Bimodal Peaks of Liver Stiffness in a Case of Drug-Induced Liver Injury

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

gamma-glutamyltransferase (γ-GTP), shear wave velocity (SWV), total bilirubin (T-BIL), virtual touch tissue quantification (VTQ), white blood cell (WBC)

CONSENT

Written informed consent was obtained from the patient for the publication of this case report and any accompanying images. A copy of the written consent form is available for review from the Editor of this journal.

Abstract

A 69-year-old male complained of general fatigue and presented with elevation of liver enzymes without any cause of liver injury. We diagnosed him with hepatocellular drug-induced liver injury (DILI). Liver stiffness, which was evaluated according to the shear wave velocity (SWV) using virtual touch tissue quantification, was serially observed during hospitalization. A fast SWV was noted on the date of admission, indicating a "hard" degree of liver stiffness. The SWV gradually decreased until the 20th hospital day. However, the patient's liver enzymes again became elevated on the 20th hospital day, and the SWV simultaneously increased in association with a rise in the total bilirubin level. The laboratory data for the second peak of the SWV indicated mixed-type DILI; therefore, the patient's pathological state transitioned from the hepatocellular type to the mixed type. A liver biopsy performed before discharge revealed a state of recovery from acute inflammation without fibrotic changes. We conclude that the second peak of the SWV may be affected by the presence of intrahepatic cholestasis. We herein report the occurrence of bimodal peaks of liver stiffness in a patient with DILI. In such cases, each peak of liver stiffness

may be the result of a different pathological mechanism, i.e., acute inflammation versus acute intrahepatic cholestasis. Although the detailed mechanisms underlying the development of liver stiffness due to intrahepatic cholestasis remain unclear, this case presented a limitation of virtual touch tissue quantification for evaluation of liver stiffness as fibrosis marker in the liver with intrahepatic cholestasis.

Introduction

Liver biopsies are the gold standard for evaluating the degree of liver fibrosis, which affects the selection of the therapeutic strategy in patients with chronic hepatitis (1). The degree of liver stiffness measured using virtual touch tissue quantification (VTQ) with Acoustic Radiation Force Impulse (ARFI) technology reflects the grade of liver fibrosis in chronic liver disease patients; therefore, the level of liver stiffness has received attention as a new parameter of liver fibrosis (2, 3). The degree of liver stiffness measured using VTQ is presented as the shear wave velocity (SWV). Severe liver fibrosis, which indicates a high level of liver stiffness, manifests as faster SWV values. Patients with various types of liver disease have been evaluated for liver stiffness, the results of which have demonstrated that other causes, in addition to fibrosis, affect the amount of liver stiffness. For example, one patient with congestive liver presented with a “hard” degree of liver stiffness. The liver stiffness in that case recovered following successful treatment of the congestive liver (4, 5). Furthermore, patients with acute liver failure and/or acute hepatitis also exhibit a high degree of liver stiffness (6-8). Intriguingly, one surviving patient with acute liver failure demonstrated a

serial recovery in liver stiffness (7). These results suggest that inflammation and/or congestion influence the development of liver stiffness. However, whether acute intrahepatic cholestasis increases the amount of liver stiffness remains unclear, as most patients with intrahepatic cholestasis, such as those with primary biliary sclerosis, also present with sustained inflammation and/or fibrosis in the liver. In contrast, familial intrahepatic cholestasis presents as “pure” intrahepatic cholestasis that is irreversible. Therefore, it is difficult to assess the simple effects of acute intrahepatic cholestasis without inflammation on the degree of liver stiffness.

We herein report the occurrence of bimodal peaks of liver stiffness in a case of drug-induced liver injury (DILI). In the present case, there was evidence of fibrosis in the first biopsy specimen obtained from the liver. However, only mild elevation of transaminase was noted around the second peak of liver stiffness. Therefore, the second peak of liver stiffness may have been affected by acute intrahepatic cholestasis as well as the persistence of fibrotic changes and mild inflammation. Although the degree of liver stiffness can be influenced by complicated mechanisms, including fibrosis and inflammation, the presence of acute intrahepatic cholestasis can also

increase liver stiffness. Therefore, liver stiffness evaluated by ARFI has a limitation in the patients with acute intrahepatic cholestasis. Accumulation of these data will provide new insight of influence of intrahepatic cholestasis to liver stiffness.

Case report

A 69-year-old male who had been treated for prostate cancer at another hospital for two months visited our facility for a further evaluation of icterus detected by the patient himself. He complained of general fatigue and icterus in the conjunctiva of the eyes and presented with elevation of liver enzymes without any cause of liver injury, such as obstructive jaundice, viral infection or autoantibodies (Supplemental figure 1 and Table 1). Based on a Roussel Uclaf Causality Assessment Method score of 10 and a Japan Digestive Disease Week score of 8, we diagnosed him with DILI (Table 2). Considering the laboratory data on admission, the patient was classified as having the hepatocellular type of DILI (Tables 1 and 3). Figure 1 shows the clinical course observed in the present case. Following the discontinuation of anti-cancer agents for prostate cancer, including flutamide and lupulin, the levels of alanine aminotransaminase (ALT) and alkaline phosphatase (ALP) decreased. However, the ALT level became slightly elevated from the 12th to the 21st hospital day, and a liver biopsy was performed to confirm the liver pathology on the 23rd hospital day. In accordance with the biopsy results, treatment with glycyrrhizin was started on the 23rd hospital day

(Supplemental figure 2). Although the ALT level decreased on the 25th hospital day, the ALP and total bilirubin levels gradually increased until the 32nd hospital day. After the 32nd hospital day, the ALP levels remained high until the 41st hospital day. The patient's liver injury was reassessed on the 34th hospital day, and he was classified as having the mixed type of DILI. The total bilirubin level peaked on the 30th hospital day, after which the total bilirubin levels gradually decreased without treatment with glycyrrhizin. The patient was subsequently discharged on the 70th hospital day after a second liver biopsy confirmed a recovery from the liver injury (Supplemental figure 2). The first liver biopsy had revealed inflammation of the liver with the expansion of fibrosis in the portal area and short fibrotic septa. In contrast, the second liver biopsy demonstrated regeneration of hepatocytes without fibrotic changes (Supplemental figure 2).

In this case, liver stiffness was serially observed from admission to discharge using virtual touch tissue elastography (Figure 1). The SWV values were obtained by the same examiner using an Acuson S2000 scanner with a 4.5-MHz convex-type probe (Siemens Medical Solutions, Mountain View, CA) throughout the patient's clinical course. The region of interest was

set in the area 2 cm from the surface of liver segment 5, through the intercostal space. The SWV was measured six times consecutively. The mean SWV value, excluding outliers, was regarded to be the final liver stiffness measurement. The degree of liver stiffness, as measured according to the SWV, gradually decreased by the 20th hospital day. However, the level of liver stiffness increased again in association with repeat elevation of the total bilirubin level on approximately the 21st hospital day. The peak of the newly increased SWV was observed on the 42nd hospital day.

Discussion

Organ stiffness is one tissue characteristic that can be evaluated using ultrasound (9). This modality has been reported to be effective for distinguishing patients with different grades of liver fibrosis (10, 11). However, the degree of liver stiffness has also been evaluated in patients with various liver diseases, which revealed that liver stiffness is affected by causes other than fibrotic changes, such as inflammation and congestion (6-8). Therefore, liver stiffness is caused by multiple factors.

Intrahepatic cholestasis is often detected in patients with liver diseases, including congenital metabolic disorder, live cirrhosis, acute liver injury and acute liver failure (12). However, the simple effects of intrahepatic cholestasis on the development of liver stiffness have not been thoroughly evaluated, as most patients with intrahepatic cholestasis also present with inflammation, fibrosis and/or other complications. Hence, whether acute intrahepatic cholestasis affects the degree of liver stiffness remains unclear.

DILI is classified into three patterns based on the laboratory data: the hepatocellular type, the cholestasis type and the mixed type (Table 3). The three patterns of DILI can manifest in one patient from the onset of

DILI to a cure. In such cases, the hepatocellular type is usually detected first, followed by the mixed type and the cholestasis type. Indeed, the present patient initially exhibited the hepatocellular type, then subsequently developed the mixed type during the course of hospitalization (Figure 1). The degree of liver stiffness in the present case was due to an acute reaction without chronic injury to the liver, as neither liver biopsy showed any evidence of fibrotic changes in liver specimen of second biopsy. A repeat increase in the SWV occurred around the 21st hospital day following repeat increases in the level of total bilirubin. Importantly, the elevation of the SWV that followed the repeat increase in the total bilirubin level gradually decreased in association with improvements in the levels of both total bilirubin and alkaline phosphatase. This finding demonstrates that the patient's acute intrahepatic cholestasis transiently affected the SWV.

Extrahepatic cholestasis-induced increases in liver stiffness have been previously described (13, 14). We hypothesize that intrahepatic cholestasis-induced SWV elevation involves the same mechanism as that observed in extrahepatic cholestasis-induced SWV elevation. Millonig et al. demonstrated that experimental bile duct ligation in pigs for 120 minutes

increases liver stiffness, while restoring the bile duct decreases liver stiffness; therefore, increased hydrostatic pressure in the liver due to an impaired bile flow results in an increase in liver stiffness (14).

We recognize that there are several possible limitations associated with this study. First, the initial liver biopsy revealed fibrotic expansion of the portal area with short fibrotic septa. These reparative changes may have persisted for a long period in this case. In addition, the ALT level observed on the 42nd hospital day remained high, although it was decreased compared to the previous values. Therefore, the second peak of SWV may have been the result of the complicated influences of several factors, including acute cholestatic changes, reparative fibrotic changes and/or liver injury. Because no histological findings were available for the date corresponding to the second peak of the SWV, we were unable to completely exclude the effects of these factors on the SWV values in this case. Furthermore, glycyrrhizin was used to treat the patient's DILI, which likely affected the ALT level. The effects of glycyrrhizin therapy on the SWV remain unclear, and the possible influence of this drug cannot be completely excluded in this case.

Although the effects of cholestasis on liver stiffness have not been

quantified as of yet, the present case suggests that acute cholestasis without inflammation affects the degree of liver stiffness. The present case therefore provides a limitation of VTQ at evaluation for liver stiffness as fibrosis marker in the patients with intrahepatic cholestasis. Further accumulation of evidence with respect to the SWV in patients with intrahepatic cholestasis is needed in order to enhance understanding regarding the detailed mechanisms underlying elevation of the SWV in the setting of intrahepatic cholestasis.

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FIGURE LEGENDS

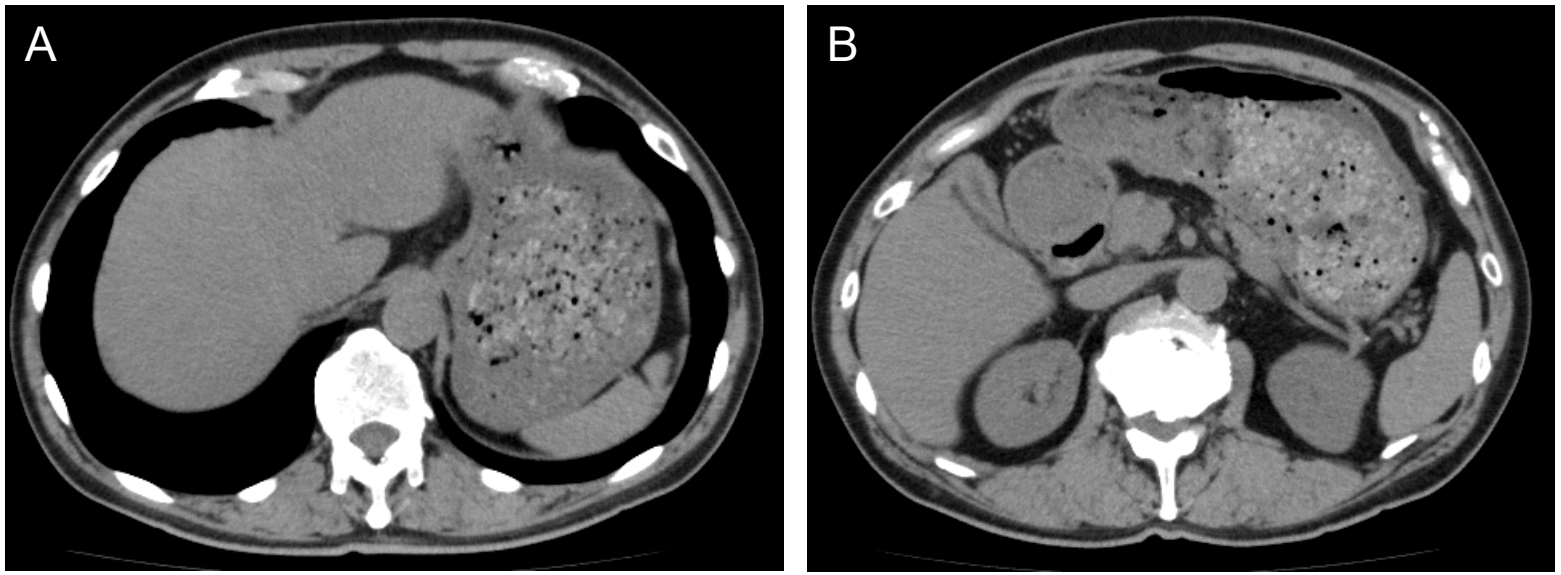
Figure 1. Time course of the laboratory data of the present patient with drug-induced liver injury. The upper line chart shows several biochemical parameters, including the levels of gamma-glutamyltransferase (γ-GTP), alkaline phosphatase (ALP), alanine aminotransferase (ALT) and total bilirubin (T-BIL). The lower line chart shows the share wave velocity (SWV) and prothrombin activity (PT). The bar chart indicates the duration of each disease type. The gray arrows show the dates of the liver biopsies.

Supplemental Figure Legends

Supplemental figure 1. Imaging findings of the present patient with drug-induced liver injury on the first hospital day. A and B: Abdominal computed tomography (CT) showed no evidence of obstructive jaundice or cholelithiasis.

Supplemental figure 2. Histological findings of the present patient with drug-induced liver injury on the 23rd and 68th hospital days. A, B, C and D: Liver specimens were obtained in the present case on the 23rd hospital day (A and B) and 68th hospital day (C and D). The microscopic findings are presented at x100 magnification. A and C: Liver specimens stained with hematoxylin and eosin. B and D: Liver specimens stained with silver impregnation.

Supplemental Figure. 1



Supplemental Figure. 2

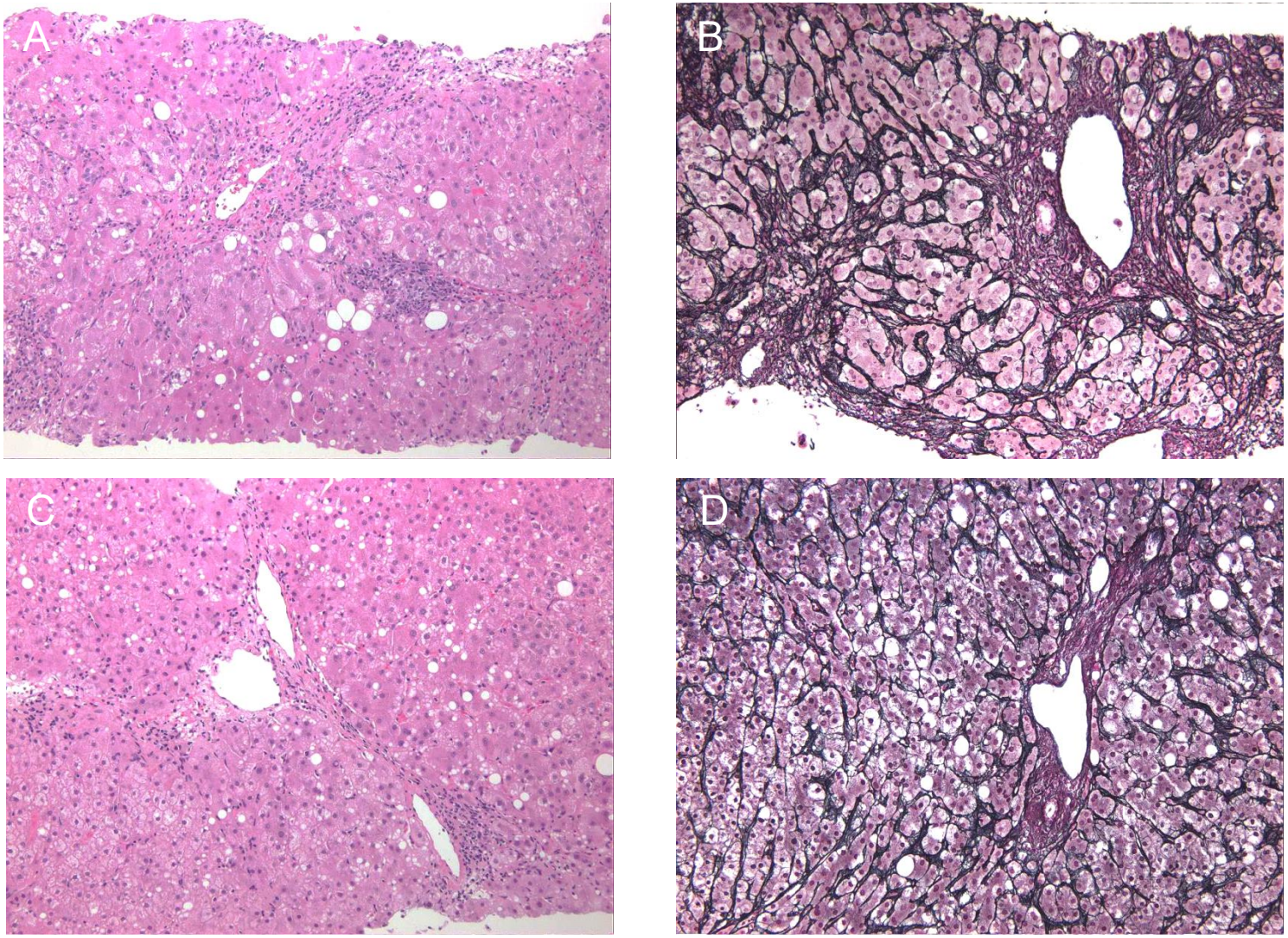


Figure. 1

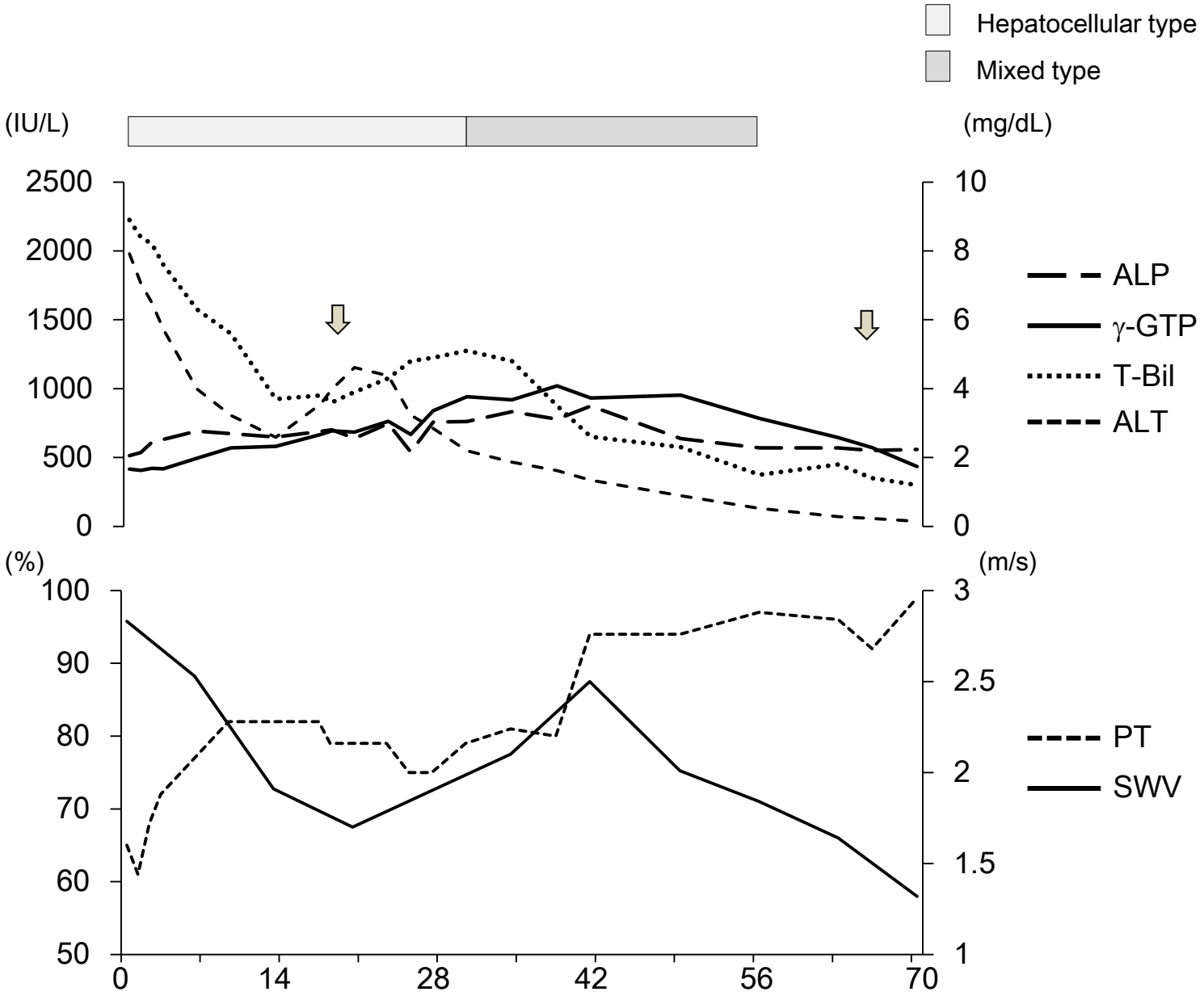

Hepatocellular type
Mixed type
(IU/L)
(mg/dL)
2500
2000
1500
1000
500
0
10
8
6
4
2
0
ALP
γ-GTP
T-Bil
ALT
(%)
(m/s)
100
90
80
70
60
50
3
2.5
2
1.5
1
PT
SWV
0
14
28
42
56
70

Table. 1 Laboratory data of the present patient with drug-induced liver injury on the first and 34th hospital days.

1st hospital day						34th hospital day					
Hematology			**Blood coagulation**			**Hematology**			**Blood coagulation**		
WBC	4.66	10^3/mL	PT	65	%	WBC	3.1	10^3/mL	PT	81	%
Neutro	56.4	%	HPT	54	%	Neutro	44	%	HPT	79	%
Lympho	32.5	%	Fib	643	mg/dL	Lympho	33.1	%	Fib	213	mg/dL
Mono	7.3	%	FDP	11.8	mg/mL	Mono	8	%	FDP	2.1	mg/mL
Eosino	1.2	%				Eosino	11.1	%			
Baso	0.4	%	**Virus markers**			Baso	3.8	%			
RBC	426	10^6/mL	HBsAg	(-)		RBC	447	10^6/mL			
Hb	13.3	g/dL	HCVAb	(-)		Hb	14.4	g/dL			
Plt	166	10^3/mL	IgM HA	(-)		Plt	369	10^3/mL			
			CMV IgM	(-)							
Blood chemistry			CMV IgG	(+)		**Blood chemistry**					
TP	6.9	g/dL	EBVCA IgG	(-)		TP	6.3	g/dL			
Albumin	3.9	g/dL	EBVCA IgM	(-)		Albumin	3.0	g/dL			
T-Bil	8.9	mg/dL	EBNA Ab	(-)		T-Bil	4.8	mg/dL			
D-Bil	6.1	mg/dL				D-Bil	3.8	mg/dL			
AST	1825	IU/L	**Autoantibodies**			AST	384	IU/L			
ALT	1979	IU/L	ANA	<x40		ALT	467	IU/L			
γ-GTP	416	IU/L	AMA	(-)		γ-GTP	833	IU/L			
ALP	514	IU/L				ALP	918	IU/L			
BUN	19.2	mg/dL	**Tumor markers**			BUN	18.2	mg/dL			
Cre	0.84	mg/dL	AFP	29.5	ng/mL	Cre	0.98	mg/dL			
AMY	63	IU/L				AMY	97	IU/L			
NH3	93	mg/dL				NH3	89	mg/dL			
CRP	0.46	mg/dL				CRP	0.26	mg/dL			

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; 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;; AFP, α-fetoprotein.

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	-	0
DLST	negative or unavailable	Negative	0
		Total	8

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

Table. 3 Type of disease in drug-induced liver injury

Hepatocellular type	ALT>2UNL + ALP<=UNL or ALT/ALP >= 5
Cholestasis type	ALT>2UNL + ALP>2UNL or ALT/ALP<=2
Mixed type	ALT>2UNL + ALP>UNL and 2<ALT/ALP<5

UNL, Upper normal limit