Role of ascitic prostaglandin E2 in diagnosis of spontaneous bacterial peritonitis and prediction of in-hospital mortality in patients with decompensated cirrhosis

Junli Luo, MS, Xianmei Wu, MS, Yu Zhang, MS, Wenxiang Huang, MS, Bei Jia, PhD

Abstract
Spontaneous bacterial peritonitis (SBP) is one of the most frequent and severe complications in patients with decompensated cirrhosis. Early antibiotic therapy is extremely important for successful treatment and reducing mortality. Prostaglandin E2 (PGE2) is a regulator of the immune response and infection. This study aimed to explore whether ascitic PGE2 could be used as a marker for diagnosing SBP and predicting in-hospital mortality.

Patients with cirrhosis and ascites undergoing abdominal paracentesis were enrolled in our study. Demographic, clinical, and laboratory parameters were recorded at the time of paracentesis and ascitic PGE2 levels were determined by ELISA. The correlation between ascitic PGE2 level and SBP as well as in-hospital mortality were analyzed.

There were 224 patients enrolled, 29 (13%) patients diagnosed as SBP based on the current guideline criteria. The ascitic PGE2 level of patients with SBP (32.77 (26.5–39.68) pg/mL) was significantly lower than that of patients without SBP (49.72 (37.35–54.72) pg/mL). In ROC analysis, the AUC of ascitic PGE2 for the diagnosis of SBP was 0.75, and the AUC of ascitic PGE2 combined with WBC and ascitic PGE2 combined with neutrophils were 0.90 and 0.90, respectively, which were significantly higher than that of ascitic PGE2. In multivariate analysis, ascites PGE2<32.88 pg/mL (OR: 9.39; 95% CI: 4.14–20.84, P=.026), hepatic encephalopathy (OR: 18.39; 95% CI: 3.00–113.13, P=.002) and a higher MELD score (OR: 1.25; 95% CI: 1.05–1.38, P=.026) remained independent predictors of in-hospital mortality.

Ascitic PGE2 level is likely to be a valuable marker in prediction of in-hospital mortality in patients with decompensated cirrhosis, and its value in diagnosis of SBP was not superior to other inflammatory indicators.

Abbreviations: ALT = alanine aminotransferase, AST = aspartate aminotransferase, AUC = area under the curve, C-reactive protein, GI = gastrointestinal, INR = international normalized ratio, PCT = procalcitonin, PGE2 = prostaglandin E2, PMN = polymorphonuclear leukocytes, ROC = receiver operating characteristic, SAAG = ascitic albumin gradient, SBP = spontaneous bacterial peritonitis, WBC = white blood cell.

Keywords: ascites, cirrhosis, diagnosis, mortality, prostaglandin E2, spontaneous bacterial peritonitis

1. Introduction
Cirrhosis is the progressive proliferation of liver fibrous tissue caused by virus, alcohol, fat, autoimmune attack, drug, and so on.[1] Patients with decompensated cirrhosis are more susceptible to bacterial infection because of intrinsic and acquired immunodefi ciency, and the most common infection is spontaneous bacterial peritonitis (SBP).[2] SBP, which frequently induces liver failure, hepatic encephalopathy, hepatorenal syndrome, and coagulopathy, is a vicious and recurrent infection of ascites. The prevalence of SBP ranges from 10% to 30%[3,4] and mortality from 10% to 32% in hospitalized patients.[1,6] Patients with SBP present with variable symptoms, some with signs of systemic inflammation, some with worsen liver function, some with hepatic encephalopathy, some with renal failure, some with gastrointestinal bleeding, and some even may be asymptomatic.[7] Diagnosis of SBP is based on ascitic fluid polymorphonuclear leukocytes (PMN) count ≥250/mm³ or ascitic fluid culture is positive.[1] Early antibiotic therapy is extremely important for the successful treatment of SBP and reducing mortality.[8]

Nevertheless, ascitic fluid PMN count is measured manually, which is operator-dependent, making quality control difficult, thus delaying the diagnosis,[9] also ascites culture is negative in approximately 60% of patients, even those with clinical manifestations suggestive of SBP and increased ascitic PMN,[2,3] due to incorrect sampling, inadequate conservation of samples, low microbial concentration in ascites, and the involvement of hardly growing bacteria.[10] Additionally, previous studies have
been shown infection threshold values of acute inflammatory phase protein markers such as C-reactive protein (CRP) and procalcitonin (PCT) in patients with SBP had high volatility and low reliability.\textsuperscript{11–13} Therefore, the development of new biomarkers to diagnose SBP or predict the mortality is significant for improving the prognosis of patients with SBP.

Prostaglandin E2 (PGE2), a substance with molecular mass of 352 Da, is a product of cyclooxygenase in the arachidonate cascade, and it has been shown to play a role as a chemical mediator in inflammation and as a regulator of the immune response.\textsuperscript{14,15} PGE2 is generally considered a proinflammatory mediator,\textsuperscript{16} promoting local vasodilatation and local attraction and activation of neutrophils, macrophages, and mast cells at early stages of inflammation.\textsuperscript{17} On the other hand, PGE2 has been demonstrated to inhibit the production of multiple proinflammatory cytokines.\textsuperscript{17,18} One study by O’Brien et al indicated that the concentrations of PGE2 from the plasma in patients with acute decompensation of cirrhosis were more than 7 times as high as in healthy volunteers.\textsuperscript{19} However, it is not yet clearly verified whether PGE2 level is increased or decreased during infection.

Although there were some works on the correlation between plasma PGE2 and decompensated cirrhosis,\textsuperscript{19–21} the value of ascitic PGE2 in SBP as well as the correlation between ascitic PGE2 and other inflammatory indicators including WBC, neutrophils, CRP, PCT have not been well characterized. Thus, our study sought to investigate the role of ascitic PGE2 to identify patients with SBP and predict in-hospital mortality of patients with decompensated cirrhosis.

2. Materials and methods

2.1. Study design

Consecutive patients with decompensated cirrhosis and ascites admitted to the Gastroenterology Unit and Infectious Disease Unit of a Chinese tertiary teaching hospital from July 2016 to June 2018 were prospectively eligible for enrollment. The inclusion criteria: the diagnosis of cirrhosis was made after liver biopsy or on the basis of a combination of imaging, laboratory, and clinical evidence; the presence of ascites accessible to biopsy; and clinical evidence; the presence of ascites accessible to abdominal paracentesis; and the age was over 18 years old. The exclusion criteria: ascites induced by diseases other than cirrhosis; intra-abdominal source of infection; and recent abdominal surgery. Patients were prospectively followed from admission until death or discharge. This protocol was approved by the Hospital Ethics Committee and informed consent was obtained from all patients enrolled in the study.

2.2. Patient data and samples

The following variables were collected at study inclusion: age; gender; body temperature, aetiology of cirrhosis; comorbidities; WBC; percent of neutrophils; serum creatinine, serum bilirubin; serum albumin; prothrombin time with international normalized ratio (INR); PCT; CRP; MELD and Child–Pugh scores. The ascites analysis included cell counts and ascitic fluid total protein quantification. SBP was defined by ascitic PMN $\geq 250$ cells/mm\(^3\), and excluding secondary peritonitis, acute pancreatitis, and tuberculous peritonitis.

Ascitic samples were collected on the day of paracentesis. Aliquots of ascitic fluid (2 mL) were centrifuged at 1000 g for 10 minutes and the supernatants were frozen at $-80^\circ$C. The levels of PGE2 were determined by enzyme-linked immunosorbent assay (ELISA) kits (Elabscience, Wuhan, China).

2.3. Statistics

Kolmogorov–Smirnov test was used to identify parametric or nonparametric distribution, accordingly parameters reported as mean $\pm$ standard deviation or median (interquartile range). Student t test or Mann–Whitney U test was applied for group comparisons. Categorical variables were compared by $\chi^2$ test or Fisher exact test. Receiver operating characteristic (ROC) curves were generated to determine the sensitivity and specificity of a number of clinical variables. Univariate and multiple logistic regression models were used to evaluate the diagnosis value of SBP and predictor value of in-hospital mortality of decompensated cirrhosis. Backward stepwise binary logistic regression was used for multivariable analysis. A P value of less than .05 indicated that the difference was statistically important. The SPSS v. 23.0 (IBM SPSS Statistics for Windows, Armonk, NY), MedCalc v. 15.0 (MedCalc for Windows, Ostend, Belgium) and GRAPHPAD RISM 5 (GRAPHPAD RISM for Windows, CA) was used for analysis.

3. Results

3.1. Patients characteristics

There were 224 patients enrolled in this prospective study, including 29 (13%) patients with SBP. Among those, none of the ascites samples was cultured positive. The median onset age and gender between 2 groups were not significantly different. Nearly 70% of patients suffered from cirrhosis with SBP were male. HBV infection (65.5% vs. 63.1%) was the main aetiology of liver cirrhosis. Patients with SBP presented with significantly higher percentage of fever (24.15% vs. 9.2%, P$=0.039$), abdominal pain (44.8% vs. 24.1%, P$=0.019$), and hepatic encephalopathy (24.1% vs. 10.8%, P$=0.042$). All patients with SBP were treated with antibiotics, and 168 out of 195 (86.2%) patients without SBP were treated with antibiotics. When comparing biochemical parameters with patients without SBP, the white blood cells (P$=0.001$), neutrophils (P$<0.001$), creatinine level (P$=0.037$), INR (P$=0.007$), and PCT level (P$=0.01$) were significantly elevated, while serum sodium (P$=0.002$) and serum ascitic albumin gradient (SAAG) (P$=0.021$) were significantly lower in patients with SBP. In addition, the higher incidence of Child–Pugh stage C (P$=0.005$) and higher MELD score (P$=0.016$) were found in the SBP group. There were no significant differences regarding platelets, albumin, total bilirubin, aspartate- and alanine-aminotransferases, CRP, and asotic total protein (Table 1).

3.2. Ascitic PGE2 as a biomarker for SBP

The median level of ascitic PGE2 of all the 224 ascites sample was 39.68 pg/mL, and the median level of ascitic PGE2 of patients with SBP was 32.77 (26.5–39.68) pg/mL, which was lower than that of patients without SBP [49.72 (37.35–54.72) pg/mL], and the difference was statistically different (P$<0.001$) (Fig. 1). In ROC analysis, the AUC for ascitic PGE2 was 0.75, and the best cutoff for diagnosis of SBP was 40.3 pg/mL, the corresponding sensitivity was 93.1%, and the specificity was 58.5%. And the AUC for WBC, neutrophils, PCT, CRP was 0.83, 0.77, 0.76, 0.57, respectively. There was no significant difference when comparing the AUC for ascitic PGE2 with that for the above 4
parameters \((P = .413, P = .832, P = .192, \text{ respectively})\) (Fig. 2A) When combining ascitic PGE2 with neutrophils and with WBC, the AUC were 0.90 and 0.90 respectively, which were elevated significantly compared with that of ascitic PGE2 \((P = .022, P = .029, \text{ respectively})\). When combined ascitic PGE2 with CRP and with PCT, the AUC were 0.79 and 0.83 respectively, and there was no significant difference when comparing with that of ascitic PGE2 (Fig. 2B).

### 3.3. Ascitic PGE2 as predictor for in-hospital mortality

Fifteen patients (7%) died during hospitalization, and the rest 209 patients were alive. The causes of death included liver failure, hepatic encephalopathy, hepatorenal syndrome, infection, and multiorgan failure.

Ascitic PGE2 were significantly lower in in-hospital death patients group than that in survival patients group \([32.88 (24.22–45.01) \text{ vs. } 47.00 (36.84–54.01) \text{ pg/mL}, P = .005]\) (Fig. 3). The ROC curve indicated the ascitic PGE2 concentration of 32.88 pg/mL as a best cutoff for predicting in-hospital mortality with a sensitivity of 83.7% and a specificity of 53.3%. The AUC for predicting in-hospital mortality was 0.72 for ascites PGE2, and 0.85 for MELD score; however, there were no statistically significant difference between the 2 parameters \((P = .129)\). When combined ascitic PGE2 with MELD score, the AUC was 0.87, which elevated significantly compared with ascitic PGE2 alone \((P = .048)\) (Fig. 4).

In univariate analysis, the level of ascitic PGE2 lower than or equal to 32.88 pg/mL was associated with an OR of 5.88 (95% CI: 2.00–17.30) for in-hospital mortality \((P = .001\), Table 2). And other parameters associated with increased in-hospital mortality were hepatic encephalopathy \((\text{OR: } 31.06, P < .001)\), elevated PCT \((\text{OR: } 9.11, P = .038)\), a higher MELD score \((\text{OR: } 1.18 \text{ per 1 point, } P < .001)\), and a higher Child-Pugh score \((\text{OR: } 2.98 \text{ per 1 point, } P < .001\) (Table 2). In multivariate analysis, ascitic PGE2 ≤32.88 pg/mL \((\text{OR: } 9.39, 95\% \text{ CI: } 1.41–67.44, P = .026)\), hepatic encephalopathy \((\text{OR: } 18.39, 95\% \text{ CI: } 3.00–113.13, P = .002)\), and a higher MELD score \((\text{OR: } 1.25; 95\% \text{ CI: } 1.05–1.40, P = .009\) remained independent predictors for in-hospital mortality.

### 4. Discussion

SBP is a frequent and severe complication in patients with cirrhosis and ascites. Because of rarely positive culture of ascites
and manually measurement of PMN in ascites, the diagnosis of SBP is not always reliable, which leads to delays in diagnosis and treatment with antibiotics. Therefore, to optimize the diagnosis of SBP, a large number of studies have been put into attempt in recent decades, including searching for new biomarkers, such as ascitic fluid lactoferrin, soluble urokinase plasminogen activator receptor, ascites neutrophil gelatinase-associated lipocalin, and ascitic calprotectin[9,22–24] and using nonculture technique, for example, reagent strips, polymerase chain reaction (PCR) detecting bacterial DNA in ascites, in situ hybridization, gas chromatography-mass-spectrometry (GCH-MS), and next-generation sequencing (NGS).[25–29] However, some of these biomarkers were reported with poor sensitivity or specificity,[30] and some of the nonculture methods were expensive and time-consuming. In our study, we attempted at looking for an easy-to-be-detected and time-saving biomarker for early diagnosis of SBP and predicting mortality, which was essential for improving the prognosis of patients and using antibiotics rationally and effectively.

In this study, there were 29 (12.9%) cases of SBP of the 224 patients enrolled, a rate similar to previous studies.[3] Ascitic PGE2 level was detected by ELISA kits, which were commercially...
available, and the entire process took only 2 hours. We found that ascitic PGE2 levels in patients with SBP decreased significantly, which may serve as a biomarker of indicating SBP (the cut-off value was 40.3 pg/mL). An AUC of 0.75 suggested that ascitic PGE2 was an intermediate biomarker for diagnosis of SBP. Ascitic PGE2 was a better biomarker indicating SBP than serum CRP, and not superior to WBC, neutrophils, and serum PCT. When combining ascitic PGE2 with serum PCT or CRP, the diagnostic efficacies were not improved significantly. However, when combining ascitic PGE2 with WBC or neutrophils, the AUC could improve to 0.90, indicating that ascitic PGE2 combined with WBC or neutrophils were with higher diagnostic value in SBP. Thus, the diagnostic value of ascitic PGE2 was not higher than that of WBC, neutrophils, or serum PCT, and it needed to be combined with other inflammatory markers, such as WBC, neutrophils, etc., while, the measures of WBC and neutrophils in clinical were faster and easy-to-be-detected. Therefore, based on our results, ascitic PGE2 was not a more easy and effective biomarker for diagnosis of SBP.

PGE2, a key mediator of immunopathology in infections, is mainly produced by inflammatory cells, including neutrophils and macrophages.\textsuperscript{[17,31]} It is widely known that cells in ascites of patients with cirrhosis are mainly lymphocytes and macrophages.\textsuperscript{[12,33]} Thus, the PGE2 produced in ascites was also reduced. In Weiler’s study, PGE2 tissue levels were significantly decreased in inflamed gastric antral mucosa of patients with cirrhosis in the presence of portal hypertension, and further decreased in inflamed gastric antral mucosa of patients with ulcers.\textsuperscript{[31]} While Shahed and Shoskes\textsuperscript{[34]} observed a higher level of PGE2 in prostatic secretions of men with symptomatic chronic prostatitis. Actually no data were disclosed on the comparison of the ascitic PGE2 concentration in the decompensated cirrhotic patients with or without SBP. From these previous studies, we hypothesize that PGE2 may exert immunomodulatory effects in a compartmental regulation way, although all diseases involving in inflammation, the level of PGE2 is varied depending on different organ, tissue, and samples. However, the mechanism of this phenomenon is still unclear and requires further research.

Previous published studies showed that higher MELD score and the development of hepatic encephalopathy are the independent predictors for in-hospital mortality in patients with decompensated cirrhosis.\textsuperscript{[35,36]} Especially, the MELD score was confirmed as an independent predictor for mortality with wide availability and stability.\textsuperscript{[37,38]} In our study, of the 224 patients enrolled, there were 15 (7.0\%) cases of death during hospitalization, corresponding with the previous studies.\textsuperscript{[39]} There were no significant differences between the AUC for ascitic PGE2 and MELD score in predict in-hospital mortality. When using ascitic PGE2 was combined with MELD score, the AUC increased compared with ascitic PGE2 or MELD score, separately. Thus, ascitic PGE2 may play a role in predict in-hospital mortality, although it was not superior to the MELD score. Additionally, the multivariate analysis indicated ascitic PGE2≤32.88 pg/mL, hepatic encephalopathy and MELD score were the independent predictors for in-hospital mortality. In our univariate analysis, PCT and Child–Pugh score were with OR of 9.11 and 2.98 separately to predict in-hospital mortality; however, they were removed from model in multiple logistic regression model analysis, this may be related to the MELD score was more reliable than the Child–Pugh score in predict mortality.\textsuperscript{[40]} and comparing with serum PCT, ascitic PGE2 level was a more valuable parameter in predict mortality.

In our study, none of the ascites specimens have been cultured available, and the entire process took only 2 hours. We found that ascitic PGE2 levels in patients with SBP decreased significantly, which may serve as a biomarker of indicating SBP (the cut-off value was 40.3 pg/mL). An AUC of 0.75 suggested that ascitic PGE2 was an intermediate biomarker for diagnosis of SBP. Ascitic PGE2 was a better biomarker indicating SBP than serum CRP, and not superior to WBC, neutrophils, and serum PCT. When combining ascitic PGE2 with serum PCT or CRP, the diagnostic efficacies were not improved significantly. However, when combining ascitic PGE2 with WBC or neutrophils, the AUC could improve to 0.90, indicating that ascitic PGE2 combined with WBC or neutrophils were with higher diagnostic value for SBP. Thus, the diagnostic value of ascitic PGE2 was not higher than that of WBC, neutrophils, or serum PCT, and it needed to be combined with other inflammatory markers, such as WBC, neutrophils, etc., while, the measures of WBC and neutrophils in clinical were faster and easy-to-be-detected. Therefore, based on our results, ascitic PGE2 was not a more easy and effective biomarker for diagnosis of SBP.
There are some limitations to our study. This is a single-center study, most patients came from the adjacent regions which limited the generalizability of our results. The small number of patients with SBP in our study makes it difficult to determine a definitive cut-off value. Unfortunately, we were unable to collect the serum samples corresponding to each patient, for this reason, we cannot understand the status of serum PGE2 and cannot compare with the case of ascitic PGE2 in our study. Because only a few patients with SBP underwent paracentesis after antibiotics treatment, it is difficult to evaluate the role of ascitic PGE2 in monitoring antibiotics treatment outcomes. A larger number of patients and more complete body fluid specimen are warranted to characterize the alteration of ascitic PGE2 level from the early stage of decompensated cirrhosis to the end stage of liver disease.

In summary, the level of ascitic PGE2 in patients with SBP was decreased than that of patients without SBP; however, its value in diagnosing SBP was not superior to other inflammatory indicators, simultaneously, ascitic PGE2 level lower than or equal to 32.88 pg/mL may independently predict the in-hospital mortality of patients with decompensated cirrhosis. Thus, ascitic PGE2 is likely to be a valuable biomarker for prognosis prediction of decompensated cirrhosis. However, due to the limited number of samples, our conclusions need to be verified by a larger sample size in the future.

Author contributions
Conceptualization: Wenxiang Huang, Bei Jia.
Data curation: Xianmei Wu, Wenxiang Huang, Bei Jia.
Formal analysis: Wenxiang Huang, Bei Jia.
Funding acquisition: Bei Jia.
Investigation: Junli Luo, Xianmei Wu, Yu Zhang, Bei Jia.
Methodology: Junli Luo, Xianmei Wu, Yu Zhang.
Software: Junli Luo, Xianmei Wu, Yu Zhang.
Validation: Wenxiang Huang, Bei Jia.
Visualization: Wenxiang Huang, Bei Jia.
Writing – original draft: Junli Luo.
Writing – review & editing: Bei Jia.

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