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Adjusted Intensive Care Infection Score ($ICIS_{\Delta}$)—A new approach for prediction of ascitic fluid infection in patients with cirrhosis

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ABSTRACT

Background: Early and accurate diagnosis is the key to improving survival in cirrhotic patients with ascitic fluid infection.
Aims: To investigate the usefulness of adjusted Intensive Care Infection Score ($ICIS_{\Delta}$) for diagnosis of ascites infection in cirrhotic patients.
Methods: Cirrhotic patients with ascites (n = 125) were enrolled, and the efficacy of ICIS and $ICIS_{\Delta}$ for predicting ascites infection was evaluated. $ICIS_{\Delta}$ was created by using the weighted variation of each ICIS parameter.
Results: The area under the curves (AUCs) of ICIS for the diagnosis of ascites infection were 0.90 (95% CI: 0.84–0.95), 0.85 (95% CI: 0.79–0.90), and 0.87 (95% CI: 0.81–0.93), for SBP, culture-negative SBP, and combined SBP/culture-negative SBP, respectively. ICIS was optimized and diagnostic accuracy was obviously improved. $ICIS_{\Delta}$ had high AUCs of 0.99 (95% CI: 0.93–1.00) for SBP, 0.98 (95% CI: 0.83–1.00) for culture-negative SBP, and 0.98 (95% CI: 0.94–1.00) for the combination group. The optimal cutoff was identified as $ICIS_{\Delta} > 2$, which had >97.8% sensitivity and 100% specificity for diagnosis of both SBP and culture-negative SBP. The $ICIS_{\Delta}$ had significantly higher AUCs than PCT and CPR in both groups ($P = 0.002$–0.008). $ICIS_{\Delta}$ kinetics could differentiate between SBP and culture-negative SBP patients. From sterile ascites, through culture-negative SBP to SBP, three $ICIS_{\Delta}$ parameters showed an increasing trend.
Conclusions: ICIS and $ICIS_{\Delta}$ are simple, rapid, accurate and cost-effective methods for the diagnosis of ascites infection in cirrhotic patients.

1. Introduction

Ascitic fluid infection is a life-threatening complication in patients with cirrhosis [1,2]. Early diagnosis is key to improving patient survival. Diagnosis of ascitic fluid infection is generally established by analysis of ascitic fluid obtained by paracentesis [3]. This is an invasive procedure requiring patient consent and cannot be used repeatedly for monitoring. Furthermore, in hemorrhagic ascites and bacterascites the polymorphonuclear leukocyte count (PMNL) may not be a reliable indicator of infection [4–7]. In bacterascites, ascitic culture is confirmatory, but is by no means a necessary prerequisite for starting antibiotic therapy. In fact, it is considered a "fatal" mistake to wait 48 h for culture results before initiating therapy in cases where it is indicated [8]. Although the leukocyte reagent strip is a highly sensitive point-of-care screening tool for spontaneous bacterial peritonitis (SBP), its specificity is reported to be as low as 57.1% [4,9,10]. There is, therefore, a pressing need to develop sensitive, specific, and easy-to-apply methods to diagnose ascitic fluid infection.

A scoring system, the Intensive Care Infection Score (ICIS), which is based on early immune reactions in systemic infection, has been shown to be able to reliably differentiate sepsis from non-infectious systemic inflammation [11–13]. An important advantage of ICIS over procalcitonin (PCT) and C-reactive protein(CRP) is its low cost and simplicity—it requires only the machine used for white blood cell count counting, which means that it can be performed in almost all laboratories and even at the bedside. More details about the ICIS are presented in Supplementary data 1-1.

Pancytopenia is a serious complication in patients with cirrhosis [14]. All five parameters of ICIS are derived from blood cells, which raises doubts about whether ICIS could be a reliable marker for ascites infection in patients with cirrhosis. Moreover, pancy-

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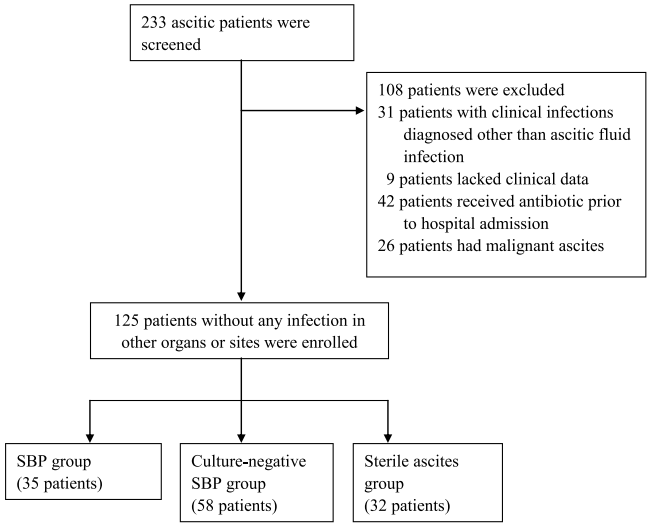

Fig. 1. Study algorithm. SBP, spontaneous bacterial peritonitis.

topenia in patients with cirrhosis varies in form and degree, and the previously determined cutoff values of ICIS may not be applicable in the presence of cirrhosis. However, previous reports have shown that infection biomarker variation over time might help circumvent this drawback, and elevation of infection biomarker level over the previous day's value is a reliable indicator of infection [15].

Systemic inflammatory response to ascites infection is complex, and a single indicator is insufficient for diagnosis; a diagnostic score derived from a combination of different parameters would be more accurate. The aim of this prospective study was to establish a bioscore for early and accurate diagnosis of ascites infection, using easily available hematology indicators. An adjusted ICIS—$ICIS_{\Delta}$—was established and the diagnostic values of both ICIS and $ICIS_{\Delta}$ in cirrhotic patients with proven ascitic fluid infection were assessed.

2. Methods

2.1. Study design and patients

This was an add-on, non-interventional prospective comparative study of inpatients selected from three 48-bed Medical Departments in the 302 Military Hospital, Beijing, China, between April 2016 and May 2017. The study was approved by the Ethics Committee of the 302 Military Hospital of China (Beijing; Permit Number 2017-008). Finally, a total of 125 cirrhotic inpatients with ascites were enrolled (Supplementary data 1-2; Fig. 1).

2.2. Classification of ascitic fluid infection

The presence of ascitic fluid infection was determined and classified as SBP, culture-negative SBP, or bacterascites according to the criteria described by Wiest et al. [16].

2.3. Definition of abnormal hematologic indices

Abnormal hematologic index was defined as: anemia (baseline hemoglobin $\leq$11.5 g/dL for women or $\leq$13.5 g/dL for men); leucopenia (WBC $\leq 4.0 \times 10^9$/L); and/or thrombocytopenia (platelet count $\leq 150 \times 10^9$/L) [17].

2.4. Bone marrow analysis

Of the 125 patients, 32 underwent bone marrow core biopsy from the posterior iliac crest. The aspirate was examined after Leishman staining, and in each sample at least 500 cells were counted, in line with current World Health Organization recommendations.

2.5. Paracentesis and culture techniques

All patients underwent diagnostic paracentesis and ascitic fluid culture. Leukocyte counts, biochemistry, and bacterial identification were performed according to standard protocols [1].

2.6. Assessments

Blood samples were collected every morning at 06:30. Every patient was assessed daily for features of ascitic fluid infection; when infection was suspected, samples were collected for bacteriological culture. The criteria for suspected infection was based on available guidelines [18,19]; these included abdominal pain, and/or fever (temperature >37.5 °C), and/or abdominal tenderness and rebound tenderness. Day 0 (D_0) was the day of diagnosis of ascitic fluid infection (for the SBP group) or the day of first suspicion of infection or the day of first ascitic fluid culture (for culture-negative SBP and sterile ascites groups). Serial monitoring of PCT, CRP, WBC, ICIS, and $ICIS_{\Delta}$ from one day prior to day 0 (D_{0-1}) up to 3 days after day 0 (D_3) were performed in all patients.

The ICIS is based on five blood-cell derived parameters [11]. Reference values of ICIS parameters in healthy individuals are antibody secreting lymphocyte count (ASL#): 0–30 cells/μL, total segmented neutrophil count (sN#): 1950–7935 cells/μL, mean fluorescence intensity of mature (segmented) neutrophils (sNFI): 420 ± 19.3 FIch, immature granulocyte count (aIG#): 0–60 cells/μL, difference in hemoglobin concentration between newly formed and mature red blood cells (dCHC): 0.4–7.0 pg. The $ICIS_{\Delta}$ is based on adjusted versions of the five ICIS parameters. The adjusted values, depicted as ΔASL#, ΔsN#, ΔaIG#, ΔsNFI, and ΔdCHC, were calculated by deducting the D_{0-1} value from the value of any given day. For example, for patients in whom ΔASL# was obtained on D_0, the $\Delta ASL\#D_0$ (i.e., $ASL\#D_0 - ASL\#D_{0-1}$) was calculated. The values of $\Delta ASL\#D_1$ (i.e., $ASL\#D_1 - ASL\#D_{0-1}$), $\Delta ASL\#D_2$ (i.e., $ASL\#D_2 - ASL\#D_{0-1}$) and $\Delta ASL\#D_3$ (i.e., $ASL\#D_3 - ASL\#D_{0-1}$) were similarly calculated.

WBC and ICIS in whole blood and ascitic fluid samples were quantified by fluorescence flow cytometry (XE-5000; Sysmex, Kobe, Japan). PCT level in serum was measured by the electrochemiluminescence immunoassay method (Roche Diagnostics, Mannheim, Germany) with a detection limit of 0.02 ng/mL using an immunology analyzer (Cobas E601; Roche Diagnostics, Mannheim, Germany). Clinical chemistry indicators in serum or ascitic fluid were measured with an AU680 analyzer (Beckman Coulter, Fullerton, USA).

2.7. Statistical analysis

SAS, version 9.3 (SAS Institute Inc., Cary, NC, USA) was used for data analysis. Data were summarized as mean ± standard deviation (SD) or percentages. Sample distribution was assessed by the Kolmogorov-Smirnov test. The Mann–Whitney or Kruskall–Wallis tests were used for comparison of continuous variables between groups, and the chi-square test or Fisher's exact test were used for comparison of categorical variables. ROC curves were constructed, and optimum cutoff values were determined by Youden index. AUCs (with 95% confidence intervals) were calculated to assess the

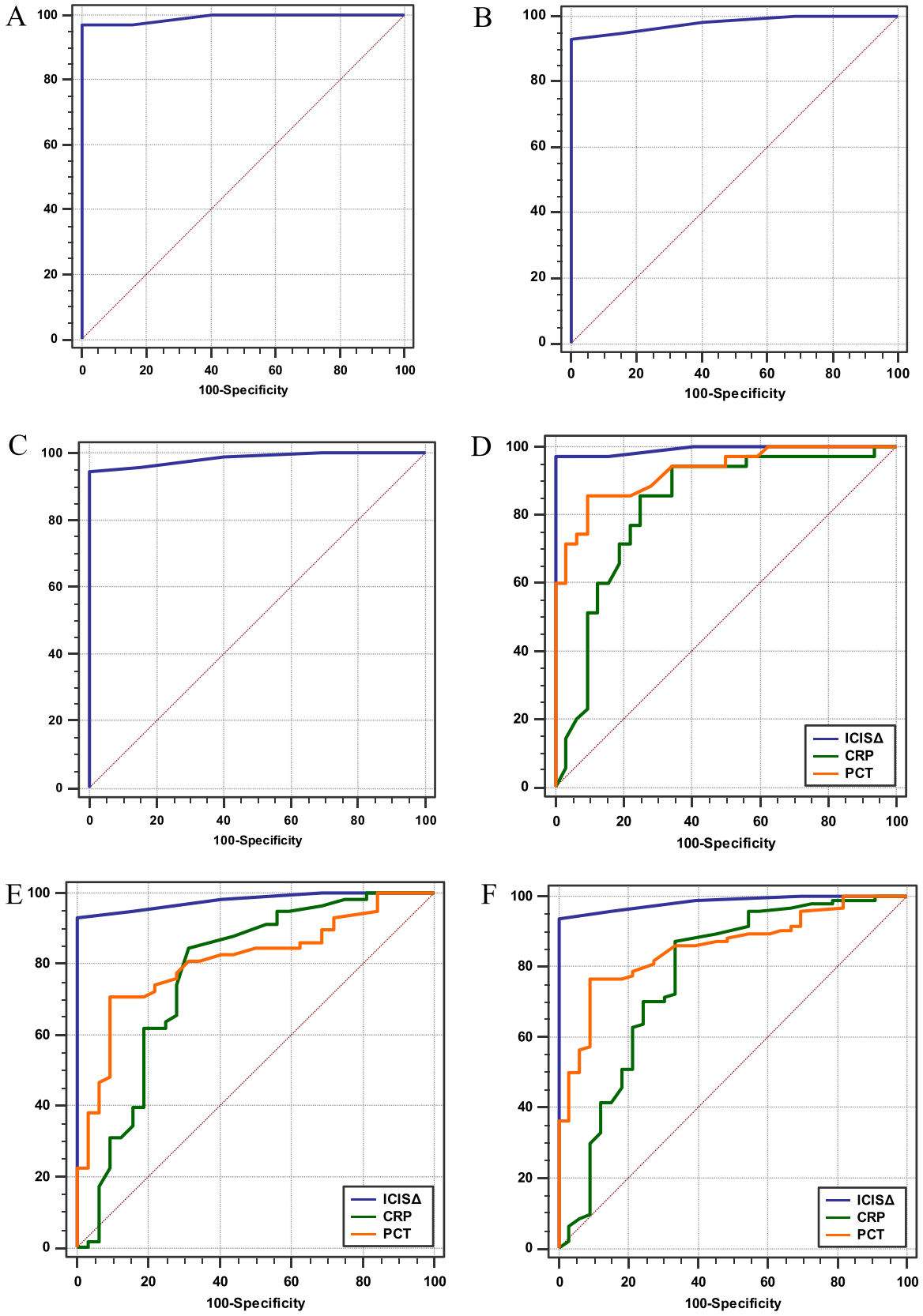

Fig. 2. ROC curve of $ICIS_{\Delta}$ for diagnosis of ascitic fluid infection.
A. ROC curve of $ICIS_{\Delta}$ for diagnosis of SBP. The AUC was 0.99 (95% CI: 0.93–1.00; P < 0.001). The optimal cutoff value for $ICIS_{\Delta}$ was >2. The sensitivity, specificity, PPV, and NPV of $ICIS_{\Delta}$ for diagnosis of SBP were 97.1%, 100%, 100%, and 97.0%, respectively.
B. ROC curve of $ICIS_{\Delta}$ for diagnosis of culture-negative SBP. The AUC was 0.98 (95% CI: 0.83–1.00; P < 0.001). The optimal cutoff value for $ICIS_{\Delta}$ was >2. The sensitivity, specificity, PPV, and NPV of $ICIS_{\Delta}$ for diagnosis of culture-negative SBP were 93.1%, 100%, 100%, and 88.9%, respectively.

Table 1
Baseline demographic data and clinical variables of the enrolled patients.

Mean (SD) or number (%)	Total (N = 125)	SBP (n = 35)	Culture-negative SBP (n = 58)	Sterile ascites (n = 32)
Physical examination				
Age (years)	54.3 (10.2)	55.2 (11.5)	49.3 (14.1)	53.6 (8.2)
Sex (male)	71 (56.8)	20 (57.1)	32 (55.2)	19 (59.4)
Temperature at D_0 (°C)	37.8 (1.2)	38.7 (0.6)	38.3 (0.4)	37.1 (0.8)
Etiology of liver cirrhosis, n (%)				
HBV-related cirrhosis	48 (38.4)	12 (34.2)	23 (39.6)	13 (40.6)
HCV-related cirrhosis	33 (26.4)	9 (25.7)	15 (25.9)	9 (28.1)
Alcoholic cirrhosis	16 (12.8)	5 (14.3)	7 (12.1)	4 (12.5)
Autoimmune cirrhosis	15 (12.0)	5 (14.3)	7 (12.1)	3 (9.4)
Cryptogenic cirrhosis	13 (10.4)	4 (11.4)	6 (10.3)	3 (9.4)
Serum analysis, mean (±SD)				
ALB (g/L)	30.7 (4.5)	27.3 (3.4)	28.1 (4.2)	31.5 (3.7)
ALT (U/L)	50.3 (37.7)	59.2 (40.2)	53.7 (25.3)	46.8 (38.1)
AST (U/L)	67.6 (49.8)	68.1 (35.3)	71.3 (45.5)	60.5 (29.7)
TBIL (μmol/L)	70.6 (51.7)	82.7 (50.2)	76.7 (46.4)	56.2 (32.4)
Child-Pugh class, n (%)				
Child A	0	0	0	0
Child B	41 (32.8)	9 (25.8)	18 (30.0)	14 (43.7)
Child C	84 (67.2)	26 (74.2)	40 (70.0)	18 (56.3)
MELD score, mean (±SD)	17.1 (9.8)	18.7 (8.0)	17.5 (7.3)	15.1 (6.6)
Complications, n (%)				
Hepatic encephalopathy (grade ≥ 2)	61 (48.8)	25 (71.4)	36 (62.1)	0
Hepatorenal syndrome (type 1 or 2 or both)	22 (17.6)	7 (22.9)	15 (25.9)	0
Septic shock	12 (9.6)	5 (14.3)	7 (12.1)	0
Ascitic fluid analysis, mean (±SD)				
WBC ($\times 10^9$ cells/L)	2.8 (0.6)	5.1 (1.1)	2.8(0.9)	0.2(0.04)
PMNL ($\times 10^9$ cells/L)	1.9 (2.0)	4.8 (1.3)	2.5 (1.1)	0.1 (0.08)
ALB (g/L)	7.8 (2.5)	8.2 (1.9)	8.0 (1.7)	7.1 (2.6)
Glu (mg/dL)	107.7 (7.8)	115.1 (18.5)	107.5 (12.1)	101.0 (11.7)
LDH (mU/mL)	821.6 (768.7)	2384.4 (167.6)	276.4 (81.5)	99.5 (18.1)
Protein (g/L)	16.8 (3.2)	16.7 (2.8)	16.9 (1.9)	17.1 (3.2)
Length of hospital stay (days), mean (±SD)	15.2 (10.6)	19.6 (9.5)	17.3 (4.6)	12.4 (5.8)
30-day mortality, n (%)	7.0 (5.6)	5 (14.3)	2 (3.4)	0

SBP: spontaneous bacterial peritonitis, ALB: albumin, ALT: aminotransferase, AST: aspartate aminotransferase, TBIL: total bilirubin, MELD score: Model for End-Stage Liver Disease Score, WBC: white blood cell, PMNL: polymorphonuclear leukocyte count, Glu: glucose, LDH: lactate dehydrogenase.

diagnostic values of the tests; AUCs >0.70 were considered clinically relevant [20]. Statistical significance was set at $P \leq 0.05$.

3. Results

3.1. Baseline population characteristics

A total of 125 inpatients (mean age, 54.3 ± 10.2 years; 56.8% males) were included in the study; 35/125 (28.0%) had SBP, 58/125 (46.4%) had culture-negative SBP, and 32 (25.6%) had sterile ascites (Fig. 1). Child-Pugh stage C disease was present in 84/125 (67.2%) patients (Table 1). Culture was positive in 35/125 (28%) patients, and *Escherichia coli* was the most commonly identified organism (12/35; 34.3%) (Table S-1-1).

3.2. Hematologic indices and bone marrow findings

Cytopenia was present in 115/125 (92.0%). Single or multiple cell line abnormalities were present in 32/125 patients and constituted the primary indications for bone marrow testing. The most common diagnosis from bone marrow analysis was hypersplenism (18/32; 56.3%) (Table S-2).

3.3. ICIS parameters

At baseline, the five ICIS parameters were comparable between patients with SBP, culture-negative SBP, and sterile ascites ($P > 0.05$; Table S-3). However, at D_0, patients with SBP and culture-negative SBP showed significant increase in ASL#, sN#, aIG#, and sNFI, and significant decrease in dCHC, compared to baseline ($P < 0.01$ or $P < 0.05$; Table S-3).

C. ROC curve of $ICIS_\Delta$ for diagnosis of the combination of SBP + culture-negative SBP. The AUC was 0.98 (95% CI: 0.94–1.00; P < 0.001). The optimal cutoff value for $ICIS_\Delta$ was >2. The sensitivity, specificity, PPV and NPV of $ICIS_\Delta$ for diagnosis of SBP + culture-negative SBP were 93.6%, 100%, 100%, and 86.5%, respectively.
D. For diagnosis of SBP, the AUCs of $ICIS_\Delta$, PCT, and CRP were 0.99 (95% CI: 0.93–1.00). 0.90 (95% CI: 0.82–0.99), and 0.78 (95% CI: 0.72–0.90), respectively. The AUC of $ICIS_\Delta$ was significantly larger than the AUCs of PCT (P = 0.008) and CRP (P = 0.002). The AUC of PCT was significantly larger than the AUC of CRP (P = 0.075).
E. For diagnosis of culture-negative SBP, the AUCs of $ICIS_\Delta$, PCT, and CRP were 0.98 (95% CI: 0.83–1.00), 0.83 (95% CI: 0.74–0.91), and 0.75 (95% CI: 0.67–0.87), respectively. The AUC of $ICIS_\Delta$ was significantly larger than the AUCs of PCT (P = 0.004) and the AUC of CRP (P = 0.003). The difference between the AUCs of PCT and CRP was not statistically significant; (P = 0.553).
F. For diagnosis of SBP + culture-negative SBP, the AUCs of $ICIS_\Delta$, PCT, and CRP were 0.98 (95% CI: 0.94–1.00), 0.85 (95% CI: 0.71–0.94), and 0.76 (95% CI: 0.68–0.89), respectively. The AUC of $ICIS_\Delta$ was significantly larger than the AUC of PCT (P = 0.008) and the AUC of CRP (P = 0.005). The difference between the AUCs of PCT and CRP was not statistically significant (P = 0.160).
AUC, area under the curve; ROC, receiver operating characteristic; SBP, spontaneous bacterial peritonitis.

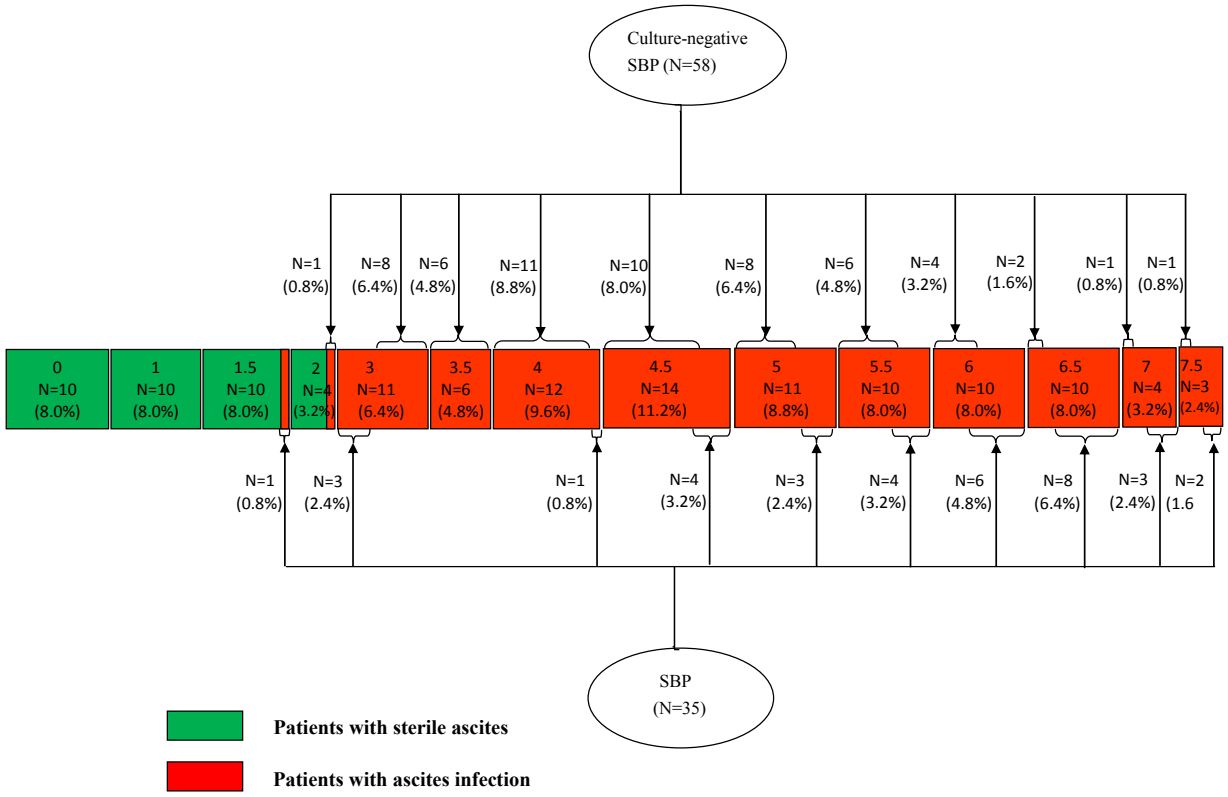

Fig. 3. Classification of the patients by $ICIS_{\Delta}$.

SBP: spontaneous bacterial peritonitis.

3.4. Cutoff values for ICIS and $ICIS_{\Delta}$

ROC curves were analyzed to identify the optimum cutoff values. The cutoff values that would provide specificity >90% were also separately calculated (Table S-4).

To identify the cutoff value that would provide the optimum combination of sensitivity and specificity we used a score of 1 above the cutoff value for the best AUC, and a score of 1.5 above the cutoff value with specificity above 90% at D_0 (Table S-4), as previously described [11]. Thus, the sum of the weighted values of the five ICIS or $ICIS_{\Delta}$ parameters could reach a maximum of 7.5 [11].

The ICIS cutoff value was 3 for diagnosis of SBP, culture-negative SBP, and combined SBP/culture-negative SBP. The AUCs were 0.90 (95% CI: 0.84–0.95), 0.85 (95% CI: 0.79–0.90), and 0.87 (95% CI: 0.81–0.93), for SBP, culture-negative SBP, and combined SBP/culture-negative SBP, respectively. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were 86.2%, 91.3%, 94.1% and 82.6% for SBP; 81.4%, 88.9%, 88.6% and 80.5% for culture-negative SBP; and 83.1%, 89.2%, 89.9% and 81.8% for combined SBP/culture-negative SBP.

In the three groups, the extent of variation of each parameter was different. We hypothesized that changes in the ICIS parameters would not be affected or would only be partially affected by cirrhosis. The cutoff value for $ICIS_{\Delta}$ was >2 between D_{0-1} and D_0 for the diagnosis of SBP, culture-negative SBP, and combined SBP/culture-negative SBP, respectively. AUCs for SBP, culture-negative SBP, and combined SBP/culture-negative SBP were 0.99 (95% CI: 0.93–1.00), 0.98 (95% CI: 0.83–1.00), and 0.98 (95% CI: 0.94–1.00), respectively. The sensitivity, specificity, PPV, and NPV were 97.1%, 100%, 100%, and 97.1% for SBP; 98.2%, 100%, 100%, and 97.1% for culture-negative SBP; and 97.8%, 100%, 100%, and 94.1% for combined SBP/culture-negative SBP (Table S-5).

3.5. $ICIS_{\Delta}$ versus PCT and CRP for prediction of ascitic fluid infection

AUCs of $ICIS_{\Delta}$, PCT, and CPR in the different groups were compared. At D_0, in the SBP and culture-negative SBP groups, the AUC was larger for $ICIS_{\Delta}$ than for PCT and CRP (0.99 vs. 0.86; $P = 0.008$; 0.99 vs. 0.78; $P = 0.002$; and 0.98 vs. 0.83; $P = 0.004$; 0.98 vs. 0.75; $P = 0.003$; Fig. 2 and Table S-5). Similarly, in the combination group, at D_0, the AUC of $ICIS_{\Delta}$ was significantly larger than the AUCs of PCT and CRP (0.98 vs. 0.84; $P = 0.008$; 0.98 vs.0.76; $P = 0.005$; Fig. 2 and Table S-5).

3.6. Validation of $ICIS_{\Delta}$

To evaluate and validate the diagnostic value of $ICIS_{\Delta}$ in clinical practice, the 125 patients were classified according to $ICIS_{\Delta}$ at D_0; thus, three categories were created: (1) *bioscore 0*: 10/125 (8.0%) patients; all 10 patients did not have ascites infection; (2) *bioscore 1–2*: 24/125 (19.2%) patients; 22/24 patients did not have ascites infection; however, 2 patients with this score had infection (1 SBP and 1 culture-negative SBP); and (3) *bioscore 3–7.5*: 91/125 (72.8%) patients; all patients with this score had ascites infection (Fig. 3). $ICIS_{\Delta} > 2$ was seen in 97.8% of patients with ascitic fluid infection vs. 0% of patients without ascitic fluid infection. Thus, from a clinical point of view, the $ICIS_{\Delta}$ was useful in 98.4% of patients.

3.7. Time-dependent analysis of $ICIS_{\Delta}$

Time-dependent analysis of PCT, CRP, and $ICIS_{\Delta}$ from D_{0-1} to D_3 was performed (Fig. 4). $ICIS_{\Delta}$ and PCT kinetics could both differentiate between SBP and culture-negative SBP from D_{0-1} to D_2. However, CRP variations were not significantly different between the two groups (Fig. 4).

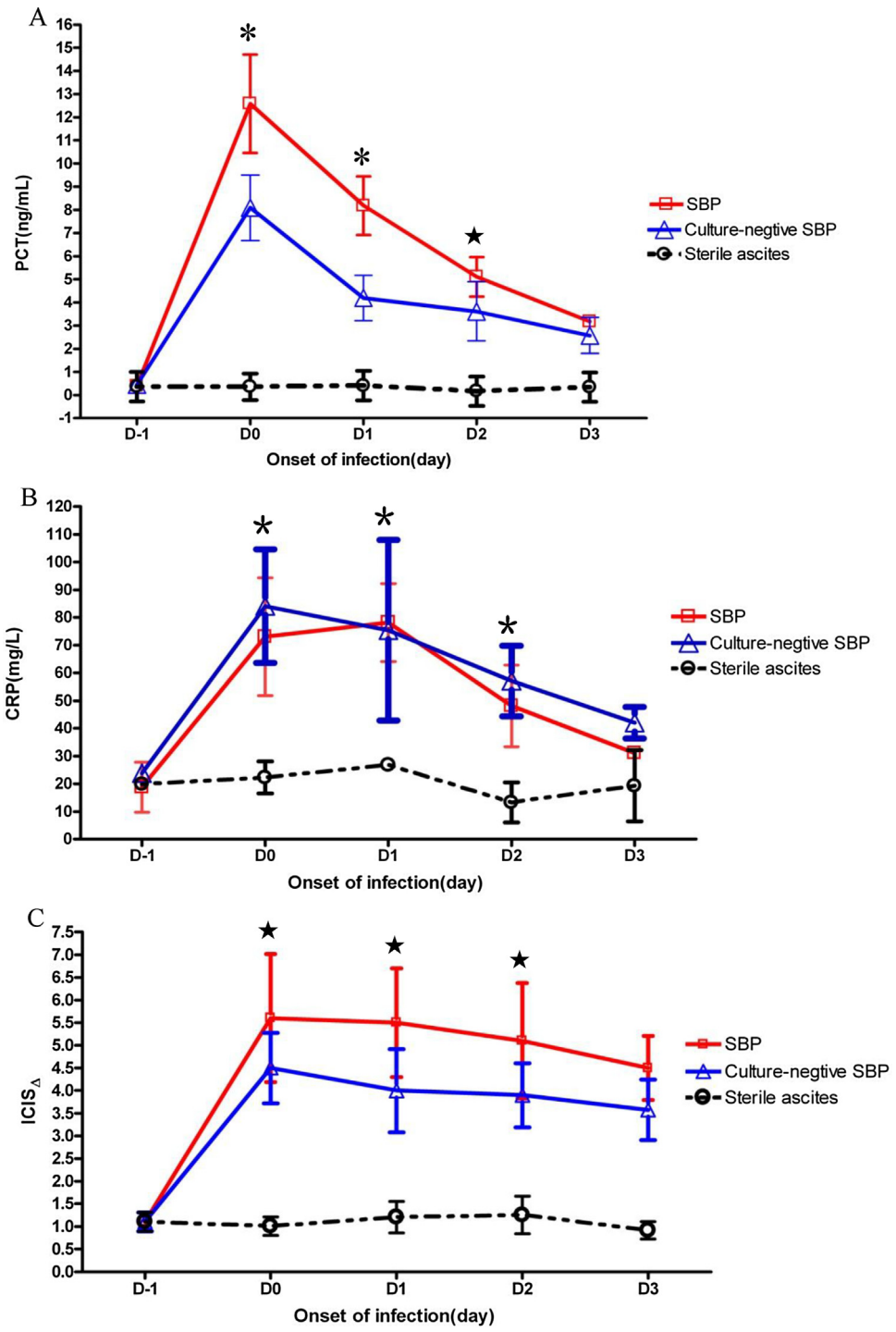

Fig. 4. Time-dependent variations of ICIS$_\Delta$, PCT, and CRP in SBP, culture-negative.
SBP, and sterile ascites. A. PCT; B. CRP; C. ICIS$_\Delta$. SBP: spontaneous bacterial peritonitis, PCT: procalcitonin; ICIS: Intensive Care Infection Score; CRP: C-reactive protein. *P < 0.05. *P < 0.01 (vs. culture-negative SBP), $_{*}$ P > 0.05 (vs. culture-negative SBP). At D_0, ICIS$_\Delta$ was significantly higher in SBP patients than in culture-negative SBP patients (5.6 ± 1.5 vs. 4.5 ± 0.8; P = 0.028). ICIS$_\Delta$ at D_1 and D_2 was also significantly different between the two groups (5.5 ± 1.2 vs. 4.0 ± 0.9; P = 0.020; and 5.1 ± 1.3 vs. 3.9 ± 0.7; P = 0.039). PCT showed a similar trend. However, CRP did not demonstrate significant difference between these two groups of patients from D_0 to D_2.

3.8. Comparison of total score distribution of the five ICIS$_\Delta$ parameters

To compare the total score distribution of the five ICIS$_\Delta$ parameters at D_0, the total score of each ICIS$_\Delta$ parameter at D_0 was calculated. In patients with culture-negative SBP, the ΔsNFI, ΔdCHC, and ΔASL# were all higher than the ΔsN# (52.0 vs. 50.5, 53.0 vs. 50.5, and 52.0 vs. 50.5, respectively; *P* > 0.05) and the ΔaIG# (52.0 vs. 48.0, 53.0 vs. 48.0, and 52.0 vs. 48.0, respectively; *P* > 0.05) (Fig. 5). Differently, in patients with SBP, the ΔsNFI, ΔdCHC, and ΔASL# were significantly higher than the ΔsN# (44 vs. 35, P < 0.01; 42 vs. 35, P < 0.01; and 39 vs. 35, respectively; P < 0.05) and the ΔaIG# (44 vs. 33; P < 0.01; 42 vs. 33; P < 0.01; 39 vs. 33, respectively; P < 0.05) (Fig. 5).

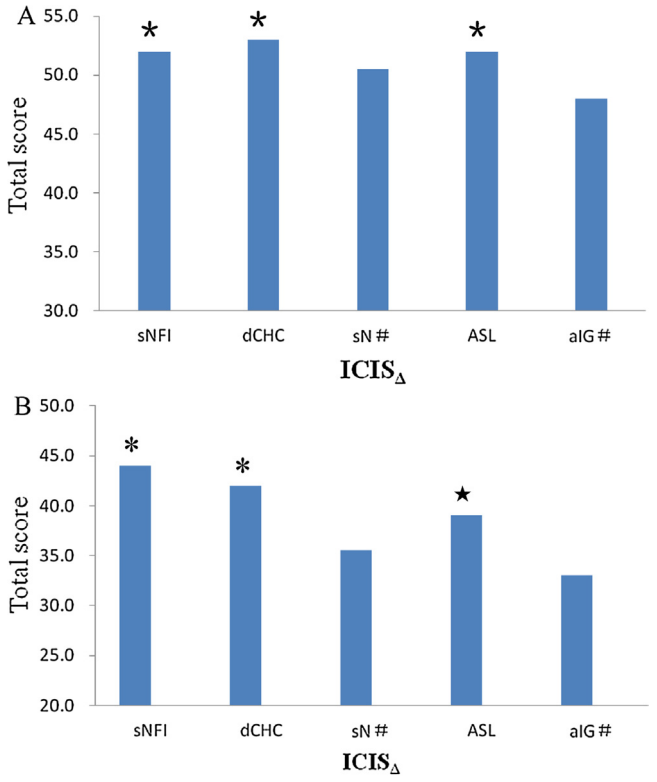

Fig. 5. Total score of the five ICIS$_\Delta$ parameters in patients with ascitic fluid infection. A. culture-negative SBP; B. SBP.
* P > 0.05 (vs. sN# and vs. aIG#), *P < 0.01 (vs. sN# and vs. aIG#), *P < 0.05 (vs. sN# and vs. aIG#).

4. Discussion

Early diagnosis of ascitic fluid infection continues to be a problem for clinicians. This is the first report on the use of a bioscore for diagnosis of ascitic fluid infection. The ICIS, especially the ICIS$_\Delta$, demonstrated impressive accuracy for the diagnosis of ascitic fluid infection. Our study found that the ICIS was a useful tool for early diagnosis of ascites infection, while the ICIS$_\Delta$ could be a powerful tool for day-to-day monitoring of ascites infection in hospitalized patients. It is easy to use and inexpensive, requiring no more than a whole blood cell analyzer to derive all values. This study also opens the door for the use of blood-cells derived parameters of the early innate immune inflammatory response for diagnosis of ascitic fluid infection.

In the present study, 39.9% (93/233) of enrolled cirrhotic patients had ascitic fluid infection; this prevalence is comparable to three recent reports [7,21,22]. Culture was positive in 28% (35/125) of the sample; this is lower than the incidence of 40%–60% in most previous reports [23].

There were no patients with bacterascites in the present study. However, three patients with bacterascites were excluded during initial screening: two because they had been diagnosed with hepatocellular carcinoma and another because antibiotic treatment had been administered for a pulmonary infection prior to hospital admission. According to previous reports, bacterascites is seen in only 3%–4% of cirrhotic patients with ascites [6,7]. Bacterascites is a controversial entity, and little is known about its evolution. It is still controversial whether bacterascites requires immediate initiation of antibiotics therapy [3,16]. Further studies with large samples are necessary to determine the diagnostic accuracy and usefulness of ICIS (and ICIS$_\Delta$) in bacterascites.

In the current study, the discriminative capabilities of PCT and CRP for ascites infection in cirrhotic patients were consistent with previous reports [21,24–28]. The AUC of ICIS was 0.85–0.90, which was a little higher than the AUCs of PCT and CRP. This value was also higher than that reported in three previous studies [11–13]. Early diagnosis of ascites infection is essential to improve short-term survival [29,30]. Cirrhotic patients with acute decompensation and suspected community-acquired infections (SBP) are usually admitted to the emergency department. In this setting, rapid diagnosis is essential, and the ICIS would be a more useful tool than PCT and CRP, or diagnostic paracentesis.

As is well known, hematologic indices are frequently abnormal in patients with cirrhosis, and the baseline values of the ICIS parameters may vary with the patient. This may be the reason for the relatively poor accuracy of ICIS for prediction of ascitic fluid infection. In the current study, 92% of the enrolled patients had some form of cytopenia, probably because of the presence of decompensated cirrhosis, which is more prone to be associated with abnormal hematological indices than compensated cirrhosis and noncirrhotic liver disease [17,31] (Table S-2). In the present study, the levels of individual ICIS parameters (except dCHC) at baseline were significantly different from that found in a previous study on intensive care unit patients with sepsis (Table S-3) [11]. At D_0 there was significantly increased ASL#, sN#, aIG#, and sNFI, as well as reduced dCHC, compared with baseline in all infected ascites patients. The magnitude of the variation of each parameter of ICIS differed significantly between SBP and culture-negative SBP patients (Table S-3).

In view of the above, we speculate that in ascites infection, the magnitude of variation in each parameter of ICIS in cirrhotic patients is unaffected or only partially affected by cirrhosis. Moreover, inflammatory biomarker levels may be affected by a previous episode of sepsis or infection, the course of infection, localized infection, or even other diseases [15,32,33]. The ICIS$_\Delta$ was designed to overcome these problems [15]. Variations of each parameter of ICIS were calculated. We developed the ICIS$_\Delta$ with a score of 1 above the cutoff value for the best AUCs. The optimal cutoff value of ICIS$_\Delta$ was >2. Interestingly, the specificity of ICIS$_\Delta$ was 100% in all the three groups.

We also recalculated the cutoff values of ICIS$_\Delta$ parameters that would ensure specificity >90%. We found that every patient with proved ascites infection had at least two ICIS$_\Delta$ parameters above the cutoff value. Therefore, to obtain the optimum balance of sensitivity and specificity, we further developed the ICIS$_\Delta$ by using a score of 1.5 above the cutoff value with specificity above 90% at D_0 (Table S-4). Overall, an ICIS value $\geq$3 (1.5 points each for at least 2 parameters or 1 point each for 3 parameters) showed diagnostic accuracy of 100% for identifying ascitic fluid infection in patients with cirrhosis. On ROC analysis, the AUC of ICIS$_\Delta$ for early diagnosis of ascitic fluid infection was 0.98–0.99, which was significantly larger than the AUCs of PCT and CRP (Fig. 2; Table S-5). The ICIS$_\Delta$ also showed better diagnostic performance than other markers used to diagnose SBP, such as calprotectin or lactoferrin in ascitic fluid which serve as surrogate markers for PMN count [34,35].

From a clinical point of view, the ICIS$_\Delta$ was useful in 98.4% of patients with cirrhosis. However, the ICIS$_\Delta$ failed to identify ascites infection in 2 patients who had SBP or culture-negative SBP (Fig. 3). We investigated the efficacy of ICIS$_\Delta$ in the diagnosis of different types of ascitic fluid infection using time-dependent analysis. Similar to PCT, but different from CRP, ICIS$_\Delta$ kinetics could differentiate between SBP and culture-negative SBP patients (Fig. 4). Furthermore, from sterile ascites, through culture-negative SBP group to SBP group, ΔsNFI, ΔdCHC, and ΔASL# values showed an increasing trend (Fig. 5). These results highlight the importance of sNFI, dCHC, and ASL in the infection process and in deciding the severity of

ascites infection in patients with cirrhosis. The results also suggest that sNFI, dCHC, and ASL may associate with different mechanisms of ascitic infection. ΔsN# and ΔaIG# were relatively lower than the other three parameters, probably because patients with chronic liver disease often have deficient neutrophil recruitment due to pancytopenia [36].

This study has some limitations. First, external validation on another independent cohort of patients with cirrhosis would have provided more powerful evidence in support of this bioscore. Second, the sample size was small; however, this study serves as a pilot study to define the cutoff values of ICIS and $ICIS_{\Delta}$ for diagnosis of ascites infection. Third, calculation of the adjusted values of five different parameters may not be simple, especially at the bedside. Next, we will analyze the data further to attempt to reduce the number of parameters to optimize the $ICIS_{\Delta}$. We also plan to examine whether other blood-cells derived parameters of early innate immune response could be used to create a more powerful scoring system.

In conclusion, the study results show that the proposed $ICIS_{\Delta}$ has higher sensitivity, specificity, and accuracy than all other biomarkers presently used for diagnosis of ascites infection. ICIS determination is a rapid and easy point-of-care method that requires only 150 μL of whole blood, it does not require sophisticated equipment and results are available within 1 min [11,12]. The medicoeconomic benefits make the $ICIS_{\Delta}$ particularly suitable for use in developing countries.

Conflict of interest

None declared.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.dld.2018.06.006.

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