Bacterial DNA and its consequences in patients with cirrhosis and culture-negative, non-neutrocytic ascites

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The detection of bacterial DNA in serum and ascitic fluid (AF) from patients with liver cirrhosis and ascites is interpreted as molecular evidence of intestinal bacterial translocation (BT) and considered sufficient to activate the cellular immune response leading to greater cytokine synthesis. We studied 34 patients with liver cirrhosis and culture-negative, non-neutrocytic ascites [22 patients without bacterial DNA (group I) and 12 patients with bacterial DNA (group II)]. History and clinical examination were done with the following investigations at first admission and followed up for 24 weeks: serum and AF tumour necrosis factor-alpha (TNF-\(\alpha\)), AF polymorphonuclear leukocytes, AF cultivation and detection of blood and AF bacterial DNA. Serum and AF TNF-\(\alpha\) were significantly higher in patients with bacterial DNA compared to those without bacterial DNA at first admission [54.5 ± 22.56 vs 35.2 ± 17.97 pg ml\(^{-1}\) (\(P<0.02\))] and 123.2 ± 49.32 vs 82.6 ± 29.58 pg ml\(^{-1}\) (\(P<0.005\)), respectively]. These changes became highly significant at the end of follow-up of both groups [119.3 ± 27.19 vs 40.2 ± 16.08 pg ml\(^{-1}\) (\(P<0.001\)) and 518.8 ± 91.11 vs 97.6 ± 17.81 pg ml\(^{-1}\) (\(P<0.001\)), respectively]. In group II, there was a significant increase in serum and AF TNF-\(\alpha\) at the end of follow-up compared to at first admission (\(P<0.001\)). The relative risk of death, hepatorenal syndrome (HRS) and spontaneous bacterial peritonitis (SBP) was higher in patients with bacterial DNA compared to those without bacterial DNA. We conclude that cirrhotic patients with culture-negative, non-neutrocytic ascites and bacterial DNA have a significantly higher level of serum and AF TNF-\(\alpha\) and higher risk of HRS, SBP and mortality compared to those without bacterial DNA, suggesting that bacterial DNA and TNF-\(\alpha\) are implicated in these complications of liver cirrhosis.

INTRODUCTION

Bacterial infections are frequently observed complications arising in patients with cirrhosis and ascites. Among these, spontaneous bacterial peritonitis (SBP) is probably the most relevant (Fernández et al., 2002). The accepted pathogenic theory of SBP postulates that bacteria of enteric origin cross the intestinal wall to reach the mesenteric lymph nodes in a process called bacterial translocation (BT), and from there reach the systemic circulation (Such & Runyon, 1998). The molecular evidence of BT is the simultaneous presence of bacterial DNA fragments in both blood and ascitic fluid (AF) in patients with advanced cirrhosis (Such et al., 2002a). Francés et al. (2004a) have reported the presence of bacterial DNA in the blood and AF in patients with advanced cirrhosis. This allows the study of BT in patients without evidence of infection, thus becoming a useful tool to investigate the steps preceding a fully developed infection.

Bacterial DNA is characterized by the presence of short repeated sequences of unmethylated CpG dinucleotides (Wagner, 2002). Experimental studies have shown the immunomodulatory role of these fragments, which are capable of inducing a similar immune response to that produced by a complete micro-organism, thus becoming a potent activator of cells of the innate immune system (Wagner, 2002).

Such et al. (2001) have shown that patients with liver cirrhosis and ascites who subsequently develop SBP have a higher baseline AF TNF-\(\alpha\) level than patients who do not

Abbreviations: AF, ascitic fluid; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BT, bacterial translocation; HRS, hepatorenal syndrome; NO, nitric oxide; PMNL, polymorphonuclear leukocyte; SBP, spontaneous bacterial peritonitis; TNF-\(\alpha\), tumour necrosis factor-alpha.
develop SBP. Francés et al. (2004a) demonstrated that peritoneal macrophages from patients with cirrhosis and bacterial DNA in serum and AF were markedly activated, as evidenced by increased nitric oxide (NO) synthesizing ability and enhanced cytokine production.

The aim of our study was to explore the risk of SBP, hepatorenal syndrome (HRS) and mortality in patients with liver cirrhosis and culture-negative, non-neutrocytic ascites who are positive for bacterial DNA compared to those who are negative for bacterial DNA and to study whether bacterial DNA and TNF-α are implicated in these complications.

**METHODS**

**Patients.** Our study comprised 34 patients with liver cirrhosis and culture-negative, non-neutrocytic ascites (24 males, 10 females). They were classified into two groups according to the absence or presence of bacterial DNA in both AF and serum. Group I (absence of bacterial DNA) comprised 22 patients aged $48.3 \pm 7.85$ years; group II (presence of bacterial DNA) comprised 12 patients aged $49.7 \pm 6.5$ years. The patients were recruited from the in-patients of Mansoura Specialized Medical Hospital. The diagnosis was based on established clinical, biochemical and ultrasonographic criteria. The Ethical Committee of Mansoura University Hospitals approved our work and all subjects gave informed consent to the work.

Patients included in our study were subjected to full history, complete clinical examination and investigations at first admission and subsequent admissions during follow-up for 24 weeks.

**Investigations done.** The following were investigated: serum alanine aminotransferase (ALT), serum aspartate aminotransferase (AST) (bioMe´rieux), serum creatinine, complete blood count, and investigations done. The following were investigated: serum alanine aminotransferase (ALT), serum aspartate aminotransferase (AST) (bioMe´rieux), serum creatinine, complete blood count, serum alanine aminotransferase (ALT), serum creatinine, complete blood count, and investigations done.

**Measurement of TNF-α.** The EASIA ELISA kit (BioSource) was used for measurement of TNF-α.

**DNA isolation.** A QIAamp DNA Mini kit (Qiagen) was used for DNA isolation. Five millilitres of AF was centrifuged at 3000 r.p.m. for 3 min and 200 μl precipitate was used for DNA isolation.

**PCR.** PCR was done according to Such et al. (2002b). Two microlitres of DNA, 1 μl sense primer and 1 μl antisense primer were added to 46 μl master mix containing 3 μl MgCl2 solution (1.5 mM), 5 μl 10 × PCR buffer, 1 μl dNTP mixture (10 mM μl$^{-1}$) and 0.4 μl Ampli Taq DNA polymerase (5 U μl$^{-1}$) all in 36.6 μl distilled water. A PerkinElmer thermal cycler was used. There was an initial denaturing step at 94 °C for 3 min, then up to 35 PCR cycles were performed, each consisting of three steps: a denaturing step (30 s at 94 °C), annealing step (30 s at 55 °C) and primer extension step (60 s at 72 °C). These were followed by a final extension step at 72 °C for 5 min. Aliquots were taken from each tube and mixed with gel loading buffer and run on a 2 % agarose gel in Tris-borate buffer.

**Primer.** A universal eubacterial primer was developed from a region of the 16S rRNA gene (Such et al., 2002b). The primers used to amplify the 16S rRNA gene were 5′-AGA GTT TGA TCG TGG CTC AG-3′ and 5′-ACC GCG ACT ACT GCT GTC AG-3′.

**Clinical course.** During follow-up of our patients for 24 weeks, we found that, in group I, two patients died from terminal liver failure, one patient developed HRS and one patient developed SBP; while in group II, three patients died from terminal liver failure, three patients developed HRS and two patients developed SBP. Three patients were not followed up and were lost from connection with our centre: two patients from group I and one from group II.

HRS was diagnosed using the criteria proposed by the International Ascites Club (Arroyo et al., 1996): (1) low glomerular filtration rate, as indicated by a serum creatinine level greater than 1.5 mg dl$^{-1}$; (2) absence of shock, ongoing bacterial infection, fluid losses and treatment with nephrotoxic drugs; (3) no improvement of renal function following diuretic withdrawal and plasma volume expansion; (4) proteinuria lower than 300 mg per day; and (5) no ultrasonographic evidence of parenchymal renal disease or urinary tract obstruction. Moreover, patients had low urine output, very low urinary sodium (<10 milli-equivalents l$^{-1}$), and urine osmolality greater than plasma osmolality.

SBP was diagnosed on the basis of a PMNL count in the AF equal to or greater than 250 cells mm$^{-3}$ in the absence of clinical, radiological or laboratory data suggesting secondary peritonitis or other abdominal disorders resembling SBP (e.g. haemorrhage into AF, peritoneal tuberculosis or carcinomatosis). Because many cases of SBP are culture-negative, the isolation of the responsible organisms was not considered essential for the diagnosis of SBP (Runyon & Hoefs, 1984).

**Exclusion criteria.** The exclusion criteria were the presence of culture-positive blood or AF, neutrocytic AF (≥250 PMNLs ml$^{-1}$), signs and symptoms of systemic inflammatory response syndrome (≥two of the following: temperature >38 °C or <36 °C, heart rate >90 beats min$^{-1}$, respiratory rate >20 breaths min$^{-1}$, a leucocyte count >12 × 10$^9$ l$^{-1}$) according to previously published criteria (Rangel-Frausto et al., 1995), upper gastrointestinal bleeding or intake of antibiotics in the preceding 2 weeks, hepatocellular carcinoma and/ or portal vein thrombosis, a previous episode of SBP, HRS and patients receiving drugs which could affect the TNF-α level (e.g. pentoxyphylline, steroid and non-steroid anti-inflammatory drugs or immunosuppressive drugs).

**Statistical analysis.** Statistical analysis was carried out using the Statistical Package for Social Science (SPSS) program v. 10. All our data were of the continuous type. Results were expressed as mean ± SD. The comparison between means was done using the two-tailed Student’s t-test. Fisher’s exact test was used to compare both groups regarding mortality, HRS and SBP. We used the Pearson’s correlation test to determine how one variable changes in response to other variables. Statistical significance was assumed when probability was less than 0.05.
RESULTS AND DISCUSSION

Bacterial DNA in blood and AF of patients with cirrhosis and culture-negative, non-neutrocytic ascites

Our study detected the presence of bacterial DNA in both serum and AF of 12 of 34 (35.29%) consecutively age matched admitted patients with liver cirrhosis and culture-negative, non-neutrocytic ascites. This finding is in agreement with Francès et al. (2004b), who reported that BT is a common event in patients with advanced and decompensated cirrhosis, because 7 of 17 consecutively admitted patients showed the asymptomatic presence of bacterial DNA in both blood and AF. This percentage is also similar to that reported by Albillos et al. (2003), who found that patients with advanced cirrhosis and ascites demonstrated increased serum levels of lipopolysaccharide-binding protein (which has been considered to be indirect evidence of BT in those patients), and Such et al. (2002b) reported the presence of bacterial DNA in blood and AF in 32% of patients with cirrhosis and culture-negative, non-neutrocytic ascites. Also, BT has been found in 30% of Child C cirrhotic patients (Cirera et al., 2001) and 62% of patients with obstructive jaundice (Sakrak et al., 2003).

Comparison of clinical and laboratory parameters in patients with and without bacterial DNA

Although a trend towards more advanced liver disease (lower serum albumin and prothrombin percentage, and higher serum bilirubin, ALT and AST) was observed in patients with bacterial DNA compared to those without bacterial DNA, these differences did not reach significance (P > 0.05). As regards serum and AF TNF-α, there were significant increases in both serum and AF TNF-α in patients with bacterial DNA compared to those without bacterial DNA (54.5 ± 22.56 vs 35.2 ± 17.97 pg ml⁻¹ and 123.2 ± 49.32 vs 82.6 ± 29.58 pg ml⁻¹, respectively) (Table 1). After 24 weeks, patients with bacterial DNA had more advanced liver disease (lower serum albumin and prothrombin percentage and higher serum bilirubin, ALT and AST) compared to patients without bacterial DNA and these differences reached statistical significance (P < 0.05). Also, there were significant increases in heart rate and decrease in both systolic and diastolic blood pressure in patients with bacterial DNA compared to those without (P < 0.05). The increase in serum and AF TNF-α became highly significant in patients with bacterial DNA compared to those without at the end of follow-up (P < 0.001) (Table 2). There was no significant change in the most studied clinical and laboratory parameters in group I at first admission compared to at the end of follow-up (Table 3). However, there was significant deterioration of clinical and laboratory parameters in group II at the end of follow-up compared to at first admission (Table 4). In cirrhotic rats with BT, Wiest et al. (1999) found splanchnic vascular hyporesponsiveness to vasoconstrictors and a lower mean arterial blood pressure than in normal rats. Also Wiest & Rath (2003) suggested that BT could cause deterioration of the already altered circulatory state in cirrhosis and these haemodynamic changes were closely related to an increased production of NO and TNF-α. These results provide further insight into very early events in the pathogenesis of SBP. Our results concur with some studies that concluded

Table 1. Clinical and laboratory characteristics of the studied patients according to absence (group I) or presence (group II) of bacterial DNA in serum and AF at first admission

<table>
<thead>
<tr>
<th></th>
<th>Group I (n=22) mean ± SD</th>
<th>Group II (n=12) mean ± SD</th>
<th>t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>48.3 ± 7.85</td>
<td>49.7 ± 6.5</td>
<td>0.6024</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>15/7</td>
<td>9/3</td>
<td>0.6767</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>123.2 ± 17.13</td>
<td>118.6 ± 12.26</td>
<td>0.4182</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>66.7 ± 5.79</td>
<td>63.1 ± 4.98</td>
<td>0.0788</td>
</tr>
<tr>
<td>Heart rate (beats min⁻¹)</td>
<td>79.3 ± 6.82</td>
<td>81.8 ± 6.87</td>
<td>0.3159</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>36.9 ± 0.31</td>
<td>37.1 ± 0.29</td>
<td>0.0754</td>
</tr>
<tr>
<td>White blood cell count (mm⁻³)</td>
<td>6250 ± 1169.12</td>
<td>6427 ± 1001.47</td>
<td>0.661</td>
</tr>
<tr>
<td>Bilirubin (mg dl⁻¹)</td>
<td>2 ± 0.43</td>
<td>2.25 ± 0.25</td>
<td>0.0746</td>
</tr>
<tr>
<td>ALT (U l⁻¹)</td>
<td>58.2 ± 11.91</td>
<td>60.6 ± 10.09</td>
<td>0.5587</td>
</tr>
<tr>
<td>AST (U l⁻¹)</td>
<td>69.3 ± 12.64</td>
<td>72.6 ± 14.95</td>
<td>0.5</td>
</tr>
<tr>
<td>Albumin (g dl⁻¹)</td>
<td>2.8 ± 0.59</td>
<td>2.58 ± 0.75</td>
<td>0.1715</td>
</tr>
<tr>
<td>Creatinine (mg dl⁻¹)</td>
<td>1.02 ± 0.23</td>
<td>1.08 ± 0.23</td>
<td>0.7619</td>
</tr>
<tr>
<td>Prothrombin percentage</td>
<td>64.2 ± 11.86</td>
<td>59.08 ± 8.4</td>
<td>0.09786</td>
</tr>
<tr>
<td>Serum TNF-α (pg ml⁻¹)</td>
<td>35.2 ± 17.97</td>
<td>54.5 ± 22.56</td>
<td>0.0222</td>
</tr>
<tr>
<td>AF PMNLs (ml⁻²)</td>
<td>77.21 ± 12.3</td>
<td>81.47 ± 16.59</td>
<td>0.4007</td>
</tr>
<tr>
<td>AF total protein (g dl⁻¹)</td>
<td>1.8 ± 0.51</td>
<td>1.6 ± 0.49</td>
<td>0.2763</td>
</tr>
<tr>
<td>AF TNF-α (pg ml⁻¹)</td>
<td>82.6 ± 29.58</td>
<td>123.2 ± 49.32</td>
<td>0.005</td>
</tr>
</tbody>
</table>
that BT increases TNF-α production in cirrhosis (Genescà et al., 2003; Francés et al., 2005).

In our study, there was a significant positive correlation between both serum and AF TNF-α with serum creatinine and PMNLs, and a significant negative correlation with serum albumin and prothrombin percentage (Table 5). Our findings are in accordance with those of Wiest & Rath (2003), who found a significant elevation in local TNF-α concentration in cirrhotic rats with ascites. Bacterial DNA mediated macrophage activation leads to TNF-α synthesis (Lipford et al., 1997), enhanced TNF-α production and upregulation of the transcription of the inducible NO synthetase gene (MacMicking et al., 1997) leading to an increase in macrophage NO synthesis capability. Francés et al. (2004b) stated that cirrhotic patients with bacterial DNA in both blood and AF show a marked increase in basal production of TNF-α with increased NO synthesis in comparison to patients without bacterial DNA.

**Patients with bacterial DNA had a higher risk of SBP, mortality and HRS**

In our study, we found that patients with bacterial DNA had higher risk of SBP, mortality and HRS after 24 weeks follow-up; however, the differences were not significant ($P > 0.05$) (Table 6). These results are in line with those of Guaran & Soriano (1997), who reported that as cirrhosis develops in animals, Gram-negative bacteria increase in

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**Table 2.** Clinical and laboratory characteristics of the studied groups after 24 weeks follow-up

Student’s t-test was used to calculate probability $P$.

<table>
<thead>
<tr>
<th></th>
<th>Group I ($n=18$)</th>
<th>Group II ($n=8$)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (beats min$^{-1}$)</td>
<td>76.4 ± 4.99</td>
<td>86.7 ± 6.42</td>
<td>0.0002</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>120.3 ± 13.92</td>
<td>95.6 ± 10.77</td>
<td>0.0002</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>67.2 ± 5.21</td>
<td>56.4 ± 5.11</td>
<td>0.0001</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>36.6 ± 0.59</td>
<td>37.3 ± 0.82</td>
<td>0.0207</td>
</tr>
<tr>
<td>White blood cell count (mm$^{-3}$)</td>
<td>6230 ± 1184</td>
<td>6390 ± 1322</td>
<td>0.7614</td>
</tr>
<tr>
<td>Bilirubin (mg dl$^{-1}$)</td>
<td>2.12 ± 0.54</td>
<td>2.85 ± 0.37</td>
<td>0.002</td>
</tr>
<tr>
<td>ALT (U l$^{-1}$)</td>
<td>60.3 ± 11.41</td>
<td>70.6 ± 10.31</td>
<td>0.0382</td>
</tr>
<tr>
<td>AST (U l$^{-1}$)</td>
<td>65.2 ± 10.31</td>
<td>79.4 ± 9.01</td>
<td>0.0026</td>
</tr>
<tr>
<td>Albumin (g dl$^{-1}$)</td>
<td>2.71 ± 0.55</td>
<td>2.21 ± 0.48</td>
<td>0.0363</td>
</tr>
<tr>
<td>Creatinine (mg dl$^{-1}$)</td>
<td>1.21 ± 0.61</td>
<td>2.19 ± 0.82</td>
<td>0.0023</td>
</tr>
<tr>
<td>Prothrombin percentage</td>
<td>60.3 ± 7.72</td>
<td>50.7 ± 6.17</td>
<td>0.005</td>
</tr>
<tr>
<td>Serum TNF-α (pg ml$^{-1}$)</td>
<td>40.2 ± 16.08</td>
<td>119.3 ± 27.19</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>AF PMNLs (ml$^{-3}$)</td>
<td>113.7 ± 39.62</td>
<td>170.3 ± 57.08</td>
<td>0.0073</td>
</tr>
<tr>
<td>AF total protein (g dl$^{-1}$)</td>
<td>1.8 ± 0.25</td>
<td>1.5 ± 0.32</td>
<td>0.016</td>
</tr>
<tr>
<td>AF TNF-α (pg ml$^{-1}$)</td>
<td>97.6 ± 17.81</td>
<td>518.8 ± 91.11</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

**Table 3.** Clinical and laboratory characteristics of group I at first admission and after 24 weeks

Student’s t-test was used to calculate probability $P$.

<table>
<thead>
<tr>
<th></th>
<th>At admission ($n=22$)</th>
<th>After 24 weeks ($n=18$)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>123.2 ± 17.13</td>
<td>120.3 ± 13.92</td>
<td>0.5664</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>66.7 ± 5.79</td>
<td>67.2 ± 5.21</td>
<td>0.7779</td>
</tr>
<tr>
<td>Heart rate (beats min$^{-1}$)</td>
<td>79.3 ± 6.82</td>
<td>76.4 ± 4.99</td>
<td>0.141</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>36.9 ± 0.31</td>
<td>36.6 ± 0.59</td>
<td>0.0457</td>
</tr>
<tr>
<td>White blood cell count (mm$^{-3}$)</td>
<td>6250 ± 1169.1</td>
<td>6230 ± 1184</td>
<td>0.9576</td>
</tr>
<tr>
<td>Bilirubin (mg dl$^{-1}$)</td>
<td>2 ± 0.43</td>
<td>2.12 ± 0.54</td>
<td>0.4386</td>
</tr>
<tr>
<td>ALT (U l$^{-1}$)</td>
<td>58.2 ± 11.91</td>
<td>60.3 ± 11.41</td>
<td>0.5752</td>
</tr>
<tr>
<td>AST (U l$^{-1}$)</td>
<td>69.3 ± 12.64</td>
<td>65.2 ± 10.31</td>
<td>0.2753</td>
</tr>
<tr>
<td>Albumin (g dl$^{-1}$)</td>
<td>2.8 ± 0.59</td>
<td>2.71 ± 0.55</td>
<td>0.6237</td>
</tr>
<tr>
<td>Creatinine (mg dl$^{-1}$)</td>
<td>1.02 ± 0.23</td>
<td>1.21 ± 0.61</td>
<td>0.1846</td>
</tr>
<tr>
<td>Prothrombin percentage</td>
<td>64.2 ± 11.86</td>
<td>60.3 ± 7.72</td>
<td>0.2372</td>
</tr>
<tr>
<td>Serum TNF-α (pg ml$^{-1}$)</td>
<td>35.2 ± 17.97</td>
<td>40.2 ± 16.08</td>
<td>0.3648</td>
</tr>
<tr>
<td>AF PMNLs (ml$^{-3}$)</td>
<td>77.21 ± 12.3</td>
<td>113.74 ± 39.62</td>
<td>0.0002</td>
</tr>
<tr>
<td>AF total protein (g dl$^{-1}$)</td>
<td>1.8 ± 0.51</td>
<td>1.8 ± 0.25</td>
<td>1</td>
</tr>
<tr>
<td>AF TNF-α (pg ml$^{-1}$)</td>
<td>82.6 ± 29.58</td>
<td>97.6 ± 17.81</td>
<td>0.0668</td>
</tr>
</tbody>
</table>
numbers in the gut, and stated that BT is currently considered a key step in the pathogenesis of gut-derived bacterial infections, mainly SBP. The gut of animals and patients with advanced cirrhosis is more permeable to bacteria than the normal gut and more permeable than the gut in less-advanced cirrhosis (Cirera et al., 2001; Runyon et al., 1994). If the ability of the AF to assist macrophages and neutrophils in killing the errant bacteria is deficient, uncontrolled growth and SBP occur (Runyon, 1988).

Although most episodes of SBP are resolved when rapidly diagnosed and treated, a significant number of patients develop infection-associated complications such as hepatic encephalopathy, septic shock or progressive renal failure, leading to an irreversible HRS and death in some cases. Indeed, bacterial infection is the main cause of renal failure in cirrhotic patients (Gines et al., 2003). Thus SBP is the result of failure of the gut to contain bacteria and failure of the immune system to kill the virulent bacteria once they have escaped the gut. Innate defenders against bacterial invasion synthesize proinflammatory cytokines and effector molecules which assist in killing bacteria.

Unfortunately, patients with advanced cirrhosis have been reported to have defects and dysfunction in many of these protection systems (Fiuza et al., 2000). Therefore, these patients are vulnerable to infection by their own gut flora. To make matters worse, some of the effector molecules and cytokines that help in killing the bacteria have undesired side effects. NO is one of these effector molecules and TNF-α is one of the relevant cytokines. Bacterial infection leads to further elevation of these molecules (Such & Runyon, 1998). NO and TNF-α are important mediators that often accompany SBP (Ruiz-del-Arbol et al., 2003).

The development of functional renal failure in cirrhosis has been related to an overproduction of NO and pro-inflammatory cytokines such as TNF-α (Such et al., 2001; Navasa et al., 1998). Based on our information, we could predict that the presence of bacterial DNA in serum and AF of patients with advanced cirrhosis would have consequences (for example, stimulation of immune defences, increase the effector molecules and cytokines). These effector molecules and cytokines are two-edged swords. They can protect from bacterial infection but they can also

<p>| Table 4. Clinical and laboratory characteristics of group II at admission and after 24 weeks |</p>
<table>
<thead>
<tr>
<th>At admission (n=12)</th>
<th>After 24 weeks (n=8)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (beats min⁻¹)</td>
<td>81.8 ± 6.87</td>
<td>86.7 ± 6.42</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>118.6 ± 12.26</td>
<td>95.6 ± 10.77</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>63.1 ± 4.98</td>
<td>56.4 ± 5.11</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>37.1 ± 0.29</td>
<td>37.3 ± 0.82</td>
</tr>
<tr>
<td>White blood cell count (mm⁻³)</td>
<td>6427 ± 1001.47</td>
<td>6390 ± 1322</td>
</tr>
<tr>
<td>Bilirubin (mg dl⁻¹)</td>
<td>2.25 ± 0.25</td>
<td>2.85 ± 0.37</td>
</tr>
<tr>
<td>ALT (U l⁻¹)</td>
<td>60.6 ± 10.09</td>
<td>70.6 ± 10.31</td>
</tr>
<tr>
<td>AST (U l⁻¹)</td>
<td>72.6 ± 14.95</td>
<td>79.4 ± 9.01</td>
</tr>
<tr>
<td>Albumin (g dl⁻¹)</td>
<td>2.58 ± 0.75</td>
<td>2.21 ± 0.48</td>
</tr>
<tr>
<td>Creatinine (mg dl⁻¹)</td>
<td>1.08 ± 0.23</td>
<td>2.19 ± 0.82</td>
</tr>
<tr>
<td>Prothrombin percentage</td>
<td>59.08 ± 8.4</td>
<td>50.7 ± 6.17</td>
</tr>
<tr>
<td>Serum TNF-α (pg ml⁻¹)</td>
<td>54.5 ± 22.56</td>
<td>119.3 ± 27.19</td>
</tr>
<tr>
<td>AF PMNLs (ml⁻¹)</td>
<td>81.47 ± 16.59</td>
<td>170.3 ± 57.08</td>
</tr>
<tr>
<td>AF total protein (g dl⁻¹)</td>
<td>1.6 ± 0.49</td>
<td>1.5 ± 0.32</td>
</tr>
<tr>
<td>AF TNF-α (pg ml⁻¹)</td>
<td>123.2 ± 49.32</td>
<td>518.8 ± 91.11</td>
</tr>
</tbody>
</table>

P, Pearson’s correlation.

<p>| Table 5. Correlation between both serum and AF TNF-α with serum creatinine, AF PMNLs, serum albumin and prothrombin percentage at the end of follow-up |</p>
<table>
<thead>
<tr>
<th>Serum TNF-α</th>
<th>AF TNF-α</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum creatinine</td>
<td>0.590</td>
</tr>
<tr>
<td>AF PMNLs</td>
<td>0.499</td>
</tr>
<tr>
<td>Albumin</td>
<td>−0.644</td>
</tr>
<tr>
<td>Prothrombin percentage</td>
<td>−0.548</td>
</tr>
</tbody>
</table>

<p>| Table 6. Relative risk (RR) of death, HRS and SBP in the studied groups during follow-up for 24 weeks |</p>
<table>
<thead>
<tr>
<th>Group I (n=20)</th>
<th>Group II (n=11)</th>
<th>RR in group II compared to group I</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deaths</td>
<td>2</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>HRS</td>
<td>1</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>SBP</td>
<td>1</td>
<td>5</td>
<td>2</td>
</tr>
</tbody>
</table>

Fisher’s exact test was used to calculate probability P.
initiate a sequence of events on haemodynamics, renal function and survival.

We conclude that cirrhotic patients with culture-negative, non-neutrocytic ascites and bacterial DNA have a significantly higher level of serum and AF TNF-α and higher risk of HRS, SBP and mortality compared to those without bacterial DNA during follow-up for 24 weeks, which could suggest that both bacterial DNA and TNF-α are implicated in these complications of liver cirrhosis. However, further study is needed to conduct a randomized controlled trial of selective intestinal decontamination versus placebo in the bacterial DNA-positive group and determine whether SBP, HRS and mortality can be prevented.

REFERENCES


