Elevation of Serum Cytokines Preceding Elevation of Liver Enzymes in a Case of Drug-Induced Liver Injury

Keisuke Kakisaka [1)2)]
Yasuhiro Takikawa [2)]

1) Department of Gastroenterology, Kazuno Kosei Hospital,
Kazuno, Japan
2) Division of Gastroenterology and Hepatology, Department of Internal Medicine,
Iwate Medical University,
Morioka, Japan

Key words: IL-1β, TNF-α, MCP-1, Bio-Plex

Address for correspondence: Keisuke Kakisaka, M.D., Ph.D.
School of Medicine
Iwate Medical University
19-1 Uchimaru Morioka
Iwate, JAPAN 0208505
Tel.: +81-19-651-5111
Fax: +81-19-652-6664
E-mail:keikaki@iwate-med.ac.jp

Running title: Serial Changes in the Cytokines Levels during DILI

Number of:
Figures - 2
Table -2
Supplemental figure -1
References - 12

Abbreviations: alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), drug-induced liver injury (DILI), **drug lymphocytes**

stimulation test (DLST), gamma-glutamyltransferase (γ-GTP), interleukin (IL), monocytic chemotactic protein 1 (MCP-1), macrophage inflammatory protein-1 beta (MIP-1β), total bilirubin (T-BIL), white blood cell (WBC)

Abstract

A 50-year-old male who was being treated for both pneumonia and type 2 diabetes mellitus complained of abdominal distention on the 16th hospital day. Liver enzyme elevation without symptoms was detected on the 17th hospital day. Based on a Roussel Uclaf Causality Assessment Method score of 10 and a Japan Digestive Disease Week score of 9, we diagnosed the patient with drug-induced liver injury (DILI). Simultaneous assays of the levels of cytokines revealed that the elevation of the levels of interleukin (IL) - 1β, IL-10, IL-12, IL-13 and tumor necrosis factor alpha preceded the elevation of the serum liver enzymes. This case suggests that some cytokines or related molecules are potentially useful as early-phase biomarkers for DILI.

Introduction

Drug-induced liver injury (DILI) is the most common cause of death from acute liver failure in the United States (1) and has become a serious health problem. In order to predict and treat DILI, the detailed mechanisms underlying its development must be clarified. However, the pathogenesis of DILI remains unclear because the diagnosis is usually retrospective.

A subset of patients with DILI present with clinical findings associated with allergic reactions, such as rashes or eosinophilia (2). These reactions in patients with DILI are

associated with several cytokines (3, 4). Therefore, cytokine interactions may play an important role in the pathogenesis of DILI.

Case report

A 50-year-old male who was being treated for type 2 diabetes mellitus and alcoholic liver injury with insulin by a general physician visited our department complaining of dyspnea and pyrexia. Moist rales were detected in the left lower lung. Cardiac and abdominal examinations were unremarkable. The laboratory data revealed leukocytosis, liver injury and hyperbilirubinemia: white blood cell (WBC) count: 14,700/mL, alanine aminotransferase (ALT): 225 IU/L and gamma-glutamyltransferase (γ-GTP): 1,090 IU/L. Chest radiography revealed an infiltrative shadow accompanied by an air bronchogram in the right upper lobe. The patient was diagnosed with alcoholic liver injury and pneumonia. The pneumonia was treated with several antibiotics: tazobactam/ piperacillin (TAZ/ PIPC, 9 g/day) from the 1st hospital day to the 7th hospital day, micafungin (MCFG, 75 mg/day) from the 8th hospital day to the 17th hospital day and levofloxacin (LVFX, 500 mg/day) from the 8th hospital day to the 17th hospital day. On the 15th hospital day, the pneumonia improved and the liver enzyme level returned to normal. However, the patient complained of right upper abdominal distention on the 16th hospital day. Although this symptom rapidly disappeared after four hours,

asymptomatic liver injury was detected on the 17th hospital day: ALT: 666 IU/L, γ-GTP: 621 IU/L and alkaline phosphatase: 2,113 IU/L (Figure 1 and Table 1). No causes of acute liver injury, such as cholelithiasis, viral infection or autoimmune disease, were detected (Supplemental Figure 1 and Table 1). Therefore, a diagnosis of DILI due to antibiotics was suspected, and all medications were discontinued, except for insulin. The liver enzyme elevation improved by the 22nd hospital day without specific therapy, and the patient was discharged on the 26th hospital day. **Although drug lymphocytes stimulation test (DLST) was performed to TAZ/ PIPC, MCFG and LVFX, DLST for all these medicines was negative.**

The Roussel Uclaf Causality Assessment Method score in this case was 10 and the Japan Digestive Disease Week score was 9 (Table 2). According to the patient's clinical course, the antibiotics were considered to be the causal drugs (Figure 1). Serum samples were collected on the 15th hospital day, when the serum liver enzyme levels were within the normal limits and it was two days before marked elevation in the liver enzymes levels was observed. Serial changes in the cytokine levels were simultaneously evaluated with the Bio-Plex 200 (BioRad, Tokyo, JAPAN), and the values were calculated using the Bio-Plex manager software program, version 5.0 (BioRad, Tokyo, JAPAN). The levels of IL-1β, IL-10, IL-12, IL-13 and TNF-α were elevated before the

liver enzyme elevation (Figure 2). The levels of IL-4, IL-5, IL-6, IL-8, IL-17, monocytic chemotactic protein 1 (MCP-1) and macrophage inflammatory protein-1 beta (MIP-1β) immediately became elevated after the liver enzyme elevation and then dramatically decreased two to three days after peaking (Figure 2).

Discussion

DILI is classified into two types: the intrinsic type and the idiosyncratic type (2, 5, 6). Most cases of DILI are idiosyncratic, accounting for 13% of cases of acute liver failure in the US (1). In order to prevent and treat idiosyncratic DILI, the pathogenesis of the condition must be understood. However, because DILI is usually diagnosed retrospectively, the detailed mechanisms underlying the development of DILI remain unclear. Because a subset of idiosyncratic DILI patients present with rashes, fever or eosinophilia, the disease is considered to be associated with the immune response (2). Therefore, cytokine interactions may play an important role in the pathogenesis of DILI.

We first reported the simultaneous evaluation of serial changes in the levels of several cytokines before the initiation of liver injury in humans and found that the elevation of the IL-1β, IL-10, IL-12, IL-13 and TNF-α levels preceded the liver enzyme elevation. These findings suggest that these cytokines play important roles in the initial stage of

DILI. Because alcoholic liver injury and pneumonia were present as preexisting diseases in this case, the levels of several cytokines were not within the normal ranges on the 15th hospital day, although the patient was asymptomatic. Because we didn't store any samples before the 15th hospital day in this case, we were not able to ascertain what cytokine was initiation cytokine in onset of DILI. Sustained high levels of several cytokines in patients with pneumonia or alcoholic liver injury after treatment have been previously reported (7-10). Therefore, the influence of alcoholic liver injury and pneumonia on the cytokine levels cannot be completely excluded in this case. In fact, the levels of most cytokines in this case were high to normal even in 52 day after the administration of treatment (11). However, the levels of several cytokines that were high before onset immediately decreased after the administration of antibiotics was discontinued as shown the profile of IL-1β. Therefore, these cytokines acted as preconditioning cytokines in this case.

We hypothesized the following mechanism of liver injury in the present case. Because IL-1β and TNF-α, which are proinflammatory cytokines, were at a high level before the elevation of liver enzymes, these cytokines functioned as preconditioning cytokines. IL-10, an anti-inflammatory cytokine, was also at a high level at that time. The IL-10 level may be elevated as a reaction to high levels of proinflammatory cytokines. The

IL-1β, TNF-α and IL-10 levels were decreased after the administration of antibiotics was discontinued. In contrast, the elevation of IL-12, which is secreted from activated hepatocytes, and IL-13, which is secreted from Th2 cells, was sustained at a high level for several days after the elevation of the liver enzymes. However, the role of sustained high levels of IL-12 and IL-13 remains unclear. Several cytokines originating from Th2 cells, such as IL-4, IL-5 and IL-6, in addition to IL-17 from Th17 cell, and several chemokines, such as IL-8, MCP-1 and MIP-1β, were increased following the elevation of liver enzymes and rapidly decreased after several days of elevation of liver enzymes. Therefore, these cytokines and chemokines may have been elevated as enhanced or inhibited factors due to the influence of IL-1β, TNF-α and IL-10. Because IL-1β, TNF-α and IL-10 were secreted from macrophage or antigen-presenting cell, these cells played an initial important role in the development of liver enzyme elevation in this case.

Intriguingly, the inhibition of IL-1β was found to attenuate liver damage in an animal model of DILI (12). IL-10 and TNF-α polymorphisms are associated with DILI (4). The identification of early-phase biomarkers for DILI is urgently needed because the prognosis of patients with overt idiosyncratic DILI remains very poor. The present case report therefore suggests that early-phase cytokines or some related molecules may be potentially useful as early-phase biomarkers for DILI. Although interaction between

preceding inflammatory diseases and the cytokines at the onset of DILI and the mechanisms through which cytokines interact in patients with DILI remain unclear, this case report may provide new insight into the initial stages of DILI.

Acknowledgements

The authors who have taken part in this study declared that they do not have anything to declare regarding funding from industry or conflict of interest with respect to this manuscript.

References

1. Ostapowicz G, Fontana RJ, Schiodt FV, Larson A, Davern TJ, Han SH, McCashland TM, et al. Results of a prospective study of acute liver failure at 17 tertiary care centers in the United States. Ann Intern Med 2002;137:947-954.

2. Tujios S, Fontana RJ. Mechanisms of drug-induced liver injury: from bedside to bench. Nat Rev Gastroenterol Hepatol 2011;8:202-211.

3. Malatjalian DA, Ross JB, Williams CN, Colwell SJ, Eastwood BJ. Methotrexate hepatotoxicity in psoriatics: report of 104 patients from Nova Scotia, with analysis of risks from obesity, diabetes and alcohol consumption during long term follow-up. Can J Gastroenterol 1996;10:369-375.

4. Pachkoria K, Lucena MI, Crespo E, Ruiz-Cabello F, Lopez-Ortega S, Fernandez MA, Romero-Gomez M, et al. Analysis of IL-10, IL-4 and TNF-alpha polymorphisms in drug-induced liver injury (DILI) and its outcome. J Hepatol 2008;49:107-114.

5. Bell LN, Chalasani N. Epidemiology of idiosyncratic drug-induced liver injury. Semin Liver Dis 2009;29:337-347.

6. Grant LM, Rockey DC. Drug-induced liver injury. Curr Opin Gastroenterol 2012;28:198-202.

7. Antunes G, Evans S, Lordan J, Frew A. Systemic cytokine levels in community-acquired pneumonia and their association with disease severity. The European respiratory journal 2002;20:990-995.

8. Felver M, Mezey E, McGuire M, Mitchell M, Herlong H, Veech G, Veech R. Plasma tumor necrosis factor alpha predicts decreased long-term survival in severe alcoholic hepatitis. Alcoholism, clinical and experimental research 1990;14:255-259.

9. Hart JP, Broadwater G, Rabbani Z, Moeller BJ, Clough R, Huang D, Sempowski GA, et al. Cytokine profiling for prediction of symptomatic radiation-induced lung injury. Int J Radiat Oncol Biol Phys 2005;63:1448-1454.

10. Kellum J, Kong L, Fink M, Weissfeld L, Yealy D, Pinsky M, Fine J, et al. Understanding the inflammatory cytokine response in pneumonia and sepsis: results of the Genetic and Inflammatory Markers of Sepsis (GenIMS) Study. Archives of internal medicine 2007;167:1655-1663.

11. Yasumi Y, Takikawa Y, Endo R, Suzuki K. Interleukin-17 as a new marker of severity of acute hepatic injury. Hepatology research : the official journal of the Japan Society of Hepatology 2007;37:248-254.

12. Yano A, Higuchi S, Tsuneyama K, Fukami T, Nakajima M, Yokoi T. Involvement of immune-related factors in diclofenac-induced acute liver injury in mice. Toxicology 2012;293:107-114.

FIGURE LEGENDS

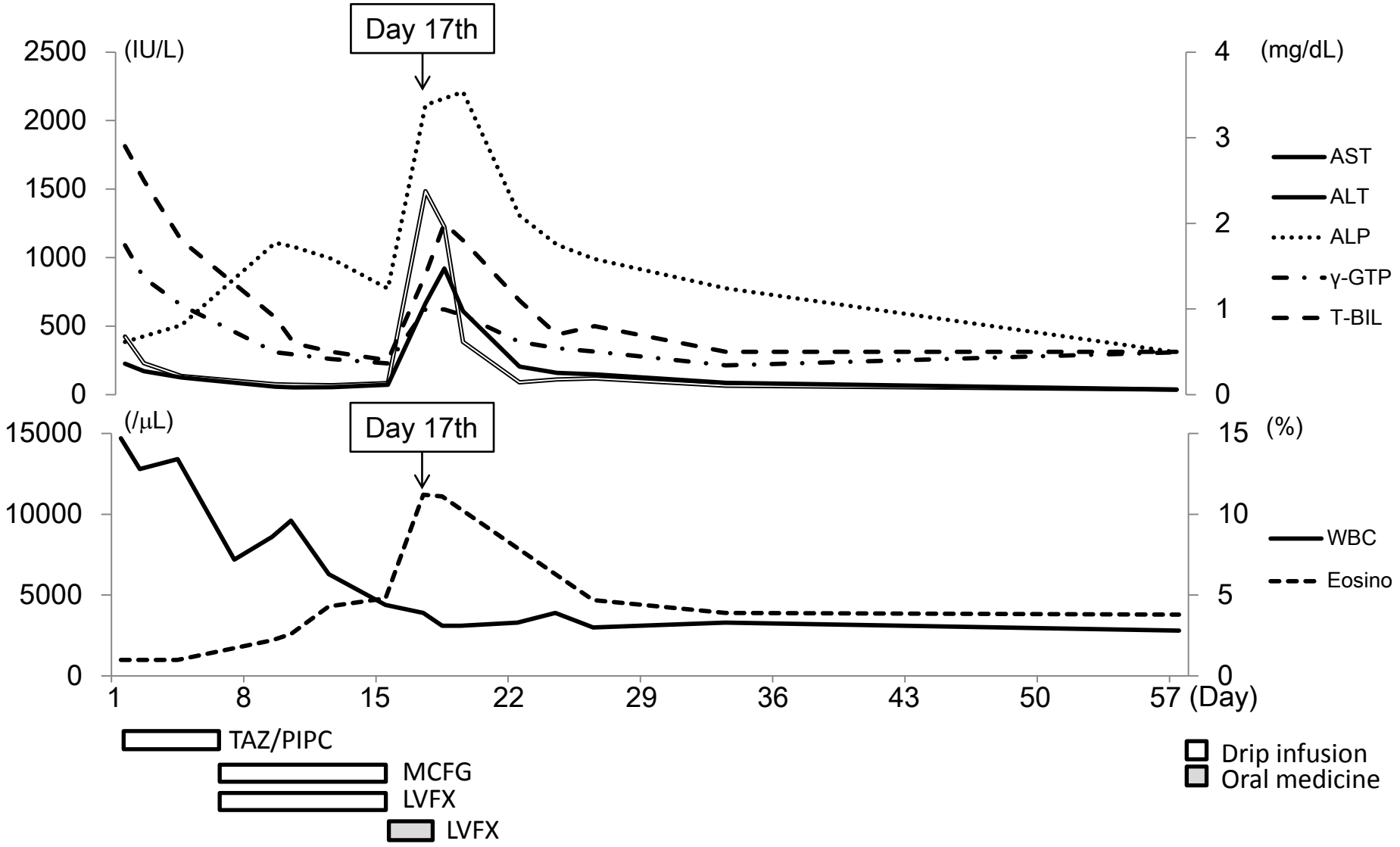
Figure 1. Time course of the laboratory data of the present patient with drug-induced liver injury. The upper line chart presents several biochemical parameters, including the levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyltransferase (γ-GTP) and total bilirubin (T-BIL). The lower line chart presents the white blood cell (WBC) count and the proportion of cells exhibiting eosinophilia relative to the total number of WBCs. The bar chart indicates the duration of each antibiotic.

Figure 2. Simultaneous evaluation of serial changes in the levels of several cytokines before the onset of drug-induced liver injury. The line charts indicate the serial changes in the levels of cytokines evaluated using BioPlex, including interleukin (IL)-1β, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IL-13, IL-17, monocytic chemotactic protein 1 (MCP-1), macrophage inflammatory protein-1 beta (MIP-1β) and tumor necrosis factor alpha (TNF-α). We collected serum samples on the 15th, 17th, 18th, 19th, 22nd, 23rd, 24th, 26th, 33rd and 52nd days. The normal ranges of several cytokines have been previously reported by us. The normal range of IL-1β, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IL-13, IL-17 and TNF-α is up to 2.0 pg/mL. The normal range of MCP-1 or MIP-1b is up to 174.8 pg/mL or up to 159.3 pg/mL, respectively.

Supplemental Figure Legend

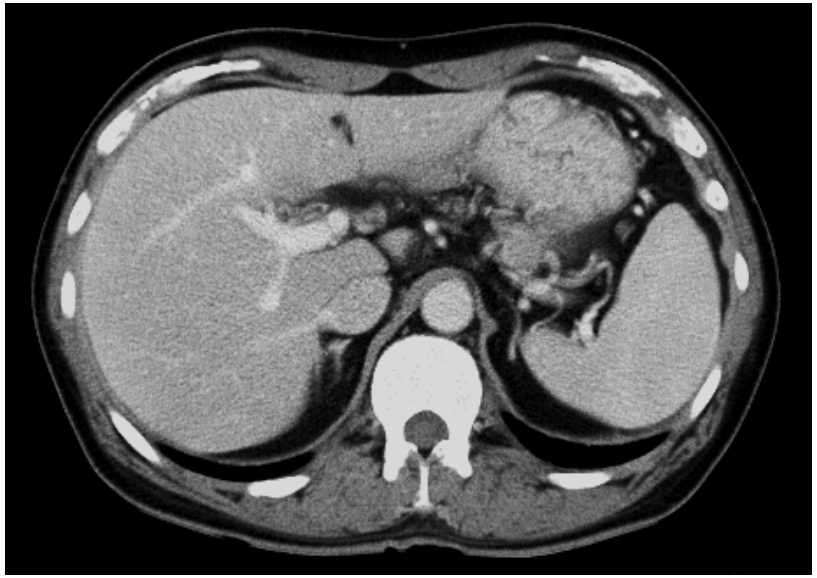
Supplemental figure 1. Imaging findings of the present patient with drug-induced liver injury on the 17th hospital day. (a) Abdominal computed tomography (CT) showed mild splenomegaly and without dilatation of the intrahepatic biliary ducts. (b) Magnetic resonance cholangiopancreatography showed no evidence of obstructive jaundice or cholelithiasis .

Figure 1.


Day 17th
(IU/L)
2500
2000
1500
1000
500
0
4
3
2
1
0
(mg/dL)
AST
ALT
ALP
γ-GTP
T-BIL
Day 17th
(/μL)
15000
10000
5000
0
15
10
5
0
(%)
WBC
Eosino
1
8
15
22
29
36
43
50
57
(Day)
TAZ/PIPC
MCFG
LVFX
LVFX
Drip infusion
Oral medicine

Supplemental figure 1.

(a)



(b)

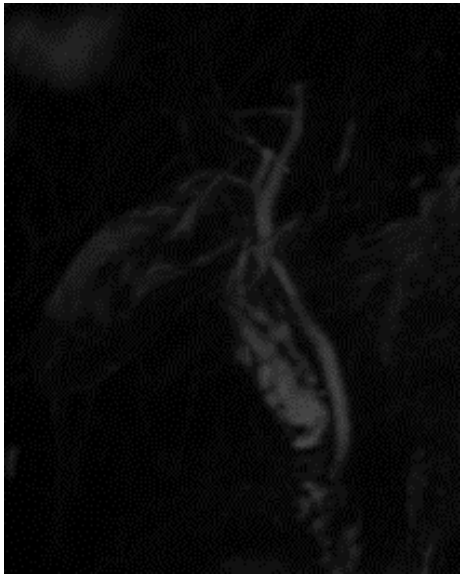


Table. 1 Laboratory data of the present patient with drug-induced liver injury on the 1st and 17th hospital days.

Administration						17th hospital day		
Hematology			**Virus markers**			**Hematology**		
WBC	14.7	10^3/mL	HBsAg	(-)		WBC	3.1	10^3/mL
Neutro	81.2	%	HCVAb	(-)		Neutro	44.0	%
Lympho	16.6	%	IgM HA	(-)		Lympho	33.1	%
Mono	1.9	%	HSV IgM	(-)		Mono	8.0	%
Eosino	0.1	%	HSV IgG	(+)		Eosino	11.1	%
Baso	0.1	%	CMV IgM	(-)		Baso	3.8	%
RBC	412	10^6/mL	CMV IgG	(+)		RBC	447	10^6/mL
Hb	13.9	g/dL	EBVCA IgG	(-)		Hb	14.4	g/dL
Plt	99	10^3/mL	EBVCA IgM	(-)		Plt	369	10^3/mL
			EBNA Ab	(-)				
Blood chemistry						**Blood chemistry**		
TP	6.6	g/dL	**Autoantibodies**			TP	6.8	g/dL
Albumin	3.3	g/dL	ANA	<x40		Albumin	3.4	g/dL
T-Bil	2.9	mg/dL	AMA	(-)		T-Bil	1.2	mg/dL
AST	424	IU/L				AST	1484	IU/L
ALT	225	IU/L	**Tumor markers**			ALT	666	IU/L
ALP	385	IU/L	CEA	2.1	ng/mL	ALP	2113	IU/L
γ-GTP	1090	IU/L	CA19-9	17.3	U/mL	γ-GTP	621	IU/L
ChE	138	IU/L	AFP	2.3	ng/mL	ALP	2113	IU/L
BUN	22.6	mg/dL	PIVKA-2	21	mAU/mL	BUN	10.8	mg/dL
Cre	1.23	mg/dL				Cre	1.03	mg/dL
AMY	33	IU/L				AMY	97	IU/L
NH3	57	mg/dL				NH3	36	mg/dL
CRP	36.13	mg/dL				CRP	0.26	mg/dL
Blood coagulation						**Blood coagulation**		
PT	65	%				PT	72	%
HPT	54	%				HPT	64	%
Fib	643	mg/dL				Fib	406	mg/dL
FDP	11.8	mg/mL				FDP	2.1	mg/mL

WBC, white blood cells; RBC, red blood cells; Hb, hemoglobin; Plt, pletelets; TP, total protein; T-Bil., total bilirubin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; γ-GTP, γ-glutamyl transpeptidase; ChE, choline esterase; BUN, blood urea nitrogen; Cre, creatinine; AMY, amylase; CRP, C-reactive protein; PT, prothrombin time; HPT, hepaplastin test; Fib, fibrinogen; FDP, fibrin degradation products; Ig, immunoglobulin; Ab, antibody; Ag, antigen; HB, hepatitis B virus; HCV, hepatitis C virus; HA, hepatitis A virus; HSV, herpes simplex virus; CMV, cytomegalovirus; EB, Epstein–Barr virus; ANA, anti-nuclear antibody; AMA, anti-mitochondrial antibody; CEA, Carcinoembryonic antigen; CA19-9, carbohydrate antigen 19-9; AFP, α-fetoprotein; PIVKA-II, protein induced by vitamin K absence/antagonist-II.

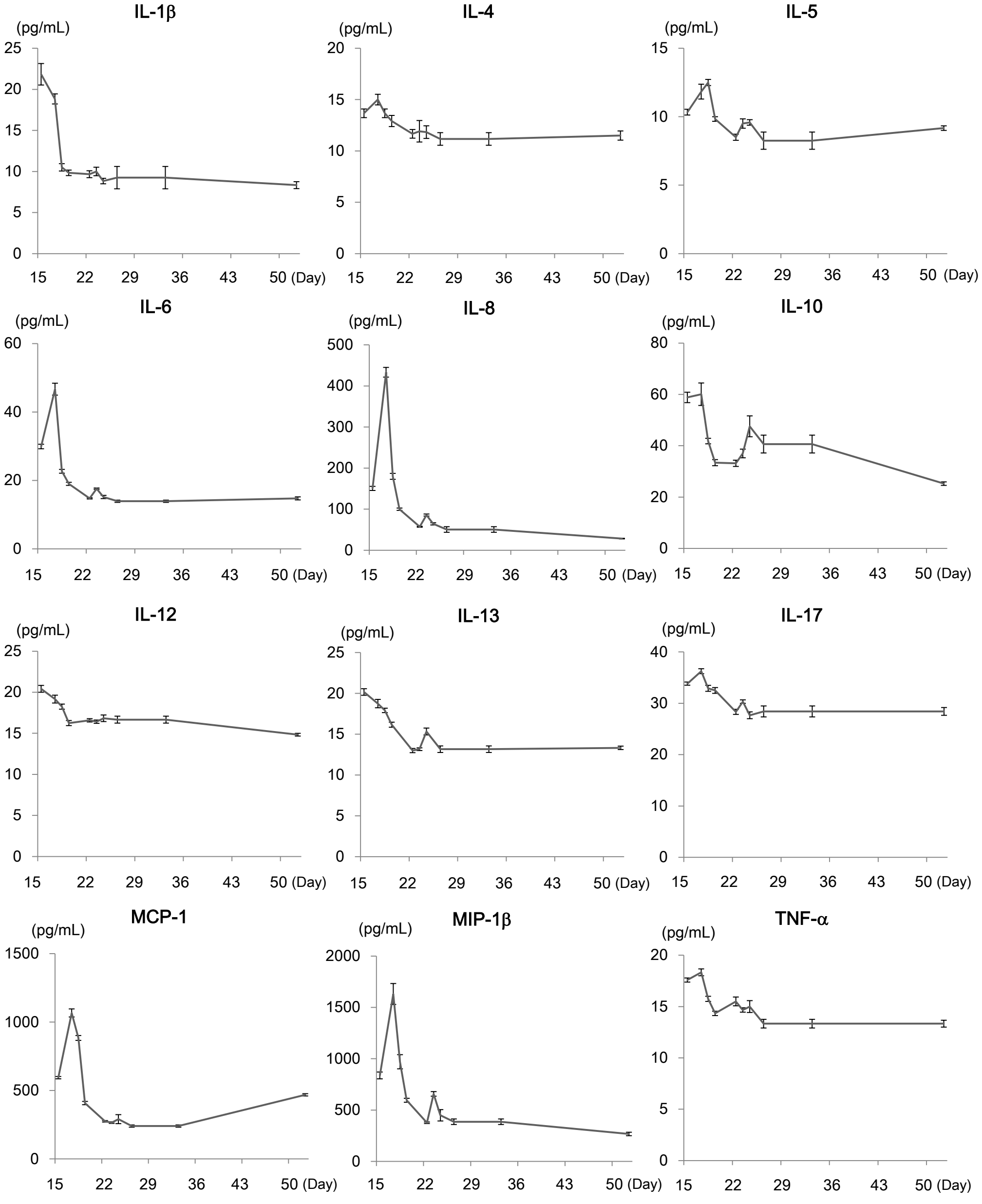
Table. 2 Assessments used to diagnose drug-induced liver injury

RUCAM score		
Type of liver injury	Cholestatic/mixed	
Time of onset of the event	First exposure	
Time from drug intake until reaction onset	5 to 90 days	2
Alcohol or pregnancy risk factor	Present	1
Age risk factor	≥55 years	1
Course of the reaction	≥50% improvement 180 days	2
Exclusion of non drug-related causes	Rule out	2
Previous information on hepatotoxicity	Reaction labeled in the product's characteristics	2
	Total	10

J-DDW score			
Type of liver injury	Cholestatic/mixed		
Time of onset of the event	Initial treatment		
Time to onset	After cessation of the drug from the beginning of the drug	5 to 90 days	2
Risk factors	Presence of ethanol or pregnancy	Alcohol	1
After cessation of the drug	Difference between the peak of ALP and upper limit of normal value	Decrease >50% within 180 days	2
Search for non drug causes	All causes – groups I and II – reasonably ruled out	Ruled out	2
Previous information on hepatotoxicity	Reaction labelled in the product characteristics	+	1
Eosinophilia (>6%)	With eosinophilia	+	1
DLST	negative or unavailable	Negative	0
		Total	9

RUCAM, The Roussel Uclaf Causality Assessment Method; J-DDW, Japan Digestive Disease Week; DLST, drug lymphocyte stimulation test.

Figure 2.


IL-1β
(pg/mL)
25
20
15
10
5
0
15 22 29 36 43 50 (Day)
IL-4
(pg/mL)
20
15
10
5
0
15 22 29 36 43 50 (Day)
IL-5
(pg/mL)
15
10
5
0
15 22 29 36 43 50 (Day)
IL-6
(pg/mL)
60
40
20
0
15 22 29 36 43 50 (Day)
IL-8
(pg/mL)
500
400
300
200
100
0
15 22 29 36 43 50 (Day)
IL-10
(pg/mL)
80
60
40
20
0
15 22 29 36 43 50 (Day)
IL-12
(pg/mL)
25
20
15
10
5
0
15 22 29 36 43 50 (Day)
IL-13
(pg/mL)
25
20
15
10
5
0
15 22 29 36 43 50 (Day)
IL-17
(pg/mL)
40
30
20
10
0
15 22 29 36 43 50 (Day)
MCP-1
(pg/mL)
1500
1000
500
0
15 22 29 36 43 50 (Day)
MIP-1β
(pg/mL)
2000
1500
1000
500
0
15 22 29 36 43 50 (Day)
TNF-α
(pg/mL)
20
15
10
5
0
15 22 29 36 43 50 (Day)