

Article

High Concentrations of Presepsin in the Bile and Its Marked Elevation in Biliary Tract Diseases: A Retrospective Analysis

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Abstract: Presepsin is a diagnostic and prognostic biomarker of sepsis; however, elevated presepsin levels have also been documented without sepsis. This study aims to retrospectively analyze the laboratory parameters and Sequential Organ Failure Assessment (SOFA) score affecting presepsin levels in 567 patients. Some patients with elevated presepsin levels exhibited renal dysfunction or elevation of biliary enzymes despite a low SOFA score. The univariate regression analysis revealed a close correlation between presepsin levels and SOFA score, serum creatinine (CRE), blood urea nitrogen, and biliary enzymes. In addition, a multivariate regression analysis revealed that SOFA score, alkaline phosphatase (ALP), and CRE independently affected presepsin levels significantly. The analysis of covariance (ANCOVA) revealed that presepsin levels were significantly higher in patients with hepatobiliary disease. Besides, we found that patients who presented with the dilatation of intra- or extrahepatic bile ducts and the elevation of ALP or total bilirubin exhibited remarkable high presepsin levels in the bile. Furthermore, the presepsin production in the liver's Kupffer cells was established by immunostaining in patients who received surgical liver resection. Overall, this study elucidates that biliary enzymes' elevation affects presepsin levels, presepsin exists in high concentrations in the bile, and is positive in Kupffer cells.

Keywords: presepsin; sepsis; Sequential Organ Failure Assessment (SOFA) score; alkaline phosphatase; bile

1. Introduction

CD14, a 55-kDa glycoprotein, is expressed in macrophages, monocytes, and granulocytes, along with their cell membranes [1]; it serves as a receptor for lipopolysaccharide-binding protein complexes. In addition, CD14 activates a series of signal transduction pathways and inflammatory cascades against microorganisms [1,2]. The glycoprotein exists in both membrane-bound and soluble (sCD14) forms. During inflammation, proteases cleave sCD14 in the blood, creating a truncated subtype, called presepsin [3,4].

Presepsin is an established biomarker of sepsis. To date, numerous studies have reported a correlation between presepsin levels and both sepsis severity and outcomes of patients with sepsis. Thus, presepsin has been considerably explored in patients receiving critical care [5–9]. Presepsin is filtered by the glomerulus, reabsorbed and catabolized within proximal tubular cells; consequently, its levels are high in patients with renal failure [10–12].

Previously, presepsin levels have been reported to be markedly elevated in other diseases without sepsis, including hemophagocytic syndrome (HPS) [13] and acute ST-elevation myocardial infarction (STEMI) [14]. Indeed, we also experienced a case of TAFRO (i.e., thrombocytopenia, anasarca, fever, renal failure or reticulin fibrosis, and organomegaly) syndrome that exhibited a remarkable elevation of presepsin levels without an apparent infectious disease (under submission). This study aims to investigate the clinical laboratory parameters that could affect the plasma presepsin levels for conditions other than renal dysfunction and examine the new mechanisms leading to the elevation of presepsin.

2. Experimental Section

2.1. Study Population and Ethical Statement

We retrospectively examined 611 consecutive patients who were suspected of some infections and got their plasma presepsin levels measured between January 2017 and April 2018. Of these, we excluded 10 patients who were unable to calculate the Sequential Organ Failure Assessment (SOFA) score. As we analyzed only the results of first blood sampling, 34 patients who had been measured more than once were also excluded. Hence, we enrolled 567 patients in the study. This study protocol was approved by the Institutional Review and Ethics Committee of Sakura Hospital, School of Medicine, Toho University (Chiba, Japan; No. S18036); the study was conducted from July 2018 following the rules of the Declaration of Helsinki of 1975 revised in 2013.

In addition, we enrolled 11 patients with either of the following conditions: a malignant biliary tract or pancreatic tumor or benign biliary tract diseases from October 2018 to August 2019. All patients received bile samples collection for cytological examination by percutaneous transhepatic gallbladder drainage, percutaneous transhepatic gallbladder aspiration, endoscopic retrograde biliary drainage, endoscopic nasobiliary drainage, and percutaneous transhepatic cholangial drainage. The studies mentioned above were conducted in compliance with the principles of the Declaration of Helsinki, then performed with an explanation to patients. Moreover, a website with additional information and including an opt-out option was created for these studies (No. S18059).

2.2. SOFA Score Calculation

We used the SOFA score, developed by a group of critical care physicians in December 1994 [15], to assess the severity of organ dysfunctions. SOFA comprises scores from six organ systems, graded from 0 to 4 based on the degree of dysfunction/failure; it scores 1–4 points for each of the six organ systems (respiratory, circulation, renal, neurologic, hepatogenic, and coagulation) [16].

2.3. Measurement of Presepsin in Plasma

The plasma presepsin levels were measured immediately after blood sampling using a fully automated PATHFAST Presepsin Assay System (LSI Medience Corporation, Tokyo, Japan), as described previously [4,17].

2.4. Sampling and Measurement of Presepsin in the Bile

All samples were centrifuged at 1500 rpm for 5 min. The supernatants were immediately frozen and stored at -80°C until use. For measurement, the supernatants were diluted to the appropriate range and measured as stated above.

2.5. Immunostaining

We used monoclonal rabbit antibodies directed against CD14 (1:100 dilution; ab183322, Abcam plc, Cambridge, UK) and polyclonal rabbit presepsin antibody (1 $\mu\text{g/mL}$; Mochida Pharmaceutical Co., Ltd, Fujieda, Japan) for immunostaining, as described previously [13]. Antibodies for presepsin were purchased from Mochida Pharmaceutical Co., Ltd (Fujieda, Japan). Sections (3–4 μm thick) of paraffin-embedded surgical specimens were prepared, deparaffinized, and hydrated. The sections were subsequently treated at 95°C for 10 min in citrate buffer (pH 6) for antigen retrieval. Immunohistochemical reactions were performed with an autostainer (the EnVisionTM FLEX, Dako, Glostrup, Denmark). Endogenous peroxidase activity was blocked by treatment with EnvisionTM Flex peroxidase-blocking solution (Dako). After washing with EnvisionTM Flex Wash Buffer (Dako), the slides were incubated with monoclonal rabbit CD14 antibody and polyclonal rabbit presepsin antibody at room temperature in a moist chamber for 20 min. After washing, the slides were treated with EnvisionTM Flex HRP (Dako) for 20 min, followed by color development in EnvisionTM Flex DAB+ Chromogen with Substrate Buffer (Dako). Finally, the slides were counterstained with hematoxylin.

2.6. Statistical Analyses

In this study, data were presented as means \pm SD. Using both univariate and multivariate analyses, we analyzed the correlation between presepsin levels and other clinical laboratory parameters [i.e., white blood cells (WBC), hemoglobin, platelets, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase (ALP), gamma-glutamyl transferase (γ -GT), lactate dehydrogenase (LDH), total bilirubin (T-BIL), serum creatinine (CRE), blood urea nitrogen (BUN), C-reactive protein (CRP), total protein, and serum albumin]. In addition, differences in plasma presepsin levels among groups were analyzed using the analysis of covariance (ANCOVA), with relevant covariates. All statistical analyses were performed using JMP software version 9.0 (SAS, Cary, NC). We considered $P < 0.05$ as statistically significant.

3. Results

3.1. Patients' Characteristics and Clinical Parameters

Table 1 presents the patients' characteristics and numerous clinical laboratory parameters. We examined 567 patients (345 males and 222 females; mean age: 66.7 ± 18.0 years). The presepsin level ranged 6.89–16,837 (median: 707.7 ± 1549.9) pg/mL . Of note, all samples were within the reference range of the PATHFAST system.

Table 1. Patients' characteristics and clinical parameters.

Clinical condition	<i>n</i>	Ratio (%)	Variable	Mean \pm SD
Total	567	100	Age (years)	66.7 ± 18.0
Urological disease	161	28.4	Gender (m/f)	345/222
Respiratory disease	121	21.3	WBC ($10^3/\mu\text{L}$)	10.5 ± 5.5
Gastrointestinal disease	84	14.8	Hemoglobin (g/dL)	11.9 ± 2.4
Hepatobiliary and pancreatic disease	57	10.1	Platelets ($10^4/\mu\text{L}$)	22.6 ± 11.5

Central nervous system disease	10	1.8	AST	(IU/L)	61.1 ± 177.2
Cardiovascular disease	7	1.2	ALT	(IU/L)	41.2 ± 99.2
Skin and connective tissue disease	7	1.2	ALP	(IU/L)	334.6 ± 363.8
Musculoskeletal disease	11	1.9	γ -GT	(IU/L)	78.1 ± 131.8
Hematological disease	15	2.6	LDH	(IU/L)	273.1 ± 244.0
Gynecological disease	12	2.1	T-BIL	(mg/dL)	1.1 ± 2.2
Autoimmune disease	26	4.6	CRE	(mg/dL)	1.11 ± 1.26
Others	56	9.9	BUN	(mg/dL)	21.7 ± 18.6
			Total protein	(g/dL)	6.8 ± 0.9
			Serum albumin	(g/dL)	3.8 ± 0.7
			Presepsin	(pg/mL)	707.7 ± 1549.9
			CRP	(mg/dL)	9.17 ± 7.74
			SOFA score		2.03 ± 2.68

WBC, white blood cells; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; γ -GT, gamma-glutamyl transferase; LDH, lactate dehydrogenase; T-BIL, total bilirubin; CRE, serum creatinine; BUN, blood urea nitrogen; CRP, C-reactive protein; SOFA, Sequential Organ Failure Assessment.

3.2. SOFA Score

Figure 1 shows the correlation between plasma presepsin levels and SOFA score. The patients' score ranged 0–17. The patients' number and presepsin level in each group were as follows (SOFA score, *n*, median \pm SD of plasma presepsin): 0, 207, 190 ± 280 pg/mL; 1, 109, 300 ± 344 pg/mL; 2, 89, 442 ± 775 pg/mL; 3, 46, 569 ± 809 pg/mL; 4, 41, 502 ± 1525 pg/mL; 5, 25, 697 ± 1060 pg/mL; 6, 16, 728 ± 1313 pg/mL; 7, 6, 3417 ± 4666 pg/mL; 8, 7, 501 ± 1419 pg/mL; 9, 5, 470 ± 147 pg/mL; 10, 5, 745 ± 4819 pg/mL. For each SOFA score from 11 to 17, there were only one or two patients. As shown in Figure 1, the presepsin level significantly and positively correlated with the SOFA scores ($r = 0.525$; $P \leq 0.0001$); however, several patients exhibited abnormally high presepsin levels even with a low SOFA score.

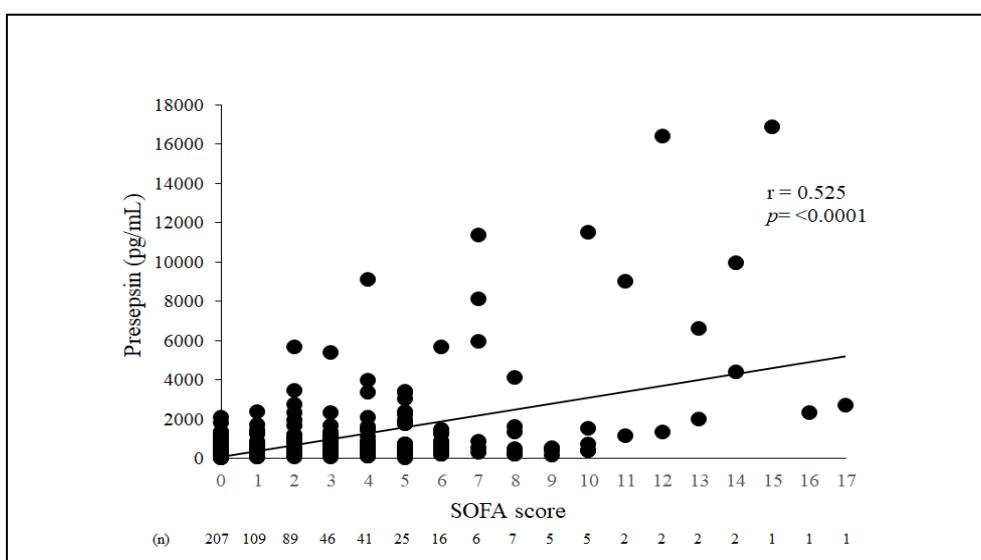


Figure 1. Correlation between presepsin and Sequential Organ Failure Assessment (SOFA) score.

In addition, we analyzed the clinical and laboratory data in patients who revealed highest presepsin levels in SOFA score from 0 to 5 (SOFA score, disease, plasma presepsin level, ALP, CRE). The data were as follows: 0, acute cholangitis, 2081 pg/mL, 2510 IU/L, 0.82 mg/dL; 1, acute cholangitis, 2392 pg/mL, 1117 IU/L, 1.29 mg/dL; 2, urinary-tract infection, 5665 pg/mL, 232 IU/L, 1.68 mg/dL; 3, prostate cancer, 5371 pg/mL, 678 IU/L, 2.19 mg/dL; 4, acute hepatitis, 9075 pg/mL, 460 IU/L, 3.32 mg/dL; 5, chronic renal failure, 3425 pg/mL, 306 IU/L, 5.27 mg/dL.

3.3. Correlation between Presepsin Levels in Plasma and Various Parameters

Univariate regression analysis was performed to analyze the correlation between presepsin levels and several clinical parameters. As shown in Figure 2, presepsin levels significantly and positively correlated with the following parameters: ALP ($r = 0.465, P < 0.0001$; Figure 2a), γ -GT ($r = 0.312, P < 0.0001$; Figure 2b), T-BIL ($r = 0.385, P < 0.0001$; Figure 2c), and CRE ($r = 0.594, P < 0.0001$; Figure 2d). Table 2 shows other that parameters positively correlated as follows: SOFA ($r = 0.525, P < 0.0001$); LDH ($r = 0.304, P < 0.0001$); BUN ($r = 0.485, P < 0.0001$).

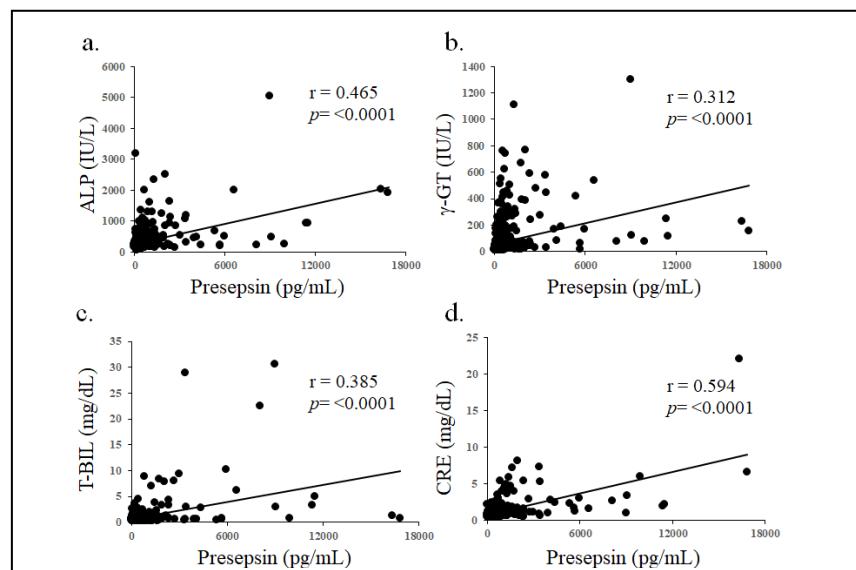


Figure 2. Correlation between presepsin values and alkaline phosphatase (ALP), gamma-glutamyl transferase (γ -GT), total bilirubin (T-BIL), and serum creatinine (CRE). Presepsin versus γ -GT, ALP, T-BIL, and CRE plots (a–d) and linear regressions. [Note: other abbreviations are shown in Table 2.]

In addition, Table 2 summarizes the results of the multivariate regression analysis for the correlation between presepsin and other clinical parameters. As the SOFA score, ALP, LDH, CRE, CRP, and WBC were correlated in the univariate analysis, they were included in the model. Of note, some variables were removed from the regression model because of collinearity with other variables (i.e., γ -GT and T-BIL were removed because of collinearity with ALP; BUN was removed because of collinearity with CRE). We observed that the SOFA score (β -coefficient: 0.246498, $P < 0.0001$), ALP (β -coefficient: 0.359898, $P < 0.0001$), and CRE (β -coefficient: 0.425216, $P < 0.0001$) were independent predictors of presepsin levels (Table 2).

Table 2. Univariate and multivariate analysis of correlation with presepsin in all patients ($n = 567$).

Univariate regression analysis			Multivariate regression analysis			
Variable	r	P	Variable	β -coefficient	SE	P

Age	0.078	0.0633	SOFA score	0.246498	20.0653	<0.0001
SOFA	0.525	<0.0001	ALP	0.359898	0.12688	<0.0001
AST	0.128	0.0023	LDH	-0.03834	0.20528	0.2362
ALT	0.167	<0.0001	CRE	0.425216	42.2233	<0.0001
ALP	0.465	<0.0001	CRP	0.001169	5.9836	0.9688
γ -GT	0.312	<0.0001	WBC	0.05949	0.00844	0.0457
LDH	0.304	<0.0001				
T-BIL	0.385	<0.0001				
BUN	0.485	<0.0001				
CRE	0.594	<0.0001				
Total protein	-0.190	<0.0001				
Serum albumin	-0.252	<0.0001				
CRP	0.145	0.005				
WBC	0.148	0.0004				
Hemoglobin	-0.124	0.0031				
Platelets	-0.1.02	0.0155				

WBC, white blood cells; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; γ -GT, gamma-glutamyl transferase; LDH, lactate dehydrogenase; T-BIL, total bilirubin; CRE, serum creatinine; BUN, blood urea nitrogen; CRP, C-reactive protein; SOFA, Sequential Organ Failure Assessment; SE; standard error.

Model: $r^2 = 0.546195$, $P < 0.0001$.

3.4. Comparison of Adjusted Plasma Presepsin Levels among Groups with Various Clinical Conditions

Figure 3 shows a comparison of adjusted plasma presepsin levels among groups with various clinical conditions. Among all groups, the plasma presepsin level adjusted by the SOFA score and CRE was the highest in the group with hepatobiliary and pancreatic disease (1497.3 ± 311.2 pg/mL). A post-hoc analysis revealed significant differences between adjusted plasma presepsin levels with hepatobiliary and pancreatic disease and those with other diseases, except for cardiovascular disease (912.7 ± 488.2 pg/mL), skin and connective tissue disease (1233.5 ± 630.1 pg/mL), and musculoskeletal disease (805.4 ± 218.3 pg/mL).

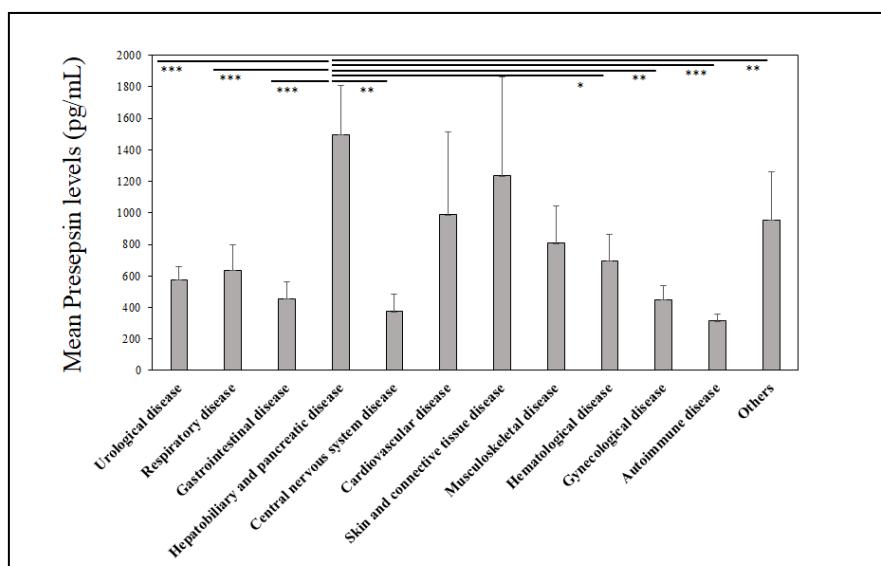


Figure 3. Comparison of adjusted plasma presepsin levels among groups with various clinical conditions. The plasma presepsin level was adjusted by the Sequential Organ Failure Assessment

(SOFA) score and serum creatinine (CRE). Data are represented as mean \pm standard error (SE). *P < 0.05, **P < 0.01, ***P < 0.001: one-way ANOVA followed by the Bonferroni multiple comparison test.

3.5. Bile Presepsin and Clinical Conditions

We evaluated bile presepsin levels in 11 patients (8 males and 3 females; mean age: 76.0 ± 9.1 years) from whom we collected the bile. Table 3 presents the sampling method of bile. Of 11 patients, 8 had biliary tract diseases, 2 had pancreatic cancer, 1 had malignant lymphoma. The presepsin levels in the bile and plasma were $117,610.0 \pm 230,936.9$ and 1495.0 ± 1919.1 pg/mL, respectively. We observed no correlation between presepsin levels in the bile and plasma because of the time lag of sampling (Table 3). In addition, ALP and T-BIL were measured on the same day of bile sampling. The presepsin levels in the bile revealed an abnormal elevation in almost all patients, excluding Cases 4 and 6. Of note, Case 3 exhibited the highest level at 638,100 pg/mL.

Moreover, we assessed the dilatation of intra- and extrahepatic bile ducts by abdominal ultrasonography and computed tomography. As shown in Table 3, patients who exhibited the dilatation of intra- or extrahepatic bile ducts and the elevation of ALP or T-BIL levels displayed remarkably high presepsin levels in the bile.

Table 3. Patients' characteristics and clinical data of 11 patients.

Case	Age	Sex	Disease	Presepsin (pg/mL)		Day *	Procedure	Findings of the bile duct dilatation and ALP, T-BIL		
				Bile	plasma			Intrahepatic c/ extrahepatic c	ALP (IU/L)/ T-BIL (mg/dL)	
1	76	M	Acute gallstone cholecystitis	2678	304	-1	PTGBD	-/-	200/1.3	
2	81	M	Acute gallstone cholecystitis	12,614	7407	-1	PTGBD	+/-	793/3.1	
3	54	M	Acute cholangitis	638,100	1084	-11	ERBD	-/+	401/1.2	
4	64	F	Acute gallstone cholecystitis	148	663	0	PTGBA	-/-	453/1.1	
5	81	F	Acute common bile duct stone and gallstone cholecystitis	3119	512	-3	PTGBD	-/-	147/0.9	
6	72	M	Acute cholangitis and gallstone cholecystitis	73.6	2412	-2	ENBD	-/-	192/0.9	
7	79	M	Acute common bile duct stone and gallstone cholecystitis	405,500	705	+2	ERBD	-/+	374/5.2	
8	64	M	Choledocholithiasis cholangitis	586,300	1495	-2	ENBD	-/+	614/7.2	
9	75	M	Pancreatic head cancer	139,500	1575	-3	ERBD	-/+	274/0.6	
10	84	F	Pancreatic head cancer	117,600	1831	-4	ERBD	+/-	1743/5.9	

11	83	M	Malignant lymphoma	223,40	3147	0	PTCD	+/+	823/3.2
Media	76			117,61	1495	-2			401/1.3
n				0					
SD	9.1			230,93	1919	3.0			438/2.2

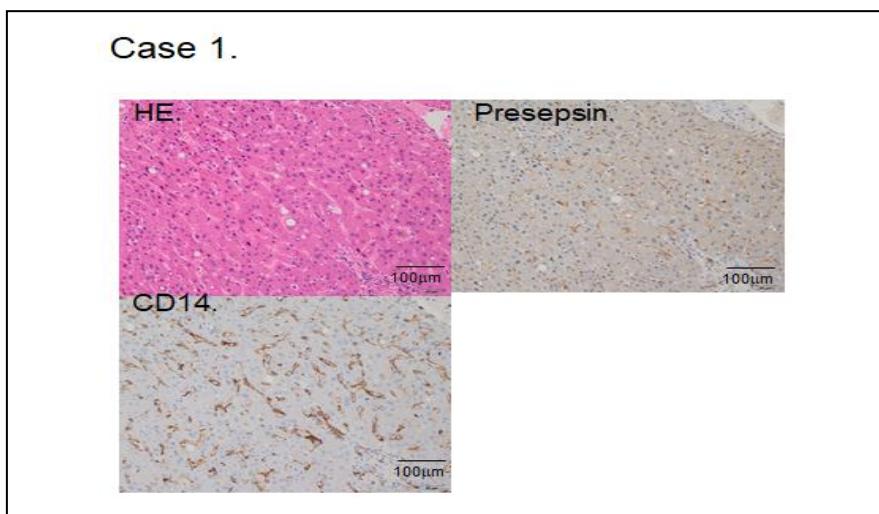
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PTGBD, percutaneous transhepatic gallbladder drainage; PTGBA, percutaneous transhepatic gallbladder aspiration; ERBD, endoscopic retrograde biliary drainage; ENBD, endoscopic nasobiliary drainage; PTCD, percutaneous transhepatic cholangial drainage.

Day*, Interval between plasma collection and bile collection.

3.6. Immunostaining of presepsin in the liver

In this study, 2 patients had their liver tissues examined by immunostaining for CD14 and presepsin. While Case 1 (age: 65 years, male) was operated for metastatic colon cancer in the liver in August 2018, Case 2 (age: 66 years, male) was operated for moderately differentiated hepatocellular carcinoma with a chronic hepatitis B infection in February 2019. As shown in Figure 4, CD14 was positive in Kupffer cells in the sinusoids of the liver's parenchyma without carcinoma invasion, and presepsin was also positive in Kupffer cells.



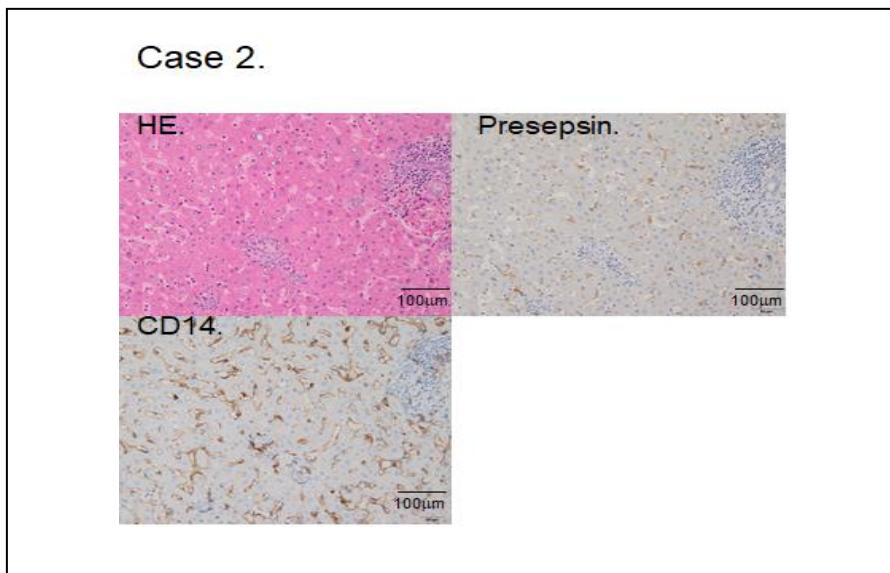


Figure 4. Immunostaining of the liver parenchyma without carcinoma invasion from 2 patients with carcinomas. Hematoxylin–eosin (H&E), CD14, and presepsin staining in 2 patients. Case 1 presented with metastatic colon cancer in the liver, while Case 2 presented with moderately differentiated hepatocellular carcinoma.

4. Discussion

Presepsin is regarded as useful for not only sepsis diagnosis but also as a prognostic predictor [5–9]. Behnes et al. reported that presepsin levels by criteria for the diagnosis of sepsis severity in the intensive care unit treatment were as follows: \geq sepsis, cutoff: 530 pg/mL; \geq severe sepsis, cutoff: 600 pg/mL; and \geq septic shock, cutoff: 700 pg/mL; moreover, presepsin levels exhibited a significant prognostic value for 30 days and 6 months for all-cause mortality [5]. In addition, Liu et al. reported that the median values of presepsin in sepsis, severe sepsis, and septic shock were 325, 787, and 1084 pg/mL, respectively [8].

Lately, the significance of the SOFA score has increased manifold and it has been incorporated in the latest surviving sepsis campaign as a tool to describe and detect sepsis [18]. In our study, there was no bias of disease state, which is recognized in six organ systems (i.e., respiratory, circulation, renal, neurologic, hepatogenic, and coagulation). As we included many mild infectious patients, the median of the SOFA score was 2.03; however, the median of the plasma presepsin level was as high as 707.7 pg/mL, accompanied by a wide variation. Hence, there is a possibility that independent factors without apparent infection affect the levels of plasma presepsin levels.

Figure 1 shows that patients with high presepsin levels, although with a low SOFA score, and those with renal dysfunction or acute cholecystitis showing ALP elevation revealed a remarkable elevation of plasma presepsin levels. In addition, Table 2 and Figure 2 explain a strong correlation between biliary enzymes and presepsin levels, following univariate regression analysis. Furthermore, presepsin levels strongly correlated with ALP in a multivariate regression analysis (Table 2). Moreover, presepsin levels were significantly higher in hepatobiliary disease in the ANCOVA analysis adjusted by the SOFA score and CRE (Figure 3).

Elefsiniotis et al. reported that plasma presepsin levels were elevated in uncomplicated cirrhotic patients; they demonstrated that patients with decompensated liver disease (DLD) exhibited higher presepsin levels than patients with compensated liver disease (CLD; 441.0 ± 442.5 vs. 262.0 ± 179.0 pg/mL). In addition, patients with DLD exhibited higher T-BIL than patients with CLD (3.1 ± 4.0 vs. 0.9 ± 0.9) [19]. Based on these results, our study focused on presepsin levels in the bile. As shown in Table 3, the median level of presepsin in the bile (117,610 pg/mL) was 100 times higher than plasma presepsin levels (1495 pg/mL) in 11 patients. Moreover, we noted a tendency that patients with remarkable elevation of presepsin in the bile show the dilatation of intra- or extrahepatic bile ducts and the elevation of ALP or T-BIL.

Su et al. reported that CD14 is highly expressed in hepatocytes, similar to macrophages and monocytes [20]. Pan et al. reported that human sCD14 is regulated differently in monocytes and hepatocytes [21]. As presepsin is a truncated N-terminal fragment of CD14 [4], we speculated that the overexpression of CD14 in hepatocytes could affect the elevation of presepsin. Thus, we focused on Kupffer cells, macrophages in the liver, as the cells of presepsin production. Our findings established that presepsin was positive in Kupffer cells (Figure 4). Although the liver tissues of the two cases did not exhibit any dilatation of the bile duct, presepsin expressed predominantly in Kupffer cells. Overall, these findings might suggest that increase of the bile duct pressure results in the presepsin overexpression of Kupffer cells, leading to the elevation of presepsin in the bile and plasma.

Previously, we reported a case of TAFRO syndrome that exhibited a remarkable elevation of presepsin level (2741 pg/mL) without an apparent infectious disease (under submission). TAFRO syndrome is a systemic inflammatory disorder and considered to be a variant of multicentric Castleman's disease [22,23]. Our case revealed remarkably high presepsin levels, accompanied by the ALP elevation. Likewise, Tokunaga et al. reported that a TAFRO syndrome case revealed elevation of presepsin (1482 pg/mL) without sepsis [24]. Indeed, to date, several reports have been published regarding the elevation of ALP in TAFRO syndrome [23,25–31]. Furthermore, Nagai et al. reported that liver biopsy exhibited cholangitis in TAFRO syndrome [32].

Arai et al. reported elevated presepsin levels in patients who developed HPS after allogeneic hematopoietic cell transplantation (HCT); besides, they found that patients with higher presepsin levels exhibited inferior overall survival rates, with a median presepsin level on day 28 following HCT (2786 pg/mL) [13]. Caglar et al. reported that presepsin levels in patients with STEMI were elevated, with a median presepsin level of 1988.89 pg/mL [14]. As the studies mentioned above did not evaluate multiple organ failure, we surmised that presepsin levels described in these reports could have been affected by both hepatobiliary tract and renal dysfunction.

5. Conclusions

This study suggests that presepsin levels increase with the elevation of biliary enzymes in patients without renal dysfunction and sepsis. Furthermore, presepsin exists at high concentrations in the bile with the dilatation of intra- or extrahepatic bile ducts and is positive in Kupffer cells.

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