Table 1 Intrahepatic expression of Toll-like receptors (TLR) on human liver cells

Kind of liver cells	Expressed TLR
Hepatocyte	TLR2,[5] TLR3,[6] TLR4[7]
Biliary epithelial cell	TLR1–6, 9[8]
Hepatic stellate cell	TLR1–9[9]
Sinusoidal endothelial cell	TLR2[10]
Kupffer cell	TLR2,[5] TLR3,[6] TLR4[5]

results in persistent inflammation and contributes to the development of chronic liver diseases. Singh *et al.*[12] reported that bacterial translocation comparably occurs in both normal and diseased livers such as primary biliary cirrhosis (PBC) and non-alcoholic steatohepatitis (NASH) although the expression of TLR2 and TLR4 is enhanced in the diseased livers than normal. In normal biliary epithelial cells (BEC), repeated LPS-stimuli induced hyporeactivity to LPS.[13] However, BEC from PBC patients show hyperreactivity to LPS.[14]

Herein, we review the association of gut microbiota with the pathogenesis of chronic liver diseases such as NASH, primary sclerosing cholangitis (PSC) and PBC.

NON-ALCOHOLIC STEATOHEPATITIS

NON-ALCOHOLIC FATTY LIVER disease (NAFLD) is recognized as a common liver disorder that represents the hepatic manifestation of metabolic syndrome, and encompasses a spectrum of hepatology, ranging from simple steatosis to cirrhosis.[15,16] NASH is the progressive form of liver injury and characterized by steatosis, lobular inflammation, hepatocyte ballooning, Mallory's hyaline and fibrosis. The histological findings of NAFLD and NASH are similar to the lesions caused by alcoholic liver disease.

The "two-hit" model is a widely accepted theory of the pathogenesis of NASH.[17] According to this theory, the first hit is an imbalance in fatty acid metabolism leading to hepatic steatosis, and the secondary hits are oxidative stress/metabolic stress and dysregulated cytokine production. In NASH patients, hepatic TLR4 expression is increased.[18] TLR4 deficiency ameliorates hepatic steatosis induced by high-fat diets.[19] Activation of TLR4 takes a role in the first hit. Next, as components potentially involved in the secondary hits, the gut microbiota have been investigated. In patients with NAFLD, intestinal permeability and the prevalence of small intestinal bacterial overgrowth are increased.[20] In NAFLD models, the translocation of bacterial components promotes tumor necrosis factor (TNF)-α release from Kupffer cells and induces hepatic inflammation through TLR4 and TLR9 signaling.[21,22] High-fat diets induce the deposition of toxic lipids such as diacylglycerol and sphingolipid in Kupffer cells and promote the secretion of TNF-α, interferon (IFN)-γ, IL-6 and IL-1β from Kupffer cells via LPS stimulation.[23] Furthermore, hepatic NKT cell numbers have been shown to be decreased.[24] High-fat diets reduce hepatic NKT cell numbers through hepatic IL-12 production, which results in increases in the hepatic production of pro-inflammatory cytokines such as TNF-α and IFN-γ and the exacerbation of inflammation in the liver.[25] Modification of gut microbiota with probiotics has been found to increase hepatic NKT cell numbers and reduce the hepatic expression of TNF-α and inflammation.[24,26,27] In NASH patients, 24-week treatment with *Bifidobacterium longum* and fructo-oligosaccharides improves insulin resistance and reduces histological NASH activity.[28] Various findings to date support an association of gut microbiota with the pathogenesis of NASH. A breakdown in TLR tolerance seems to be significantly associated with the progression of NASH. On the other hand, in NASH patients, hepatic NKT cell number has been reported to increase.[29] Thus, there may be partial differences in the pathogenesis between NASH patients and animal models. Further studies in NAFLD patients are required.

Recently, the contribution of inflammasomes to the pathogenesis of NAFLD was reported.[30] Inflammasomes are groups of protein complexes that recognize a diverse set of inflammation-inducing stimuli, including PAMP and damage-associated molecular patterns (DAMP), and that directly activate caspase-1, resulting in the production of important pro-inflammatory cytokines such as IL-1β and IL-18 and a type of cell death called "pyroptosis".[31] Csak *et al.*[30] reported that saturated fatty acid, but not unsaturated fatty acid, increases the expression of PYD domain-containing protein 3 (NLRP3) in hepatocytes, and that activation of NLRP3 by LPS stimuli via TLR4 leads to IL-1β release from hepatocytes. Furthermore, hepatocytes exposed to saturated fatty acid release danger signals that activate macrophages by the upregulation of NLRP3. The activation of hepatic macrophages leads to an exacerbation of hepatic inflammation.

PRIMARY SCLEROSING CHOLANGITIS

PRIMARY SCLEROSING CHOLANGITIS (PSC) is a chronic cholestatic liver disease characterized by inflammation, obliteration and fibrosis of the intrahe-

patic and/or extrahepatic biliary ducts.[32] Although the etiology of PSC remains unknown, gut microbiota are considered to play an important role in the pathogenesis of PSC. Sumitran-Holgersson *et al.*[33,34] reported that approximately 60% of PSC patients have serum antibodies to BEC (anti-BEC), and stimulation of BEC with the immunoglobulin (Ig)G of anti-BEC$^+$ PSC patients induces the expression of TLR4, whereas unstimulated or normal IgG-stimulated BEC do not express TLR4. TLR4-expressing BEC produce high levels of IL-1β, IL-8 and IFN-γ when stimulated with LPS. Mueller *et al.*[35] reported that BEC from end-stage PSC liver show marked expression of TLR4, increased activation of the myeloid differentiation protein 88/IL-1 receptor-associated kinase signaling cascade, and a loss of immune tolerance to endotoxin after repeated endotoxin exposure. However, the expression of TLR4 in BEC from the early-stage PSC liver is similar to that of healthy liver. Increased expression of TLR4 and a loss of immune tolerance to endotoxin in BEC may coordinate autoimmunity in the progression of PSC.

Approximately 1–3% of patients with ulcerative colitis (UC) had concurrent PSC,[36–38] and approximately 68–75% of PSC patients had UC.[39–41] UC patients with PSC more frequently have total colonic involvement than UC patients without PSC (68–85% vs 44–45%).[36,42] In UC patients, the extent of disease is positively correlated with plasma concentrations of endotoxin.[43] Thus, endotoxin concentrations in the portal vein are expected to be higher in UC patients with PSC than in UC patients without PSC. Furthermore, in the bile of PSC patients, enteric bacteria such as *Escherichia coli* are frequently detected.[44] Thus, in PSC patients, the liver constantly confronts abundant gut bacterial antigens such as endotoxin, and reinforced confrontation with these antigens is considered to be among the causes of PSC.

Serum perinuclear antineutrophil cytoplasmic antibodies (pANCA), which are frequently seen in patients with UC, have been detected in approximately 80% of PSC patients.[45,46] By indirect immunofluorescence study, pANCA in PSC show a heterogeneous rim-like staining in the nuclear periphery (atypical pANCA),[47] unlike classical pANCA, which show peripheral rim-like staining without intranuclear staining in patients with systemic vasculitis. Recently, the autoantigen of this atypical pANCA has been reported to be β-tubulin isotype 5.[48] Furthermore, this atypical pANCA cross-reacts with FtsZ, which is present in almost all bacteria of the gut microbiota. These phenomena reflect abnormal immune responses to gut bacterial antigens in PSC.

Recently, *caspase recruitment domain-containing protein 9* (*CARD9*), *v-rel reticuloendotheliosis viral pmcogene homolog* (*REL*) and *IL-2*, which are associated with the susceptibility to UC,[49] have been reported as candidate genes for PSC.[50] Of these genes, *CARD9* and *REL* are associated with innate immunity. Importantly, REL takes part in nuclear factor (NF)-κB functions. CARD9 is the adaptor molecule essential for the control of fungal infection. Gross *et al.*[51] reported that all CARD9-deficient mice died within 5 days after infection with *Candida albicans*, whereas more than 50% of control mice survived for more than 12 days. β-Glucan is initially recognized by dectin-1, a type II transmembrane protein expressed in various inflammatory cells such as macrophages, monocytes, dendritic cells, neutrophils, a subpopulation of T cells, B cells, mast cells and eosinophils. After the recognition of β-glucan by dectin-1, Syk signaling leads to the complex formation of CARD9, Bcl-10 and mucosa-associated lymphoid tissue translocation gene 1 and results in the release of IL-1β.[51–54] *Candida* is detected in the bile of approximately 10% of PSC patients, and a finding of *Candida* in the bile worsens the prognosis.[44] Polymorphisms of the *CARD9* gene may influence innate immunity to *Candida* in PSC patients. In addition, the activation of inflammasomes such as NLRP3 is involved in the process of IL-1β production by dectin-1 signaling. Silencing of NLRP3 expression partially impairs the processing of pro-IL-1β. Inflammasomes may be associated with the pathogenesis of PSC and are worth investigating in order to reveal the pathogenesis of PSC.

PRIMARY BILIARY CIRRHOSIS

PRIMARY BILIARY CIRRHOSIS is an autoimmune liver disease characterized by intrahepatic bile duct destruction, particularly chronic non-suppurative destructive cholangitis, cholestasis, and presence in the serum of antimitochondrial antibodies (AMA). AMA are detected in approximately 95% of PBC patients.[55] In particular, M2 antibodies (M2Ab) against E2 components of pyruvate dehydrogenase complex (PDC-E2) are specific to PBC and are detected in nearly 80% of patients.

Increased expression of TLR4 is shown in the liver of PBC. TLR4 expression levels in the BEC and periportal hepatocytes of PBC are augmented.[7] Especially, the BEC of PBC patients clearly express TLR4, regardless of disease stage. On the other hand, the role of TLR in the pathogenesis of PBC has been investigated also using PBMC obtained from PBC patients. Compared to

those from healthy controls, the monocytes from PBC patients produce high amounts of pro-inflammatory cytokines, particularly IL-1β and IL-6, in response to bacterial components such as LPS, flagellin and CpG, but not in response to viral components such as polyinosinic–polycytidylic acid (polyI:C).[56] LPS stimulation increases the expression of both TLR4 and MyD88 in monocytes from PBC patients.[57] Similarly, CpG-stimulated memory B cells from PBC patients express TLR9 and produce high levels of IgM.[58] However, the surface expression levels of TLR4 and TLR9, respectively, in unstimulated monocytes and B cells are similar in PBC patients and healthy controls. These findings raise the question of how innate immunity participates in the pathogenesis of PBC *in vivo*. Shimoda *et al.*[59] recently reported that when in the presence of IFN-α from polyI:C-stimulated monocytes, LPS-stimulated natural killer (NK) cells destroy autologous BEC. The activation and cross-talk of monocytes with NK cells are suggested to contribute to the pathogenesis of PBC. The various findings to date generally support the contribution of mechanisms of innate immunity in the pathogenesis of PBC.

The concept of molecular mimicry has been proposed as the cause of PBC. AMA in PBC serum cross-react with bacterial components. AMA have been reported to react with proteins of *E. coli* isolated in stool specimens from PBC patients.[60] $HRPA_{153-167}$ and $MALE_{95-109}$ of *E. coli* share 80% and 73% sequential similarity, respectively, with human $PDC\text{-}E2_{212-226}$, and M2Ab in approximately 30% of PBC patients cross-reacts with $HRPA_{153-167}$ and/or $MALE_{95-109}$ of *E. coli*.[61] In addition, approximately 50% of PBC patients harbor IgG3 antibodies that cross-react with β-galactosidase (BGAL) of *Lactobacillus delbrueckii*, a probiotic microorganism essential to starter cultures and yogurt production.[62] $BGAL_{266-280}$ of *L. delbrueckii* shares 67% similarity with human $PDC\text{-}E2_{212-226}$. In approximately 25% of PBC patients, the serum reacts in a highly directed and specific manner to proteins of *Novosphingobium aromaticivorans* from fecal specimens.[63]

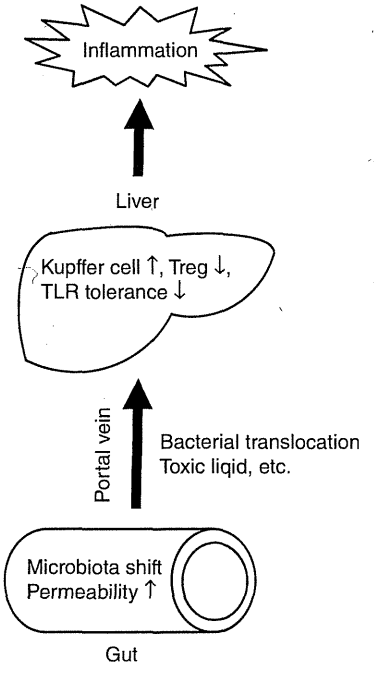

Figure 1 Concept of gut–liver axis in liver diseases. TLR, Toll-like receptors; Treg, regulatory T cells.

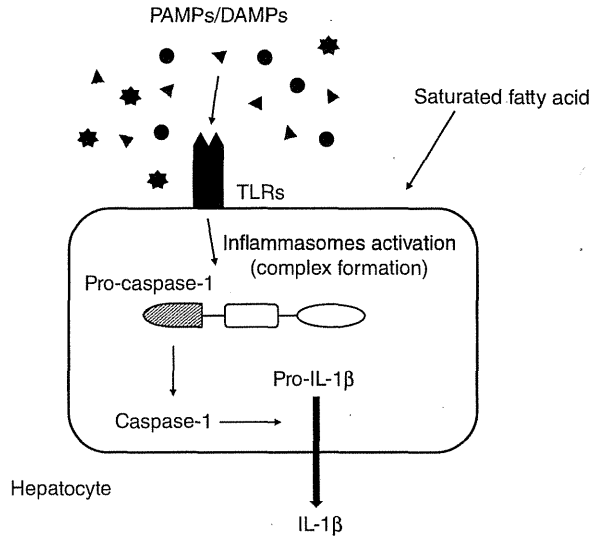

Figure 2 Relation of Toll-like receptors and inflammasomes. DAMP, damage-associated molecular patterns; IL, interleukin; PAMP, pathogen-associated molecular patterns; TLR, Toll-like receptors.

PROBIOTICS AND THE LIVER

GUT MICROBIOTA SHIFTS influence hepatic inflammation. In a model of liver injury induced by ischemic reperfusion, intestinal *Enterococcus* spp. and Enterobacteriaceae increase, while *Lactobacillus* spp., *Bifidobacter* spp. and *Bacterioides* spp. decrease. Supplementation with *Lactobacillus paracasei* decreases *Enterococcus* spp. and Enterobacteriaceae and increases *Lactobacillus* spp., *Bifidobacter* spp. and *Bacterioides* spp.,

Table 2 Intrahepatic condition of liver cells, TLR, cytokines and causative microbes in the chronic liver diseases

	Treg	NKT	HSC	Kupffer cell	TLR	Cytokine	Microbe
NASH	↑[72]	↑[29]	↑[73]	↑[72]	TLR4 ↑[18]	TNF-α ↑,[74] IFN-γ ↑,[74] IL-1β ↑,[74] IL-6 ↑,[74] IL-17 ↑[75]	*Porphyromonas gingivalis*[76]
PSC	→[77]	ND	ND	↑[78]	TLR4 ↑,[35] TLR9 ↑[34]	TNF-α ↑,[35] IFN-γ ↑,[34] IL-6 ↑,[33] IL-8 ↑[35]	*Escherichia coli*,[44] *Helicobacter pylori*,[79] *Chlamydia* spp.,[80] *Candida*[44]
PBC	↑[77]	↑[81]	↑[82]	↑[78]	TLR3 ↑,[6] TLR4 ↑,[7] TLR7 ↑,[6] TLR9 ↑[6]	IFN-α ↑,[6] IFN-β ↑,[6] IFN-γ ↑,[6] IL-5 ↑,[83] IL-6 ↑,[83] IL-8 ↑,[84] IL-12 ↑,[83] IL-17 ↑[75]	*Escherichia coli*,[60] *Lactobacillus delbrueckii*,[62] *Novosphingobium aromaticivorans*,[63] *Mycoplasma pneumonia*,[85] *Streptococcus intermedius*,[86] *Propionibacterium acnes*[87]

HSC, hepatic stellate cells; IFN, interferon; IL, interleukin; NASH, non-alcoholic steatohepatitis; ND, no data; NKT, natural killer T cells; PBC, primary biliary cirrhosis; PSC, primary sclerosing cholangitis; TLR, Toll-like receptors; TNF, tumor necrosis factor; Treg, regulatory T cells.

which result in reduced levels of expression of TNF-α, IL-1β and IL-6 and amelioration of necroinflammation in the liver.[64] In liver injury induced by chemical substances or alcohol, probiotic supplementation with species such as *Lactobacillus* spp. and *Bifidobacterium* spp. decreases bacterial translocation to the liver through decreased concentrations of aerobic bacteria such as *E. coli* as well as due to increased intestinal stability (i.e. reduced intestinal permeability), and reduces hepatic inflammation.[65–67] Furthermore, gut microbiota shifts influence hepatic metabolism (e.g. amino acid, fatty acid, organic acid and carbohydrate metabolism) by the modulation of hepatic gene expression, without direct contact with the liver.[68,69] In cirrhotic patients with hepatic encephalopathy, intestinal *E. coli* and *Staphylococcus* spp. overgrow, and supplementation with symbiotic reduces blood ammonia levels and ameliorates hepatic encephalopathy by reducing levels of *E. coli* and increasing *Lactobacillus* spp.[70] In rats fed a high-cholesterol diet, *Lactobacillus* spp. supplementation decreases intestinal *E. coli* and increases *Lactobacillus* spp. and *Bifidobacterium* spp., which leads to reduced levels of hepatic cholesterol and triglyceride.[71] In general, gut microbiota shifts have been shown to exert a substantial impact on the liver.

CONCLUSION

MANY FINDINGS TO date support the contribution of bacterial components (e.g. endotoxins, unmethylated CpG containing DNA) to the pathogenesis of various liver diseases (Fig. 1). Innate immunity plays an important role in the hepatic response to these bacterial components, and TLR4 and TLR9 signaling has been widely investigated (Table 2). However, many questions remain regarding the relation of innate immunity to the pathogenesis of liver diseases. First, it remains unclear why TLR tolerance is disrupted in various liver diseases. Second, how do the roles of innate immunity in the pathogenesis differ between PSC and PBC? BEC are the main targets of injury in both diseases, although the histological features of PSC and PBC markedly differ. Third, the factors that control the protective or detrimental roles of NKT cells and Kupffer cells remain to be determined. Fourth, we still need to determine which probiotic will be most effective for treating which liver disease(s). Further analysis will be needed to more fully understand the association of innate immunity with disease pathogenesis in the case of each specific disease.

Recently, stimuli by TLR have been indicated to activate inflammasomes, and activated inflammasomes induce the processing of pro-IL-1β and pro-IL-18 by the activation of caspase-1 (Fig. 2). The association of IL-1β with the pathogenesis of various liver diseases has been already reported;[23,34,56] however, investigation of the association of inflammasomes with liver disease is still in the early stages. Inflammasomes warrant further analysis, which may reveal the mechanisms of innate immunity in various liver diseases.

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Serum Levels of Soluble Adhesion Molecules as Prognostic Factors for Acute Liver Failure

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Key Words
Acute liver failure · Intercellular adhesion molecule-1 · Liver transplantation · Platelet endothelial cell adhesion molecule-1 · Prognosis

Abstract
Background/Aims: In patients with septic shock, the degree of liver dysfunction is correlated with serum levels of soluble intercellular adhesion molecule (sICAM)-1. We aimed to assess the usefulness of serum levels of soluble adhesion molecules as prognostic factors for acute liver failure (ALF). ***Methods:*** Serum levels of soluble platelet endothelial cell adhesion molecule (sPECAM)-1, sICAM-3, soluble endothelial (sE) selectin, sICAM-1, soluble platelet selectin, and soluble vascular cell adhesion molecule-1 on admission were measured in 37 ALF patients and 34 healthy controls. ***Results:*** Twenty-two ALF patients (59%) reached to fatal outcomes. Serum levels of sPECAM-1, sICAM-3, sE-selectin and sICAM-1 were higher in ALF patients than healthy controls. In 37 ALF patients, by the multivariate logistic regression analysis, ratio of direct to total bilirubin (per 0.1 increase; OR 0.11, 95% CI 0.01–0.99), serum sPECAM-1 level (per 100 ng/ml increase; OR 4.37, 95% CI 1.23–15.5) and serum sICAM-1 level (per 100 ng/ml increase; OR 0.49, 95% CI 0.27–0.89) were associated with fatal outcomes. Using receiver operating characteristics curve, each area under the curve of serum sPECAM-1 and sICMA-1 levels as prognostic factors was 0.71 and 0.74, respectively. ***Conclusion:*** Serum sPECAM-1 and sICAM-1 levels may be useful for predicting the prognosis of ALF.

Introduction

Acute liver failure (ALF) is the clinical manifestation of liver cell death of a critical degree with insufficient hepatocellular regeneration and characterized by hepatic encephalopathy and coagulopathy [1]. The survival rate without liver transplantation is over 60% in patients with acetaminophen-induced ALF and 20–30% in those with nonacetaminophen-related ALF [2]. Many ALF patients rapidly progress to death from multiple organ failure (MOF). In order to save more ALF patients, it is important to accurately predict their prognosis.

Up to now, elevated serum levels of soluble adhesion molecules such as soluble intercellular adhesion molecule-1 (sICAM-1), soluble vascular cell adhesion molecule-1 (sVCAM-1), and soluble endothelial (sE) selectin have been reported to be associated with the development

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of MOF [3, 4]. Furthermore, in acute pancreatitis and severe burn, serum levels of sICAM-1 and sVCAM-1 have been shown to reflect the severity of the disease and be associated with the prognosis [5, 6].

In patients with sepsis or septic shock, serum sICAM-1 levels have been reported to be correlated with serum bilirubin levels [7, 8]. Furthermore, in patients with alcoholic liver cirrhosis, serum sICAM-1 levels have been shown to be correlated with prothrombin activities and serum bilirubin levels and be associated with the prognosis [9]. On the other hand, in an endotoxic shock model, inhibition of adhesion molecules such as platelet (P) selectin and ICAM-1 have been reported to reduce the degree of liver injury [10]. Thus, we speculated that serum levels of soluble adhesion molecules might be associated with the clinical outcomes of ALF patients.

This study aimed to investigate whether serum levels of soluble adhesion molecules were useful to predict the prognosis of ALF patients.

Methods

This study was approved by the Institutional Review Board at Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences.

Patients

The study subjects consisted of 37 ALF patients and 34 healthy controls.

In this study, patients showing prothrombin activities of 40% or less of the standardized values due to severe liver damage within 8 weeks of the onset of disease symptoms were diagnosed as ALF [11]. Hepatic coma was graded on the standard scale of I to IV [12]. However, those who showed features of chronic liver disease (splenomegaly or varices, collaterals) on computed tomography were excluded.

Etiology of ALF

A diagnosis of hepatitis A and B was made based on the presence of IgM anti-hepatitis A virus antibody, and IgM anti-hepatitis B virus core antibody or hepatitis B surface antigen, respectively [13]. A diagnosis of autoimmune hepatitis was made according to the criteria revised by the International Autoimmune Hepatitis Group in 1999 [14]. A diagnosis of drug-induced liver injury was made based on the distinctive clinical course. A diagnosis of indeterminate liver failure was established when IgM anti-hepatitis A virus antibody, IgM anti-hepatitis B virus core antibody, hepatitis B surface antigen, hepatitis C virus-RNA, antinuclear antibody and anti-smooth muscle antibody were all negative with no obvious cause such as drug, acute fatty liver of pregnancy, ischemic hepatitis, Wilson's disease, malignant infiltration, cytomegalovirus infection, Epstein-Barr virus infection and herpes simplex virus infection.

Measurement of Serum Level of Soluble Adhesion Molecule

Serum was collected when each patient admitted to our hospital, and stored at −80°C.

Serum levels of 6 soluble adhesion molecules were measured using the FlowCytomix Multiple Analyte Detection System with the Adhesion 6plex (eBioscience, San Diego, Calif., USA), according to the manufacturer's protocol. This panel consisted of soluble platelet endothelial cell adhesion molecule (sPECAM)-1, sICAM-3, sE-selectin, sICAM-1, sP-selectin and sVCAM-1. In brief, the Adhesion 6plex Standard diluted in assay buffer and samples were added to a 96-well filter plate. Antibody-coupled beads were added to all wells and incubated with phycoerythrin-conjugated second antibodies for 2 h with continuous shaking. The beads were washed twice with assay buffer and re-suspended in assay buffer. The reaction mixture was analyzed using the MACSQuant Analyzer with MACSQuantify Software v2.2 (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany). Each serum level of soluble adhesion molecule was automatically calculated by FlowCytomix Pro 2.4 software (eBioscience, San Diego, Calif., USA) using the appropriate standard curve.

Statistical Analysis

SPSS statistical program (release 11.0.1 J, SPSS, Chicago, Ill., USA) was used for the statistical analysis.

Dichotomous variables were compared by the χ^2 test. Continuous variables were expressed as median (range). The Mann-Whitney U test was used to evaluate differences in the continuous variables between two groups, and the Kruskal-Wallis test was carried out among three groups. Spearman correlation coefficient was used to evaluate the consistency in the continuous variables between two groups. To identify prognostic factors for fatal outcome (liver transplantation or death), we developed the univariate logistic regression model. The variables, which showed $p < 0.05$ by the univariate analysis, were included into the multivariate logistic regression model. The prognostic accuracy of each factor which elicited by the logistic regression analyses was evaluated based on the area under the curve (AUC) using receiver operating characteristics (ROC) curve analysis. $p < 0.05$ was considered significant.

Results

Clinical Characteristics

Table 1 shows clinical characteristics and laboratory data on admission in 37 ALF patients. Of 37 ALF patients, 28 were diagnosed with ALF with hepatic coma (≥ grade II) and 9 with ALF without hepatic coma (grade 0 or I). All patients underwent culture (blood, tracheal aspirate, and urine) and computed tomography (head, chest, and abdomen) at the diagnosis of ALF; however, none clinically developed bacterial or fungal infection.

Overall, 15 survived without liver transplantation, 14 received living donor liver transplantation, and 8 died without liver transplantation. Thus, 22 patients reached to fatal outcomes (liver transplantation or death).

Table 1. Clinical characteristics and laboratory data on admission in 37 patients with acute liver failure

	Survive	Fatal outcome	p value
Patients, n	15	22	
Age, years	37 (16–71)	38 (27–73)	0.40
Gender, female (%)	8 (53)	14 (64)	0.39
Etiology, n (%)			
Viral hepatitis	8 (53)	9 (41)	0.55
Hepatitis A virus	3	0	
Hepatitis B virus	5	9	
Autoimmune hepatitis	4 (27)	5 (23)	
Drug-induced liver injury	2 (13)	3 (13)	
Indeterminate	1 (7)	5 (23)	
Hepatic coma, n (%)			0.0001
0 or I	9 (60)	0	
II	5 (33)	16 (73)	
III or IV	1 (7)	6 (27)	
Laboratory data			
White blood cells, /mm^3	7,800 (4,200–28,000)	10,165 (2,300–25,300)	0.27
Hemoglobin, g/dl	13.1 (7.3–18.4)	13.2 (8.4–16.4)	0.86
Platelets, $\times 10^4$/mm^3	14.6 (9.0–30.9)	9.1 (2.4–40.3)	0.007
Bilirubin, mg/dl	8.9 (3.9–26.0)	11.2 (2.3–32.8)	0.44
D/T ratio	0.67 (0.57–0.72)	0.52 (0.31–0.75)	0.0003
AST, IU/l	1,036 (215–17,340)	497 (41–18,360)	0.18
ALT, IU/l	2,504 (220–7,990)	1,177 (24–10,470)	0.23
Creatinine, mg/dl	0.6 (0.4–2.6)	0.7 (0.4–4.8)	0.34
Prothrombin activity, %	32 (11–40)	23 (6–40)	0.034
Prognosis, n (%)			
Liver transplantation	0	14 (64)	
Death without liver transplantation	0	8 (36)	

ALT = Alanine aminotransferase; AST = aspartate aminotransaminase; D/T ratio = ratio of direct to total bilirubin.

Serum Level of Soluble Adhesion Molecule

Table 2 shows serum levels of 6 soluble adhesion molecules on admission in 37 ALF patients and 34 healthy controls. Serum levels of sPECAM-1, sICAM-3, sE-selectin and sICAM-1 in 37 ALF patients were higher than those in 34 healthy controls. In 37 ALF patients, 22 patients reaching fatal outcome showed higher serum sPECAM-1 levels (p = 0.030) and lower serum sICAM-1 levels (p = 0.014) than 15 survivors without liver transplantation. On the other hand, there was no difference in serum levels of 6 soluble adhesion molecules between 28 ALF patients with hepatic coma and 9 ALF patients without hepatic coma (table 3). Furthermore, in 15 survivors, there were no differences in serum levels of sPECAM-1 [577 (495–784) vs. 558 (194–898) ng/ml; p = 0.48] and sICAM-1 [1,993 (1,748–2,246) vs. 1,892 (1,264–2,737) ng/ml; p = 0.91] between 6 ALF patients with hepatic coma and 9 ALF patients without hepatic coma.

In 28 ALF patients with hepatic coma, 22 patients reaching fatal outcomes showed lower serum sICAM-1 levels [1,423 (124–2,839) vs. 1,993 (1,748–2,246) ng/ml; p = 0.036] than 6 survivors; however, there were no differences in serum levels of other 5 soluble adhesion molecules between the 2 groups.

Serum Level of Soluble Adhesion Molecule as Prognostic Factor for ALF

In the univariate logistic regression model, platelet count, ratio of direct to total bilirubin (D/T ratio), serum sPECAM-1 level and serum sICAM-1 level on admission were associated with fatal outcomes in 37 ALF patients. However, the association of prothrombin activity on admission with the prognosis was equivocal (table 4).

In the multivariate logistic regression analysis, D/T ratio (OR 0.11, 95% CI 0.01–0.99), serum sPECAM-1 level (OR 4.37, 95% CI 1.23–15.5) and serum sICAM-1 level

Table 2. Serum level of soluble adhesion molecule on admission

	Acute liver failure patients			Healthy controls	p value
	overall	survive	fatal outcome		
Patients, n	37	15	22	34	
sPECAM-1, ng/ml	664 (194–2,049)	558 (194–898)	850 (313–2,049)	338 (171–1,072)	<0.0001
sICAM-3, ng/ml	286 (43–1,170)	286 (43–674)	273 (51–1,170)	88 (8–290)	<0.0001
sE-selectin, ng/ml	422 (140–1,162)	440 (140–1,098)	360 (169–1,162)	108 (35–404)	<0.0001
sICAM-1, ng/ml	1,783 (124–2,839)	1,892 (1,264–2,737)	1,423 (124–2,839)	666 (279–1,604)	<0.0001
sP-selectin, ng/ml	185 (21–499)	139 (59–499)	192 (21–418)	201 (44–475)	0.33
sVCAM-1, ng/ml	2,468 (719–3,898)	2,415 (719–3,574)	2,442 (898–3,898)	2,208 (1,262–3,574)	0.84

Each parameter was compared between 37 patients with acute liver failure and healthy controls. E-selectin = Endothelial selectin; ICAM-1 = intercellular adhesion molecule-1; ICAM-3 = intercellular adhesion molecule-3; PECAM-1 = platelet endothelial cell adhesion molecule-1; P-selectin = platelet selectin; s = soluble; VCAM-1 = vascular cell adhesion molecule-1.

Table 3. Serum level of soluble adhesion molecule on admission in 37 patients with acute liver failure

	Hepatic coma grade		p value
	0 or I	II or higher	
Patients, n	9	28	
sPECAM-1, ng/ml	558 (194–898)	742 (313–2,049)	0.061
sICAM-3, ng/ml	167 (43–674)	298 (51–1,170)	0.13
sE-selectin, ng/ml	472 (140–1,098)	360 (165–1,162)	0.42
sICAM-1, ng/ml	1,892 (1,264–2,737)	1,676 (124–2,839)	0.15
sP-selectin, ng/ml	154 (59–499)	182 (21–418)	0.96
sVCAM-1, ng/ml	3,086 (719–3,574)	2,389 (898–3,898)	0.70

E-selectin = Endothelial selectin; ICAM-1 = intercellular adhesion molecule-1; ICAM-3 = intercellular adhesion molecule-3; PECAM-1 = platelet endothelial cell adhesion molecule-1; P-selectin = platelet selectin; s = soluble; VCAM-1 = vascular cell adhesion molecule-1.

(OR 0.49, 95% CI 0.27–0.89) were associated with fatal outcomes in 37 ALF patients (table 5).

Based on the ROC curves of serum levels of sPECAM-1 and sICAM-1 for estimating fatal outcomes in the 37 ALF patients, the AUC was 0.71 ($p = 0.007$) and 0.74 ($p = 0.005$), respectively. On the other hand, the AUC of platelet count, prothrombin activity, and D/T ratio was 0.77 ($p = 0.014$), 0.70 ($p = 0.050$), and 0.85 ($p < 0.0001$), respectively.

In 37 ALF patients, serum sPECAM-1 level was inversely correlated with prothrombin activity ($r = -0.52$, $p = 0.0017$). Serum sICAM-1 level was significantly correlated with platelet count ($r = 0.50$, $p = 0.0025$) and D/T ratio ($r = 0.53$, $p = 0.0015$).

When ALF patients showing serum sPECAM-1 level ≥650 ng/ml on admission were estimated to reach fatal outcomes, the sensitivity and specificity were 68 and 73%, respectively. On the other hand, when patients showing serum sICAM-1 level ≤1,750 ng/ml on admission were estimated to reach fatal outcomes, the sensitivity and specificity were 68 and 73%, respectively.

Discussion

Recently, the prognosis of ALF patients has been improved due to the advances in supportive intensive care; however, liver transplantation is the only effective intervention for those with fatal outcomes [15]. On the other

Table 4. Prognostic factor for acute liver failure by univariate logistic regression model

	OR	95% CI	p value
Age, per 1 year increase	1.02	0.97–1.07	0.45
Gender, female	1.53	0.40–5.81	0.53
Etiology, viral hepatitis	0.61	0.16–2.28	0.46
Hepatic coma, III or IV	5.26	0.56–50.0	0.15
White blood cells, per 100/mm^3 increase	1.01	0.99–1.02	0.30
Hemoglobin, per 1 g/dl increase	0.93	0.71–1.23	0.63
Platelets, per 1 × 10^4/mm^3 increase	0.90	0.82–0.99	0.030
Bilirubin, per 1 mg/dl increase	1.04	0.96–1.13	0.36
D/T ratio, per 0.1 increase	0.14	0.03–0.59	0.007
AST, per 100 IU/l increase	1.00	0.99–1.01	0.99
ALT, per 100 IU/l increase	0.99	0.97–1.02	0.58
Creatinine, per 1 mg/dl increase	1.95	0.68–5.60	0.22
Prothrombin activity, per 1% increase	0.94	0.88–1.00	0.064
sPECAM-1, per 100 ng/ml increase	1.39	1.04–1.88	0.029
sICAM-3, per 100 ng/ml increase	1.13	0.89–1.43	0.32
sE-selectin, per 100 ng/ml increase	0.99	0.80–1.23	0.92
sICAM-1, per 100 ng/ml increase	0.84	0.72–0.97	0.016
sP-selectin, per 100 ng/ml increase	1.17	0.67–2.04	0.58
sVCAM-1, per 100 ng/ml increase	1.01	0.94–1.08	0.75

ALT = Alanine aminotransferase; AST = aspartate aminotransaminase; D/T ratio = ratio of direct to total bilirubin; E-selectin = endothelial selectin; ICAM-1 = intercellular adhesion molecule-1; ICAM-3 = intercellular adhesion molecule-3; PECAM-1 = platelet endothelial cell adhesion molecule-1; P-selectin = platelet selectin; s = soluble; VCAM-1 = vascular cell adhesion molecule-1.

Table 5. Prognostic factor for acute liver failure by multivariate logistic regression model

	OR	95% CI	p value
Platelets, per 1 × 10^4/mm^3 increase	1.26	0.98–1.63	0.074
D/T ratio, per 0.1 increase	0.11	0.01–0.99	0.049
sPECAM-1, per 100 ng/ml increase	4.37	1.23–15.5	0.022
sICAM-1, per 100 ng/ml increase	0.49	0.27–0.89	0.020

ALT = Alanine aminotransferase; AST = aspartate aminotransaminase; D/T ratio = ratio of direct to total bilirubin; ICAM-1 = intercellular adhesion molecule-1; PECAM-1 = platelet endothelial cell adhesion molecule-1; s = soluble.

hand, in Asian countries, the donation from deceased donors is severely limited because of various cultural and social reasons [16]. Approximately 50% of patients listed for emergency liver transplantation have died while awaiting a graft because of the lack of a timely suitable donor [17, 18]. So, in order to rescue more patients in a setting of the shortage of liver grafts, prognostic factors useful to determine the suitable timing for liver transplantation are required.

In ALF, elevated serum levels of pro-inflammatory cytokines such as IL-1β, IL-6, TNF-α, and IFN-γ have been reported to be associated with the disease pathogenesis and clinical outcomes [19–22]. These pro-inflammatory cytokines promote the secretion of soluble adhesion molecules such as sICAM-1, sVCAM-1 and sE-selectin from the endothelial cells [23–25]. Thus, we speculated that some soluble adhesion molecules might be associated with the clinical outcomes of ALF.

In this study, serum levels of sPECAM-1 and sICAM-1 were significantly associated with the prognosis of ALF patients. Furthermore, the accuracy of serum levels of sPECAM-1 and sICAM-1 for predicting the prognosis of ALF seemed approximately equal to platelet count and prothrombin activity, which have been reported as important prognostic factors for ALF [26]. On the other hand, although ALF patients with hepatic coma have been reported to reach fatal outcomes more frequently than those without hepatic coma [11], there were no differences in serum levels of sPECAM-1 and sICAM-1 between these 2 groups in this study. So, we consider that serum levels of sPECAM-1 and sICAM-1 may be worth investigating as biomarkers for predicting the prognosis and determining the suitable timing for liver transplantation in ALF patients.

ICAM-1 is a member of the immunoglobulin superfamily of cell adhesion molecules and expressed on both hematopoietic and nonhematopoietic cells [27]. ICAM-1 binds to its main leukocyte ligand, lymphocyte function associated molecule (LFA)-1, and plays an important roles in the trans-endothelial migration of leukocytes to sites of inflammation and the activation of T cells [27, 28]. On the other hand, sICAM-1, which consists of the five extracellular immunoglobulin domains of the membrane-bound ICAM-1 molecule and lacks the transmembrane and cytoplasmic domains, inhibits ICAM-1 interaction with LFA-1 and attenuates inflammation [28–30]. Furthermore, sICAM-1 has been reported to promote angiogenesis [31]. Angiogenesis plays an important role in liver regeneration [32]. Thus, insufficient elevation of serum sICAM-1 levels in ALF patients may lead to the continuation of intrahepatic inflammation and be associated with the failure of liver regeneration.

Generally, serum sICAM-1 levels have been reported to be higher in patients with inflammatory disorders, especially in those with poor prognosis [3, 33, 34]. However,

this study indicated that, in ALF patients, lower serum sICAM-1 levels were associated with their fatal outcomes. Part of sICAM-1 has been reported to be secreted from hepatocytes stimulated with pro-inflammatory cytokines such as IFN-γ, IL-1β and TNF-α [35]. After hepatic resection, serum sICAM-1 levels have been shown to decrease [36]. In this study, the serum sICAM-1 level was correlated with the D/T ratio, which reflects hepatic bilirubin conjugation capacity, in ALF patients. Thus, serum sICAM-1 levels may reflect the grade of hepatic dysfunction in ALF patients. In order to confirm these findings, a further study with a larger sample size is required.

This study firstly showed the association of serum sPECAM-1 levels with the prognosis of ALF patients. PECAM-1 is a member of the immunoglobulin-superfamily of cell adhesion molecules and expressed on most cells of the hematopoietic lineage including platelets [37]. sPECAM-1 lacks the cytoplasmic and trans-membrane domains. In this study, serum sPECAM-1 level was inversely correlated with prothrombin activity. In ALF, the intrahepatic and intravascular activation of coagulation, which decreases prothrombin activity and platelet count, results in microthrombus formation and local ischemia and contributes to the progression of the disease [38]. Serum sPECAM-1 levels have been reported to be associated with the development of ischemic stroke and acute coronary syndrome [39, 40]. Thus, elevated serum sPECAM-1 levels in ALF patients are considered to reflect coagulation activation.

We consider that, in order to assess the usefulness of serum sPECAM-1 and sICAM-1 levels as biomarkers for predicting outcomes of ALF patients, the relation between the changes of these levels during the clinical course and the prognosis of ALF patients should be assessed, although, in this study, we could not because of the lack of serum collection after the introduction of treatment in ALF patients. It is necessary to clarify this point.

In conclusion, this study indicated that serum levels of sPECAM-1 and sICAM-1 on admission were associated with the prognosis of ALF patients. We consider that serum levels of sPECAM-1 and sICAM-1 may be worth investigating as biomarkers for predicting their outcomes and determining the suitable timing for liver transplantation. A further study with a large sample size is required.

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INTERNAL MEDICINE

□ ORIGINAL ARTICLE □

SIRS Score Reflects Clinical Features of Non-Acetaminophen-Related Acute Liver Failure with Hepatic Coma

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Abstract

Objective In acetaminophen-induced acute liver failure (ALF), the hepatic coma grade worsens and mortality rates increase, as the number of systemic inflammatory response syndrome components fulfilled (SIRS score) increases. This study aimed to investigate the impact of SIRS score on clinical features of non-acetaminophen-related ALF.

Methods Ninety-nine patients with non-acetaminophen-related ALF with hepatic coma who did not undergo liver transplantation were investigated. Each patient was given a SIRS score of 0, 1, 2, 3 or 4 at the time of diagnosis.

Results At the diagnosis of ALF with hepatic coma, with the increase of SIRS score, hepatic coma grade and prothrombin activity were deteriorated. After the diagnosis of ALF with hepatic coma, 25 patients (25%) developed acute respiratory distress syndrome (ARDS), 31 patients (31%) developed disseminated intravascular coagulation (DIC), and 21 patients (22%) developed acute renal failure (ARF). Thirty-eight patients (38%) developed MOF. With the increase of SIRS score, frequencies of the development of ARDS, DIC and MOF increased. ARF was more frequently developed in patients with a SIRS score of 2 or higher. Overall, 36 patients (36%) survived. Overall survival rate was 66% in 29 patients with a score of 0, 43% in 21 patients with a score of 1, 17% in 29 patients with a score of 2 and 15% in 20 patients with a score of 3 or 4.

Conclusion SIRS score will be useful for predicting not only the overall survival but also the development of complications such as ARDS, DIC and MOF in non-acetaminophen-related ALF with hepatic coma.

Key words: acute liver failure, multiple organ failure, prognosis, systemic inflammatory response syndrome

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Introduction

Liver cell death of a critical degree with insufficient hepatocellular regeneration leads to the development of acute liver failure (ALF) characterized by hepatic encephalopathy and coagulopathy. The spontaneous survival rate is over 60% in patients with acetaminophen-induced ALF and 20-30% in those with non-acetaminophen-related ALF (1).

Liver transplantation (LT) is the only effective intervention for ALF. However, one-fourth of the patients listed for emergency LT are unable to undergo LT because of rapid deterioration of the disease and often end in death from multiple organ failure (MOF) (2). Thus, in order to identify the suitable timing for LT, to evaluate not only the grade of liver failure but also the whole body state is important. Recently, systemic inflammatory response syndrome (SIRS), which reflects whole body state, has been reported to be associated with the prognosis of ALF patients. SIRS is diagnosed by the presence of two or more of the following components: i) a temperature >38℃ or <36℃, ii) tachycardia >90/minute, iii) respiratory rate >20/minute or arterial

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PaCO2 <32 mmHg, and iv) white blood cell (WBC) count >12,000/mm^3 or <4,000/mm^3 or immature forms >10% (3).

In patients with acetaminophen-induced ALF, the King's College Group (4) and the U.S. Acute Liver Failure Study Group (5) have revealed that SIRS worsens the grade of hepatic coma and increases the mortality rates as the number of SIRS components fulfilled increases. On the other hand, in Asia, viral hepatitis has been a major cause of ALF, and a frequency of acetaminophen-induced ALF has been few (1). In patients with non-acetaminophen-related ALF, the increasing of the number of SIRS components fulfilled has been reported to be associated with increased probabilities of acute renal failure (ARF) (6). However, the associations of SIRS with clinical features of patients with non-acetaminophen-related ALF have yet to be fully implemented.

This study aimed to investigate the impact of SIRS on clinical features in patients with non-acetaminophen-related ALF with hepatic coma diagnosed based on the Japanese criteria (7).

Materials and Methods

Patients

One hundred and twenty patients with non-acetaminophen-related ALF with hepatic coma, who showed hepatic coma of grade II or more within 8 weeks of the onset of the disease symptoms with prothrombin time values of 40% or less of the standardized values, or international normalized ratios of 1.5 or more (7), and were admitted to the Okayama University Hospital and 8 tertiary care centers between January 1990 and December 2009. Of these patients, 21 received living donor LT based on the Guideline of the Acute Liver Failure Study Group of Japan (8). However, recently, the predictive accuracy of this guideline was reported to decrease to 73% (8). In particular, the positive predictive value of this guideline was shown to be low. Therefore, inclusion of 21 patients who were receiving LT into this study was considered inappropriate. Thus, 99 patients who did not receive LT were included in the present analysis.

Etiology of ALF

A diagnosis of hepatitis A, B and C was made based on the presence of immunoglobulin M antibody to hepatitis A virus, immunoglobulin M antibody to hepatitis B core antigen or hepatitis B surface antigen, and hepatitis C virus RNA identifiable by nested reverse transcription-polymerase chain reaction, respectively (9). A diagnosis of autoimmune hepatitis was made according to the criteria revised by the International Autoimmune Hepatitis Group in 1999 (10). A diagnosis of Epstein-Barr virus infection was made based on the measurement of Epstein-Barr virus load in whole blood by quantitative polymerase chain reaction amplification assays (11). A diagnosis of drug-induced liver injury, acute fatty liver of pregnancy and ischemic hepatitis was made based on their distinctive clinical courses. A diagnosis of indeterminate ALF was established when all of IgM anti-hepatitis A virus antibody, IgM anti-hepatitis B virus core antibody, hepatitis B surface antigen, hepatitis C virus-RNA, anti-nuclear antibody and anti-smooth muscle antibody were negative with no obvious cause such as drug, acute fatty liver of pregnancy, ischemic hepatitis, Wilson's disease, malignant infiltration, cytomegalovirus infection, Epstein-Barr virus infection and herpes simplex virus infection.

Treatment

All patients were admitted to the Intensive Care Unit to receive supportive care through the monitoring of clinical, biochemical and hemodynamic parameters. Patients received plasma exchange and/or hemodiafiltration as artificial liver support. Plasma exchange and hemodiafiltration were performed according to the following indications: i) patients with coagulopathy were indicated for plasma exchange, ii) patients with the central nerve disorder including hepatic coma were indicated for plasma exchange only or plasma exchange combined with hemodiafiltration, and iii) patients with renal failure were indicated for hemodiafiltration (12).

Statistical analysis

SPSS statistical program (release 11.0.1 J, SPSS, Chicago, IL, USA) was used for the statistical analysis. Each patient was given a SIRS score of 0, 1, 2, 3 or 4 depending on the number of SIRS components fulfilled at the diagnosis of ALF with hepatic coma. Furthermore, concerning the prediction of patient's prognosis, we evaluated using the King's College Criteria (13).

Continuous variables were expressed as median and range. Dichotomous variables were compared by the chi-square test. The Mann-Whitney U test was used to evaluate the significance of differences in the continuous variables between two groups, and the Kruskal-Wallis U test was done among four groups. Cumulative survival curves were analyzed using the Kaplan-Meier method, and the differences in the curves were tested using the log-rank test. p value <0.05 was considered significant.

Results

Characteristics of the study population

Of 99 patients, 56 (57%) were female. The median age was 47 (14-81) years. Seven patients (7%) were diagnosed with fulminant hepatitis A, 32 patients (32%) with fulminant hepatitis B, 9 patients (9%) with autoimmune hepatitis, and 14 patients (14%) with drug-induced liver injury. On the other hand, 31 patients (32%) were diagnosed with indeterminate ALF. At the diagnosis of ALF with hepatic coma, hepatic coma grade was II in 65 patients (66%), III in 22 patients (22%) and IV in 12 patients (12%). SIRS score of 0, 1, 2, and either 3 or 4 was found in 29 patients (29%),

Table 1. Clinical Characteristics and Laboratory Data at the Diagnosis of ALF with Hepatic Coma according to the SIRS Score

	SIRS score				p value
	0	1	2	3 or 4	
Patients, n	29	21	29	20	
Age, yr	42 (16-75)	45 (19-76)	49 (14-79)	54 (20-81)	0.50
Gender, female (%)	14 (48%)	11 (52%)	19 (66%)	12 (60%)	0.57
Etiology, n (%)					0.23
HAV	2 (7%)	1 (5%)	2 (7%)	2 (10%)	
HBV	13 (45%)	6 (29%)	8 (28%)	5 (25%)	
HCV	2 (7%)	0 (0%)	0 (0%)	0 (0%)	
AIH	3 (10%)	4 (19%)	1 (3%)	1 (5%)	
Drug-induced	4 (14%)	2 (10%)	2 (7%)	6 (30%)	
Ischemic hepatitis	0 (0%)	0 (0%)	2 (7%)	0 (0%)	
Epstein-Barr virus	0 (0%)	0 (0%)	1 (3%)	0 (0%)	
Acute fatty liver of pregnancy					
	0 (0%)	0 (0%)	1 (3%)	0 (0%)	
Indeterminate	5 (17%)	8 (37%)	12 (42%)	6 (30%)	
Period from initial symptoms to the diagnosis of ALF, day					
	16 (2-49)	12 (2-38)	10 (2-347)	6 (1-42)	0.30
Hepatic coma, n (%)					
II	23 (79%)	16 (76%)	19 (66%)	7 (35%)	0.008
III or IV	6 (21%)	5 (24%)	10 (34%)	13 (65%)	
Temperature >38°C or <36 °C, n (%)					
	0 (0%)	6 (29%)	7 (24%)	13 (65%)	<0.0001
Heart rate >90 beats per minute, n (%)					
	0 (0%)	6 (29%)	26 (90%)	19 (95%)	<0.0001
Tachypnea >20 breaths per minute or $PaCO_2$ <32mmHg, n (%)					
	0 (0%)	3 (14%)	17 (59%)	13 (65%)	<0.0001
Laboratory data					
WBC, $\times 10^3/mm^3$	7.8 (4.7-12.0)	7.7 (2.4-18.7)	9.7 (2.8-36.5)	14.6 (2.2-28.0)	0.0007
Hemoglobin, g/dL	12.4 (9.0-19.0)	11.6 (7.3-18.0)	11.2 (5.9-17.4)	13.2 (8.9-16.7)	0.17
Platelet, $\times 10^4/mm^3$	13.5 (3.9-39.3)	14.0 (3.0-26.4)	8.7 (1.4-29.8)	13.5 (2.4-30.5)	0.44
T.Bil, mg/dL	11.7 (3.7-50.5)	13.1 (4.3-32.5)	13.1 (2.8-45.9)	9.6 (2.3-30.0)	0.62
T.Bil/D.Bil ratio	1.5 (1.2-2.3)	1.7 (1.2-3.6)	1.6 (1.1-3.0)	1.6 (1.2-3.2)	0.43
ALT, IU/L	867 (60-6720)	660 (41-4677)	493 (40-10159)	2074 (71-8602)	0.031
Cr, mg/dL	0.7 (0.4-4.8)	0.6 (0.1-5.2)	1.0 (0.2-6.3)	1.4 (0.4-6.4)	0.024
PT activity, %	27 (5-40)	24 (7-37)	24 (5-38)	12 (5-37)	0.024

AIH, autoimmune hepatitis; ALF, acute liver failure; ALT, alanine aminotransferase; Cr, creatinine; D.Bil, direct birilubin; HAV, hepatitis A virus; HBV, hepatitis B virus; HCV, hepatitis C virus; PT, prothrombin; SIRS, systemic inflammatory response syndrome; T.Bil, total bilirubin; WBC, white blood cell.

Table 2. Treatment

	SIRS score				p value
	0	1	2	3 or 4	
Plasma exchange	21 (72%)	18 (86%)	21 (72%)	18 (90%)	0.32
Hemodiafiltration	12 (41%)	6 (29%)	10 (34%)	11 (55%)	0.33

21 patients (22%), 29 patients (29%), and 20 patients (20%), respectively.

Table 1 shows the clinical characteristics and laboratory data at the diagnosis of ALF with hepatic coma according to SIRS score. With the increase of SIRS score, hepatic coma grade, prothrombin activity, and serum creatinine level were deteriorated. However, SIRS score was not associated with patient's age, gender and etiology of ALF.

All patients underwent culture (blood, tracheal aspirate, and urine) and computed tomography (head, chest, and abdomen) at the diagnosis of ALF with hepatic coma; however, none clinically developed bacterial or fungal infection.

Treatment

As artificial liver support, 78 patients (79%) received plasma exchange, and 39 patients (39%) underwent hemodiafiltration. In 38 patients (38%), plasma exchange combined with hemodiafiltration was performed. There was no association between SIRS score and treatment procedure (Table 2).

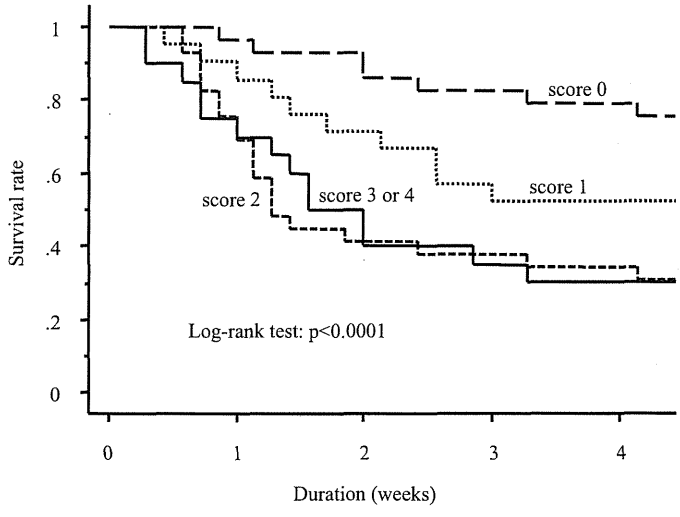
Complication after the diagnosis of ALF with hepatic coma

After the diagnosis of ALF with hepatic coma, 27 patients (27%) developed brain edema, 25 patients (25%) acute respiratory distress syndrome (ARDS), 31 patients (31%) disseminated intravascular coagulation (DIC), and 21 patients (22%) ARF. Thirty-eight patients (38%) developed MOF. With the increase of SIRS score at the diagnosis of ALF with hepatic coma, the frequencies of the development of

Table 3. Complications

	SIRS score				p value
	0	1	2	3 or 4	
Brain edema	4 (14%)	6 (29%)	9 (31%)	8 (40%)	0.21
ARDS	2 (7%)	4 (19%)	11 (38%)	8 (40%)	0.016
DIC	4 (14%)	5 (24%)	12 (41%)	10 (50%)	0.026
ARF	3 (10%)	2 (10%)	9 (31%)	7 (35%)	0.052
MOF	5 (17%)	5 (24%)	15 (52%)	13 (65%)	0.001

ARDS, acute respiratory distress syndrome; ARF, acute renal failure;
DIC, disseminated intravascular coagulation;
SIRS, systemic inflammatory response syndrome.



Patients at risk, n					
score 0	29	28	25	24	23
score 1	21	18	15	11	11
score 2	29	20	12	11	10
score 3 or 4	20	14	8	7	6

Figure 1. Kaplan-Meier curves depicting the survival rate according to the number of SIRS components fulfilled (SIRS score) at the time of the diagnosis of acute liver failure with hepatic coma.

ARDS, DIC and MOF increased (Table 3). ARF was more frequently developed in the patients with a SIRS score of 2 or higher at the diagnosis of ALF with hepatic coma than in the patients with SIRS score of 1 or 0 (33% vs. 10%, p= 0.007).

Outcome

Of the 99 patients, 36 (36%) survived without LT, and the other 63 died. Overall survival rate according to SIRS score was 66% in patients with a score of 0, 43% in those with a score of 1, 17% in those with a score of 2, and 15% in those with a score of 3 or 4 (Fig. 1). The overall survival rate was significantly lower in patients with a score of 2 than in those with a score of 0 or 1 (p<0.0001 and = 0.037, respectively). There was no difference in overall survival rate between patients with a score of 2 and those with a score of 3 or 4 (p=0.93). A difference in overall survival rate between patients with a score of 0 and those with a score of 1 was borderline (p=0.07).

Regarding short-term prognosis, there was no difference in the 2-week survival rate between patients with a score of 0 and those with a score of 1. Patients with a score of 2 showed a lower 2-week survival rate than those with a score of 1 (p=0.036). Two-week survival rate of patients with a score of 3 or 4 was similar to that of patients with a score of 2. On the other hand, the 4-week survival rate was lower in patients with a score of 1, 2, and 3 or 4, than in those with a score of 0 (p=0.039, 0.0002 and 0.0001, respectively) (Fig. 2).

Prediction of patient's death

Among the 99 patients, according to the King's College Criteria (13), the sensitivity, specificity, and positive and negative predictive values were 70%, 64%, 77%, and 55%, respectively. On the other hand, when the prognoses of patients with a SIRS score of 2 or more was predicted as death, the sensitivity, specificity, and positive and negative predictive values were 63%, 78%, 83%, and 55%, respectively.

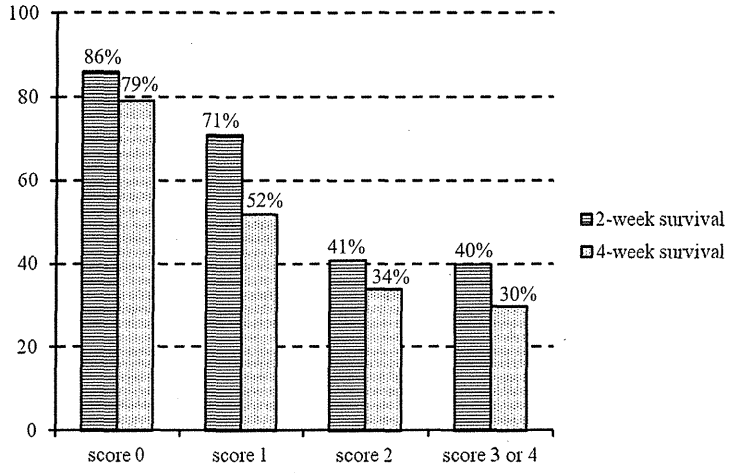


Figure 2. Two- and four-week survival rates according to the number of SIRS components fulfilled (SIRS score) at the time of the diagnosis of acute liver failure with hepatic coma.

Discussion

Complications such as ARDS, DIC, ARF and MOF reduce the opportunity for LT and the survival rates after emergency LT in ALF patients (14). In order to rescue more ALF patients, LT before the development of these complications is important, and the prognostic factor to predict the development of these complications is needed. This study suggests that, in patients with non-acetaminophen-related ALF, a higher SIRS score seems to lead to the increased probabilities of ARDS, DIC, ARF and MOF, and consequently increased mortality rates. This tendency was shown not only in patients with fulminant hepatitis B due to transient hepatitis B virus infection but also in patients with disease due to hepatitis B virus carrier (data not shown). Furthermore, predicting the prognosis of non-acetaminophen-related ALF patients according to SIRS score seems to show a better specificity and positive predictive value than when predicted according to the King's College Criteria (13). Thus, the SIRS score may be useful for predicting not only the overall survival but also the development of these complications. In particular, patients with a SIRS score of 2 or higher (in a state of SIRS) may need urgent LT.

SIRS is diagnosed by the presence of two or more of the four components (3). However, in the Japanese criteria for severity assessment of acute pancreatitis, the presence of three or more of the four components is accepted as the prognostic factor (15). It has not been investigated whether a cut-off of 2 points is appropriate for the prognostic factor for ALF patients with hepatic coma. In this study, there were differences in the overall survival rate and the frequencies of the development of complications between patients with a SIRS score of one or zero and those with a SIRS score of 2. On the other hand, there were no differences in these points between patients with a SIRS score of 2 and those with a SIRS score of 3 or 4. Thus, we consider that a cut-off of 2 points is appropriate for the prognostic factor for ALF patients with hepatic coma.

Hepatic coma grade and prothrombin activity have been accepted as major prognostic factors for ALF (16). In the present study, hepatic coma grade at the diagnosis of ALF with hepatic coma was deteriorated with the increase of SIRS score. This is similar to the reports from the King's College Group (4) and the U.S. Acute Liver Failure Study Group (5). On the other hand, this study indicated that prothrombin activity at the diagnosis of ALF with hepatic coma was reversely correlated with SIRS score. Furthermore, patients with a score of 3 or 4 showed higher serum levels of alanine aminotransferase than the others. Thus, SIRS score is speculated to reflect not only the whole body state but also the grade of liver failure. In non-acetaminophen-related ALF patients with hepatic coma in a state of SIRS, caution against the rapid progression of liver failure may be necessary.

In this study, the overall survival rate of non-acetaminophen-related ALF patients with hepatic coma was 36%. However, the 2-week and overall survival rates of 29 patients with SIRS score of 0 was 86% and 66%, respectively. Considering that the 5-year graft survival rate and patient's survival rate of ALF after receiving LT has been reported to be 61% and 70%, respectively (17), the intensive care including artificial liver support in expectation of hepatic regeneration during a few weeks from the diagnosis of ALF with hepatic coma may be permissible for those with SIRS score of 0.

In a setting of the shortage of liver grafts, artificial liver support technologies have been developed remarkably. Recently, plasma exchange combined with hemodiafiltration has been reported to be effective for recovery from hepatic coma and preventing brain edema and to be a reliable bridging procedure to LT (18). Furthermore, a recent meta-analysis has suggested that artificial liver support improves the survival of ALF patients (19). In Japan, as artificial liver support, plasma exchange and hemodiafiltration have been performed in 92% and 76%, respectively, of ALF patients

with hepatic coma (20). In this study, many patients received plasma exchange and/or hemodiafiltration; however these procedures did not reduce the development of complications. A prospective study with a larger number of patients is required to evaluate the role of artificial liver support in the treatment of ALF patients with hepatic coma.

In conclusion, this study suggests that the SIRS score may be useful for predicting not only the overall survival but also the development of these complications such as ARDS, DIC, ARF and MOF in patients with non-acetaminophen-related ALF with hepatic coma. Furthermore, SIRS score may reflect the grade of liver failure (hepatic coma grade, prothrombin activity, etc.). Patients with a SIRS score of 2 or higher (in a state of SIRS) will need urgent LT. Thus, we consider that SIRS score may be important for determining the treatment strategy including the timing for LT in non-acetaminophen-related ALF with hepatic coma. A further prospective validation study will be necessary.

The authors state that they have no Conflict of Interest (COI).

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