1 ***Serum markers for mitochondrial dysfunction and cell death are possible predictive***

2 ***indicators for drug-induced liver injury by direct acting antivirals.***

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1 ***Abstract***

2 Background & Aim: We prospectively screened patients treated with direct-acting
3 antivirals (DAAs) in order to detect and analyze serum markers that are present prior to
4 the development of drug-induced liver injury (DILI).
5 Methods: The levels of various serum markers among DILI, non-DILI, and control
6 groups were compared. The DILI group consisted of 8 patients whose alanine
7 aminotransferase (ALT) levels exceeded 32 IU/L during the DAA treatment. Eight
8 patients without DILI were selected for the non-DILI group via a matched-group design
9 based on age, gender, and disease severity. Additionally, 8 healthy volunteers were
10 employed as the controls. Serum measurements of cytokines/**chemokines**,
11 cytokeratin-18 fragment (CK-18F), and super oxidase dismutase-2 (SOD2) were
12 evaluated on the date at which HCV-RNA was absent (baseline). For patients with DILI,
13 serum measurements taken before treatment, 1 week before pronounced transaminase
14 elevation (Prominence-1W), and on the date at which pronounced elevation of
15 transaminase occurred (Prominence) were also evaluated.
16 Results: All patients treated with DAAs had normalized transaminase levels at baseline.
17 In patients with DILI, interferon-inducible protein-10 (IP-10) levels were higher at

Prominence-1W than at baseline. Those patients also had significantly higher levels of SOD2 and CK-18F at Prominence-1W than at baseline.

Conclusion: Elevated IP-10 might be a preconditioning chemokine for DAA-induced liver injury, and damage markers associated with cell death and mitochondrial dysfunction are potential predictive serum markers for DILI.

Introduction

Drug-induced liver injury (DILI), one of the most frequent side effects of medications, is divided into two categories according to pathophysiology: DILI caused by overdosage of medication, and idiosyncratic DILI [1, 2]. In the United States, overdosage of acetaminophen is the primary cause of acute liver failure (ALF), but DILI in such cases is predictable and can be treated by the detoxification agent N-acetyl cysteine[3]. In contrast, idiosyncratic DILI is relatively rare for most medications. The incidence of idiosyncratic DILI ranges between 1:10,000 and 1:100,000, and even lower for many drugs [2, 4]. Although idiosyncratic DILI is not common, it can lead to ALF, which poorly responds to treatment even after early intervention [2, 5-9]. Thus, a method for prediction or early detection of DILI is urgently needed [10, 11].

The pathophysiology of DILI has been proposed in several papers [12-14]. According to their data, single nucleotide polymorphisms (SNPs) associated with both drug metabolism and the immune system can potentially be implicated in the pathogenesis of DILI [12-14]. However, the prevalence of idiosyncratic DILI is too low to be induced by one SNP, which indicates multifactorial causes. Indeed, although genetic factors have been identified as risk factors for DILI, not all drugs are mediated by same metabolic pathway, and patients' immune responses vary. Thus, the risk factors

1 associated with the DILI development would not be same among different medications.

2 Consequently, identifying patients who might develop DILI is difficult, even if

3 screening for SNPs is performed. Because of the possibility of multifactorial

4 mechanisms and the high cost of genomic screening, predicting high risk populations

5 through genomic screening is not feasible [13-15]. Hence, the alternative strategy: early

6 detection of DILI. By identifying the conditions that exist prior to or during the initial

7 stages of DILI, early interventions such as discontinuation of the causal drug can be

8 effected[11, 16].

9 Researchers have proposed that mitochondrial damage plays a key role in the

10 development of idiosyncratic DILI [2]. Toxic metabolites inhibit biochemical activity in

11 the mitochondria, causing damaged mitochondria to accumulate in the hepatocytes. As a

12 result, hepatocytes with dysfunctional mitochondria undergo cell death [2]. Other possible

13 factors influencing the advancement of DILI are cytokines/**chemokines**. We previously

14 reported that elevation of cytokines/**chemokines** precedes the development of DILI [17],

15 indicating the presence of preconditions for DILI. That is, several parameters change

16 before transaminase elevation. We have also reported that the serum cell death marker

17 values in patients with DILI-ALF are lower than those found in patients with

1 autoimmune hepatitis (AIH)-induced ALF [6]. These data suggest that in DILI,
2 suppression of biochemical functions may occur before massive hepatocyte death.
3 In the short period since direct-acting antivirals (DAAs) have been available
4 for the treatment of hepatitis C virus (HCV) infections, many patients have been
5 treated [18]. Unfortunately, approximately 10% of patients who receive DAAs develop
6 DILI. Although many patients gain sustained viral response, some patients develop
7 elevated transaminase levels during treatment. Curiously, most patients with elevated
8 transaminase levels show an absence of HCV-RNA, and their transaminase levels
9 normalize before elevating [18]. This indicates that transaminase elevation is caused by
10 DILI, and not chronic hepatitis due to HCV infection or removal of HCV from the liver.
11 By comparing serum cytokines/**chemokines** between patients with DAA-induced
12 transaminase elevation and patients without liver injury, we can pinpoint the serum
13 parameters associated with DAA-induced liver injury.
14 Hence, we aimed to identify the preconditions of DILI. For this purpose, we
15 designed a prospective case-controlled study to compare the serum parameters in
16 patients treated with DAAs. The study groups were matched by age, gender, and HCV
17 disease stage. In addition, the same causal drugs were used to induce DILI so that the
18 pathophysiology of the DILI would be uniform and detectable.

1 ***Subjects & Methods***

2 ***Subjects:*** We prospectively collected serum from 110 patients who were administered
3 DAAs for the treatment of HCV genotype 1a infection from November 2014 to
4 November 2015. **All subjects are Japanese, and all patients were infected with HCV**
5 **genotype 1a.** Transaminase elevation was defined as serum alanine transaminase (ALT)
6 levels in excess of 32 IU/L (greater than 1.2 times the normal limit). Thirty-two patients
7 developed elevated transaminase levels during treatment (Figure 1). These patients were
8 classified as the DILI group. Patients without transaminase elevation were classified as
9 the non-DILI group. We matched 8 patients from each group for age, gender, and
10 disease stage. We also age- and gender-matched 8 volunteers for the control group.
11 Informed consent was obtained from all subjects. All of the protocols reported in this
12 paper were approved by the Institutional Review Board of Iwate Medical University
13 (approval number: H27-105).

14 ***DAA treatment for HCV infection:*** The treatment protocol was daclatasvir 60 mg/day
15 and asunaprevir 200 mg/day for 24 weeks. The inclusion criteria for treatment were as
16 follows: chronic hepatitis or liver cirrhosis due to HCV infection, and liver cirrhosis
17 classified as grade A under the Child-Pugh system. Patients were excluded if they had
18 hepatocellular carcinoma, severe renal dysfunction, or were older than 85 years old.

1 Treatment was discontinued for severe side effects such as ALT or aspartate

2 aminotransferase (AST) levels greater than 300 IU/L.

3 ***Definition of time points:*** For the patients with elevated transaminase levels, we

4 examined 4 time-points: "Before treatment" was defined as the date before starting

5 DAA treatment; "baseline" was the date on which we detected the absence of

6 HCV-RNA; "Prominence" was the date of transaminase elevation; and

7 "Prominence-1W" was defined as 1 week before the date of Prominence.

8 ***Cytokines, chemokines, super oxidase dismutase-2 and cytokeratine-18 fragment:***

9 Serum concentrations of the following products were measured: interleukin (IL)-1β,

10 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

11 fibroblast growth factor (FGF), eotaxin, granulocyte-colony stimulating factor (G-CSF),

12 interferon-gamma (IFN-γ), interferon-inducible protein-10 (IP-10), monocyte

13 chemoattractant protein-1 (MCP-1), macrophage inflammatory protein 1-alpha

14 (MIP-1α), macrophage inflammatory 1-beta (MIP-1β), platelet-derived growth factor

15 BB (PDGF-BB), RANTES (regulated on activation, normal T cell expressed and

16 secreted), tumor necrosis factor alpha (TNF-α), and vascular endothelial growth factor

17 (VEGF). A Bio-Plex Suspension Array System (Bio-Rad Laboratories, Inc.) and

18 Bio-Plex Pro Human Cytokine 27-plex Assay kit (Bio-Rad Laboratories, Inc.) were

used for those measurements as described in a previous report [6]. Super oxidase dismutase-2 (SOD2) and cytokeratine-18 fragments (CK-18Fs) were measured using an enzyme-linked immunosorbent assay (ELISA) kit (AbFrontier, Tokyo; PEVIVA, Tokyo). All assays were performed according to manufacturers' instructions.

Laboratory data: White blood cell counts, plasma prothrombin time (PT) time, and serum levels of ALT, AST, creatinine (Cre), and total bilirubin (TBil) were analyzed using an autoanalyzer (JCA-BM2250; JEOL, Tokyo, Japan).

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). Kruskal-Wallis and Friedman tests were used to evaluate the statistical significance of the results. **Binominal logistic anaylsis was used for multivariate analysis.** A two-sided p value of <0.05 was considered to be statistically significant.

Results

All patients with HCV infection had normalized liver transaminase levels after DAA treatment: Transaminase levels before treatment were significantly higher in the study groups than in the control group, but the levels did not significantly differ between the two study groups. Transaminase levels for patients in the study groups normalized at baseline (Table 1), with no significant differences between any groups. Before treatment, HCV-RNA levels did not significantly differ between the two study groups (DILI, 6.08 ± 0.34 log copies/mL; non-DILI, 6.16 ± 0.42 log copies/mL). Per our definition of "baseline," all selected patients showed an absence of HCV-RNA as measured by real-time PCR. We found that the average duration between the start of DAA therapy and baseline did not significantly differ between the study groups (4.6 weeks for both groups).

At baseline, IP-10 levels were significantly higher and RANTES levels significantly lower in the DILI group than in the control group: To confirm the baseline state, we compared several parameters among the three groups. At baseline, IP-10 levels were significantly higher for patients in the DILI group than for those in the control group (Table 2: 1617.5 ± 525.8 pg/mL vs. 510.8 ± 204.1 pg/mL, respectively). In addition, at baseline, RANTES levels were significantly lower in patients with DILI compared to

those in the control group (Table 2: 14545.0 ± 2300.6 pg/mL vs. 18469.9 ± 832.6 pg/mL, respectively). More importantly, none of the serum cytokine/**chemokine** levels significantly differed between the DILI and the non-DILI groups. **To identify risk factors of DILI development, multivariate analysis was performed. As the results, RANTES was identified independent risk factors of DILI development (Table 3).**

Patients with DILI had elevated levels of PDGF-BB and TNF-α at Prominence:

Elevated transaminase levels were found in the DILI groups at 8.5 ± 2.5 weeks after the start of DDA treatment, but the absence of HCV-RNA was sustained in all patients. Although drug lymphocyte stimulating tests were not performed, none of the patients in the DILI group showed signs of eosinophilia. After discontinuation of DAAs, transaminase levels normalized. Because other causes such as viral infections, alcohol consumption, and autoimmune diseases were absent in these patients, we concluded that the DAAs induced transaminase elevation.

To identify the dynamism of cytokines/**chemokines** when transaminase levels are elevated, we serially evaluated the serum levels of two **chemokines**. In patients with DILI, PDGF-BB and TNF-α levels were significant higher at Prominence than at baseline (Figure 2 and Table 4: 4232.8 ± 1326.7 pg/mL vs. 3075.3 ± 877.3 pg/mL, and 39.8 ± 10.8 pg/mL vs. 31.8 ± 4.0 pg/mL, respectively). These data indicated that

1 PDGF-BB and TNF-α are associated with drastic elevation of transaminase in patients

2 with DILI.

3 ***IP-10 levels were significantly higher at Prominence-1W than at baseline:*** We

4 hypothesized that the concentrations of some cytokines/**chemokines** might change

5 before elevation of transaminase, so we focused on serial changes between baseline and

6 Prominence-1W. In patients with DILI, IP-10 levels were significantly higher at

7 Prominence-1W than at baseline (Figure 2 and Table 4: 2927.9 ± 1627.4 pg/mL vs.

8 1617.5 ± 525.8 pg/mL, respectively). This indicated that elevated IP-10 levels at

9 Prominence-1W are associated with the development of DILI.

10 ***CK-18F and SOD2 were elevated at Prominence and Prominence-1W, but no***

11 ***differences were detected among the groups at baseline:*** In the search for other

12 predictive parameters of transaminase elevation, we confirmed the presence of markers

13 associated with liver injury: CK-18F, the marker for hepatocyte death; and SOD2, the

14 marker for hepatic mitochondrial dysfunction [19-22]. In patients with DILI, CK-18F and

15 SOD2 levels were higher at Prominence-1W than at baseline (468.0 ± 294.2 U/L vs.

16 108.8 ± 95.3 U/L, 3711.5 ± 573.2 pg/mL vs. 2960.1 ± 648.6 pg/mL, respectively).

17 Elevation of SOD2 level further increased at Prominence (Figure 2 and Table 4).

18

1 ***Discussion***

2 In a previous nationwide study that was performed to identify genetic risk
3 factors for idiosyncratic DILI, researchers found that SNPs in human leukocyte antigen
4 (HLA) genes are associated with SNPs in DILI [12]. However, using genetic studies to
5 ascertain the risk of DILI for each patient for each medication is costly [15]. The aim of
6 the present study was to clarify the preconditions associated with the development of
7 DILI, including identification of the cytokines/**chemokines** involved in the
8 pathophysiology of DILI that could indicate impending liver damage. We obtained the
9 following results: 1) PDGF-BB and TNF-α levels increased concurrently with elevation
10 of transaminase associated with DDA-induced liver injury; 2) baseline IP-10 and
11 RANTES levels significantly differed between the DILI and control groups; 3) IP-10
12 levels increased prior to DDA-induced liver injury; and 4) CK-18F and SOD2 levels
13 increased before elevation of transaminase.

14 We had previously reported that the serum levels of several
15 cytokines/**chemokines** climb prior to significant increases in transaminase levels [17].
16 Based on that report, we speculated that a change in cytokine/**chemokine** levels might
17 occur prior to development of DILI. Other studies have reported that TNF-α and
18 PDGF-BB (associated with injury, inflammation, and regeneration in the liver [23-25])

1 induce liver injury and fibrosis through mitochondrial dysfunction [26-28]. In the present
2 study, we found that TNF-α and PDGF-BB levels increased at the onset of DILI.
3 Because these cytokines/**chemokines** did not significantly increase at Prominence-1W,
4 we believe they enhance liver injury.
5 At baseline, IP-10 levels were significantly higher for patients with DILI than
6 for those in the control group. However, IP-10 values did not significantly differ
7 between patients with DILI and those without DILI. This phenomenon could be
8 explained by a recent study showing that serum IP-10 levels are higher in patients with
9 chronic hepatitis C [29]. Thus, high baseline IP-10 levels result from sustained liver
10 inflammation associated with HCV infections [30]. Since DAA therapy is known to
11 reduce serum IP-10 levels [29], we believe that the IP-10 levels in our study groups rose
12 prior to transaminase elevation because of DAA-induced liver injury. Considering that
13 IP-10 protects against liver injury via several mechanisms [30-32], perhaps IP-10 induction
14 was a hepatoprotective effect. However, we have not excluded the possibility that
15 elevated IP-10 levels could be a phenomenon specific to patients with HCV who
16 develop DILI. Further prospective studies might provide more details about the role of
17 IP-10 in DILI pathophysiology.

1 We previously reported that RANTES levels are significantly lower in patients
2 with DILI-ALF than in patients with AIH-induced ALF. RANTES is a chemokine
3 secreted from activated hepatic stellate cells, macrophages, and T cells. It exhibits
4 strong chemoattractant activity towards monocytes and natural killer cells [33]. Lower
5 RANTES levels will suppress inflammation. We found that baseline RANTES levels
6 were significantly lower in patients with DILI than in the control group. **Furthermore,**
7 **RANTES was independent risk factor associated with DILI development (table 3).**
8 Based on the results of the present study, we speculated about the paradoxical state of
9 chemokines: High IP-10 and low RANTES levels might be background elements
10 associated with the development of DILI. **Indeed, previous studies using genome**
11 **wide association study reported that several human leukocyte antigen (HLA); such**
12 **as HLA-B and HLA-DR, were risk factor of DILI[34-36]. Based on the above previous**
13 **reports and the present results, pathophysiology of DILI will associate with**
14 **immunity.**
15 DILI-induced transaminase elevation is believed to result from hepatocyte
16 death. In an in vitro study of drug toxicity, hepatocyte death was induced by
17 accumulation of mitochondrial stress [2, 21, 37]. In the present study, SOD2 levels (the
18 serum marker for mitochondrial dysfunction) increased prior to significant elevation of

transaminase levels (Table 4), and serum levels of the proinflammatory cytokine TNF-α increased concurrently with transaminase levels. However, the serum levels of most other cytokines/**chemokines** did not increase during the study period.

Although a growing body of evidence supports the important role of innate and adaptive immune systems in the pathogenesis of DILI [38], our results indicate that immune response to inflammation occurs after accumulation of mitochondrial dysfunction. Indeed, our data demonstrate that mitochondrial dysfunction is the primary pathophysiological cause of DILI. When elevation of transaminase occurs in idiosyncratic DILI, mitochondrial dysfunction is already present, which leads to hepatocyte death. In contrast, liver injury due to viral infection or autoimmunity is induced by an immune response to antigens on the hepatocytes [39-42]. The different pathophysiological causes of liver damage may explain the variable responses to immunosuppression therapy: AIH-induced ALI/ALF responds well to immunosuppression therapy, while drug-induced ALI/ALF does not [6].

During review process, we analyzed diagnostic efficacy of IP-10, SOD2 and CK18-F to predict DILI development using ROC analysis. Based on these results, we noticed the limitation that SOD2 did not show high accuracy (data not shown). However, concept of the present study is to identify possible parameter

1 **according to hypothetical pathophysiology of DILI. In this point of view, markers**

2 **associated with mitochondrial dysfunction might be a potential predictive**

3 **parameter of DILI.**

4 We conclude that serum cytokines/**chemokines** do not rise prior to the

5 development of DILI. In addition, although SOD2 and CK-18F could not be used to

6 distinguish between the DILI group and the non-DILI group at the point of

7 Prominence-1W (Tables 2 and 3), some serum markers for mitochondrial dysfunction

8 and cell death would make good candidates as predictive markers for DILI, which

9 would aid in the prevention of drug-induced ALF. In future studies, other markers

10 associated with mitochondrial dysfunction and cell death should be researched as

11 predictive markers for DILI, and their accuracy at predicting the onset of DILI should

12 be confirmed through testing.

13

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3 Motodate for providing excellent secretarial support. KK had full access to all the data

4 in the study and takes responsibility for the integrity of the data and the accuracy of the

5 data analysis.

Figure Legends

Figure 1. Flowchart of the subject selection process.

Figure 2. Comparison of serum PDGF-BB, TNF-α IP-10, CK-18F, and SOD2 levels during the development of DILI.

(A), (B), (C), (D) and (E): Serum levels of PDGF-BB (A), TNF-α (B), IP-10 (C), CK-18F (D) and SOD2 (E) were serially compared at baseline, Prominence-1W, and Prominence. Statistical significance was evaluated using the Friedman test, and defined as p value <0.05.

Figure 1

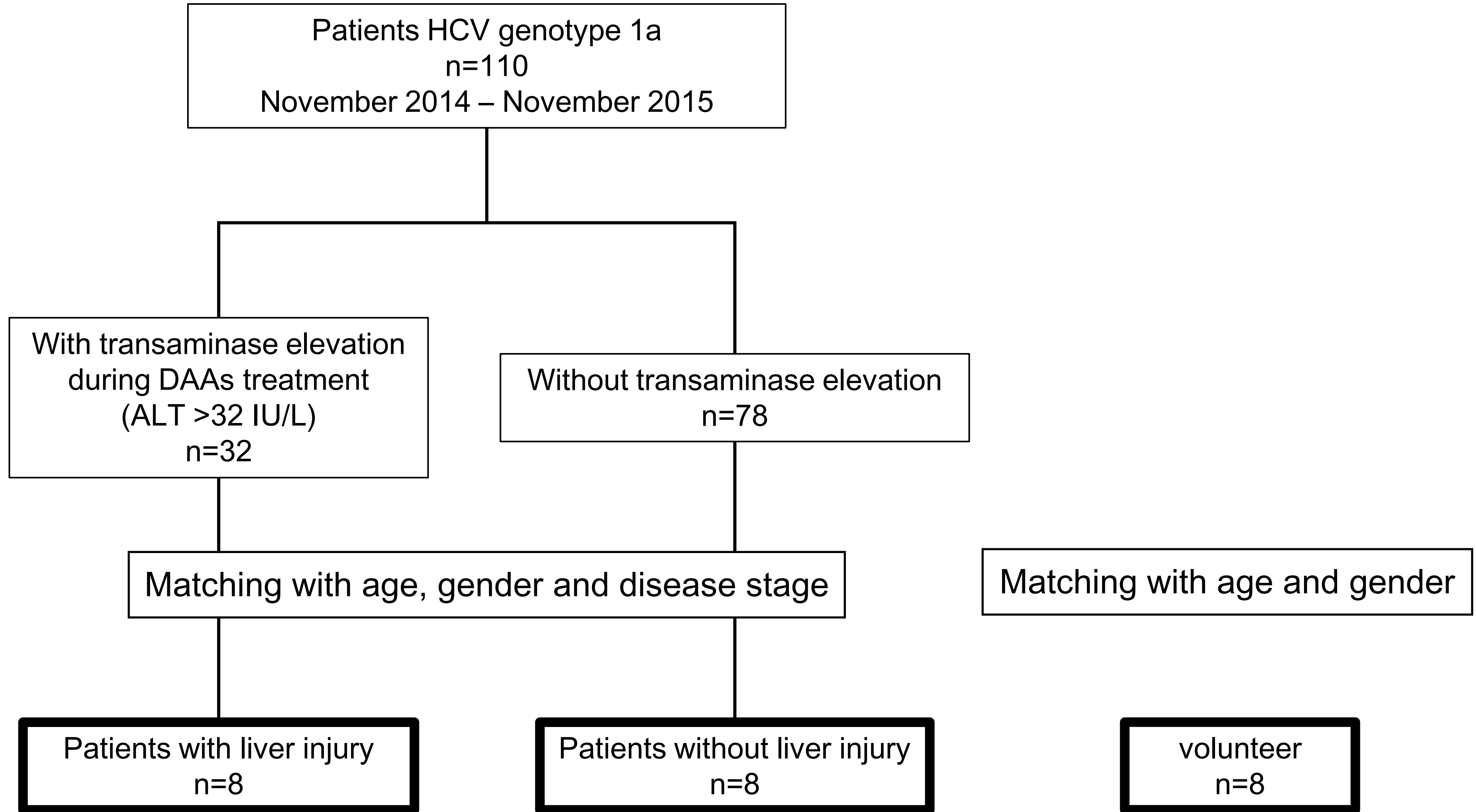
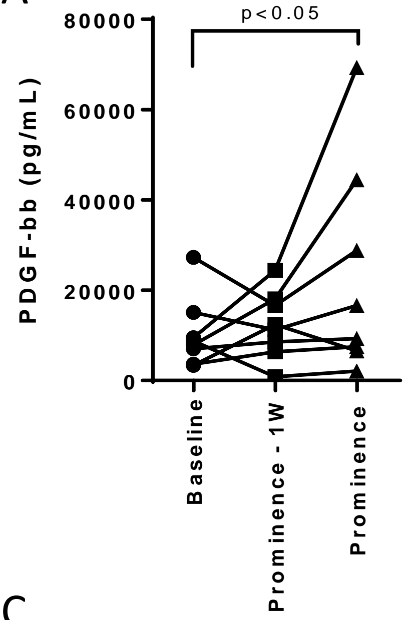
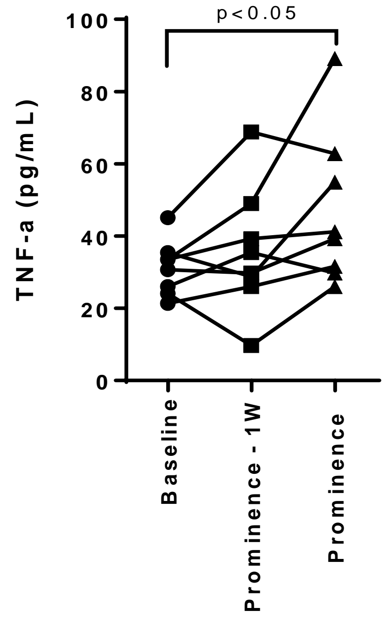
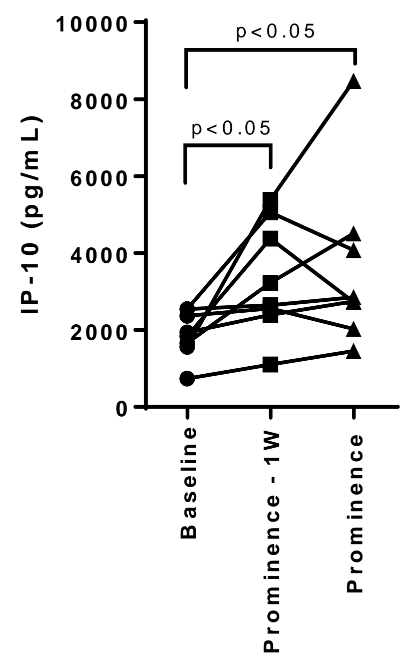
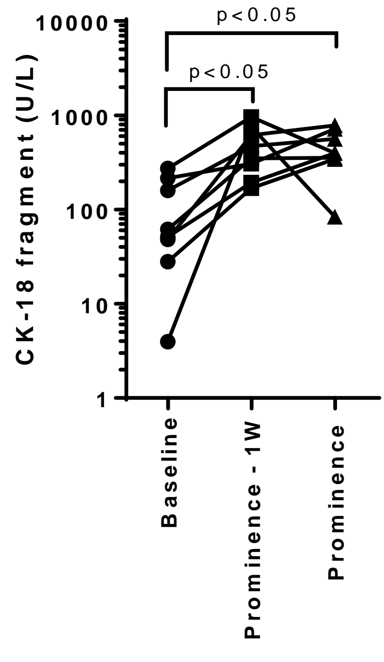


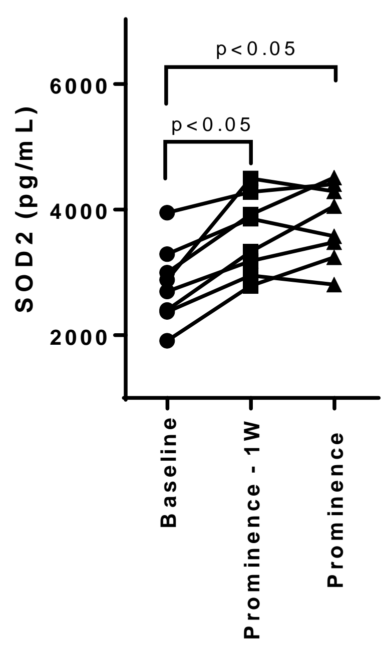
Figure 2



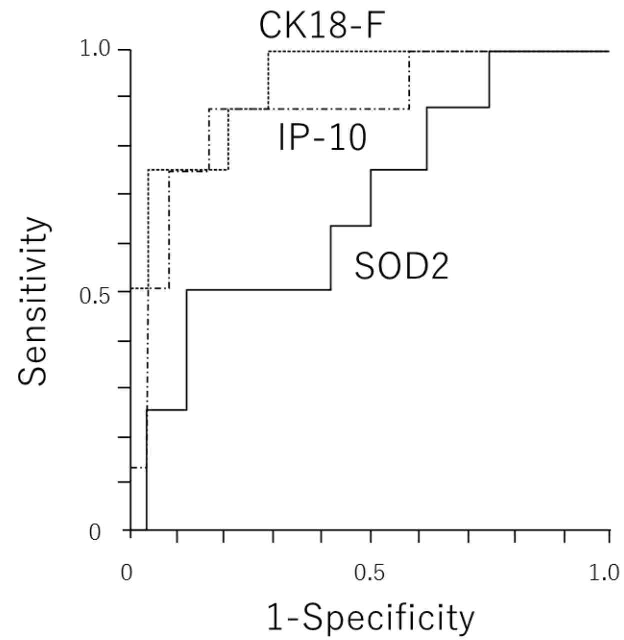








Supplemental Figure



	AUROC	Cut-off	Sensitivity	Specificity	Accuracy
CK18-F	0.927	307.1	0.750	0.958	0.857
IP-10	0.869	2396.7	0.875	0.833	0.636
SOD2	0.672	3853.4	0.500	0.875	0.571

1 Table 1. Subjects' characteristics in the present study.

	DILI	non-DILI	cont.	p
Age (y.o.)	71.0 ± 4.9	71.4 ± 5.7	71.8 ± 5.8	n.s.
Gender (M:F)	3:5	3:5	3:5	n.s.
CH: LC	7:1	7:1		n.s.
HCV-RNA[a]	6.08 ± 0.34	6.16 ± 0.42		n.s.
number of weeks until baseline	4.6 ± 0.7	4.6 ± 0.7		n.s.
number of weeks until Prominence	8.5 ± 2.6			
ALT (IU/L)				
before treatment	30.0 ± 8.3	35.4 ± 20.8		n.s.
at baseline	16.6 ± 5.8	16.0 ± 6.0	19.6 ± 5.1	n.s.
at Prominence-1W	25.0 ± 2.2			
at Prominence	95.2 ± 77.3			

2

3 Abbreviations: Drug-induced liver injury, DILI; control, cont.; p value, p; years old,
4 y.o.; not significant, n.s.; male to female ratio, M:F; chronic hepatitis, CH; liver
5 cirrhosis, LC; alanine aminotransferase, ALT.

6

7 [a]log copies/mL

8

1 Table 2. Comparison of serum cytokine/chemokine, mitochondrial marker, and cell
2 death marker levels among DILI, non-DILI and control groups at baseline.

	normal range		DILI			non-DILI			cont.			p
PDGF-BB	12794-31441	pg/mL	3075.3	±	877.3	3334.1	±	1163.6	5984.9	±	923.2	n.s.
IL-1β	0-6	pg/mL	28.1	±	6.2	27.5	±	7.3	29.7	±	4.5	n.s.
IL-1RA	136-323	pg/mL	31.4	±	6.9	31.7	±	8.0	34.6	±	5.5	n.s.
IL-2	0-3	pg/mL	43.9	±	14.0	40.4	±	8.7	43.4	±	5.5	n.s.
IL-4	13-51	pg/mL	95.2	±	30.4	98.3	±	39.8	120.6	±	26.6	n.s.
IL-5	0-6	pg/mL	17.8	±	2.6	18.5	±	4.6	19.5	±	3.7	n.s.
IL-6	8-20	pg/mL	53.5	±	17.4	45.9	±	11.7	46.0	±	7.6	n.s.
IL-7	14-52	pg/mL	30.9	±	7.0	29.6	±	6.6	29.8	±	3.6	n.s.
IL-8	15-48	pg/mL	122.6	±	127.6	75.9	±	47.1	57.8	±	7.0	n.s.
IL-9	34-86	pg/mL	113.9	±	42.0	93.4	±	20.6	105.3	±	19.9	n.s.
IL-10	0-2	pg/mL	62.6	±	10.6	63.1	±	13.3	61.0	±	12.9	n.s.
IL-12	13-55	pg/mL	85.1	±	31.4	79.1	±	21.4	103.8	±	30.2	n.s.
IL-13	0-17	pg/mL	70.6	±	52.2	44.3	±	10.0	43.1	±	10.7	n.s.
IL-17	91-228	pg/mL	158.6	±	49.9	150.3	±	44.4	191.9	±	33.0	n.s.
Eotaxin	105-342	pg/mL	151.2	±	89.0	133.0	±	47.7	119.3	±	45.4	n.s.
FGF-b	4-62	pg/mL	61.3	±	12.0	56.6	±	11.4	70.3	±	10.6	n.s.
GCSF	19-126	pg/mL	54.7	±	14.0	54.0	±	11.4	58.8	±	10.2	n.s.
GMCSF	0-0	pg/mL	121.3	±	39.8	103.1	±	50.3	96.7	±	21.6	n.s.
IFN-γ	136-822	pg/mL	31.0	±	8.8	29.4	±	7.2	33.1	±	7.1	n.s.
IP-10	1098-2616	pg/mL	1617.5	±	525.8	1581.8	±	804.9	510.8	±	204.1	**
MCP-1	22-67	pg/mL	127.3	±	62.1	121.7	±	39.0	128.8	±	48.4	n.s.
MIP-1a	0-15	pg/mL	48.3	±	18.6	54.8	±	35.7	50.8	±	15.9	n.s.
MIP-1b	112-194	pg/mL	681.4	±	390.8	560.2	±	328.3	514.1	±	271.8	n.s.
RANTES	5734-14124	pg/mL	14545.0	±	2300.6	15760.5	±	2891.3	18469.9	±	832.6	**
TNF-α	0-16	pg/mL	31.8	±	4.0	31.8	±	6.5	35.5	±	5.8	n.s.
VEGF	94-322	pg/mL	201.0	±	104.8	184.2	±	60.2	236.9	±	94.6	n.s.
SOD2	2083-3081	pg/mL	2811.0	±	626.8	3033.0	±	595.2	3551.0	±	649.0	n.s.
CK-18F	126-190	U/L	105.1	±	98.1	93.4	±	63.3	186.9	±	126.1	n.s.

3
4 **: $p<0.05$, DILI vs. cont.
5 Abbreviations: Drug-induced liver injury, DILI; control, cont.; platelet-derived growth
6 factor, PDGF; interleukin, IL, fibroblast growth factor, FGF; granulocyte-colony
7 stimulating factor, GCSF; granulocyte macrophage colony-stimulating factor, GMCSF;

1 interferon, IFN; interferon-inducible protein, IP; monocyte chemoattractant protein,
2 MCP; macrophage inflammatory protein, MIP; regulated on activation, normal T cell
3 expressed and secreted, RANTES; tumor necrosis factor, TNF; vascular endothelial
4 growth factor, VEGF; superoxide dismutase-2, SOD2; cytokeratin-18 fragment,
5 CK-18F.
6

1 Table 3. Risk factors associated with DILI development at baseline

Factor	Odds ratio	95% confidence interval	p value
IP-10	1.00	0.999-1.002	n.s.
ALT	0.87	0.621-1.216	n.s.
RANTES	0.99	0.998-0.999	$p<0.05$
Age	0.90	0.712-1.146	n.s.

2

3 Abbreviations: interferon-inducible protein, IP; not significant, n.s.; regulated on
4 activation, normal T cell expressed and secreted, RANTES; alanine aminotransferase,
5 ALT.
6
7

1 Table 4. Serial comparisons of serum cytokine/chemokine, mitochondrial marker, and
2 cell death marker levels in patients with DILI.

	normal range		baseline			Prominence-1W			Prominence			p
PDGF-BB	12794-31441	pg/mL	3075.3	±	877.3	3853.4	±	712.7	4232.8	±	1326.7	**
IL-1β	0-6	pg/mL	28.1	±	6.2	31.2	±	11.5	38.6	±	22.2	n.s.
IL-1RA	136-323	pg/mL	31.4	±	6.9	32.5	±	9.8	36.3	±	8.6	n.s.
IL-2	0-3	pg/mL	43.9	±	14.0	44.4	±	12.1	53.1	±	15.7	n.s.
IL-4	13-51	pg/mL	95.2	±	30.4	95.3	±	33.6	106.6	±	36.1	n.s.
IL-5	0-6	pg/mL	17.8	±	2.6	19.8	±	5.7	20.1	±	4.6	n.s.
IL-6	8-20	pg/mL	53.5	±	17.4	65.1	±	31.7	76.3	±	61.0	n.s.
IL-7	14-52	pg/mL	30.9	±	7.0	31.6	±	8.7	34.4	±	6.5	n.s.
IL-8	15-48	pg/mL	122.6	±	127.6	138.0	±	110.0	151.3	±	203.5	n.s.
IL-9	34-86	pg/mL	113.9	±	42.0	115.2	±	40.7	124.7	±	49.6	n.s.
IL-10	0-2	pg/mL	62.6	±	10.6	70.3	±	16.5	73.3	±	15.5	n.s.
IL-12	13-55	pg/mL	85.1	±	31.4	94.9	±	44.8	102.8	±	48.4	n.s.
IL-13	0-17	pg/mL	70.6	±	52.2	47.1	±	16.0	53.1	±	18.7	n.s.
IL-17	91-228	pg/mL	158.6	±	49.9	160.1	±	42.0	189.1	±	48.8	n.s.
Eotaxin	105-342	pg/mL	151.2	±	89.0	141.8	±	66.6	148.0	±	67.6	n.s.
FGF-b	4-62	pg/mL	61.3	±	12.0	63.4	±	14.3	72.9	±	14.7	n.s.
GCSF	19-126	pg/mL	54.7	±	14.0	52.6	±	12.2	59.1	±	11.7	n.s.
GMCSF	0-0	pg/mL	121.3	±	39.8	133.6	±	49.5	157.0	±	55.8	n.s.
IFN-γ	136-822	pg/mL	31.0	±	8.8	31.8	±	9.9	36.3	±	9.6	n.s.
IP-10	1098-2616	pg/mL	1617.5	±	525.8	2927.9	±	1627.4	2650.6	±	1082.5	*, **
MCP-1	22-67	pg/mL	127.3	±	62.1	124.8	±	28.3	143.5	±	50.4	n.s.
MIP-1a	0-15	pg/mL	48.3	±	18.6	136.5	±	253.9	354.4	±	831.2	n.s.
MIP-1b	112-194	pg/mL	681.4	±	390.8	1114.3	±	920.3	1405.8	±	1590.5	n.s.
RANTES	5734-14124	pg/mL	14545.0	±	2300.6	14260.8	±	3445.4	15680.3	±	2444.7	n.s.
TNF-α	0-16	pg/mL	31.8	±	4.0	34.1	±	9.1	39.8	±	10.8	**
VEGF	94-322	pg/mL	201.0	±	104.8	239.9	±	148.3	263.5	±	161.7	n.s.
SOD2	2083-3081	pg/mL	2960.1	±	648.6	3711.5	±	573.2	3951.5	±	465.6	*, **
CK-18F	126-190	U/L	108.8	±	95.3	468.0	±	294.2	442.2	±	233.6	*, **

3
4 *: p<0.05, baseline vs. Prominence-1W; **: p<0.05, baseline vs. Prominence
5 Abbreviations: Drug-induced liver injury, DILI; platelet-derived growth factor, PDGF;
6 interleukin, IL; fibroblast growth factor, FGF; granulocyte-colony stimulating factor,

1 GCSF; granulocyte macrophage colony-stimulating factor, GMCSF; interferon, IFN;
2 interferon-inducible protein, IP; monocyte chemoattractant protein, MCP; macrophage
3 inflammatory protein, MIP; regulated on activation, normal T cell expressed and
4 secreted, RANTES; tumor necrosis factor, TNF; vascular endothelial growth factor,
5 VEGF; superoxide dismutase-2, SOD2; cytokeratin-18 fragment, CK-18F.
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