who survived more than six months and the eleven patients who died during this period. There was a correlation between plasma IL-6 and the Child-Pugh score ($r = 0.675$; $p = 0.002$, fig 2). No relation could be established between the diagnosis of the underlying liver disorder (alcoholic, viral or PBC/PSC) and the cytokine profile (fig 3). Plasma IL-6 levels correlated with serum Ig A levels ($r = 0.649$; $p = 0.004$, Fig 4) and slightly with C reactive protein ($r = 0.49$; $p = 0.04$) but not with Ig M ($r = 0.30$; $p = 0.22$), Ig G ($r = 0.34$; $p = 0.15$), and fibrinogen ($r = -0.17$; $p = 0.53$).

Discussion

In this study we found evidence for a high production of IL-6 locally produced within the peritoneal cavity. The ascitic fluid levels were 30 times higher than plasma levels for hepatic as well as for malignant disease. Apparently, even the presence of sterile ascites generates enough inflammation of the peritoneum with an increase of locally active

monocytes and macrophages to cause these high levels of cytokines. Hepatic clearances of locally generated cytokines may be diminished in patients with cirrhosis, however this mechanism does not seem to be of much importance, as malignant ascites with an intact liver function and normal hepatic clearance generates the same high ascites/plasma ratio's. More evidence for the local production within the peritoneal cavity comes also from the fact that during bacterial inflammation of the ascitic fluid ascites levels are about ten times higher, with an ascites/plasma ratio of about 60, compared to ascitic fluid levels without infection. Ascites dynamics in patients with cirrhosis suggests that there is a continual shift between ascitic fluid and the circulation with peritoneal free-water clearances of 4-5 l/hour (15) and a high lymphe flow within the thoracic duct rich in protein and albumin content (16,17). Therefore the IL-6 is quickly cleared from the circulation as plasma levels are low and not very much influenced by the presence of bacterial peritonitis. Indeed the half-life of IL-6 has been determined to be only a few minutes (3).

High IL-6 synovial fluid levels of patients with rheumatoid arthritis with little activity in serum have been demonstrated (18) as well as high cerebrospinal fluid levels in patients with acute meningitis, but not in those with chronic or non-infectious neural disease (3). Local production of IL-6 in acute and chronic liver disease has also been demonstrated by endothelial, Kuppfer, and infiltrating mononuclear cells in liver tissue, expression being more pronounced in areas with most inflammation (19,20). Many of the activities of TNF-α, IL-1, and IL-6 indicate a local rather than a systemic function for these cytokines. IL-6 shares many of its biological activities with TNF-α and IL-1, and probably all three cytokines act in concert directing inflammatory and immunological reaction. For example, IL-1 and TNF-α can stimulate IL-6 production, but IL-6 suppresses TNF-α and IL-1β production (21). The fact that we did not find elevated TNF-α levels in plasma and ascites of most patients does not seem to concur with other studies. Most studies describe an increase in ascitic fluid levels during bacterial inflammation of both IL-6 and TNF-α (6-8). Several explanations could be given for this discrepancy. First of all there might be a difference in the methodology measuring TNF-α (22). For example Propst et al. (6) used an enzyme amplified immunoassay (EASIA) which might detect lower levels of TNF-α than the assay we used. Zeni et al. (7) used a competitive inhibition radioimmunoassay with the use of coated monoclonal antibodies to TNF-α, fixed to the inner plastic surface of the tubes. Also Devière et al. (8) made use of an immunoradiometric assay to measure TNF-α. Another explanation for the undetectable levels of TNF-α levels might be sought in the presence of high concentration of soluble TNF receptors in ascites, which might be a

potential source of interference for immunoassays (23,24). Van Zee et al. (25) found a reduction of TNF-α immunoactivity, as determined by ELISA, when soluble type I and type II TNF-α receptors were added to plasma. The low concentration of IL-1 in ascites, even during periods of acute bacterial inflammation, was also confirmed in other studies (6,7). Plasma IL-6 levels correlated with the severity of the underlying liver disease, indicated by the Child-Pugh score, but not with the etiology of the cirrhosis. This stage dependency suggests that porto-systemic shunting or impaired hepatic clearance may also contribute to the sustained elevation of endogenous cytokines. Bacterial lipopolysaccharide-induced macrophage stimulation due to prolonged endotoxaemia in patients with chronic liver disease could be responsible for activation of the cytokine cascade. A correlation between serum Ig A levels and IL-6 concentration has been described before (26) and seems to represent increased B cell maturation directly caused by IL-6. Why this correlation has repeatedly been found only for Ig A levels and not for Ig M and Ig G levels is not entirely clear. It might be that B-cells producing immunoglobulin Ig A are simply more responsive to the stimulatory effect of IL-6. In our findings there is no evidence that this effect was restricted to patients with alcoholic liver disease.

What is the potential pathogenetic role of the cytokine imbalance in patients with cirrhosis? Although locally produced IL-6 might provide stimulatory signals for the hepatocytes, resulting in liver regeneration, excess of cytokines could lead to tissue damage and fibrosis (27). Prolonged increased levels of IL-6 and TNF-α after septicaemia have been found in cirrhotics and there is some evidence suggesting that increased cytokine levels makes the host more susceptable to endotoxaemia (13). This may be related to the poor prognosis of infectious complications during cirrhosis (14). Furthermore, it does seem possible that the very high concentrations of IL-6 locally produced within the peritoneal cavity during bacterial peritonitis, is contributing to the decompensation of liver disease often found in patients with SBP.

Finally, we found evidence for high IL-6 production in the ascites in patients with and without cirrhosis. Plasma IL-6 levels in patients with cirrhosis are stage-dependant but do not correlate with the etiology. Due to the broad scatter of values in plasma as well in ascitic fluid, IL-6 can not be used to differentiate between different etiologies of ascites in the individual patient. The exact effects of IL-6, whether beneficial or harmful to the patient are at present not well understood, therefore it is too early to speculate on the therapeutic use of IL-6 antagonists, for example in patients with recurrent bacterial infection of the ascitic fluid.

Table I
Clinical and laboratory characteristics of the patients (Mean ± SD).

	Hepatic ascites group 1	Malignant ascites group 2	Bacterial peritonitis group 3
Number of patients	28	7	5
Age yrs (mean ± SD)	54 ± 15	58 ± 14	42 ± 17
Gender M/F	17/11	6/1	2/3
Child-Pugh B/C	13/15		2/3
Serum bilirubine µmol/l	84 ± 101	47 ± 87	122 ± 156[a]
Ascites leucocytes 10^9 cells/l	0.1 ± 0.1	0.7 ± 0.7[b]	8.8 ± 6.5
Ascites LDH U/l	80 ± 61[c]	295 ± 217	274 ± 253
Ascites total protein g/l	10 ± 7	36 ± 9[d]	11 ± 6
Ascites cholesterol mmol/l	0.5 ± 0.4	1.9 ± 0.9[e]	0.4 ± 0.4
Ascites triglycerides g/l	0.4 ± 0.4	0.8 ± 0.3	1.1 ± 1.8
Plasma IL-6 pg/ml	542 ± 719	559 ± 604	1637 ± 907
Ascites IL-6 pg/ml	13816 ± 15314	28138 ± 23403	133268 ± 99743[f]
Ascites/Plasma IL-6 ratio	29.2	30.3	56.7

[a] : p = 0.01 group 3 versus group 1 and 2
[b] : p = 0.002 group 2 versus group 1 and 3
[c] : p = 0.002 group 1 versus group 2 and 3
[d] : p = 0.004 group 2 versus group 1 and 3
[e] : p = 0.004 group 2 versus group 1 and 3
[f] : p = 0.002 group 3 versus group 1 and 2

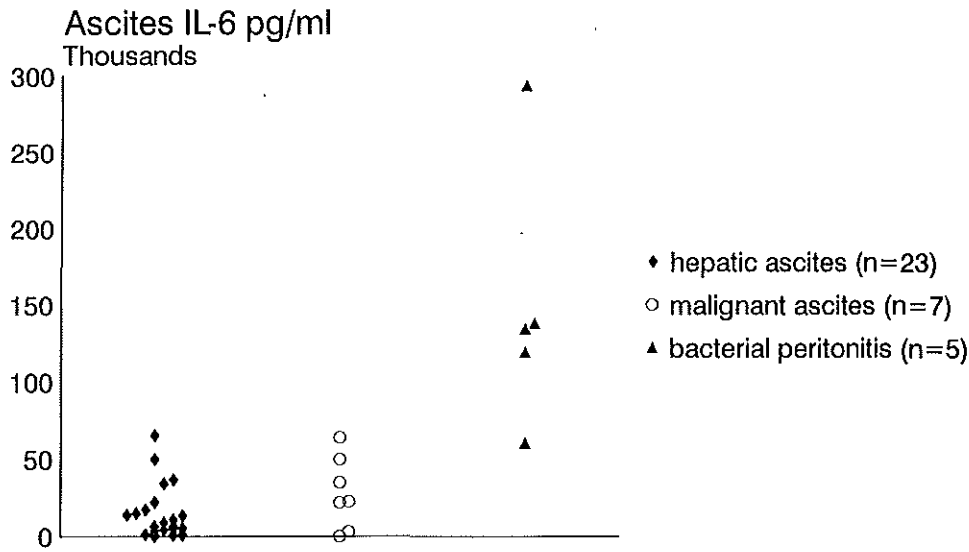
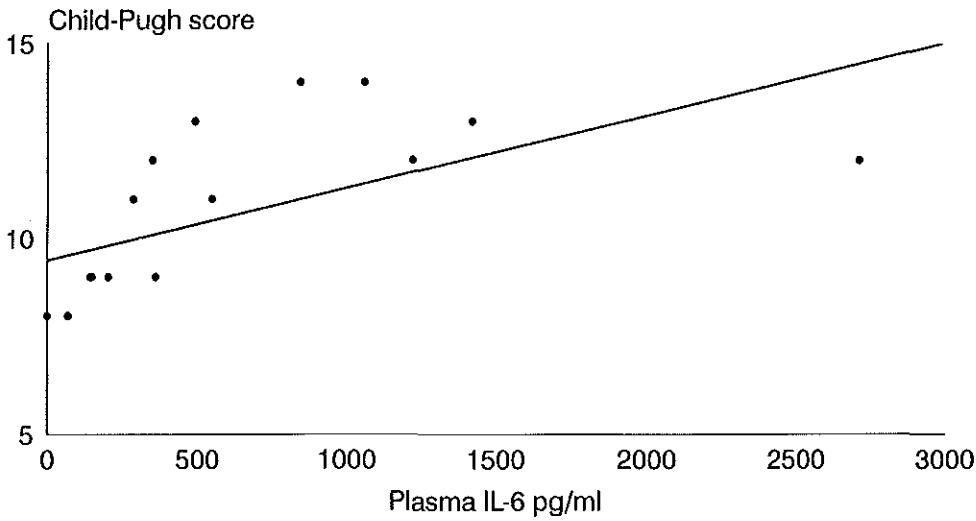


Figure 1: Ascitic fluid IL-6 levels in hepatic ascites, malignant ascites and bacterial peritonitis



r=0.675; p=0.002

Figure 2: Correlation between the Child-Pugh score and plasma IL-6 levels in patients with cirrhosis.

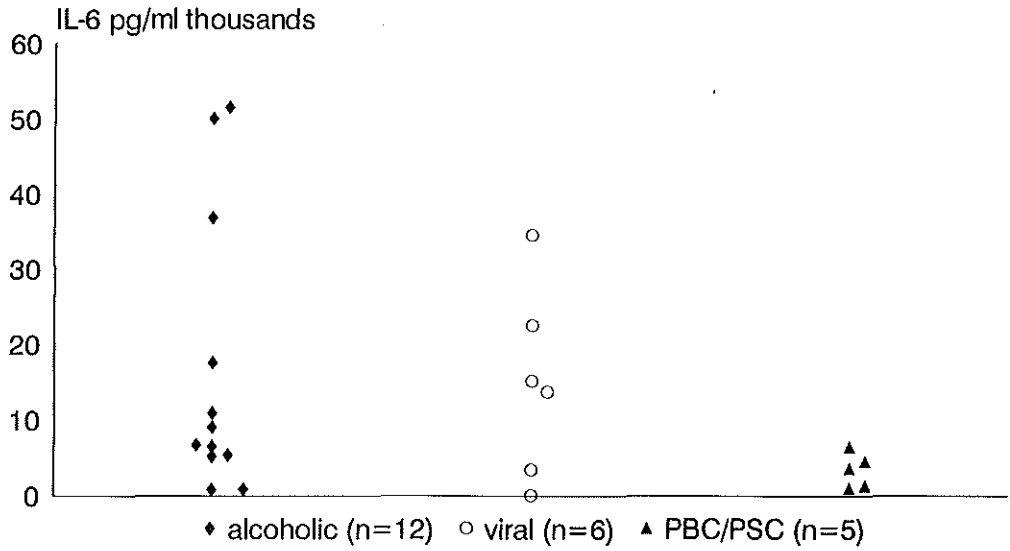
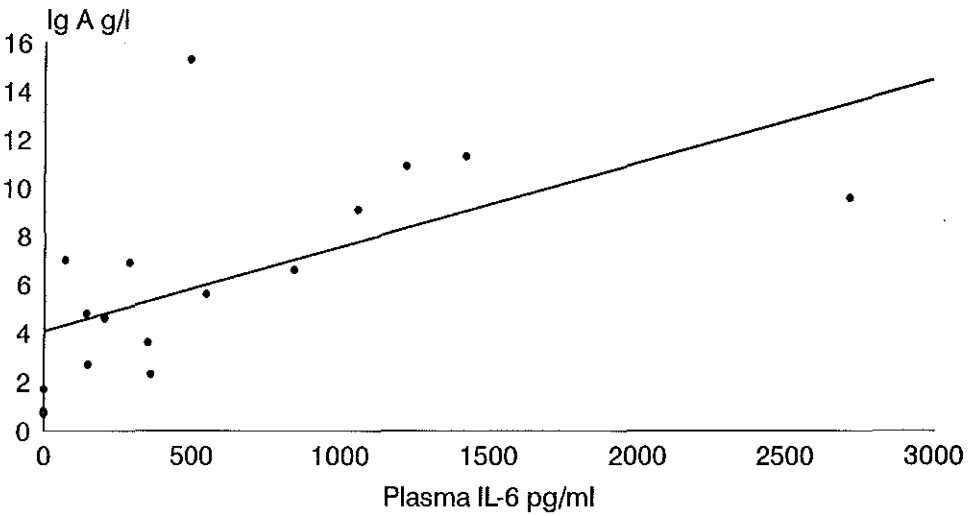


Figure 3: Ascitic fluid IL-6 levels in patients with alcoholic, viral, or primary biliary cirrhosis (PBC) and sclerosing cholangitis (PSC)



r=0649; p=0.004

Figure 4: Correlation between plasma IL-6 levels and IgA concentration.

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CHAPTER 6

SMALL BOWEL WALL FUNCTION IN PATIENTS WITH ADVANCED LIVER CIRRHOSIS AND PORTAL HYPERTENSION:

Studies on permeability and luminal bacterial overgrowth.

The contents of this chapter have been published under the same title in Eur J Gastroenterol Hepatol 1993; 5: 383-387 by the following authors: D.J. Bac, G.R. Swart, J.W.O. van den Berg and J.H.P. Wilson.

Summary

Objective: Changes in small bowel wall function could contribute to the complications of cirrhosis including malnutrition, infection and encephalopathy. To evaluate small bowel function, we studied intestinal permeability and luminal bacterial overgrowth in patients with cirrhosis and portal hypertension.

Design: The ^{14}C-glycocholic acid breath test was used to evaluate the presence of bacterial overgrowth and urine excretion of orally ingested ^{51}Cr-EDTA was used to measure intestinal permeability. Intestinal clearance of α-1-antitrypsine and faecal blood loss were also measured.

Setting: Gastroenterology and hepatology unit of a university hospital.

Patients: A total of 18 patients were studied.

Results: No evidence of small intestinal bacterial overgrowth or increased permeability of the small bowel was found. However, 28% of the patients had increased faecal blood loss.

Conclusions: Although the number of patients investigated was small, our data suggest that small bowel function is maintained to a large extent in patients with advanced liver cirrhosis and portal hypertension.

Introduction

Little information is available on small bowel function in patients with cirrhosis of the liver and portal hypertension. Indirect evidence suggests that portal hypertension might lead to changes in motility and in permeability of the small intestine. Bacterial overgrowth has been reported in liver cirrhosis [1-4], and it is possible that this might be related to changes in motor function [5,6]. The increased prevalence of endotoxaemia in alcoholic cirrhois has been suggested to be due to an increased gut permeability [7,8]; increased permeability may be an important factor in spontaneous bacterial peritonitis of cirrhosis [9]. Damage to the epithelial barrier could also result in leakage of plasma, and some conflicting reports on protein losing enteropathy in cirrhosis and ascites have been presented [10,11]. As changes in small bowel function could be a contributing factor to the complications of cirrhosis including malnutrition, infection and encephalopathy, we performed a small study in patients with cirrhosis and evidence of chronic portal hypertension to examine the following aspects of small bowel function:

(1) the occurrence of small intestinal bacterial overgrowth

(2) the permeability of the mucosal barrier documented by the transport of ^{51}Cr-EDTA from lumen to blood

(3) the permeability of the mucosal barrier as judged from the leakage of α-1-antitrypsin from the plasma into the lumen.

Subjects and methods

Patients

Eigtheen patients were included for the various tests, (5 women and 13 men). Eight patients (Table 1) gave informed consent for the ^{14}C glycocholic acid breath test and the ^{51}Cr-EDTA permeability study. From 10 other patients only faeces and serum analysis was done as described below. The diagnoses and the Child-Pugh score is given in Table 2 (class A, n = 2; class B, n = 12; class C, n = 4). All patients had histologically confirmed cirrhosis of the liver with chronic portal hypertension and oesophageal varices, splenomegaly, and/or ascites. They had no history of any other disease which could cause small bowel bacterial overgrowth such as gastrointestinal surgery, diabetes, intestinal motility disorders, inflammatory bowel disease or coeliac disease. Patients were excluded if they had used antibiotics, non-steroidal anti-inflammatory drugs or alcohol in the 2 weeks previous to the investigation, as these factors have been reported to influence small bowel permeability or bacterial overgrowth or both. The use of other medications such as diuretics and H_2 antagonists was continued.

Methods

Bacterial overgrowth was tested indirectly by the ^{14}C-glycocholic acid breath test, by screening for steatorrhoea and by measuring vitamine B_{12} levels in blood. Fasting patients were given 5μCi ^{14}C-glycocholic acid with a light breakfast. Breath samples were taken every 30 min. for 5 hours and each time 2 mmol of CO_2 was collected in hyamine for analysis of $^{14}CO_2$. A $^{14}CO_2$ concentration above $1{,}5 \times 10^{-5}$ of the administered dose/mmol CO_2, is considered evidence for bacterial overgrowth [12].

Small bowel permeability was measured using ^{51}Cr-EDTA as an indicator. For this test, fasting subjects were given 10 ml of ^{51}Cr-EDTA solution containing approximately 100 μCi (3.7 MBq), followed by 300 ml of water at 8.00 am. They fasted for a further 2 h and then they were permitted normal food and fluid intake.

Urine was collected every 3 h for the first 12 h, followed by a further 12-h collection. Urine volumes from each collection period were measured and the urine was subsequently counted for ^{51}Cr in a multichannel analyser with a solid state probe (Canberra instruments, Meriden, Connecticutt, USA). The urine portions were normalised to the same volume to

circumvent geometric problems during counting. The urinary recoveries of the orally administered ^{51}Cr-EDTA were calculated as a percentage of the dose given. Cumulative urine recoveries of ^{51}Cr -labeled EDTA exceeding 2,5% of the orally ingested dose were considered abnormal [13-18]. Intestinal clearance of serum α-1-antitrypsin (A1AT) was used as a measure of plasma protein loss into the gut. Alpha-1-antitrypsin was measured in 24-h stool collection and in a serum specimen collected during this time. The stool was weighed (wet weight) and homogenized, and an aliquot was centrifuged at 1500 x g for 30 min. Alpha-1-antitrypsin was measured in the supernatant by radial immunodiffusion (RID) (Behringwerke,Marburg/Lahn, Germany) and the clearance calculated.

The upper limit of normal is 13 ml/day in subjects without diarrhea and without fecal blood loss into the gastrointestinal tract [19-23]. Stools were collected for 3 consecutive days and in each collection A1AT was determined; mean values are presented as the A1AT concentration per 24 h. On the same stool samples fecal blood loss was assessed by using a modified Haemoquant assay [24]. Results (the mean of 3 days) are expressed in μl blood/g dry faeces (upper limit for the normal population is 83 μl blood/g dry faeces) [24].

Fat excretion in stools was assessed by a quantitative assay performed on a sample of a 24 h stool collection.

In addition to routine blood examination, vitamin B_{12}, folate, immunoglobulin G, cholesterol and triglycerides levels were measured.

Statistical analysis of data was performed using the computer program "Stata" (statistical/data analysis, Computing Resource Centre, LA, USA).

Results

None of the 8 patients under going ^{14}C bile acid breath test had a level exceeding the upper level of normal in the first 4 h, indicating the absence of small bowel bacterial overgrowth . The mean excretion was 0.38×10^{-5} of the dose/mmol CO_2; range 0.06-1.50×10^{-5}. (upper level of normal: $1,5 \times 10^{-5}$ of the dose/mmol CO_2)

In the same 8 patients the ^{51}Cr-EDTA permeability study showed a cumulative 24-h urine excretion of 1.48% ; SD $\pm$ 0.49% (range 0.78-2.16%) of the orally administrated dose ^{51}Cr-EDTA (normal $<$ 2.5%). The excretion was highest in the urine collection in the time period following 6-9 h after the intake. This is somewhat later than that found by other authors who described a maximal excretion in the period 3-6 h after ingestion of the ^{51}Cr-EDTA [14,16]. In three of these patients urine was collected untill 72 h after ^{51}Cr-EDTA

ingestion; no further excess urinary excretion of ^{51}Cr was seen after the first 24 h of observation, making temporary loss of the ^{51}Cr label into the ascites compartment unlikely. The intestinal clearance of A1AT could be determined in 16 patients (Table 1) (Two patients were excluded because of incomplete stool collection). The mean A1AT clearance was 10.2 ml plasma per day (range 1,9-61,8 ml/day). Only two patients were above the normal upper limit of 13,0 ml/day (no. 2 and 11). Patient 11 was found to have intestinal blood loss due to angiodysplastic lesions in the stomach. When omitting this patient the mean clearance is 6,8 ml/day with a range of 1,9-15,6 ml/day.

Fecal blood loss measurements showed that five patients (28%) had blood loss above the normal level (Table 1). Four of these were just above the normal level and 1 patient (number 11) with known vascular anomalies of the stomach mucosa had a strongly positive test (692 μl blood/g dry faeces). The mean fecal blood loss excluding this last patient was 56 μl blood/g dry faeces with a range from 6-98 μl blood/g dry faeces (normal < 83 μl blood/g dry faeces).

Fat excretion in a 24-h stool sample was above normal in three out of 16 patients in whom a proper stool collection was performed (upper level of normal 10 g fat/24 h). Mean fat excretion was 6,25 g/24 h with a range from 1,1 -21,0 g/24 h. Vitamin B_{12} levels were normal in all patients including those with steatorrhoea. IgG levels were raised (normal < 18 g/l) in 13 out of 18 patients, (mean 21,6 g/l; range 8,9 - 34,2 g/l). Folic acid levels were below normal values (7 ng/ml) in five out of 18 patients (Table 2). Statistical analysis did not show any significant correlation between the Child-Pugh score and the A1AT clearance ($r = 0.0070$, $p = 0.74$), the Child-Pugh score and steatorrhoea ($r = 0.022$, - $p = 0.55$), and steatorrhoea and A1AT clearance ($r = 0.0013$, $p = 0.88$).

Discussion

Although changes in the gastric mucosa in patients with chronic portal hypertension have received much attention, little is known about the effects of portal hypertension on the more distal part of the intestinal tract. Some authors have found an increased prevalence of endotoxemia in cirrhotics with ascites and/or varices and suggested that this could be due to an enhanced permeability to macromolecules of the mucosa in the upper small intestine [7,8], possibly in combination with the reported small intestinal bacterial overgrowth in patients with liver cirrhosis and chronic alcohol abuse [2,4,25].

Therefore, we studied small bowel bacterial overgrowth in patients with liver cirrhosis and

chronic portal hypertension, together with changes in the intestinal wall permeability to macromolecules shown by ^{51}Cr-EDTA excretion.

We used an indirect method, the bile acid breath test, to demonstrate bacterial overgrowth [26]. The sensitivity of the ^{14}C-bile acid breath test to detect bacterial overgrowth is about 70% [27] and it seems unlikely that lack of sensitivity is the cause of the negative test results in all eight patients. The absence of bacterial overgrowth is also supported by the normal serum levels of vitamin B_{12}, as malabsorption of vitamine B_{12} due to binding of the vitamin to the bacteria in the small bowel has been described [26]. The use of ^{51}Cr-EDTA (MW 358Da) as a relatively large (radius = 0.63 nm) hydrophilic probe to test intestinal integrity has proven to be useful in several other conditions. The permeation pathway is thougt to be through larger intercellular "channels". This is different from the "aqueous pores" through which molecules of low molecular weight such as monosaccharides and mannitol are thought to be absorbed by diffusion. Using this hydrophilic probe we were unable to demonstrate an increased permeability in the patients. We were also unable to demonstrate a significant "leak" from blood to intestinal lumen using A1AT clearance. Because the molecular weight of A1AT (54.000 daltons) is comparable to the molecular weight of albumin (61.000 daltons), excretion of A1AT in stool can serve as a measure of plasma protein leakage into the intestinal lumen [19,23]. Due to its antiproteolytic activity, A1AT that leaks into the intestinal lumen is minimally digested by intestinal proteases and therefore, mainly excreted as an intact molecule in stool. The intestinal clearance of A1AT is closely correlated to the intestinal clearance of serum albumin [20-22].

We were unable to demonstrate an increased plasma protein loss into the gut lumen in our patients. Our findings are in line with those of Iber [11] who could not demonstrate significant protein-losing enteropathy in cirrhotics using I^{131} labeled serum albumin.

A remarkable finding in this study is that 28% of the patients with liver cirrhosis and portal hypertension had increased faecal blood loss.

Increased blood loss from the stomach has been described before in those patients with portal hypertension and can probably be attributed to abnormal mucosal capillaries and venous ectasia as seen in congestive gastropathy.

Using indirect methods we have failed to find evidence of small intestinal bacterial overgrowth or increased permeability of the small bowel. Although the number of patients investigated is small, our data suggest that small bowel function is maintained to a large extent in patients with advanced liver cirrhosis and chronic portal hypertension.

Table 1 : Analysis of urine and faecal samples.

Patient no	^{51}Cr-EDTA test (%)	Faecal haem (µl/g)	A1AT clearance (ml/24h)	Fat (gr/24h)
1 (M 34)	1.02	24	7,0	21,0
2 (M 59)		6	15,6	5,3
3 (F 62)	1.42	80	5,3	1,7
4 (M 50)	2.16	49	8,5	1,1
5 (F 59)	1.01	64	8,7	7,9
6 (F 51)		45	2,1	3,1
7 (M 62)		85	9,4	2,3
8 (M 63)	0.78	79	=	=
9 (F 41)		85	3,2	2,8
10 (M 27)	1.76	24	1,9	5,0
11 (M 53)		692	61,8	3,7
12 (M 50)		35	9,1	10,0
13 (M 56)		37	2,1	4,1
14 (M 54)		23	11,6	6,2
15 (M 70)		98	3,3	14,8
16 (F 53)	2.07	67	12,3	3,0
17 (M 72)		68	2,0	8,0
18 (M 31)	1.25	94	=	=
normal values:	<2.5	<83	<13.0	<10,0

M:male, F:female, EDTA: ethylenediaminetetraacetic acid, percentage of the orally administered dose, A1AT: α-1-antitrypsin, Gender and age (years) given in brackets.

Table 2; Clinical and biochemical characteristics of the patients studied.

Patient no	Diagnosis	Child-Pugh score	Ascites	Albumin (g/l)	Ig G (g/l)	Serum folic acid levels (ng/ml)
1 (M 34)	AC	C 10	-	22	30,5	10,0
2 (M 59)	AC	C 14	+	20	31,5	12,8
3 (F 62)	AC	B 8	+	45	24,6	10,1
4 (M 50)	AC	A 6	+	38	12,9	6,1
5 (F 59)	AC	B 8	-	30	29,9	6,8
6 (F 51)	AC	B 8	+	34	19,5	10,2
7 (M 62)	AC	C 10	+	29	17,0	12,9
8 (M 63)	AC	C 11	+	34	25,0	2,6
9 (F 41)	CC	B 8	+	38	8,9	11,3
10 (M 27)	CC	A 5	-	42	20,1	10,4
11 (M 53)	CC	B 7	+	33	19,8	10,8
12 (M 60)	PVC	B 8	+	33	34,2	20,0
13 (M 56)	PVC	B 9	+	37	20,0	12,8
14 (M 64)	PVC	B 8	+	34	27,9	15,6
15 (M 70)	PVC	B 9	+	32	8,9	11,2
16 (F 53)	PBC	B 9	+	28	18,0	7,0
17 (M 72)	PBC	B 9	+	24	20,0	6,8
18 (M 31)	PSC	B 8	+	31	20,4	2,3

AC: alcoholic cirrhosis, CC: cryptogenic cirrhosis, PVC: post viral cirrhosis, PSC: primary sclerosing cholangitis, PBC: primary biliary cirrhosis, F:female, M:male, Ig: immunoglobuline, Gender and age (years) given in brackets.

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CHAPTER 7

SPONTANEOUS BACTERIAL PERITONITIS; OUTCOME AND PREDICTIVE FACTORS.

The contents of this chapter have been published under the same title in Eur J Gastroenterol Hepatol 1993; 5: 635-640 by the following authors: D.J. Bac, P.D. Siersema, P.G.H. Mulder, S. de Marie and J.H.P. Wilson.

Summary

Objective : To identify patients who were most at risk of developing spontaneous bacterial peritonitis and who might benefit from selective intestinal decontamination.

Design : prospective analysis of all patients admitted with liver cirrhosis during a 4-year period who had ascitic fluid analysis and appropriate bed-side inoculation of ascites in blood culture bottles.

Setting : Liver transplantation centre in a university hospital.

Patients : Thirty-one positive episodes and 92 negative episodes for spontaneous bacterial peritonitis were analyzed and compared in 83 patients.

Results : The 1-year survival after spontaneous bacterial peritonitis was 14% compared to 43% in the group without spontaneous bacterial peritonitis. The calculated recurrence rate was 20%. The Child-Pugh score and coagulation parameters were identified as predictive factors for the development of spontaneous bacterial peritonitis, as was a deterioration in values of liver function tests. The total ascitic fluid protein content was not predictive. Sclerotherapy for oesophageal varices may be a precipitating factor, but this requires further investigation.

Conclusions : On the basis of our results we recommend the use of selective intestinal decontamination for patients with liver cirrhosis Child-Pugh class C and ascites who develop rapidly progressive deterioration of liver function tests, patients who survived a first episode of spontaneous bacterial peritonitis and those awaiting liver transplantation.

Introduction

Spontaneous bacterial peritonitis (SBP) is a serious complication in patients with cirrhosis and ascites with an incidence of about 7-23% in patients admitted to hospital [1-3] and a reported mortality between 37% and 78%, older studies reporting a higher mortality than the more recent ones [2,4,5]. The 1-year survival following SBP has been estimated to be about 30% and the frequency of a recurrence within 1 year after a first episode of SBP varies between 11% and 69% [2,4-7].Spontaneous bacterial peritonitis is diagnosed when (a) pathogenic bacteria are cultured from ascites. (b) the ascitic polymorphonuclear (PMN) count is $>0.25x10^9$ cells/l and (c) there is no evident intra-abdominal source of infection [8]. Because of the low number of microorganisms in the ascitic fluid, ascites culture may remain negative although the clinical picture is compatible with SBP and there is an elevated PMN count. This entity is recognized as culture-negative neutrocytic ascites (CNNA) [9] and is defined as: (a) an ascitic fluid PMN count greater than $0.50x10^9$ cells/l, (b) a negative ascitic fluid culture, (c) no administration of antibiotics within 30 days (d) no other explanation for an elevated PMN count in ascitic fluid, such as pancreatitis, intraperitoneal hemorrhage, peritoneal tuberculosis or carcinomatosis. Culture-negative neutrocytic ascites is regarded as a variant of SBP which should be treated with antibiotics [9,10]. Recently it has become clear that the proportion of CNNA in patients suspected of having SBP depends on the culture method used. Bedside inoculation of ascitic fluid into blood culture bottles has been found to be superior to conventional culture methods [11-14].

As most microorganisms causing SBP are gut-derived aerobic Gram-negative bacilli, selective intestinal decontamination, eliminating Gram-negative aerobic intestinal flora has been proposed for the prophylaxis of SBP [5,15]. The aim of this study was to identify in a population of cirrhotic patients, those patients most at risk of developing SBP who might benefit from selective intestinal decontamination. Predictive factors for SBP were analyzed, including therapeutic procedures in the period before the development of SBP. In addition the survival rate after an episode of SBP, the long term prognosis and recurrence rate after an episode of SBP were investigated.

Patients and methods

Between July 1987 and April 1991, 123 consecutive abdominal paracenteses were

performed in 83 patients with a diagnosis of liver cirrhosis, ascites and portal hypertension. The indications to perform a paracentesis were (a) suspicion of SBP (b) routine paracentesis on admission or (c) large volume therapeutic paracentesis. Patients were included in this study when ascitic fluid samples were cultured in blood culture bottles as described elsewhere [14]. Ascites was obtained under sterile conditions with or without ultrasound guidance.

The total leucocytes count in ascitic fluid was done by a Sysmex NE 8000^{R} analyzer (TOA Medical Electronics, Kobe Japan). In addition, 10-20 ml of ascites was centrifuged at 2000 rpm for 5 min, the pellet stained with Giemsa stain, and at least 50 cells were examined to determine the percentage of PMN cells.

Total protein, albumin, lactate dehydrogenenase (LDH) and amylase levels were determined in ascitic fluid. Serum liver enzymes, renal function tests, leucocyte count and differentiation and the coagulation parameters antithrombin III activity and Normotest were determined by routine methods. The Normotest (Nyegard, Oslo) is a modified prothrombin time in which factor V and fibrinogen are added to the reagent. Thus, the test is only dependent on coagulation factors II,VII and X and has been reported to be a sensitive marker of hepatic synthesis [16]. The serum-ascites albumin gradient (serum albumin minus ascites albumin concentration) was calculated . Bilirubin, albumin, Normotest and creatinine values were determined at the time of the ascitic fluid paracentesis and compared with values obtained 2-4 months previously. If blood or urine cultures were performed on the same day, these results were also recorded.

All patients were classified according to the Child-Pugh score [17]. In most patients, a liver biopsy was performed. The performance of ascitic fluid drainage, fibre-endoscopy or sclerotherapy for oesophageal varices within 2 weeks before paracentesis was recorded. At the time of the paracentesis the clinical picture was assessed including the presence and duration of fever, the presence of abdominal pain, progression of jaundice and the presence and progression of encephalopathy. The severity of hepatic encephalopathy was graded on a scale of 0-4 on the basis of clinical criteria and quantitative EEG analysis [18]. The use of diuretics, antacids and antibiotics was recorded. The follow-up and the number of recurrences were recorded in all patients. Causes of death were classified as either due to liver failure or due to another cause.

Since more than one paracentesis was performed in some patients (123 in 83 patients with at least 14 days between the different episodes), the statistical analysis had to take account of these repeated observations. Testing of the mean difference of various

variables between the group with and without SBP was carried out by univariate logistic regression analysis with random effects. In the analysis, each patient has his own (random) effect, around which the repeated observations within each patient were clustered. In this way, the interdependency in the data was taken into account so that all data could be used while errors of the mean difference between the group with and without SBP are prevented from being underestimated. When comparing mortality between these two groups, the patient is considered the experimental unit and the most recent outcome in each of the 83 patients was taken as the start of the follow-up.

Results

Patient characteristics:

Table 1 summarizes the clinical characteristics of the patients in 123 episodes of SBP when a paracentesis was performed. Ninety-two paracenteses in 65 patients were negative for a diagnosis of SBP because the PMN count in ascitic fluid was $< 0.25 \times 10^9$ cells/l and the ascitic fluid culture was negative (SBP negative). Thirty-one paracenteses, in 29 patients were positive with an ascitic fluid PMN count of $> 0.50 \times 10^9$ cells/l and/or a positive ascitic fluid culture (SBP positve). There were no patients with an ascitic fluid PMN count between $0.25\text{-}0.50 \times 10^9$ cells/l. It is noteworthy that of the patients who used antibiotics at the time of the paracentesis, none were diagnosed as having SBP. Of the patients in both groups, 10% (three out of 31 in the group with SBP and nine out of 92 in the group without SBP) used immunosuppressive drugs, which did not influence the clinical presentation. Eleven patients initially had a negative ascitic fluid culture with a low PMN count (SBP negative) and developed SBP during follow-up (SBP positive).

Biochemical analysis:

The biochemical parameters are summarized in Table 2. Bilirubin, albumin, creatinine and Normotest values were compared with values taken 2-4 months before the paracentesis. This information was available in 101 of 123 (82%) episodes (SBP negative; 71, SBP positive; 30) . There was a statistically (Wilcoxon test) significant increase in bilirubin ($p = 0.005$) and creatinine ($p = 0.009$) values and a statistically significant decrease in albumin ($p = 0.001$) and Normotest ($p = 0.002$) values in both the SBP-negative and the SBP-positive groups. However, the difference was significantly greater in the SBP-positive group. Comparing the SBP-positive group with the SBP-negative group, this reached

statistical significance for the albumin decrease only ($p = 0.011$).

Ascitic fluid analysis

Within the group of positive episodes, there was a difference in leucocyte count and PMN count between culture-positive ascitic fluid and CNNA. The PMN count was higher in culture-positive ascites than in those with CNNA, but this did not reach statistical significance. (PMN count ± SD in ascitic fluid in SBP vs. CNNA: $4.2 \times 10^9 \pm 6.9$ cells/l and $1.2 \times 10^9 \pm 0.7$ cells/l;Mann-Whitney test; $p = 0.9$). Lactate dehydrogenase levels were significantly higher in the SBP-positive group compared with the SBP-negative group. Total protein and albumin concentrations in ascites were similar in the SBP-positive and in the SBP-negative group (Table 3).

Bacteriology

These results have also been described in another study comparing different culture methods [14]. In 25 of the 31 (81%) episodes classified as SBP, bacteria were cultured. Gram-negative bacilli were detected in 15 of the 25 (60%) episodes (*Escherichia coli* in eight, *Klebsiella pneumoniae* in two, *Pseudomonas aeruginosa* in two, *Bacteroides* sp. in two, *Acinetobacter* sp. in one) and Gram-positive cocci in 10 of the 25 (40%) episodes (alpha-hemolytic streptococci in three, *Enterococcus faecalis* in four, *Streptococcus pneumoniae* in one, *Streptococcus bovis* in one, *Staphylococcus epidermidis* in one).

In only two of the positive episodes did the Gram stain reveal bacteria (6%). Blood cultures were obtained during 25 episodes and were positive in nine episodes (36%), culturing the same microorganisms as from the ascitic fluid. In the patient without SBP, blood cultures were taken during 33 episodes and were positive in eight episodes (24%), while ascitic fluid cultures remained negative and the ascitic fluid PMN count was $< 0.25 \times 10^9$ cells/l.

Recurrence and survival analysis

The mean follow up time in the SBP-positive group was 194 days (range 1-1278 days). Twenty-three of the 29 patients in the SBP-positive group died (79%). Nine of these patients died within 2 weeks, most likely due to SBP, although they were treated with antibiotics. Only two patients from the SBP- positive group died due to a cause not related

to their liver disease (8%). Of the six surviving patients, one received a liver transplant and is still alive, two were lost to follow-up and the remaining three are still alive and survived 22, 30 and 42 months, respectively . The 1-year survival in the SBP- positive group was 14%. Of the 65 patients in the SBP- negative group, the mean follow-up was 300 days (range 2-1170 days) and the 1-year survival rate was 43%. Forty out of the 65 patients in the SBP-negative group died, seven were lost to follow up and 18 are still alive. By means of Cox's proportional hazards survival analysis, a hazard ratio of 1.94 ($p=0.017$) was estimated for SBP-positive group with respect to SBP-negative group (fig.1).
Within the SBP-positive group there was no significant difference in survival between those with culture-positive ($n=25$) and those with CNNA fluid (six patients). In four of the 20 patients (20%) in the SBP-positive group who survived the initial period, SBP recurred during follow-up, after 2, 6, 11 and 14 months, respectively.

Discussion

In 29 out of the 31 episodes, a diagnosis of SBP was suspected on clinical grounds because of fever (oral temperature $>37.5^{\circ}C$), abdominal tenderness, worsening of hepatic encephalopathy and/or deterioration of liver function tests (Table 1). A diagnosis of SBP in patients without any symptoms suggesting SBP, was made in only 6% of patients, which contrasts with a reported frequency of up to 33% of cases when a routine diagnostic paracentesis was performed on admission irrespective of clinical features [19]. As in other studies [11-14], ascitic fluid culture results were only positve in 81% of cases with suspected SBP, therefore, the best diagnostic test to discriminate between those with and those without SBP and to decide whom to treat, is the total amount of PMN cells in the ascitic fluid [8]. The Gram stain is rarely helpful and culture results will follow only after some days. A diagnosis of CNNA is usually defined when the PMN count in ascitic fluid is somewhere between $0.25\text{-}0.50\times10^{9}$ cells/l [8,9]. We confirmed the findings of Pelletier et al.[9] who found a lower PMN count in patients with culture-negative suspected bacterial peritonitis compared with those with culture-positive bacterial peritonitis. These authors suggested that this supports the idea that CNNA is a less severe variant of SBP. In our study, however, mortality rates were not statistically significantly different between those with culture-positive and those with culture-negative neutrocytic ascitic fluid, although numbers are small.
Invasive procedures such as fibreoptic endoscopy and ascitic fluid drainage within 2 weeks

did not show a significant relationship with the occurrence of SBP, although sclerotherapy for oesophageal varices within 2 weeks might be a risk factor ($p=0.07$). There are a number of case reports suggesting a relationship between previous sclerotherapy and SBP [20,21], but larger series are required to confirm this.

In evaluating predictive factors to identify patients at risk of developing SBP we found the Child-Pugh classification and the coagulation parameters Normotest and antithrombin III activity to be significantly different between the two groups. The importance of coagulation parameters in predicting those patients at risk has also been described by other authors [4,22, 23]. We found no difference in the total protein and albumin content of ascitic fluid and the blood-ascitic albumin gradient between patients who developed SBP and those who did not, which is in agreement with findings of Silvain et al. with regard to recurrent SBP [24]. Even in the patients with alcoholic liver disease, no such difference could be detected. By contrast, other studies have indicated that patients with a low ascitic fluid total protein concentration, reflecting a low opsonic activity, are more likely to develop SBP [22,23,25]. Runyon reported that cirrhotic patients with ascitic fluid total protein content of less than 10 g/l were 10 times more at risk to develop SBP [26]. However, the specificity of the findings was low (60%), and the predictive value was only 15% for patients with a total protein level < 10 g/l in ascites. In a recent study, the total protein content of ascitic fluid was an inclusion criterium to select patients for a study evaluating the benefit of selective intestinal decontamination in preventing SBP [15]. According to our findings, the ascitic protein content is not a predictive marker for patients at an increased risk for developing SBP.

Over 2-4 months there was a significant deterioration of liver and renal function in all cirrhotic patients admitted to our hospital. The drop in albumin levels was greater in patients who developed SBP in comparison with those who did not. This may reflect a deterioration in hepatic function mediated by endotoxaemia and increased cytokine production in those with SBP [27,28]. Another possibility is that patients with a rapidly progressive hepatic insufficiency, as suggested by decreased coagulation and protein synthesis, are more prone to develop SBP. The prognosis of patients who developed SBP was poor; 30% of patients died within 14 days and the 1-year survival rate was only 14%. Because of the high death rate, despite recovery from a first episode of SBP, a liver transplant should be considered in all patients recovering from SBP. Liver transplantation should be performed within 2 months of an episode of SBP to save more than 50% of the patients, but figures may be different in other centres. We could not confirm the high rate

of recurrence of SBP as described by Tito et al [4] (43% at 6 months and 69% at 1 year). In our population only four of the 20 patients who survived the initial event had a recurrence. An explanation for this difference could be that in our study, cirrhotic patients with more advanced liver disease were included. In patients with end-stage liver disease, complications other than SBP contribute substantially to mortality. This might also explain the higher overall mortality rate in our study.

Because enteric bacteria are the most common causative agents of SBP, it has been suggested that selective intestinal decontamination eliminating the aerobic Gram-negative intestinal flora and preserving the remaining aerobic and anaerobic flora could be useful in its prophylaxis. Two recent placebo-controlled studies have demonstrated the efficacy of oral norfloxacin in the prevention of SBP episodes without attaining a reduction in mortality [5,15]. However, in the study of Ginés et al. [5] patients with advanced liver disease defined by bilirubin (> 170 μmol/l), creatinine (> 200 μmol/l) and prothrombin time (< 25%) were excluded from the study [5]. Also in the study of Soriano et al. [15], 50% of the patients included had liver cirrhosis Child-Pugh class A or B and apparently had less hepatic insufficiency than the patients in our study. It is possible that selective intestinal decontamination with norfloxacin could lead to a decreased mortality in certain sub-groups of patients who are at high risk of developing SBP. According to our results subgroups which have the highest incidence of SBP and, therefore, who might benefit from selective intestinal decontamination, include (1) patients with Child-Pugh class C liver cirrhosis and rapidly progressive deterioration in liver function tests, (2) patients who have survived a first episode of SBP and (3) patients awaiting liver transplantation with Child-Pugh class C liver cirrhosis and ascites. Another group of patients who might benefit from antibiotic prophylaxis are patients with liver cirrhosis and ascites undergoing oesophageal sclerotherapy, but data are lacking at present. Because of the mainly Gram-positive flora in the upper gastrointestinal tract and the potential risk of bacteraemia during manipulation [29,30], antibiotic prophylaxis covering both Gram-positive and Gram-negative bacteria may be more appropriate during sclerotherapy. A category of patients who also might benefit from antibiotic prophylaxis are cirrhotics who present with an upper gastrointestinal hemorrhage [31]. Soriano et al. [15] showed in a prospective randomized study, no reduction of mortality, but a lower incidence of infections when norfloxacin was given during 7 days, mainly due to less bacteraemia and urinary infections in the treated group. More studies are needed in the above defined risk groups to assess the benefits and risks of antibiotic prophylaxis. One should be cautious of the complications of long-term

selective intestinal decontamination, such as bacterial overgrowth in the gut and secondary infection with Gram-positive and norfloxacin-resistant Gram-negative bacteria and with yeasts [32].

Liver transplantation remain to be considered as a definitive solution in patients who were already candidates for transplantation as soon as they survived an episode of SBP.

Table 1 ; Clinical characteristics of patients without (SBP-negative group) and with (SBP-positive group) SBP.

	SBP-negative	SBP + positive	P value
Episodes	92	31	
Patients	65	29	
Male %	74	74	n.s.
Age in years (range)	52 (19-75)	50 (27-74)	n.s.
Aetiology of cirrhosis :			
alcoholic %	43	61	n.s.
viral %	18	13	n.s.
other %	39	26	n.s.
Child-Pugh A/B/C%	1/37/62	0/13/87	0.03
Previous procedure :			
ascitic drainage	2 (2%)	1 (3%)	n.s
sclerotherapy	12 (13%)	9 (29%)	0.076
endoscopy	14 (15%)	4 (13%)	n.s.
Indication paracentesis :			
suspicion	9 (10%)	29 (94%)	<0.001
routine	28 (30%)	1 (3%)	<0.001
volume depletion	55 (60%)	1 (3%)	<0.001
Clinical picture :			
fever >37.5°C	7 (8%)	20 (65%)	<0.001
abdominal pain	9 (10%)	16 (52%)	<0.001
encephalopathy grade 0-4 ± SD	0.9 ± 1.1	1.6 ± 1.2	0.010
worsening encephalopathy	14 (15%)	19 (61%)	<0.001
use of diuretics	72 (78%)	21 (68%)	n.s.
use of antibiotics	21 (23%)	0(0%)	<0.001
use of antacids	37 (40%)	15 (48%)	n.s.

Table 2; Mean serum biochemical parameters of patients without (SBP-negative group) and with (SBP-positive group) SBP. (minimum, maximum values given in brackets)

	SBP-negative	SBP-positive	P value
Bilirubin (μmol/l)	118 (4, 722)	166 (12, 599)	n.s.
Albumin (g/l)	27 (17, 47)	25 (19, 31)	n.s.
Normotest (%)	45 (10, 98)	29 (13, 50)	0.004
Antithrombin III act (U/l)	0.53 (0.08, 1.37)	0.32 (0.11, 0.73)	0.018
Ammonia (mmol/l)	79 (10, 218)	71 (16, 141)	n.s.
Creatinine(μmol/l)	138 (46, 557)	158 (49, 669)	n.s.
Leucocytes(10^9/l)	7.7 (2, 34)	11.3 (3, 30)	0.023
Differences in: (values X2-X1)			
bilirubin	45 (-316, +641)	96 (-23, +486)	n.s.
albumin	-2.9 (-13, +19)	-7.2 (-19, +4)	0.011
Normotest	-6 (-54, +34)	-19 (-49, +3)	n.s.
creatinine	37 (-121, +358)	73 (-47, +664)	n.s.

values X2-X1 : bilirubin, albumin, creatinine and Normotest values at the time of the paracentesis (X2) were compared to values 2-4 months before (X1). ns; not significant.

Table 3; Mean ascitic fluid parameters of patients without (SBP-negative group) and with (SBP-positive group) SBP. (minimum, maximum values given in brackets)

	SBP-negative	SBP-positive	P value
Leucocytes (10^9/l)	0.27 (0.1, 1.5)	4.23 (0.5,27.7)	<0.001
PMN cells (10^9/l)	0.015 (0, 0.2)	3.68 (0.6,26.1)	<0.001
Total protein (g/l)	17.4 (5, 66)	17.5 (6, 52)	n.s.
Albumin (g/l)	9.1 (3, 90)	7.7 (4, 25)	n.s.
Serum ascitic-albumin gradient	18.7 (0, 37)	17.8 (2, 24)	n.s.
Lactate dehydrogenase (U/l)	72 (13, 262)	152 (34, 831)	0.007

PMN; polymorphonuclear. ns; not significant

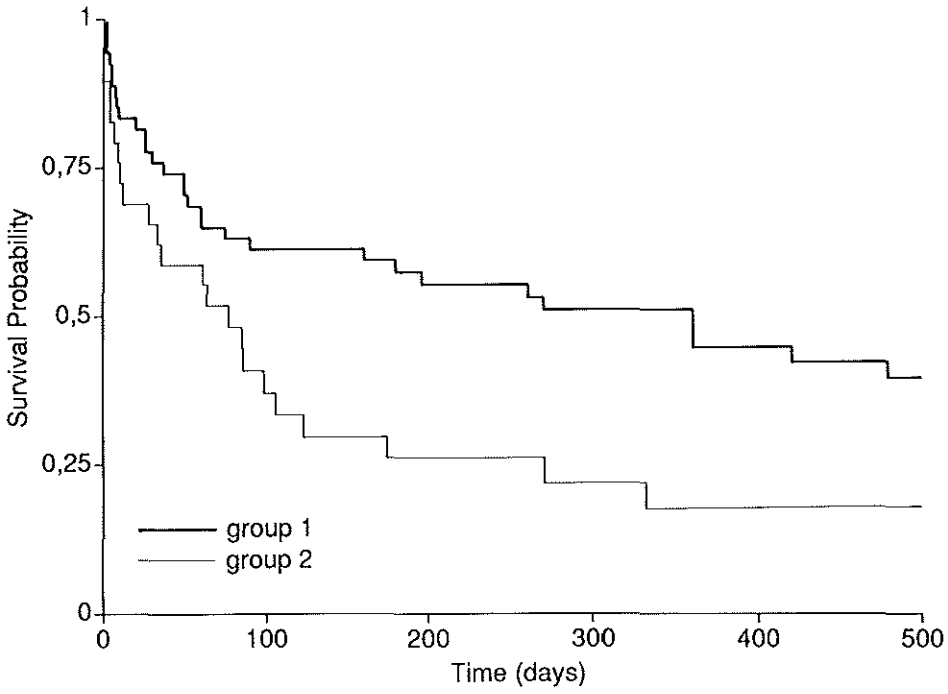

Figure 1
Survival analysis comparing patients without (group 1) and with (group 2) spontaneous bacterial peritonitis.

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CHAPTER 8

POST-SCLEROTHERAPY BACTERIAL PERITONITIS: A COMPLICATION OF SCLEROTHERAPY OR OF VARICEAL BLEEDING ?

The contents of this chapter have been published under the same title in Am J Gastroenterol 1994; 89: 859-862 by the following authors: D.J. Bac, S. de Marie, P.D. Siersema, J. Snobl and H.R. van Buuren.

Summary

To assess the risk of bacterial peritonitis following endoscopic variceal sclerotherapy (EVS), we recorded the incidence of this complication within 2 weeks of the procedure. in all patients (n = 216) undergoing 1092 sclerotherapy sessions in our hospital during a 5-year period (1987-1992). The sclerotherapy sessions were separated in prophylactic EVS (without a previous bleeding, n = 172 sessions), elective EVS (following a previous variceal bleeding, n = 720) and emergency EVS (within 24 hours of a variceal bleeding, n = 200). During the study period, 60 patients with spontaneous bacterial peritonitis were recorded. In 10 patients, peritonitis was diagnosed within 14 days after EVS. Six patients received emergency EVS and 4 elective EVS. In 7 patients Gram-negative aerobic and anaerobic microorganisms were cultured from the ascitic fluid and in 3 patients cultures were negative; however, an elevated ascitic fluid polymorphonuclear cell count of $> 0.5 \times 10^9$cells/L was present. The mean period between EVS and the diagnosis of peritonitis was 3.5 days. On average, the patients had been febrile during 2.1 days before the diagnosis was established. None of the patients who had received prophylactic EVS developed peritonitis. The calculated risk to develop peritonitis following elective EVS was 0.5% (4/742 sessions) and following emergency EVS 3% (6/200 sessions)($p = 0.019$, Fisher's exact test). Gram-negative gut-derived microorganisms were the most common pathogenic bacteria cultured from the ascites, which is different from the microbial flora causing bacteremia after EVS. This suggests that the risk for bacterial peritonitis is determined primarily by factors associated with bleeding, such as shock with increased bowel wall translocation of bacteria. These results indicate that standard antibiotic prophylaxis before EVS is not indicated, but could be considered in patients with liver cirrhosis and ascites receiving emergency EVS.

Introduction

Endoscopic variceal sclerotherapy (EVS) has an established role in the management of active variceal bleeding and in reducing the frequency of rebleeding (1).
Among a large variety of complications of EVS (2-6), a number of infectious complications, including bacteremia (7-14), meninigitis (15), subdural empyema (16), perinephric and cerebral abcesses (16,17), endocarditis (18), and bacterial peritonitis (19-22), have been reported. Although bacteremia, usually asymptomatic and involving commensals from the skin and oropharynx, has been reported to occur in up to 54% after EVS (18), most infectious complications are rare.
EVS was found to be a possible predictive factor for the development of spontaneous bacterial peritonitis by us in a previous study (23); however, the risk of bacterial peritonitis is poorly defined, as only one long-term study has been published (21), reporting a 3 % incidence of bacterial peritonitis within 72 hours of EVS. In nearly all reported patients, peritonitis developed within 48-72 hours of a variceal bleeding episode and acute EVS. This suggests that bacterial peritonitis after EVS may, at least in part, be related not merely to EVS but to the bleeding itself.
The purpose of the current study was to assess the incidence and the causative agents of bacterial peritonitis after EVS in a large group of patients, and to study the risk of this complication in relation to the indication for sclerotherapy (prophylactic and elective versus emergency treatment) and to establish the need for prophylactic measures.

Patients and methods

The records of all patients who underwent EVS from 1987 until 1992 and of all patients in whom bacterial peritonitis was diagnosed during the same period were reviewed. Bacterial peritonitis was diagnosed when bacteria were cultured from the ascitic fluid and the polymorphonuclear white blood cell count was more than 0.25×10^9 cells/L. Patients with another intra-abdominal source of infection were excluded. Patients with an elevated white blood cell count ($> 0.5 \times 10^9$ cells/L) and a negative ascitic fluid culture result were classified as culture-negative-neutrocytic-ascites. During the study period, the clinicians were attentive to the diagnosis of spontaneous bacterial peritonitis, mainly because this was a subject of research in our department. Thus, in all patients with cirrhosis and ascites and signs of possible peritonitis (e.g. fever, abdominal pain, clinical deterioration) there was

in general a low threshold for performing diagnostic paracentesis and ascitic fluid cultures. Bacterial peritonitis diagnosed within 2 weeks of EVS was classified as post-sclerotherapy bacterial peritonitis. An arbitrary period of 2 weeks was chosen because of the likeliness that esophageal ulcerations due to EVS could be a source of bacterial entry and subsequent peritonitis. Details of the endoscopic procedure were recorded, especially as to whether prophylactic (in the context of a clinical trial), elective (long-term prevention of rebleeding) or emergency EVS was performed. Emergency EVS was defined as EVS performed within 24 hours of a variceal bleeding episode. Patients were excluded from the analysis when they had received antibiotics prior to EVS.

Endoscopic sclerotherapy

During the study period, EVS was the standard treatment in our hospital both for active variceal bleeding and for the prevention of rebleeding. Patients received premedication with midazolam (0.075 mg/kg) intravenously and atropine 0.5 mg intramuscular.

Endoscopy was performed with Olympus GIF IT, Q10 or Q20 endoscopes, 25-gauge 4-mm disposable flexible injectors, and aethoxysclerol 2% or ethanolamin oleate 5% as sclerosing agents. Injections were intentionally intravariceal, maximal 2 ml /injection and 40 ml /session. Occasionally, 1 ml boluses of bovine thrombin 1000 NIH units/ml were injected when the bleeding did not respond to injection of sclerosing agents (24).

Ascitic fluid analysis

In 62% of the spontaneous bacterial peritonitis episodes, ascitic fluid was cultured using blood culture bottles with bedside inoculation of ascites into the blood culture bottles (25). In the remaining episodes, conventional culture methods were used. The total leucocyte count in ascitic fluid was counted by a Sysmex NE8000^{R} analyzer. In addition, 10-20 ml of ascites were centrifuged at 2000 rpm for 5 min, the pellet was stained with Giemsa stain, and at least 50 cells were examined to calculate the number of polymorphonuclears. Also, the total protein and albumin content was measured in the ascitic fluid.

Results

During the 5-year study period, 216 patients underwent a total of 1092 treatment sessions. Of these, 200 sessions were performed on an emergency basis and 720 sessions were performed electively. Prophylactic EVS was performed in 33 patients, who received

a total of 172 treatments. During the study period, bacterial peritonitis was diagnosed in 60 patients with liver cirrhosis admitted to our department. In 50 patients, there was no relation with EVS, and the peritonitis was classified as spontaneous bacterial peritonitis. In 10 of the 60 patients, peritonitis developed within 2 weeks of EVS (Table 1), in 6 after emergency EVS, and in 4 after elective EVS. No case of peritonitis was seen after prophylactic EVS. The calculated risk to develop bacterial peritonitis after emergency EVS was 3% (6/200 sessions) and after elective EVS 0.5% (4/720 sessions). The difference between elective and emergency EVS was statistically significant ($p = 0.019$, two sided Fisher's exact test).

The mean period between EVS and the diagnosis of bacterial peritonitis was 3.5 days (range 1-8 days). On average, the patients were febrile (temperature $> 37.5^{\circ}$C) during 2.1 days (range 1-5 days) before the diagnosis was made. In each of these 10 patients, diagnostic paracentesis was performed because of symptoms compatible with bacterial peritonitis, such as fever, abdominal pain, and worsening of encephalopathy. Nine patients were classified as Child-Pugh class C patients and 1 patient as class B. All patients had ascites at the time of the EVS. The results of the biochemical and bacteriological investigations of the ascitic fluid and the blood cultures are summarized in Table 2. In 3 patients, cultures remained negative despite immediate bedside inoculation of ascitic fluid into blood culture bottles. Based on an elevated polymorphonuclear cell count in the ascitic fluid, culture-negative-neutrocytic peritonitis was diagnosed in these patients.

Of the 10 patients with bacterial peritonitis after EVS, 7 died and 3 survived. The survival rate after 30 days and 1 year was 70% and 40 %, respectively. The causes of death of the patients who died within 30 days after bacterial peritonitis was diagnosed, were hepatic insufficiency (1), respiratory insufficiency with ARDS (1), and septicemia (1). The survival rate and the Child-Pugh score did not differ statistically between the groups of patients with post-EVS ($n = 10$) and spontaneous bacterial peritonitis ($n = 50$).

Discussion

Our study shows that patients receiving EVS on an emergency basis carry a significantly higher risk for developing bacterial peritonitis than patients who are being treated electively or prophylactically. This outcome strongly suggests that other factors, such as the clinical condition of the patient at the time of EVS, largely determine the risk of this complication. It is remarkable that the microorganisms cultured from the ascitic fluid in our patients were

different from the bacteria that have been isolated in studies evaluating post-sclerotherapy bacteremia. These bacteria, usually α-hemolytic streptococci, *Staphylococcus epidermidis*, *St. aureus* and diphteroids represent commensals of the skin and oropharynx (Table 3). Most likely, these microorganisms are introduced into the circulation via contamination of the endoscope and disruption of the mucosal barrier with the injection needle. The mainly Gram-negative pathogens isolated in our patients suggest that peritonitis following EVS frequently originates from an intestinal source, which is in agreement with some of the previously reported cases with post-sclerotherapy peritonitis (Table 4). Spontaneous bacterial peritonitis is caused in more than two-thirds of the episodes by Gram-negative aerobic bacteria originating from the gut (26). Therefore, the microorganisms identified in patients with post-sclerotherapy bacterial peritonitis do not seem to be different from the pathogens causing spontaneous peritonitis. It has been recognized that cirrhotic patients with a gastrointestinal hemorrhage carry an increased risk of infections, mainly from aerobic Gram-negative bacteria of intestinal origin (27-29). In addition, hypotensive periods during EVS, caused by sedatives or bleeding episodes, might promote bacterial translocation through the bowel wall and could increase the risk of spontaneous bacterial peritonitis. A recently published paper supports the hypothesis that acute hemorrhage promotes bacterial translocation in portal hypertensive rats (30).

The incidence of bacterial peritonitis following sclerotherapy in our study was comparable with that reported in other studies (3,20,31) and indicates that this complication can be expected to occur in 2-3 % of the patients undergoing non-elective EVS. In fact, the incidence of bacterial peritonitis is surprisingly low, given the immunocompromized state with decreased opsonisation in serum and ascitic fluid documented in most cirrhotics (32). Our data do not support the use of antibiotic prophylaxis in all patients undergoing EVS. In patients with active or recent gastrointestinal bleeding, especially in patients with decompensated cirrhosis, the use of prophylactic antibiotics could be considered before emergency EVS. Moreover, it has been argued that irrespective of diagnostic and therapeutic procedures, such patients may benefit from antibiotic prophylaxis eliminating the Gram-negative aerobic intestinal flora (26,28).

We conclude that bacterial peritonitis after EVS in some patients should be regarded as a complication of the procedure, when commensals of the skin and oropharynx are isolated from the ascitic fluid. However, in most patients reported with post-sclerotherapy peritonitis, the bacteria have an intestinal source, and probably did translocate through the bowel wall. Therapeutic procedures such as EVS could enhance this bacterial migration,

but hypotension and tissue hypoxia due to gastrointestinal blood loss probably contribute more than sclerotherapy itself. The pathogenesis of post-sclerotherapy peritonitis does not seem to be different from that of true spontaneous bacterial peritonitis. Patients with decompensated liver cirrhosis presenting with variceal bleeding are most at risk for developing this serious complication. If antibiotic prophylaxis is considered in this subgroup of patients, it is more important to cover the Gram-negative pathogens than the commensals of the skin and oropharynx, which are usually responsible for the transient bacteremia associated with the sclerotherapy.

Table 1: Clinical characteristics of the patients with bacterial peritonitis following esophageal sclerotherapy

Patient no sex/age	Etiology Cirrhosis	Emergency EVS	Diagnosis BP, Days after EVS	No of days with fever (>37,5°C)
1 F/66	Alc	+	1	1
2 M/51	Alc	+	4	2
3 F/62	PBC	+	1	1
4 M/39	Alc	-	2	1
5 M/67	Alc	-	3	2
6 M/47	Alc	-	3	2
7 M/41	Alc	+	5	3
8 M/39	Alc	-	2	1
9 M/66	HCV	+	8	4
10 M/59	Alc	+	6	5

Alc: alcoholic cirrhosis, PBC: primary biliary cirrhosis, HCV: hepatitis C viral cirrhosis. BP: bacterial peritonitis. EVS: esophageal variceal sclerotherapy

Table 2: Characteristics of culture results in patients with post-sclerotherapy peritonitis

Case	PMN's* 10^9cells/L	Ascites culture	Blood culture
1	0.5	Gram negative rod	Not done
2	1.0	Negative	Negative
3	0.7	*Escherichia coli*	*Klebsiella*
4	0.8	*Bacteroides*	Negative
5	0.6	Negative	*Staph.aureus*
6	15.0	*Bacteroides*	*Bacteroides*
7	2.0	*Pseudomonas*	Negative
8	1.0	*Escherichia coli*	*Escherichia coli*
9	8.2	*Escherichia coli*	Not done
10	0.9	Negative	Not done

* PMN's: Polymorphonuclears in ascitic fluid

Table 3: Summary of prospective studies of bacteremia after sclerotherapy

Study author (ref)	Positive episodes (%)	Gram - bacteria	Gram + bacteria	Anaerobes
Camara[7]	2/40 (5)	1	1	
Sauerbruch[8]	21/40 (53)	5	25	1
Brayko[9]	0/25 (0)			
Snady[10]	4/43 (11)	0	4	
	11/28 (39)	3	11	1
Low[11]	9/104 (9)		9	
Hegnhoj[12]	6/31 (19)		6	
Sethi[13]	8/75 (16)	1	7	
Ho[14]	7/56 (13)	3	3	1
	0/33 (0)			

Table 4: Summary of ascites culture results of cases in the literature of post-sclerotherapy peritonitis

Author (ref)	Case	Cultured microorganism
Lai[19]	1	*Klebsiella Pneumoniae*
Barnett[20]	1	*Streptococcus sanguis*
Tam[21]	1	Enterococcus
	2	Streptococcus group B
	3	*Staphylococcus aureus*
Shembre[22]	1	Negative
	2	*Escherichia coli*
	3	Negative
	4	Gram + coccus
	5	*Escherichia coli*
	6	*Citrobacter freundii*

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CHAPTER 9

SPONTANEOUS BACTERIAL PERITONITIS COMPLICATING MALIGNANCY-RELATED ASCITES. TWO CASE REPORTS AND A REVIEW OF THE LITERATURE.

The contents of this chapter have been submitted for publication to J Gastroenterol and Hepatol under the same title with the following authors: D.J. Bac, S. de Marie and M. van Blankenstein.

Summary

Objective: To study the occurrence of spontaneous bacterial peritonitis complicating ascites due to another etiology than cirrhosis of the liver.

Methods: During a five-year period (1987-1992) all cases with a diagnosis of spontaneous bacterial peritonitis were prospectively identified and analyzed.

Setting: Gastroenterology and hepatology unit in a tertiary referral centre.

Results: In 2 out of the 60 identified cases the spontaneous bacterial peritonitis occurred in malignancy-related ascites.

Both patients developed spontaneous bacterial peritonitis (SBP) in pre-existing ascites. The first patient with diffuse livermetastases of an adenocarcinoma and ascites developed an episode of spontaneous peritonitis, with *Escherichia coli* cultured from the blood and ascitic fluid. The second patient, with peritoneal adenocarcinomatosis and portal vein thrombosis had peritonitis due to *Klebsiella pneumoniae* cultured from the blood and ascitic fluid.

Conclusion: A summary is presented of the rarely reported cases of spontaneous bacterial peritonitis in malignancy-related ascites without cirrhosis of the liver. The presence of portal hypertension is recognized as an important predisposing factor.

Introduction

Spontaneous bacterial peritonitis (SBP) has been well recognized as a severe and common complication in patients with decompensated cirrhosis of the liver, resulting in a high mortality [1,2]. Knowledge about SBP has been accumulating over the last 20 years, leading to earlier diagnosis and treatment [3]. However, most studies on SBP only deal with patients suffering from decompensated cirrhosis, and SBP complicating preexistent ascites due to other etiologies has rarely been reported. We report 2 episodes of SBP in 2 patients without cirrhosis of the liver. A review of the literature is presented of all cases diagnosed as SBP without cirrhosis of the liver and its rarity in malignancy-related ascites is discussed.

Case reports

Patient 1

A 69-year-old Caucasian male was admitted because of poorly regulated non-insulin dependent diabetes mellitus, weight loss and anorexia. On physical examination the patient was cachetic and dehydrated. There was hepatomegaly without splenomegaly and no physically detectable ascites. Laboratory examination showed a raised ESR (66mm/h), an elevated glucose (26 mmol/l) and raised bilirubine 58 µmol/l (n: 2-12 umol/l), gamma glutamyl transpeptidase 332 U/l (n:25-75 U/l), lactate dehydrogenase 556 U/l (n:160-320 U/l). Serum albumin was decreased, 28 g/l (n:36-48 g/l), and the albumin gradient (serum minus ascites) was 22 g/l indicating the presence of portal hypertension. There were impaired coagulation parameters: Prothrombin Time 15,1 sec. (n:9,3-12,3 sec), Normotest 44% (n:65-110%), antithrombine III activity 0,48 E/l (n:0.85-1.20 E/l).

Ultrasonography of the liver showed massive hepatic metastases and ascites. Gastroscopy revealed mild esophagitis without esophageal varices. Histology of an ultrasound guided liver biopsy showed a well-differentiated adenocarcinoma. The surrounding liver tissue showed no histological signs of cirrhosis. No malignant cells were detected on cytological examination of the ascitic fluid.

Three weeks after admission the patient developed rigors with fever and abdominal tenderness. Ascitic fluid and blood cultures grew *Escherichia coli*. Haematological and chemical analysis of the ascitic fluid is shown in Table 1. The patient was treated with antibiotics but died 8 days later. An autopsy was not performed.

Patient 2

A 59-year-old male patient was seen in another hospital because of abdominal pains and weight loss. The patient had insulin-dependent diabetes mellitus since two years. Laboratory examination revealed cholestatic liver functions and an ultrasonography demonstrated an enlarged pancreatic head with calcifications. An ERCP showed a strictured common bile duct and an endoprosthesis was placed. The diagnosis was thought to be chronic pancreatitis with calcifications and an inflammatory reaction in the head of the pancreas.

Four months later the patient presented with increasing weight loss and abdominal pains. Physical examination revealed ascites, without abdominal tenderness. Laboratory examination showed increased cholestasis; bilirubin 11 μmol/l (n:2-12 umol/l), alkaline phosphatase 623 U/l (n: 40-85 U/l), with normal transaminases. Clotting parameters were normal with a decreased albumin level of 30 g/l (n:36-48 g/l). The endoprosthesis was exchanged but found not to be occluded. Ultrasonography showed an increasing mass within the pancreatic head, suggesting malignancy, and evidence of portal vein thrombosis. Thin needle biopsy of the mass in the head of the pancreas failed to confirm malignancy. Two weeks after admission the patient developed fever and a tender abdomen. *Klebsiella pneumoniae* was cultured from the blood and ascitic fluid. The ascitic fluid characteristics are shown in Table 1. The patient was treated for bacterial peritonitis with amoxycillin-clavulanic acid. Liver functions had remained stable during this time and there was no evidence of an occluded endoprosthesis. Because of the progressive ascites, without proof of malignancy, a laparoscopy was performed. The liver appeared normal but there were many small white dots on the peritoneal surface, biopsy of which confirmed peritoneal carcinomatosis due to an adenocarcinoma, most probably from the pancreas. Liver histology was normal. Within six weeks the patient developed progressive esophageal varices, and a bleeding episode which was treated by sclerotherapy. In spite of this the patient died from recurrent variceal bleeding 8 weeks later. An autopsy was not performed.

Discussion

Malignancy-related ascites is a heterogeneous group of conditions with wide variations in ascitic fluid analysis. Within this group peritoneal carcinomatosis, characterized by positive ascitic fluid cytology, is the most frequently reported cause of ascites formation [4]. Massive liver metastases with concomitant portal hypertension is the second most

important group. Two patients are described with a bacterial infection of the malignancy-related ascites. A review of the literature confirms that SBP complicating malignancy-related ascites has only been described in patients who had evidence of portal hypertension. Isner et al [5] described one patient with a gastric adenocarcinoma who developed SBP after chemotherapy. At autopsy 75% of this patient's liver parenchyma had been replaced by tumor. Kurtz et al [6] reviewed more than 100 patients with cytology positive ascitic fluid and found only three patients with bacterial peritonitis. The authors stated that these cases do not represent true SBP because invasive procedures in two patients (indwelling peritoneal catheter and bowel puncture at initial paracentesis) and a nadir sepsis (leucocyte count $< 0{,}3 \times 10^9$ cells/l) in one patient clearly predisposed them to bacterial peritonitis. Runyon [7] described one patient with SBP and cardiac ascites. But also in this patient there was evidence of portal hypertension with splenomegaly and esophageal varices due to liver fibrosis.

Our cases and those reported above do suggest that the presence of portal hypertension, either due to massive hepatic metastases or portal vein thrombosis is a prerequisite for malignancy-related ascites to become infected. Intact antimicrobial activity of the peritoneal fluid and functioning hepatic tissue are important determinants in clearing microorganisms from the ascitic fluid [8]. Comparing cirrhotic ascites with malignant ascites (without portal hypertension), complement concentrations C3 and C4 are about four times higher in the latter group with better opsonic and chemo-attractant activity [9]. The hepatic venous pressure gradient in patients with massive liver metastases has been found to be similar to that quantified in cirrhotic patients [10]. This is confirmed by the presence of a wide (> 11 g/l) serum-ascites albumin gradient (as a manifestation of oncotic-hydrostatic balance) which has been found in patients with malignancy-related ascites and massive hepatic metastases [11,12]. The presence of portal hypertension gives rise to a significantly lower total protein concentration in the ascitic fluid, compared to patients with peritoneal carcinomatosis without portal hypertension [9,11,12]. A low protein concentration in the ascitic fluid reflects decreased complement concentrations and decreased opsonic and bactericidal activity [9,13,14]. This would explain the susceptibility of malignant ascites to the development of SBP in the presence of portal hypertension.

However, also other factors might be involved. Lebrun et al. recently described decreased polymorphonuclear (PMN) function in cirrhotic ascitic fluid [15]. The PMN function improved with serial dilutions or by adding heat-treated malignant ascitic fluid. This suggests that phagocytosis is deficient independantly of the C3 level, and that other

inhibitory intracellular and extracellular factors that modulate PMN function are involved. In conclusion there are only sporadic case reports of SBP not related to cirrhosis of the liver. The presence of portal hypertension due to massive liver metastases or to portal vein thrombosis, appears to predispose patients with malignancy-related ascites to the development of SBP.

Table 1: Ascitic fluid parameters

patient:	1	2
leucocytesx10^9/l	2.8	29,8
polymorphonuclear %	88	90
lymphocytes %	12	10
Lactate dehydrogenase U/l	257	246
amylase U/l	45	220
total protein g/l	19	20
albumin g/l	6	12
serum-ascites albumin gradient	22	18

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Summary

This thesis describes several aspects of **spontaneous bacterial peritonitis** in patients with cirrhosis of the liver and ascites. Most of the data discussed and submitted in this thesis are based on clinical studies during the years 1987-1992 at the Department of Internal Medicine II, University Hospital Rotterdam-Dijkzigt.

Chapter 1. In this chapter definitions are provided for the diagnosis of spontaneous bacterial peritonitis which are to be used in this thesis. A historical background is given of spontaneous bacterial peritonitis, and a review of the literature concerning its prevalence and incidence in the different centers is provided. The pathogenesis of spontaneous bacterial peritonitis is discussed, with special emphasis on host factors, environmental factors and the microorganisms responsible for the peritonitis. Appropriate antibiotic treatment is discussed and the possibility of *selective intestinal decontamination* to prevent recurrences of spontaneous bacterial peritonitis.

Chapter 2. This is a review article concerning the appropriate diagnostic tests to be done on the ascitic fluid when a paracentesis is performed. The superiority of the *albumin gradient (serum albumin-minus ascites albumin concentration)* compared to the traditional "exudate-transudate" concept in clarifying the etiology of the ascites is discussed. A white blood cell count with an accurate differentiation is the single most important test to be done on ascitic fluid.

Chapter 3. Because of the low values of total protein and albumin and the low white cell count in ascitic fluid special reference values are needed in the laboratory for an accurate and precise determination of these values. A reliable and convenient procedure is presented to perform a manual differential when low numbers of white cells are present. The reliablity of the determination of total protein and albumin in ascitic fluid on a routine serum chemistry analyzer is described.

Chapter 4. Three different culture techniques of ascitic fluid are described and compared, to test sensitivity and convenience of each culture method. Inoculation of ascites into blood culture bottles at the bedside of the patient gives the highest diagnostic yield (84% positive cultures, compared to 35% positive cultures when using the conventional culture method and 46% positive cultures when using the lysis-centrifugation tubes). Moreover, the blood culture bottle method also shortened the time needed for the detection of bacterial growth. Gram-negative microorganisms were detected in 65% of the culture-

positive episodes of spontaneous bacterial peritonitis. Gram-positive cocci were cultured in the remaining 35% of the episodes.

Chapter 5. The measurements of cytokines in plasma and ascites could be of diagnostic and prognostic importance, assisting in early diagnosis of infectious complications and in the differential diagnosis of the etiology of ascites. To test this hypothesis, interleukin-6 (IL-6), interleukin 1β (IL-1β), and tumor necrosis factor-α (TNF-α) were measured in ascites and plasma in 8 patients with malignancy-related ascites and in 32 patients with decompensated cirrhosis. Five patients had an episode of bacterial peritonitis during which period one or more ascitic fluid samples were analyzed. It was found that ascitic fluid IL-6 levels are about 30 times higher compared to plasma values, indicating that IL-6 is produced in great quantities within the peritoneal cavity of patients with ascites, irrespective of the etiology of the ascites. During a period of bacterial peritonitis the IL-6 levels, but not the other cytokines, were significantly raised in the ascites, which declined after antibiotic treatment. The importance of locally generated cytokines and the possibility that this relates to the poor prognosis of patients with cirrhosis and infectious complications is discussed.

Chapter 6. In this study the presence of increased intestinal permeability and luminal bacterial overgrowth in the small bowel of patients with cirrhosis and portal hypertension is evaluated. Changes in small bowel function in patients with cirrhosis could contribute to complications, such as malabsorption and infections e.g. spontaneous bacterial peritonitis. Using the ^{14}C-glycocholic acid breath test and measuring the urinary excretion of orally ingested ^{51}Cr-EDTA, and by assessing the leakage of alpha-1-antitrypsine into the intestinal lumen, no evidence was found of small intestinal bacterial overgrowth or of increased permeability of the small bowel in a small number of patients. It is concluded that small bowel function is maintained to a large extent in patients with advanced liver cirrhosis and portal hypertension.

Chapter 7. A comparison is made between 31 positive ascitic fluid samples with 92 negative samples for spontaneous bacterial peritonitis (SBP) in 83 patients. Predictive factors for the development of SBP are the severity of the cirrhosis, as indicated by the Child-Pugh score and coagulation parameters. The prognosis for patients who had an episode of SBP is significantly worse compared to those who had sterile ascites (1 year survival respectively 14% and 43%; $p = 0.017$). The recurrence rate for those who had an episode of SBP was 20%. Because enteric microorganisms are the most common causative agents of SBP, suggestions are made for the use of *selective intestinal*

decontamination in certain subgroups of patients to prevent the occurrence of SBP.

Chapter 8. In this chapter the relationship between endoscopic variceal sclerotherapy (EVS) and "spontaneous" bacterial peritonitis is elaborated on. It was established that the risk to develop infected ascites following sclerotherapy is significantly higher after *emergency EVS* (sclerotherapy within 24 hours of a variceal bleeding) compared to *elective EVS* (respectively 3% vs. 0.5%). Gram-negative gut derived microorganisms were the most common bacteria cultured from the ascites, which is identicle to the bacteria causing SBP unrelated to sclerotherapy, but different from studies reporting on the incidence of bacteremia during sclerotherapy. It is suggested that translocation of Gram-negative microorganisms during active gastrointestinal bleeding is a more important mechanism causing bacterial peritonitis than the act of sclerotherapy itself. Antibiotic prophylaxis could be considered in patients with severe cirrhosis and ascites, presenting with a gastrointestinal bleeding.

Chapter 9. Spontaneous bacterial peritonitis complicating ascites due to another etiology than cirrhosis of the liver has rarely been reported. In 2 of the 60 episodes of SBP identified during a five-year period, the diagnosis of SBP was made in malignancy-related ascites without cirrhosis. One patient had massive liver metastases causing portal hypertension and ascites, and the other patient had peritoneal carcinomatosis with portal vein thrombosis. It is discussed why SBP is rarely described in patients without cirrhosis of the liver. The presence of portal hypertension in both these patients is recognized as an important predisposing factor for the development of SBP.

Conclusion

During the period 1987-1992 sixty episodes of spontaneous bacterial peritonitis were diagnosed in 52 patients. SBP was found to be a serious complication in patients with cirrhosis and ascites with a 1-year survival of 23% (Confidence Interval 17%-35%). Three of the 12 patients who survived more than 1 year had a livertransplant.

Clinical awareness, a lower threshold to perform paracentesis, earlier diagnosis and treatment might have resulted in a slightly improved survival compared to the results described in chapter 7.

Improved culture techniques of the ascitic fluid and more adequate leucocyte count with cell differential will add to a better diagnosis. There is evidence in the literature that the use of selective bowel decontamination in patients at high risk to develop SBP will decrease its incidence, as most causative microorganisms are of enteric origin.

However, an episode of SBP should be regarded as a symptom of hepatic insufficiency and patients who developed an episode of SBP and who are regarded as suitable candidates for a liver transplantation should have this procedure as soon as possible. As such, an episode of SBP should be one of the many factors which are to be included in the complicated decision making process that determines timing and priorization of candidates for transplantation.

Samenvatting

In dit proefschrift worden een aantal aspecten van **spontane bacteriële peritonitis** bij patienten met levercirrose en ascites behandeld. De resultaten die worden besproken zijn verkregen uit klinische studies die verricht werden gedurende de periode 1987-1992 op de afdeling Inwendige Geneeskunde II van het Academisch Ziekenhuis Rotterdam, Dijkzigt.

Hoofdstuk 1. Hierin worden definities besproken zoals die verder in dit proefschrift gehanteerd worden. Een historisch overzicht wordt gegeven betreffende het begrip spontane bacteriële peritonitis, evenals een literatuuroverzicht betreffende incidenties en prevalenties in de verschillende centra. De pathogenese van het ziektebeeld wordt besproken, waarbij de oorzakelijke microorganismen, de gastheer functie en de omgevingsfactoren verantwoordelijk voor het ontstaan van de peritonitis worden toegelicht. De optimale antibiotische behandeling wordt besproken evenals de mogelijkheid van *selectieve darm decontaminatie* ter preventie van spontane bacteriële peritonitis.

Hoofdstuk 2. Dit is een overzichtsartikel betreffende bepalingen in de ascites welke kunnnen plaats vinden bij het verrichten van een diagnostische ascites drainage. De voorkeur voor de *albumine gradiënt (serum albumine minus ascites albumine concentratie)* boven het conventionele "exudaat-transudaat" concept wordt toegelicht. Een telling van het totaal aantal leukocyten met een juiste differentiatie is de belangrijkste bepaling in de ascites.

Hoofdstuk 3. Vanwege de lage concentraties totaal eiwit en albumine, evenals het geringe aantal leucocyten in de ascites, zijn speciale referentie waarden noodzakelijk in het laboratorium voor een adequate en precieze bepaling hiervan. Een bruikbare en betrouwbare methode wordt besproken om de hand-differentiatie te verrichten bij een laag aantal leucocyten in de ascites. De betrouwbaarheid van het totale eiwit gehalte en albumine gehalte bepaling in de ascites met behulp van routine chemie analyse wordt weergegeven.

Hoofdstuk 4. Drie verschillende kweekmethoden van de ascites worden beschreven en met elkaar vergeleken. Inoculatie van ascites in bloedkweekflesjes direct aan het bed bij de patient geeft de hoogste opbrengst (84% positieve kweken, vergeleken met 35% positieve kweken met de conventionele methode en 46% positieve kweken met gebruik van de lysis centrifugatie techniek mbv. Isolator^{R} buizen). Tevens is de periode tot aan de kweekuitslag significant korter bij gebruik van de bloedkweekflesjes. Gram negatieve microorganismen werden gedetecteerd in 65% van de positieve kweken en Gram positieven coccen in de resterende 35% van alle episoden met spontane bacteriële peritonitis.

Hoofdstuk 5. Het bepalen van cytokinen in plasma en ascites zou van diagnostische en prognostische betekenis kunnen zijn, zowel bij het vroegtijdig herkennen van infectieuze complicaties alsook bij de differentiaal diagnose ter bepaling van de etiologie van de ascites. Om deze hypothese te toetsen werden zowel interleukine-6 (IL-6), interleukine 1β (IL-1β) als ook tumor necrosis factor-α (TNF-α) bepaald in plasma en ascites bij 8 patienten met ascites secundair aan een maligniteit, en bij 32 patienten met gedecompenseerde lever cirrose. Vijf patienten maakten een episode van bacteriële peritonitis door waarbij ascites verkregen kon worden voor interleukinen bepalingen. De IL-6 concentraties in ascites waren ongeveer 30 x hoger dan in plasma, passend bij een hoge intraperitoneale productie van IL-6 bij patienten met ascites, onafhankelijk van de etiologie van deze ascites. Gedurende een periode van bacteriële peritonitis was er een sterke stijging in de IL-6 concentratie in de ascites, maar niet in plasma, welke afnam na adequate antibiotische therapie. De fysiologische betekenis van deze hoge lokale productie van cytokinen en de mogelijke relatie met de slechte prognose van infectieuze complicaties bij levercirrose wordt verder besproken.

Hoofdstuk 6. In deze studie wordt de hypothese getoetst dat er sprake is van een toegenomen permeabiliteit en bacteriële overgroei in de dunne darm bij patienten met cirrose en portale hypertensie. Een veranderde darmfunctie bij cirrose zou een bijdragende factor kunnen zijn in het ontstaan van complicaties, zoals ondervoeding en infecties, bijv. spontane bacteriële peritonitis. Met behulp van de ^{14}C-glycocholzuur ademtest, het bepalen van de ^{51}Cr-EDTA excretie in de urine na orale inname, en de alpha-1-antitrypsine klaring in de faeces, kon bij een kleine patienten groep vastgesteld worden dat er geen toegenomen permeabiliteit van de dunne darm noch bacteriële overgroei in de dunne darm aantoonbaar is. De conclusie is dan ook dat bij patienten met levercirrose en portale hypertensie de dunne darm functie grotendeels gespaard blijft.

Hoofdstuk 7. Een vergelijking wordt gemaakt tussen 31 episoden van spontane bacteriële peritonitis met 92 episoden met steriele ascites in totaal 83 patienten. Voorspellende factoren voor de ontwikkeling van SBP waren de ernst van de cirrose uitgedrukt in de Child-Pugh score en de stollings parameters. De prognose van patienten die een episode van SBP hadden doorgemaakt was significant slechter dan van patienten met steriele ascites (1 jaars overleving respectievelijk 14% en 43%; $p=0.017$). De recidief kans voor patienten die een episode van SBP hadden doorgemaakt was 20%. Aangezien gram negatieve microorganismen afkomstig vanuit de darm met name verantwoordelijk zijn voor SBP, worden suggesties gedaan voor het gebruik van *selectieve darm decontaminatie* bij

geselecteerde groepen van patienten om het optreden van SBP te voorkomen.

Hoofdstuk 8. Dit hoofdstuk behandeld de relatie tussen het verrichten van endoscopische sclerotherapie van slokdarm varices (ESV) en het optreden van "spontane" bacteriële peritonitis. Het risico op de ontwikkeling van bacteriële peritonitis is significant hoger na een *spoed* ESV (sclerotherapie binnen 24 uur na een varices bloeding) vergeleken met een *electieve* ESV (respectievelijk 3% vs. 0.5%). Gram negatieve microorganismen afkomstig uit het darm lumen zijn ook na sclerotherapie verantwoordelijk voor het merendeel van de infecties, wat dus overeenkomt met de verwekkers van SBP optredend onafhankelijk van het verrichten van sclerotherapie. Dit gegeven suggereert dat translocatie van gram negatieve bacteriën vanuit het darmlumen ten tijde van een gastro-intestinale bloeding een belangrijker mechanisme is voor het veroorzaken van SBP dan het scleroseren zelf. Indien het scleroseren, met de daarbij behorende beschadiging van de mucosa de oorzakelijke factor zou zijn, zou men meer microorganismen vanuit de mond-keel holte verwachten als verwekker van de SBP. Bij patienten met cirrose en ascites met een gastro-intestinale bloeding kan het kortdurend gebruik van antibiotische profylaxe overwogen worden.

Hoofdstuk 9. Spontane bacteriële peritonitis wordt zelden gerapporteerd als complicatie van ascites welke niet het gevolg is van een gedecompenseerd leverlijden. In 2 van de 60 episoden welke gedurende een periode van 5 jaar geregistreerd werden, trad SBP op als complicatie van een maligniteit. Eén patient had uitgebreide levermetastasering met secundair hieraan portale hypertensie en ascites, de andere patient had een peritonitis carcinomatosa met vena porta thrombose. De mogelijke redenen waarom SBP zelden gediagnostiseerd wordt bij patienten zonder levercirrose worden toegelicht. De aanwezigheid van portale hypertensie in deze beide patienten wordt gezien als een belangrijke predisponerende factor voor het ontstaan van SBP.

Conclusie

Gedurende de periode 1987-1992 werden 60 episoden van spontane bacteriële peritonitis geregistreerd bij 52 patienten. SBP bleek een ernstige complicatie te zijn bij patienten met levercirrose en ascites met een hoge mortaliteit en een lage 1 jaars overleving van 23% (Betrouwbaarheids interval 17%-35%). Drie van de 12 patienten die meer dan 1 jaar overleefden ontvingen een levertransplantatie. Toegenomen alertheid en bekendheid met het ziektebeeld van SBP, en een lagere drempel om paracentese te verrichten met hierdoor vroegere behandelingsmogelijkheden hebben mogelijk geresulteerd in een iets betere overleving dan gemeld in hoofdstuk 7. Verbeterde kweek technieken en een precieze leucocyten telling met differentiatie in de ascites dragen bij tot een juiste diagnose stelling. Uit de literatuur zijn gegevens bekend dat het gebruik van selectieve darm decontaminatie, met als doel de gram negatieve flora te verminderen met het relatief sparen van de resterende anaerobe flora, bij patienten met een hoog risico op het krijgen van SBP, de incidentie van SBP doet afnemen.
Echter, een episode van SBP dient geinterpreteerd te worden als een symptoom van ernstig leverfalen en bij patienten die een dergelijke infectieuze complicatie ontwikkelen moet het verrichten van een levertransplantatie ernstig overwogen worden. Als zodanig is een doorgemaakte episode van SBP één van de vele factoren die meegenomen moeten worden in de vaak moeilijke besluitvorming over de noodzaak tot, en het tijdstip van een levertransplantatie.

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Curriculum Vitae

De auteur werd geboren op 6 november 1956 te Moerkapelle. Na het afleggen van het eindexamen Atheneum β in 1975 aan de Christelijke scholengemeenschap te Gouda, studeerde hij Geneeskunde aan de Erasmus Universiteit te Rotterdam. Er werd een student-assistentschap verricht op de afdeling Pathologische Anatomie (hoofd: Prof. Dr. R.O. van de Heul). Het doctoraal examen volgde in 1980 (Cum Laude) en het Artsexamen in 1981. Van 1982-1987 was de auteur werkzaam als medical officer en later als senior medical officer in het Donald Fraser Hospital te Venda in Zuid-Afrika. In 1983 werd het Diploma Tropical Medicine and Hygiene (DTM&H) en in 1984 het Diploma Public Health (DPH) behaald aan de Witwatersrand University te Johannesburg.
In juli 1987 werd een aanvang gemaakt met de opleiding tot internist in het Havenziekenhuis (Opleider: Prof. Dr. P.C. Stuiver) welke werd vervolgd in het Academisch Ziekenhuis Rotterdam-Dijkzigt, afdeling Inwendige Geneeskunde II (Opleider: Prof. J.H.P. Wilson).
Op 1 augustus 1992 volgde de registratie tot internist en werd begonnen met de vervolg specialisatie tot gastro-enteroloog. De auteur maakt deel uit van de Rotterdamse Levertransplantatie groep, en is als zodanig direct betrokken bij de pre- en posttransplantatie patientenzorg.