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The role of the neutrophil Fcγ receptor I (CD64) index in diagnosing spontaneous bacterial peritonitis in cirrhotic patients

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SUMMARY

Objective: To investigate the role of the neutrophil Fcγ receptor I (CD64) index in the diagnosis of spontaneous bacterial peritonitis (SBP) in cirrhotic patients.
Methods: A total of 123 cirrhotic patients with ascites who fulfilled the inclusion criteria were enrolled in this study. Ascites and blood samples were collected; the polymorphonuclear neutrophil (PMN) count, bacterial culture, and related laboratory tests were performed. The CD64 index was determined for each sample using flow cytometry.
Results: The neutrophil CD64 index results were significantly higher in cirrhotic patients with SBP than in those without SBP ($p < 0.001$). There was a positive correlation between the neutrophil CD64 index and the PMN count in ascites. In the receiver operating characteristic curve (ROC) analysis, the area under the curve (AUC) was 0.894 (95% confidence interval 0.823–0.964, $p < 0.001$). The optimal cut-off value for the neutrophil CD64 index was 2.02. The sensitivity and specificity of the neutrophil CD64 index for cirrhotic patients with SBP were 80.49% and 93.90%, respectively. The elevated neutrophil CD64 index was down-regulated by antibiotic therapy ($p = 0.002$).
Conclusions: The neutrophil CD64 index could be used as a sensitive and specific indicator for the diagnosis of SBP in cirrhotic patients with ascites and is also modulated by antibiotic therapy.

1. Introduction

Spontaneous bacterial peritonitis (SBP) is a common and serious complication in patients with ascites caused by decompensated liver cirrhosis, with an incidence of 10% to 30%.[1] Most cases are caused by bacterial infections spreading to the peritoneum across the gut wall or mesenteric lymphatics, or less frequently from hematogenous transmission, in combination with an impaired immune system and in the absence of an identified intra-abdominal source of infection or malignancy.[2]

The development of ascites in cirrhosis indicates a poor prognosis, with a mortality rate of approximately 40% in the first year after diagnosis and 50% in the second year; there is an increased risk of other liver complications, including refractory ascites, SBP, hyponatremia, and hepatorenal syndrome (HRS).[3] Studies have shown that some symptoms of SBP patients may be masked by the symptoms of liver cirrhosis and the effects of medication.[1] A weak response to inflammatory stimulation in patients with liver cirrhosis due to hypoimmunity and large amounts of ascites cause atypical abdominal signs and a less obvious rise in body temperature; therefore, the diagnosis of SBP by symptoms and signs is clinically insufficient .

Although the mortality of SBP has decreased from 90% to 20% in recent years due to the use of antibiotics, the mortality in untreated patients remains as high as 50%.[4,5] On the other hand, the empirical use of antibiotics in the clinical setting is a normal way to alleviate the condition of patients with ascites. The misuse of antibiotics causes drug resistance and has an adverse effect on the efficacy of long-term treatments. Therefore, the early and effective diagnosis of SBP, along with the prompt initiation of empiric antibiotic therapy, has been considered crucial to overall patient survival.[6]

Although a diagnostic paracentesis and appropriate ascitic fluid analysis is considered essential for all patients admitted with

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ascites, the procedure is associated with dangerous complications such as bleeding and infection.[7] Meanwhile, in the emergency setting, performing ascitic fluid culture examinations is time-consuming and not always possible. Hence, according to the current guidelines, a diagnosis of SBP is made when the ascitic fluid polymorphonuclear neutrophil (PMN) count is $\geq 0.25 \times 10^9$ cells/l . However, the dissociation of PMNs during transportation to the laboratory may lead to false-negative results. Furthermore, a large amount of ascitic fluid in cirrhotic patients and the many visible components in ascitic fluid could make cell counts inaccurate. Manual measurement of the ascitic fluid PMN count is operator-dependent, making quality control difficult, and can delay the diagnosis.[8,9]

In this regard, a new inflammatory marker that could predict bacterial infections in ascitic fluid and assist in determining the effect of therapy would be extremely useful. In recent years, several studies on Fcγ receptor I (FcγRI), otherwise named CD64, have confirmed this to be a marker of infection. In resting neutrophils, CD64 is expressed at a very low level (approximately 1400 receptors per cell on average). However, the up-regulation of CD64 on the surface of neutrophils is induced by inflammatory cytokines such as interferon gamma[10] and granulocyte colony-stimulating factor,[11] which are produced during bacterial infections. Therefore, the expression of CD64 on human neutrophils may be used as an improved test for the early diagnosis of bacterial infections. Recently, the neutrophil CD64 index – a novel indicator – has been confirmed to be efficient in predicting sepsis,[12,13] neonatal infection,[14,15] intestinal diseases,[16] surgical infection,[17] etc.

Appropriate diagnostic studies are required to identify infections of the ascitic fluid in cirrhotic patients. On account of the rapid and valid application of the neutrophil CD64 index to indicate bacterial infections, the aim of the present study was to investigate the role of the CD64 index in the diagnosis of SBP in cirrhotic patients.

2. Materials and methods

2.1. Study population

This was a prospective study conducted at Beijing You'an Hospital, Capital Medical University, China, from March 2014 to June 2015. A total of 123 patients with ascites caused by cirrhosis who fulfilled the inclusion criteria were enrolled in the study. The inclusion criteria were as follows: (1) age ≥18 years; (2) diagnosis of liver cirrhosis according to clinical, liver tissue pathology, biochemical, and imaging markers; (3) presence of ascites. The exclusion criteria were the following: (1) secondary peritonitis; (2) tuberculous peritonitis; (3) other infections except ascites infection; (4) use of immunosuppressants or other chemotherapy drugs; (5) trauma, surgery, or other vital disease except liver disease within 3 months of study entry; (6) cancers other than liver cancer. Twenty healthy subjects were also enrolled.

2.2. Diagnostic criteria for SBP

The diagnosis of SBP was based on two of the following criteria from available guidelines:[18,19] (1) abdominal pain and/or hyperthermia, and/or abdominal and rebound tenderness (excluding secondary peritonitis); (2) ascitic fluid PMN count $\geq 0.25 \times 10^9$/l; (3) positive ascitic fluid bacterial culture. The non-SBP group included patients with ascites caused by cirrhosis without SBP; the SBP group included patients with cirrhosis who had SBP.

2.3. Paracentesis and ascitic fluid culture

Diagnostic paracentesis was carried out at the bedside using a sterile method before antibiotic therapy. The aspirated ascitic fluid was collected in ethylenediaminetetraacetic acid (EDTA) tubes and analyzed within 3 h of aspiration. The ascitic fluid total and differential cell counts were performed using an optical microscope (CX41; Olympus, Japan). Bacterial cultures were obtained by bedside inoculation of 20 ml of ascitic fluid into aerobic and anaerobic bottles (BACTEC 9120; BD, USA). The bottles were incubated at 35 °C for 3 days and if it was negative, it would be discarded. Bacterial identification and antimicrobial susceptibility testing were performed using an automated microbial identification system (Phoenix 100; BD, USA).

2.4. Patient assessments

Relevant tests were performed on blood samples to assess the condition of the patient. The peripheral white blood cell (WBC) and platelet (PLT) counts were obtained using an automated blood cell analyzer (XT-4000i; Sysmex, Japan). Liver and renal biochemical parameters, such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin, albumin, blood urea nitrogen, and creatinine levels, were obtained using an automated biochemical analyzer (Au5400; Olympus, Japan).

2.5. Neutrophil CD64 index

Blood samples were collected within 6 h after paracentesis and before antibiotic therapy; they were processed within 24 h after collection for the measurement of CD64 expression on leukocytes by flow cytometry (FACSCalibur; BD, USA) using a CD14/CD64 assay kit (BD, USA). The kit includes fluorescein isothiocyanate (FITC)-conjugated anti-CD14 and phycoerythrin (PE)-conjugated anti-CD64 antibodies. The lymphocyte, monocyte, and neutrophil populations are defined by their forward and side scatter characteristics along with surface CD14 staining. The CD64 index was calculated using the following formula:

$$\text{CD64 index} = \frac{(\text{neutrophil CD64 average fluorescence intensity}/\text{lymphocyte CD64 average fluorescence intensity})}{(\text{monocyte CD64 average fluorescence intensity}/\text{neutrophil CD64 average fluorescence intensity})}$$

CD64 expression on lymphocytes served as an internal negative control, while CD64 expression on monocytes served as an internal positive control.

2.6. Statistical analysis

Data were entered into a database and analyzed using SPSS version 16.0 software (SPSS Inc., Chicago, IL, USA). Data are expressed as the number (%), mean ± standard error of the mean (SEM), median (interquartile range, IQR), or area under the receiver operating characteristic curve (AUC) with the 95% confidence interval (CI), where appropriate. The *t*-test was used for quantitative variables subordinate to the normal distribution, and the Mann–Whitney *U*-test was used for the comparison of quantitative variables with a non-normal distribution. The correlation of the neutrophil CD64 index with the WBC and PMN counts in ascites samples was assessed using the Spearman test. Receiver operating characteristic (ROC) curves