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Original Research Article

Procalcitonin and macrophage inflammatory protein-1 beta (MIP-1β) in serum and peritoneal fluid of patients with decompensated cirrhosis and spontaneous bacterial peritonitis

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ABSTRACT

Purpose: Spontaneous bacterial peritonitis (SBP) is the most frequent infection in patients with cirrhosis causing significant mortality which requires rapid recognition for effective antibiotic therapy, whereas ascitic fluid cultures are frequently negative. The aim of this study was to evaluate the SBP diagnostic efficacy of procalcitonin (PCT) and macrophage inflammatory protein-1 beta (MIP-1β) measured in serum and peritoneal fluid.

Material/methods: Thirty-two participants with liver cirrhosis and ascites were included into the study (11 females and 21 males, mean age 49.5 ± 11.9 years). The peritoneal fluid and venous blood were collected for routine laboratory examinations and measurements of PCT and MIP-1β. Patients were divided into two groups according to the ascitic absolute polymorphonuclear leukocytes count (≥250 mm^{-3} and <250 mm^{-3}).

Results: Ascites was sterile in 22 participants and SBP was diagnosed in 10 patients. Serum and ascitic levels of PCT and MIP-1β did not correlate with clinical and routine laboratory parameters. MIP-1β in the ascitic fluid was significantly higher in patients with SBP (213 ± 279 pg/ml vs. 66.3 ± 49.8 pg/ml; $p = 0.01$). The sensitivity and specificity for diagnosis of SBP with ascitic MIP-1β were 80% and 72.7%, respectively (cut-off value 69.4 pg/ml) with AUROC 0.77 (95%CI 0.58–0.96). Serum levels of MIP-1β showed lower diagnostic yield. Serum and ascitic PCT levels were not different in patients with and without SBP.

Conclusions: MIP-1β concentration in ascitic fluid may distinguish patients with and without SBP with satisfactory sensitivity and specificity. Chemokines should be further explored for diagnostic use.

1. Introduction

Spontaneous bacterial peritonitis (SBP) occurs in about 10% of patients with cirrhosis and ascites [1]. Responsible bacteria are usually aerobic or microaerophilic enteric organisms. Due to low bacterial density, up to 50% of patients with clinical signs of SBP have negative cultures of ascitic fluid. Routine diagnosis of SBP is based on absolute polymorphonuclear leukocytes (PMN) count in ascitic fluid (more than 250 cells/mm^3). Common incidence of asymptomatic SBP justifies a diagnostic paracentesis in all patients with new-onset ascites or already existing ascites requiring hospitalization [2]. Patients with recognized SBP should receive immediately empiric antibiotic therapy as delay in diagnosis and treatment has been shown to increase mortality [3,4]. Early antibiotic therapy dramatically improves prognosis in SBP, however, the 1-year mortality in these patients still approaches 50% [5].

At present, clinical diagnosis of infected ascitic fluid without paracentesis is not satisfactory and is not recommended. Although paracentesis is generally safe procedure even in patients with coagulopathy, dangerous complications may occur. Abdominal wall hematomas were reported in about 1% of patients, and more sever complications such as hemoperitoneum or bowel entry by the needle occurred in 1 case per 1000 paracenteses [6]. In rare cases skin organisms such as staphylococci can be introduced with needle to the ascites.

Procalcitonin (PCT) is a 116 amino acids peptide that is precursor of the hormone calcitonin that is produced not only by parafollicular cells (C cells) of the thyroid gland but also by the neuroendocrine cells of the lung and the intestine [7]. The level of

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PCT in the blood of healthy individuals is below the limit of detection of clinical assays, but it rises in response to proinflammatory stimuli, especially of bacterial origin. In patients with septicemia the blood levels of PCT may rise to 100 ng/ml and in such cases the PCT is produced mainly by the cells of lung and the intestine [8]. A meta-analysis reported a sensitivity of 76% and specificity of 70% in diagnosis of bacterial infection [9]. PCT is used as an early marker of sepsis, and is considered better indicator of this condition as compared with proinflammatory interleukins 2, 6 or 8, C-reactive protein (CRP) or TNF-alpha [10]. Long half-life of PCT in serum (25–30 h) makes it suitable for daily monitoring of treatment effects. The reports on significance of serum PCT levels in diagnosis of SBP are contradictory. Viallon et al. [11] showed that serum PCT with the cut-off value of 0.75 ng/ml was better marker of SBP than ascitic PMN count or serum CRP and interleukin-6 levels. In this study the mean ratio for serum to ascites PCT was 0.31 that suggests extraperitoneal release of this peptide. In another study, the serum level of PCT was significantly higher in patients with infected than sterile ascites, but their values showed wide overlap [12].

Macrophage inflammatory protein type 1 beta (MIP-1β, CCL4) belongs to the family of chemokines, best known for their chemotactic and proinflammatory effects. MIP-1β is an acidic protein composed of 69 amino acids that is produced by many cells, particularly macrophages, dendritic cells, and lymphocytes. Synthesis of MIP-1β is stimulated with bacterial endotoxins. MIP-1β is responsible for the activation of PMN and is involved in acute neutrophilic inflammation. This protein is most effective at augmenting adhesion of CD8(+) T-cells to the vascular cell adhesion molecule (VCAM-1). The diagnostic significance of MIP-1β for bacterial infections is poorly recognized and it has never been examined in cirrhotic patients with SBP.

From obvious reasons the blood is more appropriate material for routine examinations than ascitic fluid. Until now, no serum marker of SBP is sufficiently sensitive to be recommended for diagnosis of this life-threatening complication. The peritoneal cavity is not a closed space as in cirrhotic patients each day 700–900 ml of ascitic fluid is being absorbed to systemic circulation. Therefore, many inflammatory and immune-derived products may get from the infected fluid through peritoneal barrier to activate systemic neuroendocrine or reticuloendothelial cells. The aim of this study was to investigate serum and ascitic levels of PCT and MIP-1β as potential markers of SBP.

2. Material and methods

2.1. Subjects

Thirty-two consecutive patients with decompensated liver cirrhosis were included to the study (11 females and 21 males, mean age 49.5 ± 11.9 years). Sixteen patients were classified to Child–Pugh stage C, 15 patients to stage B and 1 patient to stage A. The average MELD (model for end stage liver disease) score was 20.4 ± 7.0 points. In 24 patients the cause of cirrhosis was alcohol overuse, and in others infection with HCV (2 patients), infection with HBV (2 patients), autoimmune hepatitis (2 patients), Wilson's disease (1 patient) or combined alcohol and infection with HCV (1 patient). Exclusion criteria were: current use of antibiotics, advanced encephalopathy (stages 3 and 4 according to West-Haven classification), current gastrointestinal bleeding, surgery during recent 6 months, severe heart failure and neoplastic (including hepatocellular carcinoma), inflammatory and other than SBP bacterial infectious diseases.

Appropriate informed consent was obtained from each patient enrolled in the study. The study protocol was approved by the Medical University of Silesia Ethics Committee and conformed to the ethical guidelines of the 1975 Declaration of Helsinki (6th revision, 2008).

2.2. Study protocol

On the first day of hospitalization in each patient the venous blood was sampled for routine laboratory examinations, including blood morphology with smear test, serum levels of bilirubin, alkaline phosphatase, gamma-glulamyltranspeptidase (γ-GT), alanine and aspartate aminotransferases, C-reactive protein (CRP), creatinine, electrolytes, total protein, albumin and international normalized ratio (INR). For exclusion of infectious diseases a routine urine examination with bacteriological culture, abdominal ultrasound and chest radiography were performed. Patients, who were eligible for this study underwent diagnostic paracentesis. The analysis of peritoneal fluid included the PMN count and the bacteriological culture. Peritoneal liquid was sown into liquid culture medium immediately after taking a sample, both for aerobic and anaerobic organisms (BD BACTEC Plus Anaerobic/F and Aerobic/F kit). The patients were divided into two groups according to the ascitic PMN count ($\geq$250 mm^{-3} and $<$250 mm^{-3}), i.e. with and without SBP, respectively.

The measurement of PCT and MIP-1β levels, in the serum and the peritoneal fluid, was performed with immunoluminescent and immunoenzymatic methods, respectively. PCT was measured by the "LUMItest®-PCT"-kit (Vidas B.R.A.H.M.S.-Diagnostica, Berlin, Germany) according to the instructions of the manufacturer. The minimum detectable dose of PCT was 0.2 ng/ml. MIP-1β was measured with RayBio® enzyme-linked immunosorbent assay, employing an antibody specific for human MIP-1β coated on a 96-well (RayBiotech Inc., Cat#: ELH-MIP1beta-001). The color intensity was measured spectrophotometrically at 450 nm. The minimum detectable dose of MIP-1 is 2.5 pg/ml.

Patients with SBP were treated with Ceftriaxon 1 g injected intravenously twice daily for 5–7 days. Clinical state of patients was monitored with blood morphology and serum levels of CRP, creatinine and electrolytes. In all patients the empiric antibiotic therapy was successful.

2.3. Statistical analysis

All data were analyzed using the STATISTICA package (version 8) and presented as means, medians, standard deviations and ranges. A p-value of less than 0.05 was considered statistically significant. The first step of statistical analysis was comparative analysis of demographic and laboratory data between patients with PMN count in ascitic fluid $\geq$250 mm^{-3} and $<$250 mm^{-3}. For this purpose the medians were compared using Mann–Whitney U test. The bivariate Spearman's rank correlation test was used to assess relationships between PCT and MIP-1β levels and selected clinical variables. In the second step the diagnostic value of PCT and MIP-1β for detection of SBP was assessed by constructing a receiver-operating characteristic (ROC) curve and calculating the area under the ROC curve (AUROC). From these curves, the best cut-off values were established, which minimized the error rate (i.e. the number of false positive and false negative classifications). The sensitivity, specificity, positive predictive values (PPV), and negative predictive values (NPV) for relevant cut-offs were calculated. PPV and NPV are parameters dependent on the prevalence; therefore, it was assumed that general prevalence of SBP equals to that seen in our study group.

3. Results

The clinical and laboratory characteristics of 32 patients included to the study are shown in Table 1. Ascites was sterile