International Journal of Infectious Diseases 49 (2016) 154–160

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

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

Yan Dang [a], Jinli Lou [a,*], Yan Yan [a], Yanhua Yu [a], Ming Chen [a], Guizhen Sun [a], Ning Li [b]

[a] Department of Clinical Laboratory, Beijing You'an Hospital Affiliated to Capital Medical University, No. 8 You'an Men Wai Xi TouTiao, Fengtai District, Beijing 100069, China

[b] Centre of Infectious Diseases, Beijing You'an Hospital Affiliated to Capital Medical University, Beijing, China

ARTICLE INFO

Article history:
Received 1 April 2016
Received in revised form 18 May 2016
Accepted 28 June 2016

Corresponding Editor: Eskild Petersen, Aarhus, Denmark

Keywords:
Neutrophil CD64 index
Spontaneous bacterial peritonitis
Cirrhosis
Ascites

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

* Corresponding author. Tel.: +86-13718641405; fax: +010-63054847.
E-mail address: loujinli@163.com (J. Lou).

http://dx.doi.org/10.1016/j.ijid.2016.06.021

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

were used to evaluate the diagnostic value and determine the optimum cut-off value by maximizing the sensitivity and specificity. A p-value of < 0.05 was considered statistically significant.

3. Results

3.1. Clinical and laboratory characteristics of patients

A total of 123 cirrhotic patients with ascites were enrolled in the study, including 41 patients with clinical SBP (ascitic fluid PMN count $\geq 0.25 \times 10^9$/l or positive ascitic fluid bacterial culture) and 82 patients without SBP (ascitic fluid PMN count $< 0.25 \times 10^9$/l and negative ascitic fluid bacterial culture). The etiologies of liver cirrhosis were determined by laboratory tests and clinical diagnosis. Cirrhosis was related to hepatitis B alone in 59% ($n = 73$) of the patients, hepatitis C alone in 5% ($n = 6$), alcoholism alone in 18% ($n = 22$), cholestasis alone in 3% ($n = 4$), and a combination of multiple factors in 5% ($n = 6$). The cause of the cirrhosis was unknown in 10% ($n = 12$) of the patients. The average age of the subjects was 55.13 ± 1.31 years in the SBP patient group and 54.36 ± 1.64 years in the non-SBP patient group.

There were no significant differences in age, PLT count, ALT, AST, albumin, gamma-glutamyltransferase, alkaline phosphatase, total bile acid, creatinine, or urea between the two patient groups ($p > 0.05$). There were differences between the groups in prothrombin time, international normalized ratio (INR), total bilirubin, total protein, and cholinesterase. The WBC count in the blood was significantly higher in SBP patients than in non-SBP patients ($p < 0.001$). All the clinical and laboratory characteristics of the cirrhotic patients are shown in Table 1.

Table 1
Clinical and laboratory characteristics of cirrhotic patients with and without spontaneous bacterial peritonitis; results are presented as the mean ± SEM, median (IQR), or n (%)

	SBP ($n = 41$)	non-SBP ($n = 82$)	p-Value
Sex, female/male	27/14	65/17	0.106
Age, years	54.36 ± 1.64	55.13 ± 1.31	0.726
Etiology			0.204
HBV	28 (68.30%)	45 (54.93%)	-
Alcoholism	5 (12.19%)	17 (19.72%)	-
HCV	1 (2.44%)	5 (7.04%)	-
Cholestasis	1 (2.44%)	3 (2.82%)	-
Multiple	0 (0%)	6 (8.45%)	-
Unknown	6 (14.63%)	6 (7.04%)	-
Laboratory tests			
PLT ($\times 10^9$/l)	108 (80–179)	88 (54–142)	0.073
PT (s)	17.8 (15.6–22.6)	14.3 (12.6–18.5)	<0.001
INR	1.57 (1.38–1.98)	1.27 (1.13–1.60)	<0.001
ALT (U/l)	32.3 (16.9–55.13)	29.2 (17.6–50.3)	0.699
AST (U/l)	67.9 (35.9–129.4)	63.6 (34.5–103.9)	0.679
Tbil (μmol/l)	79.5 (32.3–152.2)	45.7 (24.1–96.7)	0.029
TP (g/l)	52.6 (45.6–59.1)	56.9 (51.3–63.9)	0.037
ALB (g/l)	28.5 (25.1–30.5)	29.4 (26.7–32.3)	0.087
GGT (U/l)	73.0 (34.8–164.9)	74.3 (36.5–198.3)	0.662
ALP (U/l)	106.8 (68.5–208.9)	99.4 (70.4–157.8)	0.479
TBA (μmol/l)	36.5 (13.6–109.3)	35.0 (16.4–79.5)	0.394
CHE (U/l)	1125 (761–1594)	1641 (1217–2228)	0.005
Urea (mmol/l)	8.92 (5.37–19.58)	6.85 (4.66–11.55)	0.063
Creatinine (μmol/l)	85.4 (60.1–193.9)	78.75 (57.40–110.75)	0.251
WBC ($\times 10^9$/l)	11.18 (6.715–15.215)	4.88 (3.58–7.31)	<0.001

ALB, albumin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CHE, cholinesterase; GGT, gamma-glutamyltransferase; HBV, hepatitis B virus; HCV, hepatitis C virus; INR, international normalized ratio; IQR, interquartile range; PLT, platelets; PT, prothrombin time; SBP, spontaneous bacterial peritonitis; SEM, standard error of the mean; TBA, total bile acid; Tbil, total bilirubin; TP, total protein; WBC, white blood cell count.

3.2. Detection of ascitic fluid in cirrhotic patients

Ascites is a common complication in cirrhotic patients and ascites infection results in a poor prognosis. Although the diagnosis of SBP relies on the PMN count, microbial culture is the most reliable criterion for all kinds of infection. Patient ascitic fluid samples were collected and cultured both aerobically and anaerobically for 3 days. Thirteen of 41 patients diagnosed clinically with SBP had positive ascites cultures, a positive rate of 31.71%. Nine different types of bacteria were cultured in the ascites of these 13 patients: *Escherichia coli* ($n = 4$, 31.7%), *Klebsiella pneumoniae* ($n = 2$, 15.4%), *Staphylococcus aureus* ($n = 1$, 7.7%), *Enterococcus faecalis* ($n = 1$, 7.7%), *Enterobacter cloacae* ($n = 1$, 7.7%), *Acinetobacter baumannii* ($n = 1$, 7.7%), *Enterococcus faecium* ($n = 1$, 7.7%), *Staphylococcus epidermidis* ($n = 1$, 7.7%), and *Stenotrophomonas maltophilia* ($n = 1$, 7.7%). This shows that the diagnosis of infection in ascites cannot rely on bacterial culture alone on account of the low positive rate.

Meanwhile, using the diagnostic criteria of SBP, cells were detected in the ascites samples of cirrhotic patients. It was found that the WBC and PMN counts in the ascites of SBP patients (median 1.778, IQR 0.927–6.079 and median 1.352, IQR 0.432–4.267) were both significantly higher than those in non-SBP patients (median 0.172, IQR 0.107–0.285 and median 0.026, IQR 0.016–0.052) ($p < 0.001$).

3.3. Neutrophil CD64 index in SBP patients

Since diagnostic paracentesis is an invasive detection method, a minimally traumatic method to identify ascites infection effectively was sought. In this study, the difference in neutrophil CD64 index was explored in SBP patients, non-SBP patients, and healthy subjects.

A significant difference was found among the three groups ($p < 0.001$). The neutrophil CD64 index in SBP samples (median 6.09, IQR 2.35–15.23) was significantly higher than that in non-SBP samples (median 0.71, IQR 0.36–1.05; $p < 0.001$) and that in healthy subjects (median 0.63, IQR 0.08–1.41; $p < 0.001$). However, there was no significant difference between cirrhotic patients without SBP and healthy subjects ($p = 0.511$) (Figure 1). Neutrophil CD64 expression in the three groups is shown in Figure 2.

A tendency towards a higher neutrophil CD64 index was witnessed in culture-positive SBP patients (16.53 ± 4.06) compared with culture-negative SBP patients (8.87 ± 2.21) in the experiment

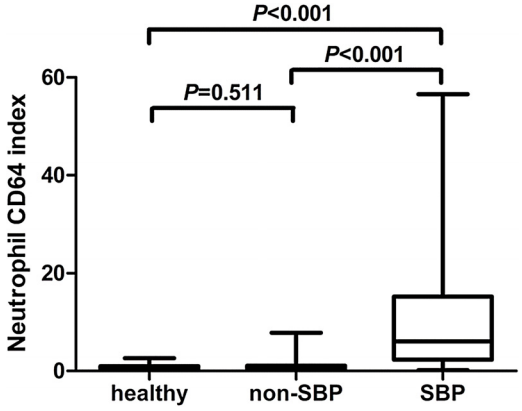

Figure 1. Neutrophil CD64 index in healthy subjects, cirrhotic patients without SBP, and cirrhotic patients with SBP. The neutrophil CD64 index in SBP samples (median 6.09, IQR 2.35–15.23) was significantly higher than that in non-SBP samples (median 0.71, IQR 0.36–1.05; $p < 0.001$) and that in healthy subjects (median 0.63, IQR 0.08–1.41; $p < 0.001$). However, there was no significant difference between cirrhotic patients without SBP and healthy people ($p = 0.511$). (IQR, interquartile range; SBP, spontaneous bacterial peritonitis.).

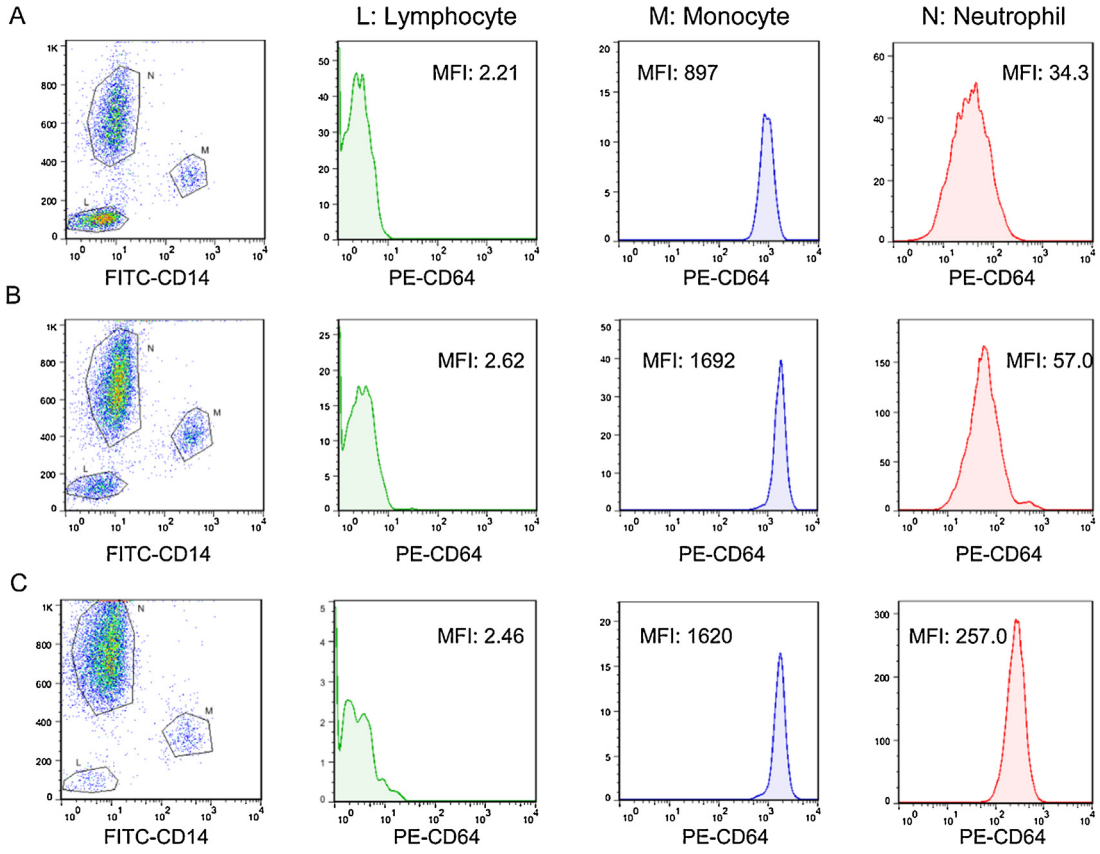

Figure 2. Neutrophil CD64 expression in the three groups. Determination of different cells in peripheral blood by double-staining using fluorescein isothiocyanate (FITC)-conjugated anti-CD14 and phycoerythrin (PE)-conjugated anti-CD64 antibodies. (A) Healthy people. (B) Patients with ascites caused by cirrhosis but without SBP. (C) SBP patients. (SBP, spontaneous bacterial peritonitis.).

presented; however, the difference was not significant ($p = 0.080$) (Figure 3).

3.4. Correlation of the neutrophil CD64 index and the PMN count in ascites

Since the main diagnostic criterion of SBP is the PMN count in ascites, the correlation of the neutrophil CD64 index and the PMN count in ascites was analyzed in order to confirm that the reason for up-regulation of the neutrophil CD64 index was ascites infection.

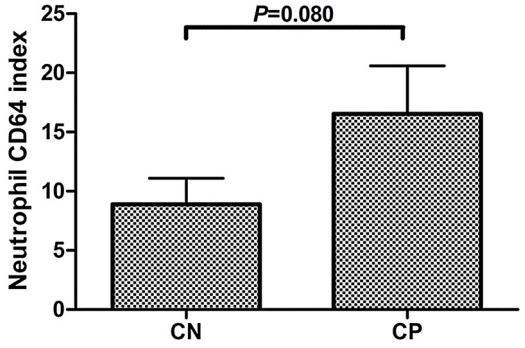

Figure 3. Neutrophil CD64 index in culture-negative SBP and culture-positive SBP patients. There was no significant difference in the neutrophil CD64 index between culture-negative SBP patients (8.87 ± 2.21) and culture-positive SBP patients (16.53 ± 4.06) ($p = 0.080$). (CN, culture-negative; CP, culture-positive; SBP, spontaneous bacterial peritonitis.).

As shown in Figure 4, there was a moderate positive correlation between the neutrophil CD64 index and the PMN count in ascites ($p < 0.001$, $r = 0.484$), as well as a positive correlation between the neutrophil CD64 index and the WBC count in ascites ($p < 0.001$, $r = 0.450$). These results indicate that the neutrophil CD64 index in blood can be regulated by ascites infection.

3.5. Clinical value of the neutrophil CD64 index in diagnosing SBP

A ROC curve was generated and showed that the neutrophil CD64 index was a valuable parameter for discriminating cirrhotic patients with SBP from those without SBP. The AUC was 0.894 (95% CI 0.823–0.964) ($p < 0.001$) (Figure 5).

Several potential cut-off values that can be considered for the diagnosis of SBP are presented in Table 2. The optimal cut-off value for the neutrophil CD64 index, with the highest combined sensitivity and specificity to distinguish SBP samples from those without SBP, was 2.02. At this cut-off level, the sensitivity, specificity, and accuracy were 80.49%, 93.90%, and 89.43%, respectively, while the negative predictive value (NPV) and positive predictive value (PPV) were 90.58% and 86.84%, respectively.

3.6. The neutrophil CD64 index is modulated by antibiotic therapy

The blood of 10 newly diagnosed SBP patients was analyzed and the neutrophil CD64 indices were compared before and after 3–5 days of antibiotic therapy. It was found that the neutrophil CD64 index is modulated by antibiotic therapy. The neutrophil CD64 index was elevated in newly diagnosed SBP patients, but this increased neutrophil CD64 index was significantly down-regulated

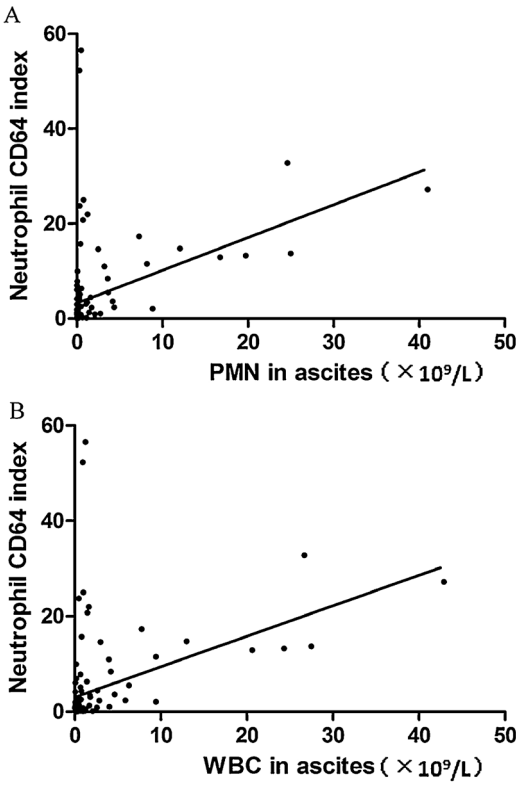

Figure 4. Correlation of the neutrophil CD64 index and cell counts in ascites. A significant positive correlation was found between the neutrophil CD64 index and the polymorphonuclear neutrophil cell count (PMN) in ascites ($p < 0.001$, $r = 0.484$), as well as a positive correlation between the neutrophil CD64 index and the white blood cell count (WBC) in ascites ($p < 0.001$, $r = 0.450$).

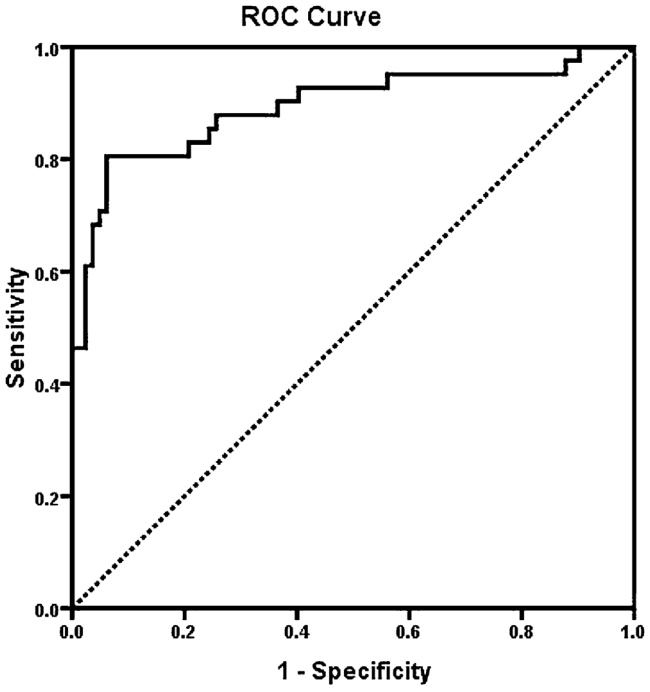

Figure 5. ROC curve of the neutrophil CD64 index in the diagnosis of SBP. The AUC was 0.894 (95% CI 0.823–0.964; $p < 0.001$). The optimal cut-off value for the neutrophil CD64 index was 2.02. The sensitivity and specificity of the neutrophil CD64 index for cirrhotic patients with SBP were 80.49% and 93.90%, respectively. (AUC, area under the curve; CI, confidence interval; ROC, receiver operating characteristic; SBP, spontaneous bacterial peritonitis.).

Table 2
Diagnostic accuracy of the neutrophil CD64 index in cirrhosis patients with spontaneous bacterial peritonitis

Cut-off value	Sensitivity (%)	Specificity (%)	Accuracy (%)	PPV (%)	NPV (%)	PLR	NLR
1.64	80.49	87.80	85.37	76.74	90.00	6.60	0.22
1.84	80.49	91.46	87.80	82.50	90.36	9.43	0.21
2.02	**80.49**	**93.90**	**89.43**	**86.84**	**90.59**	**13.20**	**0.20**
2.21	78.05	93.90	88.62	86.48	89.53	12.80	0.23
2.48	70.73	93.90	86.18	85.29	86.52	11.60	0.31

NLR, negative likelihood ratio; NPV, negative predictive value; PLR, positive likelihood ratio; PPV, positive predictive value.

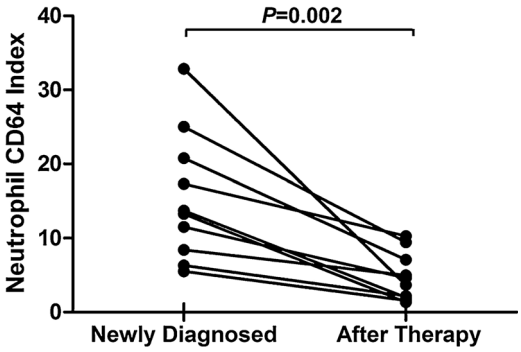

Figure 6. The neutrophil CD64 indices of SBP patients when newly diagnosed and after therapy. The neutrophil CD64 index was elevated in SBP patients at first diagnosis, but this increased neutrophil CD64 index was significantly down-regulated by antibiotic therapy ($p = 0.002$). (SBP, spontaneous bacterial peritonitis.).

by antibiotic therapy in all patients ($p = 0.002$) (Figure 6); neutrophil CD64 expression before and after therapy is shown in Figure 7.

4. Discussion

SBP is a frequent and life-threatening bacterial infection in patients with liver cirrhosis and ascites. Portal hypertension leads to translocation of the intestinal bacteria, which cannot be eliminated due to immune defects caused by liver cirrhosis. However, the positive rate of ascites culture in SBP in the laboratory is extremely low owing to dilution by large amounts of water. In the samples included in this study, 13 out of 41 had positive ascites cultures, giving a positive rate of 31.71%. *E. coli* and *K. pneumoniae* were the pathogens most frequently responsible for infection, which is in agreement with previous studies.[20–22]

On account of the low positive rate of ascites culture, the diagnosis of SBP is based on a PMN count $\geq 0.25 \times 10^9$ cells/l in ascites clinically. However, an invasive test – paracentesis – is required to obtain the PMN count in ascites; this can be dangerous and is not always readily available for antibiotic therapy. Therefore, efforts must be made to develop a rapid and reliable detection method for the auxiliary diagnosis of SBP.

CD64 is one of the receptors for the Fc portion of immunoglobulin G (IgG) and is also called Fcγ receptor I. Many studies have confirmed that the neutrophil CD64 index can be used to diagnose bacterial infections in blood with high sensitivity and specificity,[23] and to distinguish bacterial infections from active autoimmune inflammation.[24]

In this study, it was found that the neutrophil CD64 index can discriminate between SBP and non-SBP samples. There was a positive correlation between the neutrophil CD64 index and the PMN count in ascites. The neutrophil CD64 index could be applied in the auxiliary diagnosis of SBP. Furthermore, the neutrophil CD64 index at the optimal cut-off value of 2.02 had the best sensitivity

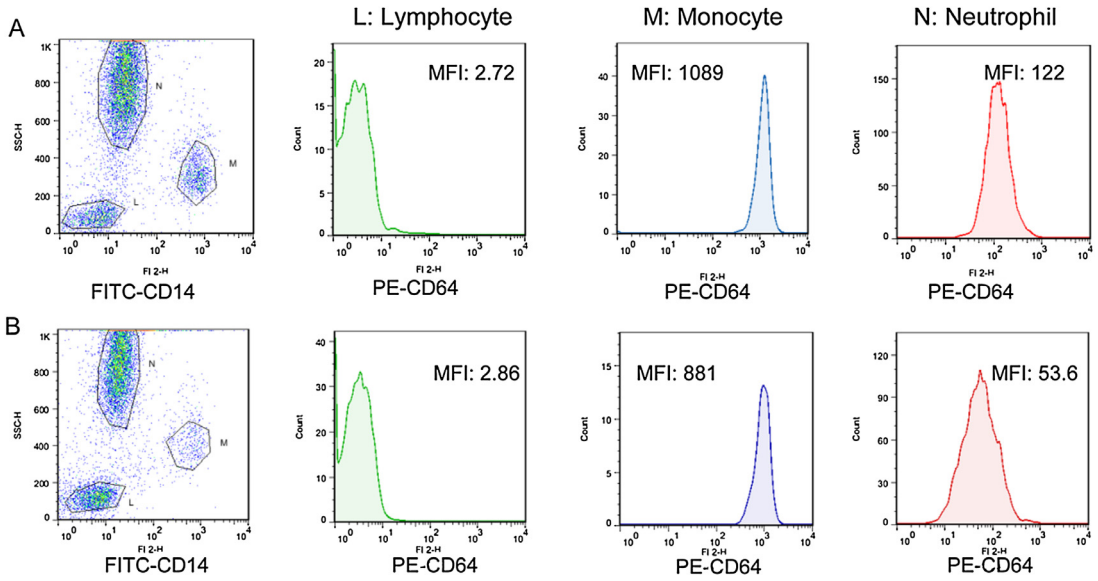

Figure 7. Neutrophil CD64 expression: (A) at first diagnosis, and (B) after antibiotic therapy.

(80.49%), specificity (93.90%), and AUC (0.894) for SBP in cirrhotic patients. These data demonstrate a similar cut-off value for the neutrophil CD64 index in diagnosing SBP to that used in the diagnosis of other diseases. Jukic et al. recommended a CD64 index value $\geq$1.3 for positive infection .[25] Motta et al. reported that the CD64 index was a marker of early-onset sepsis in very low birth weight neonates with a cut-off value of 2.4 .[26] Minar et al. found that the neutrophil CD64 index cut-off point for newly diagnosed Crohn's disease was 1.0.[16]

The difference in neutrophil CD64 index between culture-negative SBP patients and culture-positive SBP patients was not significant, thus the index could not be used to distinguish between microbiologically confirmed and clinically diagnosed bacterial infections. This is in agreement with a previous analysis of the clinical value of neutrophil cell surface CD64 expression.[27]

In the present cohort, the neutrophil CD64 index was modulated by antibiotic therapy. It was observed that the neutrophil CD64 index was elevated in SBP patients when first diagnosed with a bacterial infection, but that the increased neutrophil CD64 index was down-regulated by antibiotic therapy. Migita et al. reported the same characteristic of neutrophil CD64 expression in familial Mediterranean fever patients.[28]

Given the dangerous complications that may occur as a result of diagnostic paracentesis, such as bleeding and infection,[7] the rapid detection and auxiliary diagnosis of SBP with a blood sample is necessary to improve the survival of cirrhotic patients with ascitic fluid infections. In the present cirrhotic patients with ascites, a high specificity and sensitivity was found for the neutrophil CD64 index in SBP patients with a time cost of 1–2 h. In this regard, based on its rapid and cost-effective features, determination of the neutrophil CD64 index may play an increasingly important role in the rapid detection and identification of SBP, allowing the prompt initiation of appropriate therapy, which may help to increase the overall survival of patients at high risk of developing SBP.

In conclusion, the results of this study show that the neutrophil CD64 index could be used as a sensitive and specific indicator for the diagnosis of SBP in cirrhotic patients with ascites. In addition, the neutrophil CD64 index is modulated by antibiotic therapy and could be used to evaluate the effect of therapy in SBP patients.

The small number of patients included in this study made it difficult to define an accurate cut-off value for the neutrophil CD64 index. Therefore, a larger trial is needed before the neutrophil CD64 index can be recommended for widespread clinical use.

Acknowledgements

We thank the Department of Gastroenterology Center of Beijing You'an Hospital for the timely help given in clinical instruction and collecting the samples. This study was supported by grants from Building of Clinical Science Basis and Technology Platform for Major Infectious Diseases Prevention and Treatment with Chinese Traditional Medicine (No. 2012ZX10005010). The funders had no role in the study design, data collection and analysis, interpretation of data, decision to publish, or preparation of the manuscript.

Conflict of interest: No conflict of interest exits in the submission of this manuscript, and the manuscript was approved by all authors for publication.

References

1. Wiest R, Krag A, Gerbes A. Spontaneous bacterial peritonitis: recent guidelines and beyond. *Gut* 2011;**2**:297–310.
2. Lippi G, Danese E, Cervellin G, Montagnana M. Laboratory diagnostics of spontaneous bacterial peritonitis. *Clin Chim Acta* 2014;**430**:164–70.
3. Cekin Y, Cekin AH, Duman A, Yilmaz U, Yesil B, Yolcular BO. The role of serum procalcitonin levels in predicting ascitic fluid infection in hospitalized cirrhotic and non-cirrhotic patients. *Int J Med Sci* 2013;**10**:1367–74.
4. Volk ML. Advances in critical care hepatology. *Minerva Anestesiol* 2006;**5**:269–81.
5. Tandon P. Bacterial infections, sepsis, and multiorgan failure in cirrhosis. *Semin Liver Dis* 2008;**1**:26–42.
6. Mostafa MS, El-Seidi EA, Kassem AM, Shemis MA, Saber M, Michael MN. Detection of ascitic fluid infections in patients with liver cirrhosis and ascites. *Arab Journal of Gastroenterology* 2011;**12**:20–4.
7. De Gottardi A, Thévenot T, Spahr L, Morard I, Bresson-Hadni S, Torres F, et al. Risk of complications after abdominal paracentesis in cirrhotic patients: a prospective study. *Clin Gastroenterol Hepatol* 2009;**7**:906–9.
8. Runyon BA. Strips and tubes: improving the diagnosis of spontaneous bacterial peritonitis. *Hepatology* 2003;**37**:745–7.
9. Runyon BA. The evolution of ascitic fluid analysis in the diagnosis of spontaneous bacterial peritonitis. *Am J Gastroenterol* 2003;**28**:1675–7.
10. Quayle JA, Watson F, Bucknall RC, Edwards SW. Neutrophils from the synovial fluid of patients with rheumatoid arthritis express the high affinity immunoglobulin G receptor, Fc gamma RI (CD64): role of immune complexes and cytokines in induction of receptor expression. *Immunology* 1997;**91**:266–73.
11. Gericke GH, Ericson SG, Pan L, Mills LE, Guyre PM, Ely P. Mature polymorphonuclear leukocytes express high-affinity receptors for IgG (Fc gamma RI) after stimulation with granulocyte colony-stimulating factor (G-CSF). *J Leukoc Biol* 1995;**57**:455–61.
12. Rogina P, Stubljar D, Lejko-Zupanc T, Osredkar J, Skvarc M. Expression of CD64 on neutrophils (CD64 index): diagnostic accuracy of CD64 index to predict sepsis in critically ill patients. *Clin Chem Lab Med* 2015;**453**:e89–91.
13. Stubljar D, Skvarc M. Expression of CD64 on neutrophils can be used to predict the severity of bloodstream infection before broad range 16S rRNA PCR. *Folia Microbiol (Praha)* 2015;**60**:111–8.

14. Kipfmueller F, Schneider J, Prusseit J, Dimitriou I, Zur B, Franz AR, et al. Role of neutrophil CD64 index as a screening marker for late-onset sepsis in very low birth weight infants. *PLoS One* 2015;**10**:e0124634.
15. Lynema S, Marmer D, Hall ES, Meinzen-Derr J, Kingma PS. Neutrophil CD64 as a diagnostic marker of sepsis: impact on neonatal care. *Am J Perinatol* 2015;**4**:331–6.
16. Minar P, Haberman Y, Jurickova I, Wen T, Rothenberg ME, Kim MO, et al. Utility of neutrophil Fcγ receptor I (CD64) index as a biomarker for mucosal inflammation in pediatric Crohn's disease. *Inflamm Bowel Dis* 2014;**20**: 1037–48.
17. Gerrits JH, McLaughlin PM, Nienhuis BN, Smit JW, Loef B. Polymorphic mononuclear neutrophils CD64 index for diagnosis of sepsis in postoperative surgical patients and critically ill patients. *Clin Chem Lab Med* 2013;**4**:897–905.
18. European Association for the Study of the Liver. EASL clinical practice guidelines on the management of ascites spontaneous bacterial peritonitis and hepatorenal syndrome in cirrhosis. *J Hepatol* 2010;**53**:397–417.
19. Runyon BA. Introduction to the revised American Association for the Study of Liver Diseases Practice Guideline management of adult patients with ascites due to cirrhosis. *Hepatology* 2013;**57**:1651–3.
20. Merli M, Lucidi C, Di Gregorio V, Falcone M, Giannelli V, Lattanzi B, et al. The spread of multi drug resistant infections is leading to an increase in the empirical antibiotic treatment failure in cirrhosis: a prospective survey. *PLoS One* 2015;**10**:e0127448.
21. Lutz P, Nischalke HD, Strassburg CP, Spengler U. Spontaneous bacterial peritonitis: the clinical challenge of a leaky gut and a cirrhotic liver. *World J Hepatol* 2015;**3**:304–14.
22. Parsi MA, Saadeh SN, Zein NN, Davis GL, Lopez R, Boone J, et al. Ascitic Fluid lactoferrin for diagnosis of spontaneous bacterial peritonitis. *Gastroenterology* 2008;**135**:803–7.
23. Li S, Huang X, Chen Z, Zhong H, Peng Q, Deng Y, et al. Neutrophil CD64 expression as a biomarker in the early diagnosis of bacterial infection: a meta-analysis. *Int J Infect Dis* 2013;**17**:12–23.
24. Hoffmann JJ. Neutrophil CD64: a diagnostic marker for infection and sepsis. *Clin Chem Lab Med* 2009;**47**:903–16.
25. Jukic T, Ihan A, Stubljar D. Dynamics of inflammation biomarkers C-reactive protein, leukocytes, neutrophils, and CD64 on neutrophils before and after major surgical procedures to recognize potential postoperative infection. *Scand J Clin Lab Invest* 2015;**75**:500–7.
26. Motta M, Zini A, Regazzoli A, Garzoli E, Chirico G, Caimi L, et al. Diagnostic accuracy and prognostic value of the CD64 index in very low birth weight neonates as a marker of early-onset sepsis. *Scand J Infect Dis* 2014;**46**:433–9.
27. Nuutila J. The novel applications of the quantitative analysis of neutrophil cell surface FcgRI (CD64) to the diagnosis of infectious and inflammatory diseases. *Curr Opin Infect Dis* 2010;**23**:268–74.
28. Migita K, Agematsu K, Yamazaki K, Suzuki A, Yazaki M, Jiuchi Y, et al. Expression of CD64 on polymorphonuclear neutrophils in patients with familial Mediterranean fever. *Clin Exp Immunol* 2011;**164**:365–72.