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Association Between Serum Soluble CD14 and IL-8 Levels and Clinical Outcome in Primary Biliary Cholangitis

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List of Abbreviations: PBC, primary biliary cholangitis; UDCA, ursodeoxycholic acid; HIV, human immunodeficiency virus; HCV, hepatitis C virus; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HR, hazard ratio; LPS, lipopolysaccharide

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Abstract

Background & Aims: Primary biliary cholangitis (PBC) is an autoimmune liver disease characterized by portal inflammation and immune-mediated destruction of intrahepatic bile ducts that often leads to liver decompensation, and liver failure. Although the biochemical response to ursodeoxycholic acid (UDCA) can predict disease outcome in PBC, few biomarkers have been identified as prognostic tools applicable prior to UDCA treatment. We therefore sought to identify such indicators of long-term outcome in PBC in the Japanese population.

Methods: The pre-biopsy serum samples and subsequent clinical data of 136 patients with PBC treated with UDCA were analyzed over a median follow-up period of 8.8 years. Serum levels of biomarkers related to microbial translocation (sCD14, EndoCAb, and I-FABP) were measured along with those of 33 cytokines and chemokines and additional autoantibodies. Associations between the tested parameters and the clinical outcomes of liver decompensation and liver-related death/liver transplantation were evaluated using the Cox proportional hazards model with stepwise methods and Kaplan-Meier analysis.

Results: Elevated levels of serum IL-8, and sCD14 before UDCA therapy were significantly associated with both liver decompensation and liver-related death/liver transplantation. In multivariate analyses, IL-8 ≥ 46.5 pg/mL or sCD14 ≥ 2.0 μg/mL at enrollment demonstrated the same results. Kaplan-Meier analysis also revealed IL-8 and sCD14 to be significantly associated with a poor outcome. sCD14 was significantly correlated with IL-8. EndoCAb and I-FABP were not related to disease outcome.

Conclusions: Serum IL-8 and sCD14 levels before UDCA therapy represent noninvasive surrogate markers of prognosis in patients with PBC. (246 words)

Key words: PBC; liver-related death; transplantation; decompensation; microbial translocation

Key points box:

- Noninvasive biomarkers prior to ursodeoxycholic acid (UDCA) therapy are needed to better predict the progression of primary biliary cholangitis (PBC).
- As a marker of monocyte activation and response to lipopolysaccharide, high serum sCD14 concentrations are significantly associated with liver decompensation and liver-related death/liver transplantation in PBC.
- Elevated levels of serum IL-8 before UDCA therapy are also significantly associated with poor outcome in PBC. IL-8 is significantly correlated with sCD14.
- Positive anti-gp210 antibodies prior to UDCA therapy are associated with liver decompensation and liver-related death/liver transplantation.

Introduction

Primary biliary cholangitis (PBC) is an autoimmune liver disease characterized by portal inflammation and immune-mediated destruction of intrahepatic bile ducts that often leads to cirrhosis, liver decompensation, and liver failure.(1, 2) Although the cause of PBC remains poorly understood, this condition is a multifocal polygenic disorder suspected to be possibly caused by an environmental trigger in genetically susceptible individuals.(3-6)

Since several bacterial products have been detected in the serum or liver tissues of PBC patients,(7-12) infectious agents may play a crucial role in disease pathogenesis.(13) Recently, microbial translocation has been implicated in liver disease as well; Balagopal et al.(14) reported that human immunodeficiency virus (HIV)-related $CD4^+$ lymphocyte depletion was associated with microbial translocation and established a link between HIV-related translocation and the severity of liver disease in an HIV-hepatitis C virus (HCV) co-infected cohort. Thereafter, microbial translocation was associated with cirrhosis and found to predict progression to end-stage liver disease in patients with hepatitis B virus or HCV infection.(15) However, such a relationship remains unknown in individuals with PBC. Several highly accurate prognostic models have been developed for PBC based on large patient series (16-21) that focus on the biochemical response to ursodeoxycholic acid (UDCA) therapy, but not specifically on the use of biomarkers for estimation of prognosis and clinical outcome prior to treatment.

This investigation analyzed for relationships between microbial translocation and long-term disease progression in patients with PBC. Moreover, we assessed the serum levels of 33 cytokines and chemokines and additional autoantibodies with relation to

clinical outcomes since several biomarkers (22-26) and autoantibodies (27, 28) have already been associated with PBC pathogenesis and clinical results.

Patients and Methods

Subjects

The clinical and biochemical features of 136 PBC patients who were enrolled in this study between April 1989 and June 2015 are summarized in Table 1. PBC diagnosis was based on the criteria of the American Association for the Study of Liver Diseases.(29) All patients were treated with UDCA (10-13 mg/kg per day) following liver biopsy. No patients were positive for hepatitis B surface antigen or antibodies to hepatitis B core antigen, HCV, or HIV. Patients exhibiting evidence of other liver disease during follow-up, such as alcoholic or non-alcoholic liver disease, were excluded from the investigation. Among the 136 patients, 20 (15%) had concomitant autoimmune diseases, namely, autoimmune hepatitis (overlap syndrome) (n = 5), Hashimoto's thyroiditis (n = 4), Hashimoto's thyroiditis + Sjögren's syndrome (n = 2), Hashimoto's thyroiditis + Sjögren's syndrome + rheumatoid arthritis (n = 1), Hashimoto's thyroiditis + CREST syndrome (n = 1), Sjögren's syndrome (n = 2), Sjögren's syndrome + rheumatoid arthritis (n = 1), Sjögren's syndrome + CREST syndrome (n = 1), rheumatoid arthritis (n = 2), and systemic sclerosis (n = 1). This study was conducted in accordance with the principles of the 2013 Declaration of Helsinki after review and approval by the ethics committee of Shinshu University School of Medicine, Matsumoto, Japan (No. 2189). Informed consent was obtained from each patient included in the

study. Serum samples were obtained at the time of liver biopsy prior to UDCA treatment and stored at -30°C until testing.

Liver Biopsy and Histological Evaluation

Liver biopsies were performed by percutaneous sampling of the right lobe with a 14-gauge needle in all but 6 patients before UDCA administration, as reported previously.(30) All biopsies were 1.5 cm or more in length. Formalin-fixed, paraffin-embedded specimens were prepared and used for histopathological studies. Sections measuring 4 μm were cut from each paraffin block and stained with hematoxylin and eosin, periodic acid-Schiff after diastase digestion, and Azan-Mallory staining. For the 6 patients who did not undergo liver biopsy, nodular liver laparoscopic findings had already indicated definite cirrhosis. Histological stage was determined according to Scheuer's classification.(31) The investigators (AM, ET) involved in this part of the study were blinded to the results of other portions.

Biomarkers of Microbial Translocation, Cytokines, and Chemokines

Commercially available enzyme-linked immunosorbent assay (ELISA) kits were used to measure serum levels of I-FABP (Cell Sciences, Canton, MA), EndoCAb IgM (Cell Sciences), and sCD14 (R&D Systems, Minneapolis, MN) according to the manufacturers' protocols.

Thirty-three cytokines and chemokines (Eotaxin/CCL11, GM-CSF, GROα/CXCL1, IFNα, IFNγ, IL-1α, IL-1β, IL-1RA, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8/CXCL8, IL-9, IL-10, IL-12p70, IL-13, IL-15, IL-17A, IL-21, IL-22, IL-23, IL-27, IL-31, IP-10/CXCL10, MCP-

1/CCL2, MIP-1α/CCL3, MIP-1β/CCL4, RANTES/CCL5, SDF1α/CXCL12, TNFα, and TNFβ/LTA) were quantified using Luminex® Multiplex Cytokine Kits (ProcartaPlex Human Cytokine & Chemokine Panel 1A) from serum samples obtained before liver biopsy. All assays were conducted following the manufacturer's recommendations and according to optimized protocols applied in various earlier studies.(32-34) In addition, we performed intra-assay precision measuring 10 times for 1 serum sample each of sCD14 and IL-8. The coefficient of variation of sCD14 (3.64) and of IL-8 (2.85) demonstrated the feasibility and reproducibility of the assays to be sufficient.

Autoantibodies

The antibody titers to mitochondrial antigens MIT3 (recombinant proteins containing PDC-E2, BCOADC-E2, and OGDC-E2), gp210, centromere A&B (recombinant centromere proteins), sp100 (synthetic peptides), and chromatin (H1-stripped chromatin) were determined using ELISA kits (INOVA Diagnostics, San Diego, CA), whereby a titer of >25 U/ml was interpreted as a positive finding according to the manufacturer's protocol and instructions.

Statistical Analysis

Categorical variables were compared using the Pearson's chi-squared or Fisher's exact tests, as appropriate, and continuous variables were evaluated by the Mann-Whitney *U* test. Optimal cutoff values were determined by the Youden index. Clinical outcome as of December 2015 was recorded as either liver-related death, liver transplantation, or liver decompensation, which included ascites, esophageal varices,

hepatic encephalopathy, hepatocellular carcinoma, and total serum bilirubin level >3 mg/dL. The Kaplan-Meier method and log-rank test were used to estimate the liver decompensation and survival rates of patients. Multivariate analysis was performed using the Cox proportional hazards model with stepwise method to identify independent factors associated with liver decompensation or liver-related death/liver transplantation. Variables associated with a $P < 0.05$ in univariate analysis were included in this step. A $P < 0.05$ was considered to be statistically significant. Statistical analyses were performed using IBM SPSS Statistics 21.0 (IBM Japan Inc., Tokyo, Japan) software.

Results

Baseline Clinical Characteristics of Patients

The clinical profile of the experimental cohort is presented in Table 1. Median age was 57 years, with a female preponderance of 85%. Median follow-up was 8.8 years (interquartile range [IQR]: 5.0-14.5 years). Seropositivity for anti-gp210, anti-centromere, anti-sp100, and anti-chromatin antibodies was 23%, 33%, 10%, and 2%, respectively, in agreement with a prior Japanese study.(28)

Prediction and Risk Factors for Liver Decompensation at Diagnosis

Of the 136 patients with PBC, 19 (14%) developed liver decompensation during follow-up. As shown in Table 2, patients with decompensation had a significantly lower median serum level of albumin as compared with compensated patients. The median values of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and bilirubin were significantly higher in patients who exhibited liver decompensation.

Furthermore, these patients had a significantly higher frequency of anti-gp210 positivity and Scheuer's stage III or IV.

Serum samples obtained prior to UDCA therapy were examined for the presence of a total of 36 biomarkers (Table 3). The median baseline serum concentrations of 3 biomarkers (GROα/CXCL1; P = 0.010, IL-8; P = 0.004, and sCD14; P = 0.027) were significantly higher in patients who developed liver decompensation than in those who did not (Fig. 1). IL-8 and sCD14 were both significantly higher in the liver decompensation group after adjustment for multiple comparisons (P = 0.035 and P = 0.033, respectively).

Multivariate Cox regression analysis for factors associated with liver decompensation revealed albumin ≥ 4.0 g/dL (hazard ratio [HR] 0.08; 95% confidence interval [CI]: 0.02-0.33, P = 0.001), bilirubin ≥ 1.1 mg/dL (HR 10.44; 95% CI: 2.69-40.42, P = 0.001), IL-8 ≥ 46.5 pg/mL (HR 3.89; 95% CI: 1.05-14.46, P = 0.043), and sCD14 ≥ 2.0 μg/mL (HR 5.58; 95% CI: 1.32-23.61, P = 0.020) to be independent predictors of this outcome. Kaplan-Meyer analysis disclosed that patients with sCD14 ≥ 2.0 or IL-8 ≥ 46.5 had a significantly higher risk of decompensation (log-rank test; P = 0.038 and P < 0.001, respectively) (Fig. 2A and 2B).

Prediction and Risk Factors for Liver-related Death/Liver Transplantation at Diagnosis

We next sought to identify risk factors for mortality in patients with PBC. A total of 14 subjects (10%) experienced liver-related death (n = 11) or liver transplantation (n = 3) in our cohort. As presented in Table 4, patients with liver-related death/liver

transplantation had a significantly lower median age and serum level of albumin as compared with survivors. In contrast, the median values of ALT, AST, alkaline phosphatase, γ-glutamyltransferase, and IgM in these subjects were significantly higher. Furthermore, patients with a poor outcome had a significantly higher frequency of anti-gp210 positivity and Scheuer's stage III or IV than those with a favorable outcome.

Serum samples taken prior to UDCA therapy were examined for the presence of a total of 36 biomarkers. The median baseline serum concentrations of 2 biomarkers (IL-8; $P = 0.018$, and sCD14; $P = 0.003$) were significantly higher in patients with liver-related death/liver transplantation than in those without (Fig. 3). After adjusting for multiple comparisons, IL-8 and sCD14 both differed significantly between the groups ($P = 0.032$ and $P = 0.002$, respectively).

Multivariate Cox regression analysis for factors associated with liver-related death/liver transplantation revealed albumin ≥ 3.8 g/dL (HR 0.05; 95% CI: 0.01-0.30, $P = 0.001$), γ-glutamyltransferase ≥ 158 IU/L (HR 11.20; 95% CI: 1.89-66.43, $P = 0.008$), IL-8 ≥ 46.5 pg/mL (HR 6.35; 95% CI: 1.47-27.43, $P = 0.013$), and sCD14 ≥ 2.0 μg/mL (HR 7.02; 95% CI: 1.20-41.18, $P = 0.031$) to be significant predictors of mortality. Kaplan-Meyer analysis demonstrated that patients with sCD14 ≥ 2.0 or IL-8 ≥ 46.5 had a significantly higher risk of liver-related death or transplantation (log-rank test; $P = 0.027$ and $P < 0.001$, respectively) (Fig. 2C and 2D).

Discussion

The present investigation measured markers of the humoral immune response (EndoCAb), lipopolysaccharide (LPS) bioactivity (sCD14), and enterocyte damage (I-

FABP), 33 cytokines and chemokines, and several autoantibodies in patients with PBC prior to UDCA therapy to identify associations with the development of decompensation or liver-related death/liver transplantation.

We observed that serum IL-8 level was closely correlated with the progression of fibrosis in PBC, which was consistent with a prior study.(23) Zimmermann et al. also found that patients with Child-Pugh C had significantly higher IL-8 levels than did those with non-cirrhosis or Child-Pugh A. IL-8 can be induced by monocytes, epithelial cells, fibroblasts, and hepatocytes.(35) In PBC, serum IL-8 is higher than in other etiologies of chronic liver disease,(25) and high intrahepatic IL-8 expression has been reported.(23) IL-8 expression has also been identified in injured biliary epithelial cells of patients with cholestatic liver disease and linked to neutrophilic infiltration around reactive bile ducts.(36) Since there have been no other reports associating IL-8 levels with mortality or disease progression in PBC, further studies are needed to confirm our results worldwide.

As a marker of monocyte activation and response to LPS, sCD14 has been associated with mortality during HIV infection (37) and disease progression in chronic viral hepatitis.(15) Baseline sCD14 levels were significantly higher in patients with a poor outcome than in those whose condition was stable in this series. Microbial translocation is a contributor to persistent inflammation and fibrosis in chronic liver disease. As the disease can impair the clearance of translocating microbial products, LPS is considered to play a role in liver injury. LPS activation causes the shedding and secretion of sCD14, thereby making sCD14 an important marker of LPS bioactivity.(38-40) sCD14 may be a more relevant biomarker of disease progression since it reflects

the host response to products of microbial translocation. A high baseline sCD14 level of ≥ 2.0 μg/mL was revealed as an independent risk factor for PBC progression by Cox regression and Kaplan-Meyer analyses. sCD14 was significantly correlated with IL-8 (r = 0.253, P < 0.001). Yokoyama et al.(41) reported that human intrahepatic biliary epithelial cells constitutively expressed transcripts encoding TLR 1-6, TLR 9, and CD14. Stimulation of human intrahepatic biliary epithelial cells by LPS resulted in translocation of NF-κB subunits from the cytoplasmic to the nuclear fraction, followed by increases in IL-8. Hence, these markers might be important factors in the pathogenesis of PBC. Particularly, the correlation between sCD14 and IL-8 levels suggests an indirect relationship in which the factors that cause increased sCD14 levels, namely, microbial products, also augment the levels of inflammatory markers. Recent animal and human studies have implicated gut microbiota as potentially important players in the pathogenesis of non-alcoholic steatohepatitis.(42-45) Since our data indicate that microbial translocation may be associated with PBC progression, possible links between gut microbiota and PBC formation and progression warrant future clarification.

Lastly, several studies have proposed anti-gp210 antibody positivity to be a significant risk factor for hepatic failure and liver transplantation.(27, 28) We confirmed here that positive anti-gp210 antibodies prior to UDCA therapy were associated with liver decompensation and liver-related death/liver transplantation. Although gp-210 antibody positivity was not identified as an independent risk factor by multivariate analysis, patients who were positive for these antibodies displayed a significantly higher risk of decompensation or liver-related death/liver transplantation in Kaplan-Meyer

testing (Supplementary Figure 1A and 1B). Therefore, the detection of these autoantibodies may assist in the prognosis of PBC.

This study has several limitations. First, bias might have been introduced during case selection because the clinical indications for liver biopsy were unclear. Second, since patients with advanced fibrosis tended not to undergo liver biopsy, the proportions of subjects with severe fibrosis or cirrhosis were small in this study. Third, this study was a retrospective analysis conducted at a single institute, although the follow-up period was relatively long and all patients were recruited prior to UDCA therapy.

In conclusion, serum IL-8 and sCD14 levels before UDCA therapy represent noninvasive surrogate markers of prognosis in patients with PBC.

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Table 1. Demographic and Clinical Characteristics of Patients with PBC

Characteristic	N = 136	
Age at admission (years)	57	(51-63)
Female, n (%)	113	(83)
Follow-up (years)	8.8	(5.0-14.5)
Mayo score	4.2	(3.7-4.6)
Concomitant autoimmune diseases		
Overlap syndrome, n (%)	5	(4)
Hashimoto's thyroiditis, n (%)	8	(6)
Sjögren's syndrome, n (%)	8	(6)
Rheumatoid arthritis, n (%)	5	(4)
CREST syndrome, n (%)	2	(1)
Systemic sclerosis, n (%)	1	(1)
Laboratory data at admission		
Platelet count ($\times 10^9$/L)	222	(179-255)
Albumin (g/dL)	4.2	(4.0-4.5)
ALT (IU/L)	41	(28-68)
AST (IU/L)	41	(30-60)
ALP (IU/L)	448	(326-607)
GGT (IU/L)	138	(86-261)
Bilirubin (mg/dL)	0.8	(0.6-1.0)
IgM (mg/dL)	290	(183-471)
IgG (mg/dL)	1630	(1363-2009)
MIT-3, n (%)	113	(83)
Anti-gp210, n (%)	32	(23)

Anti-centromere, n (%)	45	(33)
Anti-sp100, n (%)	13	(10)
Anti-chromatin, n (%)	3	(2)
Histological findings		
Scheuer's stage (I:II:III:IV)	88 (65):24 (18):14 (10):10 (7)	

Parameters are presented as median (IQR) for continuous variables and total number (%) for categorical variables.

Table 2. Comparison of Patients Exhibiting Liver Decompensation and Compensation

Characteristic	Decompensation (n = 19)		Compensation (n = 117)		*P* value
Age at admission (years)	54	(42-61)	57	(52-63)	0.969
Female, n (%)	14	(74)	99	(85)	0.238
Laboratory data at admission					
Platelet count (×10^9/L)	25.2	(18.0-30.0)	22.2	(18.1-24.9)	0.532
Albumin (g/dL)	3.9	(3.4-4.2)	4.3	(4.1-4.5)	<0.001
ALT (IU/L)	70	(37-122)	40	(28-62)	0.034
AST (IU/L)	69	(42-109)	39	(30-55)	0.001
ALP (IU/L)	515	(336-1658)	451	(326-611)	0.438
GGT (IU/L)	244	(137-327)	128	(86-247)	0.111
Bilirubin (mg/dL)	1.1	(0.6-5.0)	0.8	(0.6-0.9)	0.001
IgM (mg/dL)	404	(189-827)	295	(199-467)	0.551
IgG (mg/dL)	1751	(1449-2064)	1610	(1357-1954)	0.385
AMA-positive, n (%)	17	(90)	96	(82)	0.423
Anti-gp210, n (%)	9	(47)	23	(20)	0.008
Anti-centromere, n (%)	4	(21)	41	(35)	0.229
Anti-sp100, n (%)	0	(0)	13	(11)	0.127
Anti-chromatin, n (%)	1	(5)	2	(2)	0.892
Histological findings					
Scheuer's stage III+IV, n (%)	9	(64)	19	(16)	<0.001

Parameters are presented as median (IQR) for continuous variables and total number (%) for categorical variables.

Table 3. Associations Between Quantitative Serum Marker Levels and Disease Progression

Variable	Total	Liver decompensation	Liver compensation	*P* value	Death	Survival	*P* value
Eotaxin	52.0 (18.4-88.1)	60.8 (19.9-111.4)	46.7 (16.4-79.1)	0.067	40.3 (22.8-65.6)	51.0 (16.3-85.5)	0.949
GM-CSF	17.4 (17.4-17.4)	17.4 (17.4-17.4)	17.4 (17.4-17.4)	0.967	17.4 (17.4-17.4)	17.4 (17.4-17.4)	0.708
GRO-α	27.3 (17.6-33.1)	39.1 (26.2-55.1)	32.7 (27.1-38.7)	**0.010**	35.1 (27.9-56.0)	32.9 (27.3-39.3)	0.116
IFN-α	0.5 (0.5-0.5)	0.5 (0.5-0.5)	0.5 (0.5-0.5)	0.844	0.5 (0.5-0.5)	0.5 (0.5-0.5)	0.881
IFN-γ	7.1 (7.1-7.1)	7.1 (7.1-7.1)	7.1 (7.1-7.1)	0.836	7.1 (7.1-7.1)	7.1 (7.1-7.1)	0.861
IL-10	1.6 (1.6-1.6)	1.6 (1.6-1.6)	1.6 (1.6-1.6)	0.788	1.6 (1.6-1.6)	1.6 (1.6-1.6)	0.960
IL-12p70	4.6 (4.6-4.6)	4.6 (4.6-4.6)	4.6 (4.6-4.6)	0.687	4.6 (4.6-4.6)	4.6 (4.6-4.6)	0.735
IL-13	1.5 (1.5-1.5)	1.5 (1.5-1.5)	1.5 (1.5-1.5)	0.872	1.5 (1.5-1.5)	1.5 (1.5-1.5)	0.654
IL-15	2.2 (2.2-2.8)	2.2 (2.2-2.7)	2.2 (2.2-3.2)	0.978	2.2 (2.2-3.2)	2.2 (2.2-3.8)	0.877
IL-17A	3.5 (3.5-3.5)	3.5 (3.5-3.5)	3.5 (3.5-3.5)	0.897	3.5 (3.5-3.5)	3.5 (3.5-3.5)	0.546
IL-1α	3.2 (1.2-8.7)	2.2 (0.9-7.1)	3.5 (1.2-9.7)	0.529	3.5 (1.6-8.5)	3.2 (1.2-8.7)	0.525
IL-1β	2.1 (2.1-2.1)	2.1 (2.1-2.1)	2.1 (2.1-2.1)	0.753	2.1 (2.1-2.6)	2.1 (2.1-2.1)	0.365
IL-1RA	212.6 (108.9-383.2)	315.3 (128.8-537.3)	209.9 (108.9-381.6)	0.593	242.2 (130.0-371.9)	212.6 (108.9-401.5)	0.606
IL-2	5.1 (5.1-5.1)	5.1 (5.1-12.2)	5.1 (5.1-5.1)	0.133	5.1 (5.1-8.7)	5.1 (5.1-5.1)	0.609
IL-21	22.2 (17.0-147.7)	23.3 (17.0-172.0)	17.0 (17.0-101.7)	0.396	40.7 (17.0-127.3)	22.2 (17.0-168.3)	0.476
IL-22	41.0 (41.0- 41.0)	41.0 (41.0- 41.0)	41.0 (41.0- 41.0)	0.586	41.0 (41.0- 83.0)	41.0 (41.0- 41.0)	0.644
IL-23	17.6 (17.6-17.6)	17.6 (17.6-17.6)	17.6 (17.6-17.6)	0.980	17.6 (17.6-17.6)	17.6 (17.6-17.6)	0.722
IL-27	18.0 (18.0-18.0)	18.0 (18.0-23.8)	18.0 (18.0-18.0)	0.725	18.0 (18.0-26.8)	18.0 (18.0-18.0)	0.363
IL-31	10.5 (10.5-10.5)	10.5 (10.5-21.6)	10.5 (10.5-15.8)	0.650	10.5 (10.5-17.3)	10.5 (10.5-15.9)	0.747
IL-4	9.7 (9.7-9.7)	9.7 (9.7-9.7)	9.7 (9.7-9.7)	0.926	9.7 (9.7-9.7)	9.7 (9.7-9.7)	0.711
IL-5	5.3 (5.3-5.3)	5.3 (5.3-5.3)	5.3 (5.3-5.3)	0.330	5.3 (5.3-5.3)	5.3 (5.3-5.3)	0.186
IL-6	7.5 (7.5-7.5)	7.5 (7.5-12.0)	7.5 (7.5-7.5)	0.935	7.5 (7.5-17.6)	7.5 (7.5-7.5)	0.755
IL-7	12.3 (8.7-17.1)	11.3 (6.7-24.5)	12.5 (9.3-17.0)	0.965	12.0 (5.7-21.4)	12.5 (9.3-17.1)	0.699
IL-8	20.0 (13.0-35.4)	41.5 (14.7-100.7)	18.8 (12.7-32.6)	**0.004**	48.6 (16.4-141.5)	18.6 (12.7-32.8)	**0.018**
IL-9	11.8 (11.8-11.8)	11.8 (11.8-11.8)	11.8 (11.8-11.8)	0.687	11.8 (11.8-11.8)	11.8 (11.8-11.8)	0.735

IP-10	155.3 (57.7-333.1)	236.5 (82.5-402.1)	130.2 (56.1-316.3)	0.059	117.2 (69.5-236.5)	141.7 (26.6-333.1)	0.858
MCP-1	229.8 (164.8-320.5)	219.8 (165.7-335.6)	236.2 (165.0-325.2)	0.947	219.3 (157.4-331.1)	235.2 (166.6-328.6)	0.720
MIP-1α	38.7 (27.8-56.5)	49.5 (30.8-66.0)	36.7 (26.2-51.5)	0.097	44.2 (27.7-61.3)	37.4 (27.8-52.7)	0.402
MIP-1β	191.2 (145.5-270.0)	209.4 (178.7-279.2)	187.7 (143.0-271.6)	0.097	211.9 (168.9-257.0)	185.2 (144.0-280.2)	0.222
RANTES	330.5 (230.3-429.1)	307.9 (212.6-400.1)	331.6 (236.7-432.6)	0.686	369.6 (262.5-414.7)	328.6 (221.9-434.0)	0.742
SDF-1α	848.1 (611.8-1099.6)	860.9 (769.0-1078.7)	845.5 (551.4-1094.9)	0.054	831.2 (663.4-1124.6)	849.6 (584.6-1090.7)	0.756
TNF-α	5.9 (5.9-5.9)	5.9 (5.9-5.9)	5.9 (5.9-5.9)	0.735	5.9 (5.9-6.7)	5.9 (5.9-5.9)	0.084
TNF-β	3.3 (3.3-3.3)	3.3 (3.3-3.3)	3.3 (3.3-3.3)	0.289	3.3 (3.3-3.3)	3.3 (3.3-3.3)	0.927
sCD14	1.9 (1.5-2.2)	2.1 (1.7-2.6)	1.9 (1.6-2.2)	**0.027**	2.3 (2.0-2.6)	1.9 (1.5-2.2)	**0.003**
EndoCab	813.5 (642.5-990.3)	709.5 (591.8-873.3)	825.0 (670.0-1005.0)	0.112	825.0 (601.0-948.0)	813.5 (655.0-993.8)	0.629
I-FABP	340.3 (174.2-744.3)	292.1 (155.3-369.2)	338.4 (167.4-811.2)	0.663	283.0 (161.1-453.9)	335.6 (166.8-901.1)	0.501

All data except sCD14 (μg/mL) are expressed as pg/mL.

Table 4. Comparison of Liver-related Death/Liver Transplantation and Survival

Characteristic	Death (n = 14)		Survival (n = 122)		*P* value
Age at admission (years)	48	(38-54)	58	(52-64)	0.012
Female, n (%)	10	(71)	103	(84)	0.219
Laboratory data at admission					
Platelet count (×10^9/L)	23.5	(14.7-31.1)	22.4	(18.3-25.3)	0.750
Albumin (g/dL)	3.7	(3.7-4.2)	4.3	(4.1-4.5)	0.001
ALT (IU/L)	71	(55-96)	39	(28-63)	0.018
AST (IU/L)	81	(55-107)	40	(30-57)	0.001
ALP (IU/L)	636	(457-2086)	439	(320-591)	0.003
GGT (IU/L)	297	(173-466)	127	(79-248)	0.003
Bilirubin (mg/dL)	1.0	(0.6-3.7)	0.8	(0.6-0.9)	0.057
IgM (mg/dL)	532	(345-702)	283	(194-444)	0.008
IgG (mg/dL)	2017	(1474-2102)	1597	(1354-1922)	0.110
AMA-positive, n (%)	12	(86)	101	(83)	0.782
Anti-gp210, n (%)	10	(71)	22	(18)	<0.001
Anti-centromere, n (%)	2	(14)	43	(35)	0.114
Anti-sp100, n (%)	0	(0)	13	(11)	0.199
Anti-chromatin, n (%)	1	(7)	2	(2)	0.713
Histological findings					
Scheuer's stage III+IV, n (%)	11	(79)	17	(14)	<0.001

Parameters are presented as median (IQR) for continuous variables and total number (%) for categorical variables

Figure Legends

Figure 1. Serum biomarkers related to liver decompensation. Boxes represent the interquartile range of the data. The lines across the boxes indicate median values. The hash marks above and below the boxes indicate the 90th and 10th percentiles for each group, respectively. Open circles indicate outliers. Baseline serum GROα/CXCL1 (A), IL-8 (B), and sCD14 (C) were evaluated in 19 patients who developed liver decompensation and 117 patients who did not.

Figure 2. Cumulative survival rates were analyzed using the Kaplan-Meier method according to baseline sCD14 and IL-8 levels. Progression to liver decompensation was significantly higher in patients when (A) sCD14 level was ≥ 2.0 μg/mL and (B) IL-8 level was ≥ 46.5 pg/mL. Survival was significantly lower in patients when (C) sCD14 level was ≥ 2.0 μg/mL and (D) IL-8 level was ≥ 46.5 pg/mL.

Figure 3. Serum biomarkers related to mortality. Boxes represent the interquartile range of the data. The lines across the boxes indicate median values. The hash marks above and below the boxes indicate the 90th and 10th percentiles for each group, respectively. Open circles indicate outliers. Baseline serum IL-8 (A) and sCD14 (B) were measured in 14 patients progressing to liver-related death/liver transplantation and 122 patients who did not.

Supplementary Figure 1. Cumulative survival rates were analyzed using the Kaplan-Meier method according to anti-gp210 positivity. Progression to liver decompensation

was significantly higher in patients with PBC when (A) anti-gp210 was positive. Survival was significantly lower in patients when (B) anti-gp210 was positive.