Necrotic cell death and suppression of T-cell immunity characterized acute liver failure due to drug-induced liver injury

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Abstract

Background & Aims: The aim of this study was to investigate the clinical characteristics and pathophysiology of drug-induced liver injury (DILI) - acute liver failure (ALF). Methods: The patients with acute liver injury (ALI) including ALF from 2009 to 2014 were analyzed. The hepatic encephalopathy (HE) development rate was compared with the findings from a national survey in Japan. The serum cytokines levels and the findings of a liver function test were evaluated in the DILI patients. Results: The HE development rate substantially decreased for autoimmune hepatitis (AIH) - and undetermined cause-induced ALI owing to the early prediction system, but not in DILI-ALI. Among the DILI-ALF and AIH-ALF cases, the CK-18 fragment (1480.1 U/L, 3945.4 U/L), IL-8 (82.9 pg/ml, 207.5 pg/ml), IP-10 (1379.6 pg/ml, 3731.2 pg/ml) and MIP-1β (1017.7 pg/ml, 2273.3 pg/ml) levels were lower in the DILI-ALF cases. Among the DILI-ALI and DILI-ALF cases, IL-4 (19.8 pg/ml, 25.4 pg/ml) and RANTES (14028.0 pg/ml, 17804.7 pg/ml) were higher in DILI-ALI, and HMGB-1 (397.1 pg/μl, 326.2 pg/μl) and HGF (2.41 ng/ml, 0.55 ng/ml) were higher in DILI-ALF. We observed that HGF independently associated with DLI-ALF development. Conclusions: Despite the low grade apoptosis and inflammation, DILI patients progressed to ALF comparable with that of the AIH patients.

Abbreviations:, acute liver failure (ALF), acute liver injury (ALI), autoimmune hepatitis (AIH), AIH-induced ALF (AIH-ALF), AIH-induced ALI (AIH-ALI), drug-induced liver injury (DILI), DILI-induced ALF (DILI-ALF), DILI-induced ALI (DILI-ALI), hepatitis A virus (HAV), hepatitis C virus (HCV), hepatic encephalopathy (HE), hepatitis E virus (HEV), model for end-stage liver disease (MELD), prothrombin time-international normalized ratio (PT-INR)

Key words: ALF, acute liver failure, DILI, drug-induced liver injury, HGF

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Introduction:

Most patients with acute liver injury (ALI) can recover without intensive care [1]. However, a few patients with ALI can progress to acute liver failure (ALF) [2, 3]. Acute liver failure patients can be divided into two groups: those with and without hepatic encephalopathy (HE) [4]. Hepatic encephalopathy is the result of severe hepatocyte dysfunction, which morphologically presents as progressive liver atrophy [2]. The mortality rate of ALF with HE is approximately 70% even if the patients receive intensive care other than liver transplantation [2].

Drug-induced liver injury (DILI) can lead to jaundice, ALF or death [5] and it is the most frequent adverse drug reaction that leads to withdrawal of approved medications from markets [6]. Despite its negative effects on patient care and the pharmaceutical industry, DILI has not yet been eliminated. The low incidence of DILI and the variety of drug reactions among individuals thus makes it difficult to predict the occurrence of DILI [5]. It is important to note that DILI is one of the most frequent causes of ALF in Japan and also worldwide [1, 2]. Thus, the pathophysiology of DILI needs to be clarified, with the ultimate aim of preventing DILI-induced ALF (DILI-ALF).

The development of HE in the ALF patients leads to a critical illness because

the hepatocytes of the patients with ALF and HE have already been badly damaged and are resistant to available therapies. To prevent HE development in ALF, a method to accurately predict HE development needed to be established. Therefore, we have previously investigated a potential early prediction system for the short-term development of HE before the occurrence of progressive liver atrophy induced by the destruction of hepatocytes [7]. The Japan HE prediction model (JHEPM) scores were calculated for each patient based on PT **(Prothrombin time)**, serum total bilirubin, age and the etiology of liver failure on admission [7]. The detailed formula that was used is described in the “Materials and methods” section. Although the clinical utility of the JHEPM score for the patients with liver failure has been reported[7, 8], the clinical significance of the JHEPM for each etiology of ALF has never been elucidated.

The first aim of the present study was to investigate the clinical characteristics of these ALF patients among the variously encountered etiologies according to the response to early intervention by means of an early predictive system for HE development: JHEPM (Figure 1). The results of this evaluation led us to our second aim. The development of HE in DILI-ALF was not prevented by an early intervention initiated based on the results of the prediction system. To investigate the pathophysiology of DILI, we analyzed several cytokines, cell death markers or

laboratory data in two datasets: DILI-ALF vs. AIH-ALF and DILI-ALI vs. DILI-ALF. for the DILI-ALI cases.

Materials and methods:

Subjects:

Study 1. From January 2004 to May 2014, 314 patients were followed for ALI using our early prediction system. The criteria for enrollment in our prediction system have been previously reported [7]. Briefly, patients with mild coagulopathy (less than 80% of prothrombin activity or a prothrombin time-international normalized ratio (PT-INR) value more than 1.2) and elevation of transaminase were registered. The patients were transferred to our hospital when the JHEPM score (predicted the probability for HE development) was more than 20%. As the coagulopathy progressed (less than 60% of prothrombin activity or a PT-INR value more than 1.3), the patients were administered a steroid treatment, which included steroid pulse therapy and 0.5-1.0 mg/kg of prednisolone, according to the diagnosis of the patients. The patients with pre-existing symptomatic chronic liver disease and those with alcoholic liver injury alone were excluded. The etiology of ALI was classified as follows: hepatitis A virus (HAV) infection, acute HBV infection, HBV flare-up, *de novo* HBV-related hepatitis, acute hepatitis C virus (HCV) infection, hepatitis E virus (HEV) infection, DILI, autoimmune hepatitis (AIH) and undetermined or shock/sepsis. The usual criteria for serologic diagnosis of acute viral hepatitis, types A, B, C and E, were used as previously

reported [9, 10]. The hepatic encephalopathy (HE) development rate was compared with the results from a national survey in Japan conducted between 1997 and 2003 [11]. The DILI was diagnosed using the Roussel Uclaf Causality Assessment Method, with a score of 6 or higher indicating DILI.

Study 2-A and 2–B. To understand the pathophysiology of DILI, we conducted two comparisons: one comparison to determine the characteristics of patients with DILI-ALF among DILI-ALF and AIH-induced ALF (AIH-ALF) and another to investigate the factors associated with the severity of the liver injury among DILI-ALI and DILI-ALF (Figure 2). As the rate of the HE development in AIH-ALF was drastically decreased compared with that of ALF induced by other causes (Figure 1), AIH-ALF was used for the comparisons with DILI-ALF. There were 11 cases of DILI-ALF and 10 cases of AIH-ALF in the first comparison (study 2-A), and 11 DILI-ALF cases and 29 cases of DILI-ALI in the second comparison (study 2-B).

All protocols reported in this paper were approved by the Institutional Review Board of Iwate Medical University (approval number: H20-36), and informed consent was obtained from all participants.

Laboratory data: The plasma PT-INR value and serum levels of alpha fetoprotein (AFP), alanine transaminase (ALT), aspartate transaminase (AST) albumin,

creatinine, gamma glutamate transaminase, hepatocyte growth factor (HGF), immunoglobulin G, lactate dehydrogenase, total bilirubin and total protein were analyzed using an autoanalyzer (JCA-BM2250, JEOL, Tokyo, Japan).

Measurement of cytokines, CK-18 fragment and high-mobility group 1: The concentration of interleukin (IL)-1β, IL-1ra, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12, IL-13, IL-17, **basic fibroblast growth factor (FGF), eotaxin, granulocyte macrophage colony-stimulating factor (G-CSF), interferon gamma (IFN-γ), interferon inducible protein 10 (IP-10), monocyte chemotactic protein 1 (MCP-1), macrophage inflammatory protein (MIP)-1α, MIP-1β, platelet-derived growth factor (PDGF)-BB, regulated on activation, normal T cell expressed and secreted (RANTES), tumor necrosis factor (TNF)-α and vascular endothelial growth factor (VEGF)** in the serum was measured using the Bio-Plex suspension array system (BioRad Laboratory Inc.) and the Bio-Plex Pro human cytokine 27-plex assay (BioRad Laboratory Inc.) as described in a previous report[12].

The serum cytokeratin (CK)-18 fragment was measured using the M30-Apoptosense ELISA kit (Peviva; Alexis, Grunwald, Germany) and serum high-mobility group 1 (HMGB-1) was measured using the HMGB-1 ELISA kit II (Shino-test, Sagamihara, Japan) according to the manufacturers' instructions.

Evaluation of prognostic models for ALF: The model for end-stage liver disease (MELD) and JHEPM scores were calculated for each patient based on the results of a hematological examination and the reported etiology of the liver failure on admission. The detailed formulas that were used are as follows:

MELD [13]= 9.57 loge [Cre (mg/dL)] + 3.78 loge [Tbil (mg/dL)] + 11.20 loge [PT-INR] + 6.43,

JHEPM [7]; λ = [0.692 loge (1 + Tbil (mg/dL)] - 0.065 PT(%) + [1.388 Age (years)] + [0.868 Etiology] - 1.156; where Age is 1 in patients older than 50 years and Etiology is 1 when the cause is DILI, flare-up of type B hepatitis, AIH or unknown, and 0 for the other causes. The JHEPM value is calculated as $p = 100/ (1 + e^{-\lambda})$.

Statistical analysis: The results are expressed as the mean and standard deviation. All statistical analyses were performed using the SPSS 17.0 software program (SPSS Inc., Chicago, IL, United States). The statistical differences were determined using the two-sided Mann-Whitney U test. A two-sided p value of <0.05 was considered to be statistically significant. A test of parallel lines was used to confirm the equality of the slopes in regression lines according to a linear regression analysis. A logistic regression analysis was used for the multivariate analysis. The predictive

performance of the JHEPM for ALF was assessed in patients with DILI-ALF / ALI using the receiver–operator curves (ROC) method. The cut-off value for the early prediction of DILI-ALF was estimated using the area under the ROC (AUROC) method.

Results:

Study 1: The HE development rate due to DILI-ALF did not improve in the present population compared with a previously performed national study. The incidence of HE based on the etiology of the present study was compared with that observed in a national study conducted from January 1997 to December 2003 [11]. The characteristics of the patients were summarized in Table 1. **Laboratory data of the hepatitis patients were summarized in Table 2 for comparison to the data of the national study [7]. According to the comparison of the laboratory data between the national survey and our data, two data sets revealed similar findings (Table2)[7].** The rate of HE significantly decreased after the introduction of the early prediction system, except for DILI (Figure 1). These data indicated that the HE development in the DILI-ALF patients was not prevented by the treatment initiated based on our early prediction system. Accordingly, HE would develop in patients with severe DILI-ALF. In contrast, the HE development was drastically decreased in AIH-induced ALI (AIH-ALI). Although the reason for these results remains unclear, the HE development in the AIH-ALI might be prevented by the treatment initiated based on the early prediction system.

Study 2-A: Proinflammatory cytokines and the CK-18 fragment in sera were

significantly lower in DILI-ALF than in AIH-ALF. To prevent the HE development in the DILI-ALF patients, the pathophysiology of DILI-ALF needs to be clarified. Therefore, we compared the laboratory data, several cytokines and cell death markers among DILI-ALF and AIH-induced ALF (AIH-ALF) patients because the HE development in AIH-ALF was decreased by an early intervention based on the JHEPM (Figure 1 and Table 3). According to the results from the measurement of cytokines and cell death markers, IL-8 (82.9 pg/ml, 207.5 pg/ml), IP-10 (1379.6 pg/ml, 3731.2 pg/ml), MIP-1β (1017.7 pg/ml, 2273.3 pg/ml) and CK18 fragment (1480.1 U/L, 3945.4 U/L) were significantly lower in DILI-ALF than in AIH-ALF (Figure 3). There were no differences in laboratory data, such as the results for AST, ALT or PT-INR, between the two groups.

Study 2-B: Cytokines associated with T-cell immunity and the necrosis marker were related to the severity of DILI. To determine the factors associated with DILI disease severity, we compared the laboratory data, **causal drugs,** several cytokines and cell death markers between the DILI-ALF and DILI-ALI patients **(supplemental table** and Table 4). **There was no tendency of causal drugs among both groups.** The level of PT-INR, Alb, T-Bil, AFP and the MELD score were higher in DILI-ALF than in DILI-ALI, which indicated the disease severity of DILI-ALF. The values for IL-4 (19.8

pg/ml, 25.4 pg/ml) and RANTES (14028.0 pg/ml, 17804.7 pg/ml) were significantly lower in DILI-ALF than in DILI-ALI. In contrast, the values for HGF (2.41 ng/ml, 0.55 ng/ml) and HMGB-1 (397.1 pg/μl, 326.2 pg/μl) were higher in DILI-ALF than in DILI-ALI (Figure 4).

HGF was found to be independently associated with the development of DILI-ALF, and it is thus considered to be a predictive marker of severe DILI. To determine the critical factors for DILI-ALF development, the factors and the patients' age were analyzed using a multivariate logistic regression analysis. We observed that HGF independently associated with DLI-ALF development (Table 5). We then evaluated the cutoff value of HGF for DILI-ALF development. The HGF value was evaluated using a ROC analysis to detect DILI-ALF development. The area under the curve of the ROC was 0.949 and the cutoff HGF value by Youden`s index was 0.69 ng/mL (Figure 5). Sensitivity and specificity of the cutoff value were 1.000 and 0.814, respectively.

Discussion:

DILI is a growing problem in health care because it is one of the most common reasons drugs are withdrawn from the market [6]. Furthermore, DILI can cause ALF and readministration of a drug that causes DILI has resulted in death in 7% of cases [2, 14]. As the rate of incidence is too low to be due to a single nucleotide polymorphism, the pathophysiology of DILI is believed to be multifactorial, including factors such as allergy, immunity or metabolism [5, 15]. Therefore, accurately predicting whether patients have DILI is difficult, and effective treatments for DILI are needed. The current treatment for most DILI cases involves withdrawing the causal drug[16]. However, patients with DILI require intensive care when DILI progresses to ALF.

To determine the clinical importance of DILI, we confirmed the rate of HE development in DILI-ALI, including DILI-ALF. Importantly, the early treatment system based on the HE prediction by the JHEPM did not decrease the rate of HE development in the patients with DIL-ALI, although the system remarkably decreased the rate of HE development in ALI induced by other etiologies (Figure 1). **We recognized limitation in comparison between the present data and the Japan national survey because detail information, such as treatment, was absent. However, the data of the Japan national survey was collected from major medical center for ALF treatment and**

there was problem according to shortage of liver donor in Japan. Treatment strategy to the ALF would be similar in both data sets. Although there was limitation we mentioned above, we considered that these data demonstrated that early intervention based on the JHEPM results did not prevent HE development in DILI-ALF.

According to these results, we hypothesized that liver injury in DILI was distinct from the liver injury induced by other conditions, and that the pathophysiology of DILI-ALI was associated with resistance to therapies. To confirm the hypothesis, we compared DILI-ALF with AIH-ALF because AIH drastically decreased the rate of HE development (Figure 1). **Comparing of several chemokines between these patients, IL-8, IP-10 and MIP-1b were significantly lower in DILI-ALF than in AIH-ALF. IL-8 played chemoattractant which inducted neutrophilia infiltration. Histological finding of the liver with biliary atresia revealed neutrophilia infiltration, and IL-8 level of the patients with biliary atresia was significantly higher than normal control. IP-10 has been shown to worsen hepatic inflammatory conditions in patients with several liver diseases such as viral hepatitis and autoimmune liver diseases, as well as Con A-induced liver injury. MIP-1β is expressed by the portal vessel endothelium and recruit macrophages into the liver. According to these**

previous studies, low levels of these cytokines in DILI-ALF patients might not lead these pro-inflammatory cytokines-induced inflammation in the liver. Interestingly, the cell death marker; the CK-18 fragment, was significantly lower in DILI-ALF than in AIH-ALF although there was no significant difference of disease severity between DILI-ALF and AIH-ALF. **These findings suggested that ALF might be occurred with both less hepatocyte death and less inflammation response in DILI-ALF compared with AIH-ALF.** Taken together, the functional failure of the hepatocytes in DILI-ALF may precede cell death. Because the serum AFP level correlated with prevalence of the liver stem/progenitor cells (LPCs), we also focused on the difference in the serum AFP level among both groups. There was no significant difference among DILI-ALF and AIH-ALF although the AFP value exceeded the normal limits. These findings suggested that LPCs might therefore be induced in both groups.

We then determined the factors associated with disease progression by comparing DILI-ALF with DILI-ALI because **gender difference and** several cytokines might affect DILI pathogenesis [12, 17]. **Four of the 10 DILI-ALF patients were female. Although female gender was less than male in DILI-ALF, number of the patients with HE was much in female gender compared with male. We suspected**

female gender was worsen factor in DILI although number of subjects in our study was quite small. The levels of cytokines associated with the Th2-cell, IL-4 and RANTES, were higher in DILI-ALI than in DILI-ALF (Figure 4). The level of the necrosis marker, HMGB-1, was lower in DILI-ALI than in DILI-ALF (Figure 4). These data indicated that the suppression of Th2-cell immunity and accumulation of necrosis occurred during the progression of the disease. According to the comparison between DILI-ALF and AIH-ALF and between DILI-ALI and DILI-ALF, DILI-ALF was characterized by a lower immune response, less apoptosis, more aggressive necrosis and hepatocyte dysfunction. These data may indicate the reason why obtaining immune suppression using steroids was not always clinically effective in DILI. Indeed, a previous study reported that a steroid therapy for ALF did not improve the survival rate in the patients with DILI-ALF [18].

Several markers were compared between DILI-ALF and DILI-ALI, and HGF was identified as a predictive parameter for the development of DILI-ALF. The HGF was reported as a mitogenic protein for hepatocytes [19], and enhanced hepatocyte proliferation in an *in vivo* model of acute liver injury [20]. However, HGF suppressed cell proliferation at high doses in several cell types [21-23]. Furthermore, high level of HGF was approved as a marker of poor prognosis in patients with ALF although the

detail mechanism of HGF in ALF had never elucidated. Based on the results from previous studies and the clinical findings of the present study, we determined that a high level of HGF in sera might not affect the acceleration of hepatocyte proliferation. Although a lower HGF level demonstrated high biological activity for hepatocyte proliferation [24, 25], HGF in DILI-ALF was increased. The microenvironment of DILI-ALF would be inadequate for lower HGF levels to efficiently function as a factor for hepatocyte proliferation. These data indicated that the impairment of the HGF effect was due to some inhibitory factors or subclinical liver damage. To prevent the progression of ALI to ALF in the patients with DILI, an adequate microenvironment that can enable the recovery of liver function should be identified.

We investigated whether serum proteins were candidates for detecting the biological dysfunction of hepatocytes. We identified HMGB-1 as a candidate because it was significantly more abundant in DILI-ALF than in DILI-ALI (Figure 4). However, serum HMGB-1 levels did not correlate with HE development, severity of the disease or prognosis (data not shown). A previous study reported that levels of HMGB-1 in culture medium did not increase until the onset of secondary necrosis in a human hepatoma cell line [26]. Thus, the level of HMGB-1 in sera did not reflect intracellular conditions, such as mitochondrial dysfunction.

In conclusion, the present study indicates that DILI-ALF develops in an independent manner of inflammation, and thereafter it tends to be steroid resistant. Therefore, it is urgently needed to develop new types of hepatocyte-protective therapy, which can restore microenvironment to an adequate condition for HGF.

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Table 1. Characteristics of the patients with ALI in the cohort study during 2004-2014

Etiology	n	Patients with the HE development (%)
Hepatitis		
HAV	9	0 (0%)
Acute HBV infection	21	2 (9.5%)
Flare of HBV carrier	29	3 (10.3%)
HBV de novo	4	2 (50%)
HEV	11	1 (9.1%)
Non-hepatitis virus	11	0 (0%)
Drug reaction	40	4 (10%)
AIH	36	1 (2.8%)
Hepatitis of unknown etiology	98	7 (7.1%)
Non-hepatitis		
Shock/Sepsis	42	
Malignant infiltration	6	
Others	7	

Autoimmune hepatitis, AIH; hepatitis A virus infection, HAV; hepatitis B virus infection, HBV; hepatic encephalopathy, HE; hepatitis E virus infection, HEV.

Table 2. Laboratory data of the hepatitis patients in the cohort study during 2004-2014

	HE						
	Non-developed (n=239)			Developed (n=20)			
Age	48	±	18	60	±	13	p<0.05
Platelet (×10^3/μL)	135	±	80	131	±	39	n.s.
TBil (mg/dL)	5.9	±	5.8	10.5	±	7.3	p<0.05
Cholineesterase (IU/L)	257	±	169	162	±	77	p<0.05
TC (mg/dL)	160	±	55	110	±	48	p<0.05
TP (g/dL)	6.95	±	0.84	6.05	±	0.79	p<0.05
Alb (g/dL)	3.75	±	0.59	3.28	±	0.87	n.s.
NH3 (μg/dL)	46.2	±	24.5	107	±	112.8	p<0.05
Prothrombin time (%)	73	±	22	32	±	21	p<0.05
PT-INR	1.29	±	0.43	3.46	±	1.63	p<0.05

Albumin, Alb; total bilirubin, TBil; total cholesterol, TC; total protein, TP; prothrombin time-international normalized ratio, PT-INR

Table 3. The patients' characteristics, laboratory data and cytokines were compared between DILI-ALF and AIH-ALF.

	normal range		DILI (11)			AIH (10)			
Age		y.o.	58.5	±	18.5	63.8	±	11.3	n.s.
JHEPM			24.9	±	21.6	34.9	±	27.7	n.s.
MELD			21.9	±	4.7	21.0	±	6.7	n.s.
IAIH						14.1	±	0.9	
RUCAM			8.6	±	0.2				
Hepa : Mix : Chole			2 : 4 : 5						
PT-INR			1.9	±	0.3	1.9	±	0.4	n.s.
TP	6.5-8.2	g/dL	6.1	±	0.8	6.7	±	0.5	n.s.
Alb	4.3-5.4	g/dL	3.0	±	0.5	3.2	±	0.5	n.s.
CRNN	0.29-1.14	mg/dL	0.6	±	0.1	0.8	±	0.3	n.s.
TBil	0.2-1.2	mg/dL	12.5	±	9.3	14.5	±	7.6	n.s.
AST	10-32	IU/L	793.4	±	776.5	1299.0	±	774.4	n.s.
ALT	7-27	IU/L	1113.5	±	932.6	1385.5	±	864.4	n.s.
LDH	118-257	IU/L	483.1	±	327.4	516.2	±	222.8	n.s.
γGTP	5-55	IU/L	426.1	±	332.7	251.1	±	164.9	n.s.
IgG	793-1846	mg/dL	1533.7	±	495.6	2012.6	±	778.6	n.s.
AFP	0.0-7.0	ng/mL	281.8	±	574.5	157.4	±	422.9	n.s.
HGF	-0.4	ng/mL	2.4	±	2.2	1.8	±	1.2	n.s.
HMGB1		ng/mL	397.1	±	84.4	374.6	±	150.2	n.s.
CK-18 fragment	126-190	U/L	1480.1	±	879.6	3945.4	±	1400.9	$p<0.05$
PDGFbb	12794-31441	pg/mL	1214.2	±	723.2	1567.1	±	678.6	n.s.
IL1β	0-6	pg/mL	11.8	±	6.5	10.4	±	2.4	n.s.
IL1ra	136-323	pg/mL	21.5	±	24.7	18.7	±	8.0	n.s.
IL2	0-3	pg/mL	22.5	±	18.6	14.3	±	10.8	n.s.
IL4	13-51	pg/mL	19.8	±	5.8	23.5	±	10.8	n.s.
IL5	0-6	pg/mL	7.0	±	9.5	4.9	±	1.6	n.s.
IL6	8-20	pg/mL	57.9	±	65.5	38.1	±	17.2	n.s.
IL7	14-52	pg/mL	14.5	±	10.3	13.9	±	5.5	n.s.
IL8	15-48	pg/mL	82.9	±	33.8	207.5	±	144.8	$P<0.05$
IL9	34-86	pg/mL	73.8	±	34.1	110.2	±	132.8	n.s.

IL10	0-2	pg/mL	34.1	±	27.9	57.2	±	25.3	n.s.
IL12	13-55	pg/mL	49.7	±	48.6	60.7	±	25.7	n.s.
IL13	0-17	pg/mL	15.8	±	11.3	13.7	±	8.0	n.s.
IL17	91-228	pg/mL	21.0	±	23.1	17.8	±	7.9	n.s.
Eotaxin	105-342	pg/mL	42.8	±	35.7	45.8	±	14.8	n.s.
FGFbasic	4-62	pg/mL	21.6	±	26.1	15.8	±	7.0	n.s.
GCSF	19-126	pg/mL	17.2	±	18.5	14.0	±	4.5	n.s.
GMCSF	0-0	pg/mL	87.6	±	91.1	72.3	±	75.5	n.s.
IFNγ	136-822	pg/mL	13.5	±	6.4	12.2	±	4.5	n.s.
IP10	1098-2616	pg/mL	1379.6	±	1262.8	3731.2	±	2094.1	$p<0.05$
MCP1	22-67	pg/mL	189.0	±	266.2	130.1	±	51.7	n.s.
MIP1α	0-15	pg/mL	20.9	±	21.3	15.1	±	6.8	n.s.
MIP1β	112-194	pg/mL	1017.7	±	403.8	2273.3	±	1328.8	n.s.
RANTES	5734-14124	pg/mL	14028.0	±	3491.5	14760.8	±	2000.6	$p<0.05$
TNFα	0-16	pg/mL	14.7	±	11.4	11.9	±	3.9	n.s.
VEGF	94-322	pg/mL	155.6	±	180.2	194.3	±	103.3	n.s.

Alpha fetoprotein, AFP; alanine aminotransferase, ALT; albumin, Alb; aspartate transaminase, AST; cholestasis type, Chole; cytokeratin-18, CK-18; creatinine, CRNN; fibroblast growth factor, FGF; gamma-glutamyl transpeptidase, γGTP; granulocyte-colony stimulating factor, GCSF; granulocyte macrophage colony-stimulating factor, GMCSF; immunoglobulin G, IgG; hepatocellular type, Hepa; hepatocyte growth factor, HGF; high mobility group box 1, HMGB1; international autoimmune hepatitis score, IAIH; Interleukin, IL; interferon gamma, IFNγ; interferon inducible protein 10, IP10; Japan hepatic encephalopathy prediction model, JHEPM; lactate dehydrogenase, LDH; monocyte chemotactic protein 1, MCP1; model for end-stage liver disease, MELD; mixture type, Mix; macrophage inflammatory protein, MIP; platelet-derived growth factor, PDGF; prothrombin time-international normalized ratio, PT-INR; regulated on activation, normal T cell expressed and secreted, RANTES; Roussel Uclaf causality assessment method, RUCAM; total bilirubin, TBil; tumor necrosis factor α, TNFα; total protein, TP; vascular endothelial growth factor, VEGF.

Table 4. The patients' characteristics, laboratory data and cytokines were compared between DILI-ALI and DILI-ALF.

	normal range		ALI (29)			ALF (11)			
Age		y.o.	55.3	±	18.7	58.5	±	18.5	n.s.
Gender	F: M		18: 11			4: 6			
HE onset	F: M		0: 0			2: 1			
JHEPM			2.8	±	6.2	24.9	±	21.6	p<0.05
MELD			12.6	±	7.0	21.9	±	4.7	p<0.05
RUCAM			8.3	±	0.9	8.6	±	0.2	n.s.
Hepa : Mix : Chole			5 : 14 : 10			2 : 4 : 5			
PT-INR			1.1	±	0.2	1.9	±	0.3	p<0.05
TP	6.5-8.2	g/dL	6.7	±	0.6	6.1	±	0.8	p<0.05
Alb	4.3-5.4	g/dL	3.6	±	0.6	3.0	±	0.5	p<0.05
CRNN	0.29-1.14	mg/dL	0.8	±	0.3	0.6	±	0.1	n.s.
TBil	0.2-1.2	mg/dL	4.1	±	5.8	12.5	±	9.3	p<0.05
AST	10-32	IU/L	502.9	±	436.2	793.4	±	776.5	n.s.
ALT	7-27	IU/L	583.9	±	510.1	1113.5	±	932.6	n.s.
LDH	118-257	IU/L	410.7	±	233.1	483.1	±	327.4	n.s.
γGTP	5-55	IU/L	225.6	±	219.2	426.1	±	332.7	n.s.
IgG	793-1846	mg/dL	1353.2	±	492.9	1533.7	±	495.6	n.s.
AFP	0.0-7.0	ng/mL	15.3	±	30.4	281.8	±	574.5	n.s.
HGF	-0.4	ng/mL	0.55	±	0.3	2.41	±	2.2	p<0.05
HMGB1		ng/mL	326.2	±	64.4	397.1	±	84.4	p<0.05
CK-18 fragment	126-190	U/L	1729.0	±	1600.8	1480.1	±	879.6	n.s.
PDGFbb	12794-31441	pg/mL	1532.2	±	657.5	1214.2	±	723.2	n.s.
IL1β	0-6	pg/mL	13.2	±	4.2	11.8	±	6.5	n.s.
IL1ra	136-323	pg/mL	16.2	±	7.0	21.5	±	24.7	n.s.
IL2	0-3	pg/mL	18.1	±	13.6	22.5	±	18.6	n.s.
IL4	13-51	pg/mL	25.4	±	8.2	19.8	±	5.8	p<0.05
IL5	0-6	pg/mL	9.8	±	5.4	7.0	±	9.5	n.s.
IL6	8-20	pg/mL	30.3	±	20.3	57.9	±	65.5	n.s.
IL7	14-52	pg/mL	16.8	±	6.0	14.5	±	10.3	n.s.
IL8	15-48	pg/mL	79.4	±	43.2	82.9	±	33.8	n.s.

IL9	34-86	pg/mL	81.8	±	26.5	73.8	±	34.1	n.s.
IL10	0-2	pg/mL	43.4	±	18.9	34.1	±	27.9	n.s.
IL12	13-55	pg/mL	65.2	±	39.6	49.7	±	48.6	n.s.
IL13	0-17	pg/mL	18.1	±	10.6	15.8	±	11.3	n.s.
IL17	91-228	pg/mL	28.9	±	13.4	21.0	±	23.1	n.s.
Eotaxin	105-342	pg/mL	48.4	±	29.7	42.8	±	35.7	n.s.
FGFbasic	4-62	pg/mL	26.3	±	16.0	21.6	±	26.1	n.s.
GCSF	19-126	pg/mL	20.2	±	7.5	17.2	±	18.5	n.s.
GMCSF	0-0	pg/mL	77.6	±	75.7	87.6	±	91.1	n.s.
IFNγ	136-822	pg/mL	16.1	±	5.9	13.5	±	6.4	n.s.
IP10	1098-2616	pg/mL	2624.7	±	2720.0	1379.6	±	1262.8	n.s.
MCP1	22-67	pg/mL	105.3	±	60.2	189.0	±	266.2	n.s.
MIP1α	0-15	pg/mL	33.2	±	27.4	20.9	±	21.3	n.s.
MIP1β	112-194	pg/mL	1376.6	±	790.0	1017.7	±	403.8	n.s.
RANTES	5734-14124	pg/mL	17084.7	±	2516.5	14028.0	±	3491.5	$p<0.05$
TNFα	0-16	pg/mL	14.3	±	4.2	14.7	±	11.4	n.s.
VEGF	94-322	pg/mL	188.9	±	128.1	155.6	±	180.2	n.s.

Alpha fetoprotein, AFP; alanine aminotransferase, ALT; albumin, Alb; aspartate transaminase, AST; cholestasis type, Chole; cytokeratin-18, CK-18; creatinine, CRNN; fibroblast growth factor, FGF; gamma-glutamyl transpeptidase, γGTP; granulocyte-colony stimulating factor, GCSF; granulocyte macrophage colony-stimulating factor, GMCSF; immunoglobulin G, IgG; hepatocellular type, Hepa; hepatocyte growth factor, HGF; high mobility group box 1, HMGB1; Interleukin, IL; interferon gamma, IFNγ; interferon inducible protein 10, IP10; Japan hepatic encephalopathy prediction model, JHEPM; lactate dehydrogenase, LDH; monocyte chemotactic protein 1, MCP1; model for end-stage liver disease, MELD; mixture type, Mix; macrophage inflammatory protein, MIP; platelet-derived growth factor, PDGF; prothrombin time-international normalized ratio, PT-INR; regulated on activation, normal T cell expressed and secreted, RANTES; Roussel Uclaf causality assessment method, RUCAM; total bilirubin, TBil; tumor necrosis factor α, TNFα; total protein, TP; vascular endothelial growth factor, VEGF.

Table 5. Laboratory data were compared for isolation of factors that correlated with onset of DILI-ALF.

	Univariate	Multivariate	
	p	p	Odds ratio (95% confidence)
Age	n.s.	n.s.	
PT-INR	p<0.01	n.s.	
TP	p=0.029	n.s.	
Alb	p<0.01	n.s.	
TBil	p<0.01	n.s.	
HGF	p<0.01	p=0.004	57.9 (3.7-907.0)
HMGB1	p<0.01	n.s.	
IL4	p=0.025	n.s.	
RANTES	p=0.019	n.s.	

Albumin, Alb; hepatocyte growth factor, HGF; high mobility group box 1, HMGB1; Interleukin, IL; prothrombin time-international normalized ratio, PT-INR; regulated on activation, normal T cell expressed and secreted, RANTES; total bilirubin, TBil.

Figure Legends

Figure 1. Comparison of the hepatic encephalopathy development rate in patients with acute liver injury from a national survey in Japan (1997-2003) and from the current study (2004-2014). The y-axis shows the percentage of hepatic encephalopathy development while the x- axis shows the etiologies of the acute liver injury. The light gray bars correspond to the data from the national survey and the dark gray bars correspond to the data from the current study.

Abbreviations: autoimmune hepatitis, AIH; drug-induced liver injury, DILI; hepatitis A virus infection, HAV; hepatitis B virus infection, HBV.

Figure 2. Overview of eligibility of the subjects in both study 2-A and 2-B.

Figure 3. Cytokines and the cytokeratin-18 fragment were compared among cases of acute liver failure due to drug-induced liver injury or autoimmune hepatitis.

The y-axis shows the serum concentration of the indicated protein while the x-axis shows the etiologies of the acute liver failure.

Abbreviations: autoimmune hepatitis, AIH; cytokeratin-18, CK-18; drug-induced liver injury, DILI; IL, interleukin; interferon-γ inducible protein 10, IP-10; macrophage inflammatory proteins 1 beta, MIP-1β.

Figure 4. Cytokines and several proteins were compared in patients with acute liver injury and acute liver failure due to DILI.

The y-axis shows the serum concentration of the indicated protein while the x-axis shows the type of drug-induced liver injury.

Abbreviations: alpha fetoprotein, AFP; interleukin, IL; hepatocyte growth factor, HGF; high mobility group box 1, HMGB1; regulated on activation, normal T cell expressed and secreted, RANTES.

Figure 5. Hepatocyte growth factor in sera distinguished acute liver failure in the patients with DILI. The area under the ROC (AUROC) value of acute liver failure was 0.949 for the HGF value in sera. The distinction for acute liver failure for sensitivity, specificity, positive predictive value and negative predictive value were 1.000, 0.814, 0.688 and 1.000, respectively, when the cutoff value of HGF was 0.690.

Highlights

- Apoptosis marker and pro-inflammatory cytokines were lower in DILI-ALF compared to AIH-ALF.
- Among the DILI cases, Th2 cytokines were higher in ALI, and necrosis marker was higher in ALF.
- HGF level independently associated with DLI-ALF development.

Supplemental table. Causal agents of DILI

ALI	n	%	ALF	n	%
Antibiotics	7	24.1%	Herbal prdocut	3	27.3%
Herbal product	4	13.8%	diuretic agent	2	18.2%
NSAIDs	4	13.8%	NSAIDS	2	18.2%
antihyperlipidemic agent	3	10.3%	antihyperlipidemic agent	1	9.1%
PPI	3	10.3%	antihypertensive agent	1	9.1%
analgesic agent	2	6.9%	antituberculosis agent	1	9.1%
antihypertensive agent	2	6.9%	hormonal drug	1	9.1%
hormonal drug	2	6.9%			
anticancer agent	1	3.4%			
antituberculosis agent	1	3.4%			

Figure 1

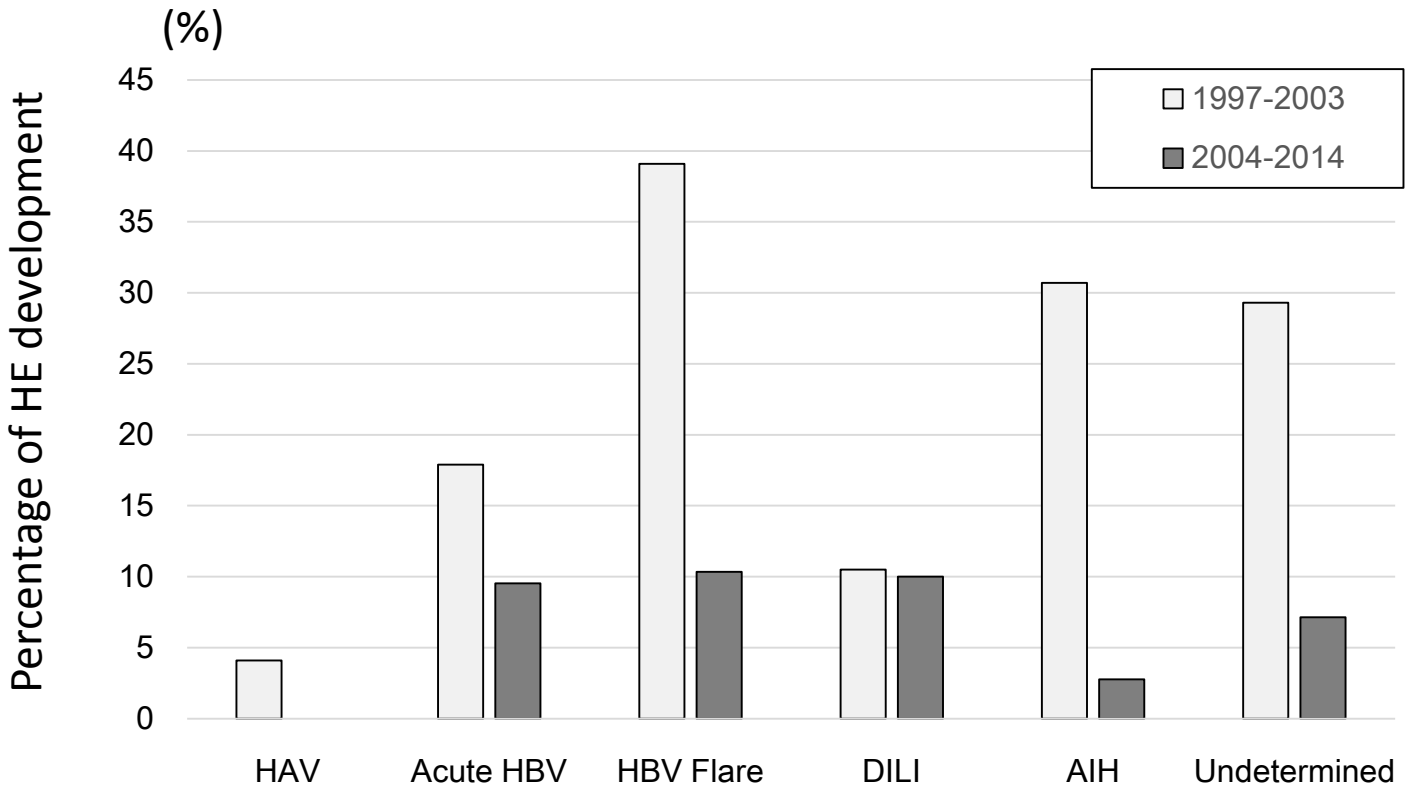

(%)
Percentage of HE development
45
40
35
30
25
20
15
10
5
0
1997-2003
2004-2014
HAV
Acute HBV
HBV Flare
DILI
AIH
Undetermined

Figure 2.

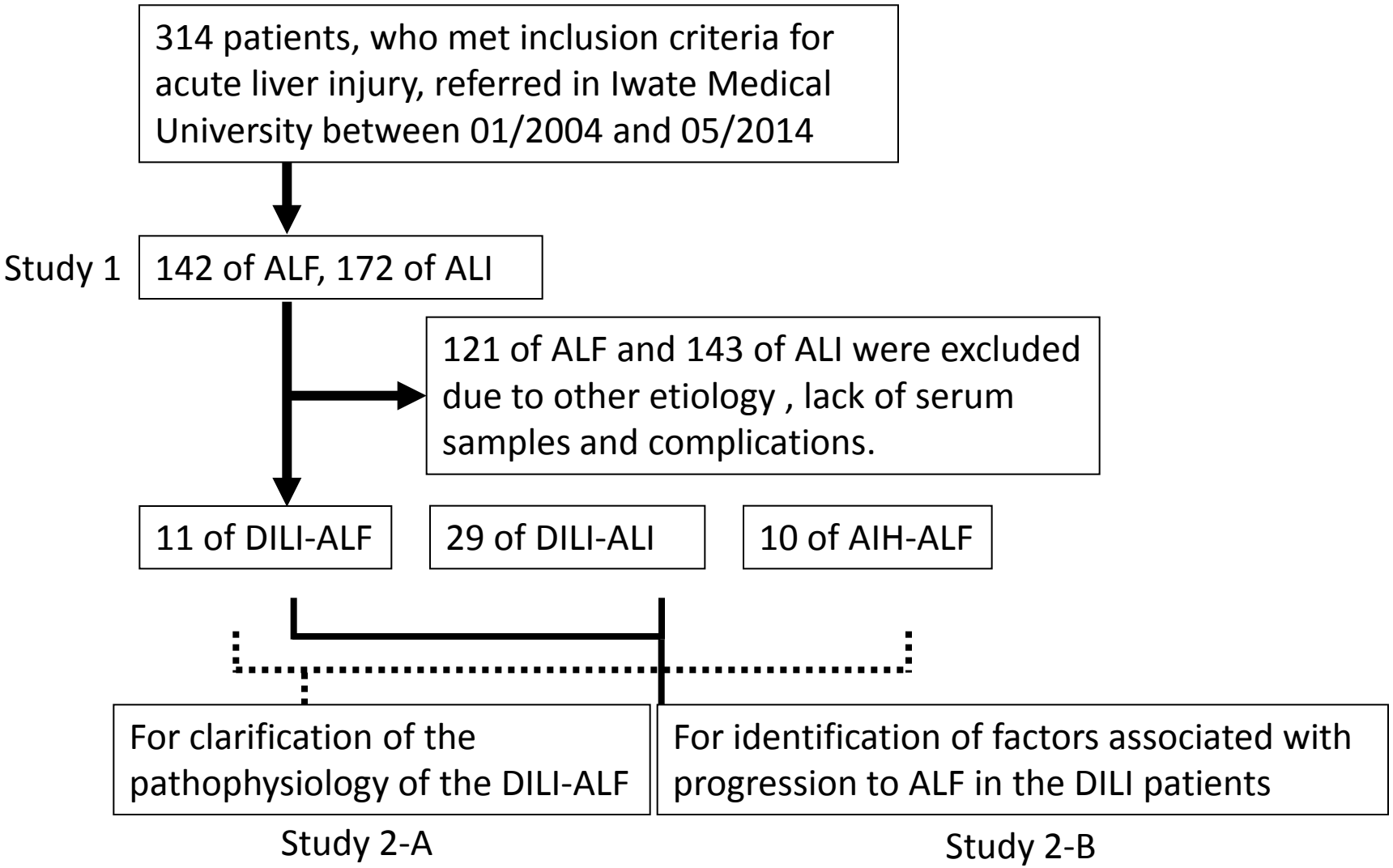

314 patients, who met inclusion criteria for acute liver injury, referred in Iwate Medical University between 01/2004 and 05/2014
Study 1
142 of ALF, 172 of ALI
121 of ALF and 143 of ALI were excluded due to other etiology , lack of serum samples and complications.
11 of DILI-ALF
29 of DILI-ALI
10 of AIH-ALF
For clarification of the pathophysiology of the DILI-ALF
For identification of factors associated with progression to ALF in the DILI patients
Study 2-A
Study 2-B

Figure 3

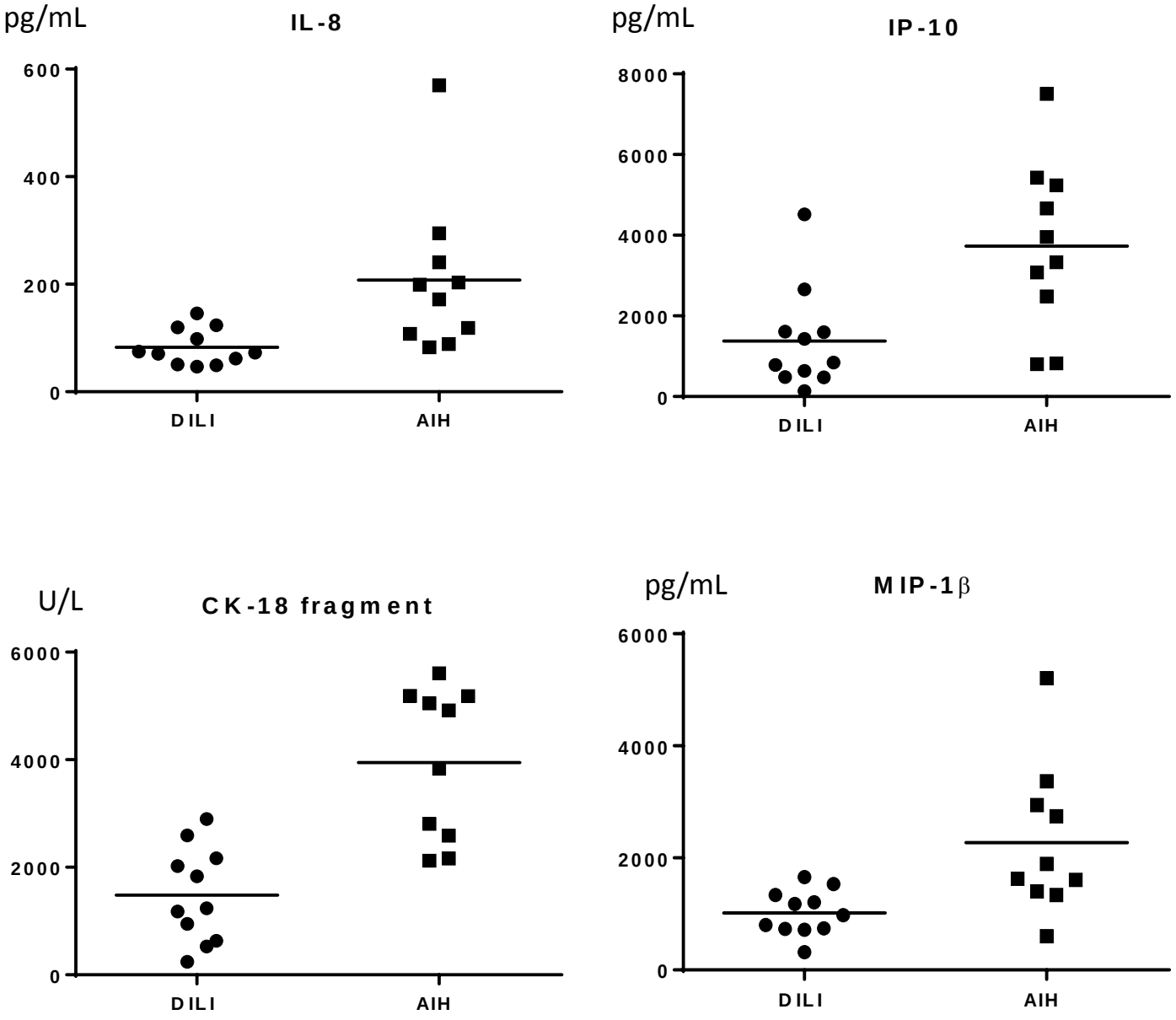

pg/mL
IL-8
600
400
200
0
DILI
AIH
pg/mL
IP-10
8000
6000
4000
2000
0
DILI
AIH
U/L
CK-18 fragment
6000
4000
2000
0
DILI
AIH
pg/mL
MIP-1β
6000
4000
2000
0
DILI
AIH

Figure 4

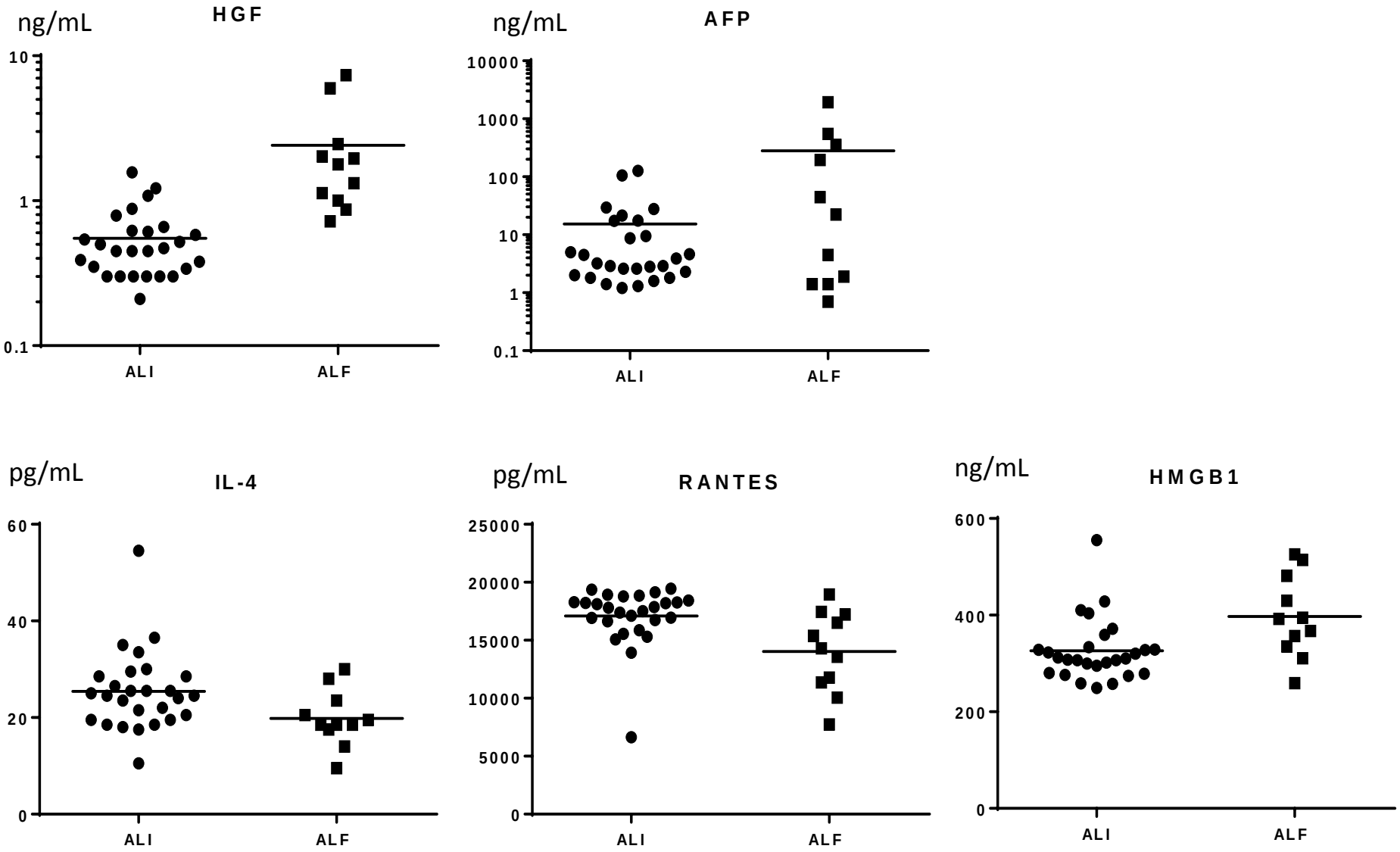
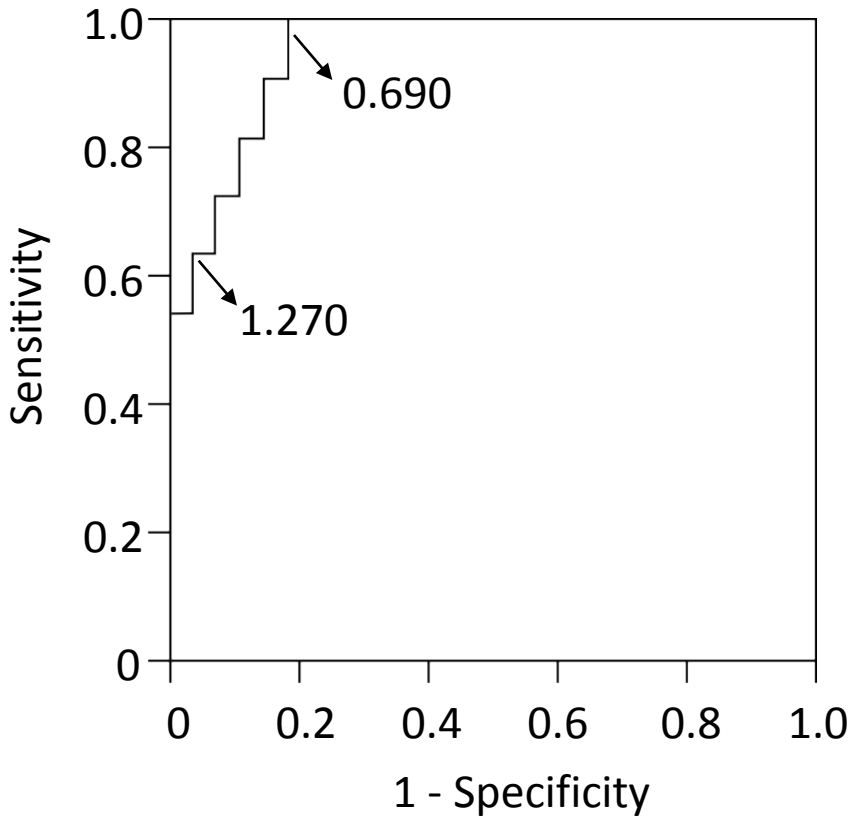

ng/mL
HGF
10
1
0.1
ALI
ALF
ng/mL
AFP
10000
1000
100
10
1
0.1
ALI
ALF
pg/mL
IL-4
60
40
20
0
ALI
ALF
pg/mL
RANTES
25000
20000
15000
10000
5000
0
ALI
ALF
ng/mL
HMGB1
600
400
200
0
ALI
ALF

Figure 5



	AUROC	p value
HGF	0.949	0.001

Cut off value (HGF)	0.690
Sensitivity	1.000
Specificity	0.814
Positive predictive value	0.688
Negative predictive value	1.000