Ascitic Fluid Interleukin-8 to Distinguish Spontaneous Bacterial Peritonitis and Sterile Ascites in Cirrhotic Patients, Cecilia Martínez-Brú,1 Cristina Gómez,2 Mariano Cortés,1 Germán Soriano,2 Carlos Guarner,2 Teresa Planella,1 and Francesc González-Sastre1 (Departments of 1 Biochemistry and 2 Gastroenterology, Hospital de la Santa Creu i Sant Pau, 08025 Barcelona, Spain; * address correspondence to this author: Servei de Bioquímica, Hospital de la Santa Creu i Sant Pau, Avgda. Pare Claret 167, 08025 Barcelona, Spain; fax 34-93-2919196, e-mail mcr@teleline.es)

Spontaneous bacterial peritonitis (SBP) is a common and potentially fatal complication of cirrhosis. The pathogenesis of spontaneous ascitic fluid infection appears to involve translocation of bacteria from the gut to the mesenteric lymph nodes, depressed reticuloendothelial phagocytic activity, and deficient ascitic fluid antibacterial activity. A high index of suspicion of this infection and a low threshold for performing an abdominal paracentesis are required to detect infection early, when survival is most likely (1). Interleukin-8 (IL-8) is a cytokine produced by a variety of cells in response to stimuli, such as bacterial lipopolysaccharide, with a strong chemotactic activity on neutrophils. Increased plasma IL-8 has been observed in patients with severe infections as well as in alcoholic and nonalcoholic cirrhotic patients (2, 3). To our knowledge, IL-8 production in ascitic fluid in cirrhotic patients developing SBP has not been determined.

The aim of this study was to evaluate (a) IL-8 production in both plasma and ascitic fluid in cirrhotic patients with SBP and sterile ascites (SA); (b) the correlation of these values with neutrophil count in ascitic fluid; (c) the effect of therapy on IL-8; and (d) the sensitivity and specificity of ascitic fluid IL-8 in cirrhotic patients with ascites for detecting SBP patients.

Thirty-three cirrhotic patients were included in the study: 11 cirrhotic patients with SBP, and 22 patients with SA. A patient was classified as a SBP patient when the neutrophil count in the ascitic fluid was ≥250 cells/μL and as a SA patient when neutrophil count in ascitic fluid was <250 cells/μL with negative ascitic fluid culture (1).

Patients with liver carcinoma or other neoplasms, with infections or recent antibacterial therapy (previous 2 weeks), and with gastrointestinal hemorrhage and under treatment with corticosteroids or pentoxifylline were excluded. Patients under prophylactic norfloxacin were not excluded. Patients classified as SBP-cirrhotic patients were treated with cefotaxime or a combination of amoxicillin-clavulanic acid.

To avoid interferences from cells, plasma and ascitic fluid samples were centrifuged immediately after collection. Blood samples were collected in EDTA tubes from a peripheral vein. Plasma was separated immediately by centrifugation (900g for 15 min) and stored at −80 °C until assayed. Ascitic fluid samples were collected in EDTA tubes and stored at −80 °C until assayed.

Plasma and ascitic fluid IL-8 concentrations were determined by a solid-phase, two-site chemiluminescent enzyme immunoassay (EURO/DPC) in an IMMULITE automated analyzer. Seven SBP patients had another IL-8 (plasma and ascitic fluid) determination at 48 h after onset of antibiotic treatment.

Statistically significant differences for IL-8 and other values in ascitic fluid and in plasma between groups were studied with the Mann–Whitney U-test. Regression analysis was carried out with the Spearman rank-order correlation. Wilcoxon analysis was used to evaluate the effects of therapy in SBP patients.

The distribution of patients by age, sex, cirrhosis etiology, prophylaxis, and alcohol consumption showed no significant differences between SBP patients and SA patients. Ascitic fluid IL-8 concentrations were greater in SBP patients than in the SA group (P < 0.000001; Table 1). Ascitic fluid IL-8 showed a statistically significant correlation with ascitic fluid neutrophil count (r = 0.57; P

Table 1. IL-8 and C-reactive protein in ascites fluid and plasma.

<table>
<thead>
<tr>
<th></th>
<th>SBP (n = 11), median (quartiles) min/max</th>
<th>Sterile ascites (n = 22), median (quartiles) min/max</th>
</tr>
</thead>
<tbody>
<tr>
<td>AF IL-8, ng/L</td>
<td>312 (128–942) 106/41260 0.1/90</td>
<td>36 (32–46) 17/87 0.1/18</td>
</tr>
<tr>
<td>AF CRP, mg/L</td>
<td>22.0 (9.2–27.7) 0.1/90</td>
<td>3.0 (0.1–6.8) 0.1/18</td>
</tr>
<tr>
<td>AF neutrophils, cells/μL</td>
<td>1832 (870–7670) 8 (2–32)</td>
<td>764/19674 0/210 5/631 5/283</td>
</tr>
<tr>
<td>Plasma IL-8, ng/L</td>
<td>18 (12–94) 5/631 7.5/96.8</td>
<td>14 (7–71) 5/283 0.1/90</td>
</tr>
<tr>
<td>Plasma CRP, mg/L</td>
<td>35.7 (21.0–76.5) 7.5/96.8</td>
<td>15.4 (10.0–24.0) 0.1/90</td>
</tr>
</tbody>
</table>

*a min, minimum; max, maximum; AF, ascitic fluid; CRP, C-reactive protein.

b P <0.000001.
c P <0.0001.
d Neutrophil count was used to define SBP (>250 cells/μL).

Fig. 1. Effects of antibiotic therapy on ascitic fluid IL-8 in seven SBP patients.

Admission, concentration at admission, before initiation of antibiotic therapy; 48 hours, concentration 48 h after initiation of antibiotic therapy.
<0.001). Wilcoxon analysis, which was used to evaluate the effects of therapy on IL-8 concentrations, showed a statistically significant decrease (P <0.05) in ascitic fluid IL-8 concentrations after 48 h of therapy. Ascitic fluid IL-8 concentrations on admission and at 48 h after the initiation of treatment are represented in Fig. 1.

A cutoff value of 100 ng/L for the ascitic fluid IL-8 concentration yielded 100% sensitivity and 100% specificity for diagnosis of spontaneous bacterial peritonitis in cirrhotic patients.

These data are the preliminary results of a larger study that is still ongoing to establish the possible prognostic value of ascitic fluid IL-8 in SBP patients. Two of 11 SBP patients developed septic shock, both patients having the highest ascitic fluid IL-8 concentrations. Up to now, we conclude that (a) IL-8 concentrations in ascitic fluid in SBP-cirrhotic patients are significantly greater than in patients with SA; (b) ascitic fluid IL-8 concentrations decrease from baseline to 48 h after the initiation of treatment in SBP patients; (c) in SBP patients, IL-8 concentrations in ascitic fluid are higher than plasma IL-8 concentrations, suggesting a local peritoneal production of IL-8 during SBP; (d) IL-8 seems to play a role in SBP in cirrhotic patients; and (e) IL-8 correctly classified SBP and SA patients.

References


Denaturing Gradient Gel Electrophoresis-based Analysis of Loss of Heterozygosity Distinguishes Nonobvious, Deleterious BRCA1 Variants from Nonpathogenic Polymorphisms, Miguel de la Hoya, Eduardo Díaz-Rubio, and Trinidad Calde± 1,2* (1) Laboratory of Molecular Oncology and Departments of 2 Immunology and 3 Oncology, San Carlos University Hospital, 28040 Madrid, Spain; * address correspondence to this author at: Laboratorio de Oncología Molecular, Planta Baja Sur, Hospital Clínico San Carlos, c/Martín Lagos s/n, 28040 Madrid, Spain; fax 34-1-3303544, e-mail uinvest2@hcsc.es

BRCA1 is a tumor suppressor gene (1) responsible for one-half of familial breast/ovarian cancer syndromes and 40% of breast-only cancer syndromes (2, 3). BRCA1 codes for a 220-kDa nuclear phosphoprotein that has been suggested to play a role in cellular processes, including DNA repair and recombination (4, 5), transcriptional regulation (6, 7), and appropriate chromosomal segregation (8). It is unclear which of BRCA1 functions are important for decreasing breast/ovarian cancer susceptibility.

BRCA1 testing and genetic counseling services are offered to families with histories of breast and/or ovarian cancer (9). The available screening methods to detect germ-line BRCA1 mutations are expensive and time consuming because the gene is large, prevalent BRCA1 mutations are not found (except in ethnic communities), and mutations are scattered throughout the coding sequence (10).

Gene screening often detects a BRCA1 variant that does not imply a frameshift or a splicing alteration but represents a missense mutation not previously reported or registered in the Breast Cancer Informative Core Database (11). To address the question of whether these mutations represent new cancer-predisposing mutations or rare polymorphisms, one must consider characteristics such as absence of the variant in a control group of sufficient size, cosegregation with cancer in some families, and occurrence in a highly conserved protein sequence or in a putative functional domain. These considerations imply the study of large-pedigree families, which very often are not available and the conclusions of which are not always compatible with genetic counseling practice. As an indication of these limitations, only BRCA1 missense mutations that abolish the BRCA1 C-terminal transcriptional activity in a transfection assay (12) or disturb the RING-finger domain structure (13) have been defined as cancer-predisposing mutations (11). The lack of complete understanding of BRCA1 makes it difficult to design a reliable BRCA1 functional test similar to those for other tumor suppressor genes such as p53 (14).

BRCA1 is a classical tumor suppressor gene that follows Knudson’s two-hits hypothesis (10). BRCA1 somatic mutations have not been detected in sporadic breast cancer (15) and are very uncommon in sporadic ovarian cancer (16). These findings indicate that selective retention of one BRCA1 allele in a breast or ovarian tumor is a good predictor of the cancer-predisposing role of this allele.

Very often, index cases from families under study in genetic counseling services are women already diagnosed as having breast or ovarian cancer, and fresh or paraffin-embedded tissue samples from their tumors are readily available. In these cases, a loss of heterozygosity (LOH) study at the BRCA1 locus is possible. Traditionally, tumor LOH has been studied with the help of microsatellite markers. Unfortunately, this method does not permit discrimination of whether the unclassified mutant allele has been the one selectively retained in the tumor DNA. Hence, additional sequencing analysis is needed to address this issue.

We have developed an analysis based on PCR-denaturing gradient gel electrophoresis (DGGE) that permits us to demonstrate the selective retention of deleterious BRCA1 alleles in DNA extracted from either fresh or paraffin-embedded breast/ovarian tumor tissues.

The extraction protocol for paraffin-embedded tissue DNA was modified from Sarkar et al. (17). Basically, two 10-mm sections from a block of paraffin-embedded tissue were placed in a 1.5-mL microcentrifuge tube after excess paraffin was removed with a scalpel. To dissolve the paraffin, the sections were immersed in 1 mL of xylene,