

[CASE REPORT]

Rapid Progression of Liver Fibrosis Induced by Acute Liver Injury Due to Immune-related Adverse Events of Atezolizumab

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Abstract:

A 72-year-old woman with advanced lung cancer had received systemic chemotherapy including atezolizumab. About three months after the initial administration of atezolizumab, her liver enzyme levels increased. The histopathological findings of the initial liver biopsy revealed acute inflammatory infiltrate, predominantly CD3⁺, CD4⁺ and CD8⁺ T lymphocytes, in the hepatic lobules. We diagnosed her with atezolizumab-induced immune-related acute hepatitis. Oral corticosteroid therapy successfully improved the elevation of serum aminotransferases. A sequential liver biopsy demonstrated the rapid progression of liver fibrosis. Because hepatocellular carcinoma occurs most often in advanced cases of chronic liver disease, we should pay close attention to immune-related acute hepatic injury when treating patients with advanced liver diseases using atezolizumab.

Key words: atezolizumab, autoimmune hepatitis, immune checkpoint inhibitor, immune-related adverse event, sequential liver biopsy, liver fibrosis

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Introduction

Immune checkpoint inhibitors (ICIs) are monoclonal antibodies targeting immune checkpoint molecules. ICIs target three main molecules: including programmed cell death receptor-1 (PD-1), programmed cell death ligand-1 (PD-L1), and cytotoxic T-lymphocyte-associated molecule-4 (CTLA-4). ICIs trigger an immune-mediated anti-tumor response by promoting the activation of cytotoxic T lymphocytes. ICIs have led to important clinical breakthroughs in the field of immunotherapy for the treatment of advanced malignancies and improved the overall survival (1).

Recently, the combination of atezolizumab, a humanized

monoclonal immunoglobulin (Ig) G1 antibody to the PD-L1, and bevacizumab, an anti-vascular endothelial growth factor (VEGF) antibody, has demonstrated efficacy and been adapted for the treatment of hepatocellular carcinoma (HCC) (2). Although ICIs have been proven to be highly effective for various malignancies, they sometimes induce significant immune-related adverse events (irAEs), which mimic autoimmune diseases, including hypothyroidism, adrenal insufficiency, thyroiditis, diabetes, enterocolitis and hepatitis. The mechanisms underlying the development of immune-related hepatitis have been presumed to be similar to those of autoimmune hepatitis (AIH) (3). However, the clinical features of immune-related hepatic injury induced by ICIs have yet to be fully clarified.

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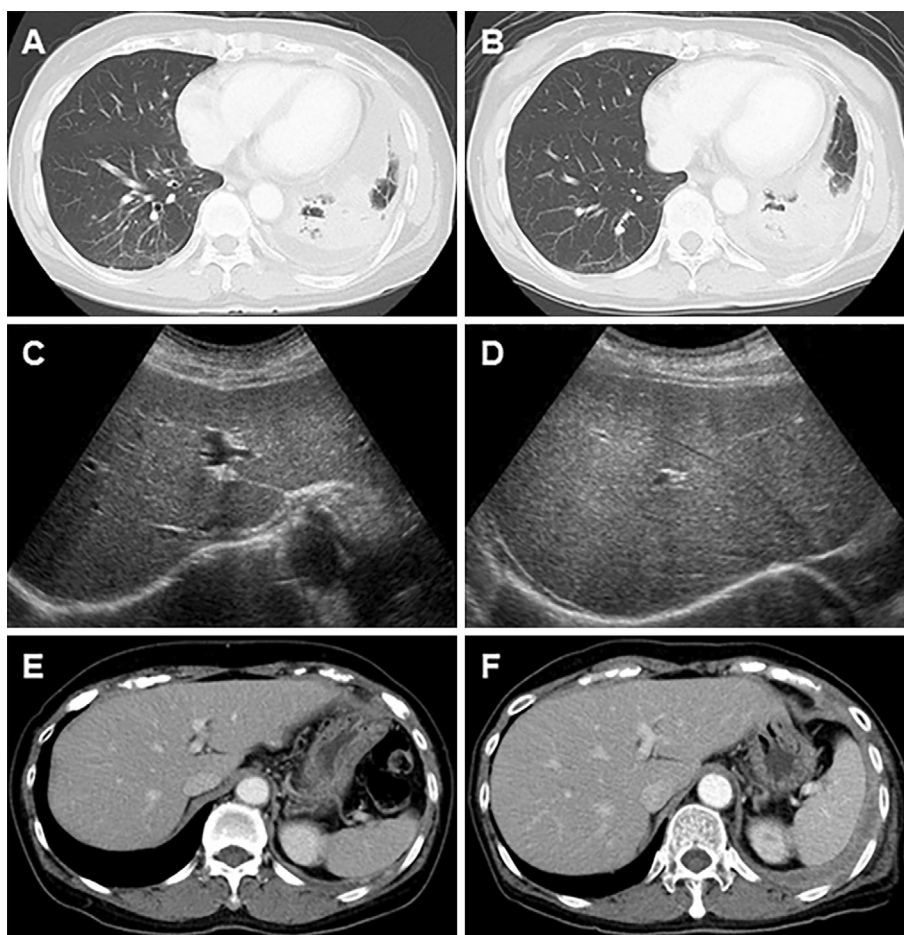


Figure 1. Imaging findings of computed tomography (CT) and abdominal ultrasonography of this patient. Chest CT demonstrated no marked changes in lung cancer by comparing the findings pre-treatment (A) and after three courses administration of atezolizumab in combination with chemotherapy (B). Abdominal ultrasonography (C, D) and contrast-enhanced-CT (F) on admission showed no liver metastasis of lung cancer. Hepatomegaly was evident on the comparison of CT findings at pre-treatment (E) and admission (F).

We herein report a Japanese patient with advanced non-small-cell lung cancer who developed acute hepatitis during treatment with the combination of atezolizumab plus bevacizumab, carboplatin and paclitaxel. Although corticosteroid therapy immediately improved the liver injury, comparing the histopathological findings between the liver biopsy at the diagnosis and two month later revealed the rapid progression of liver fibrosis.

Case Report

A 72-year-old Japanese woman was referred to our hospital due to left chest pain. She was diagnosed with non-small-cell lung cancer with pleural dissemination by a thorascopic biopsy (Fig. 1A). She had no history of acute or chronic liver diseases. She had never smoked and did not have a history of habitual alcohol consumption. There was no family history of liver diseases. Her body weight was 45.4 kg, and her height was 148.8 cm, giving a body mass index of 20.5 kg/m². She started intravenous atezolizumab (1,200 mg/body) in combination with bevacizumab (15 mg/

kg body weight), carboplatin and paclitaxel every four weeks as first-line systemic chemotherapy, receiving three courses of this regimen for three months. This regimen effectively controlled her advanced lung cancer with a Response Evaluation Criteria in Solid Tumors (RECIST) classification of stable disease (Fig. 1B), and no adverse events were observed after three courses chemotherapy.

However, at three months after the initiation of this chemotherapy, she developed liver injury. We stopped the administration of the combination of atezolizumab and other anti-cancer drugs and started treatment of ursodeoxycholic acid (UDCA) (300 mg/day). Because her liver injury gradually worsened, she was admitted to our department. A physical examination showed no remarkable abnormality. A laboratory examination (Table) showed elevated aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and gamma-glutamyl transpeptidase (γ -GTP). Hepatitis B virus (HBV) DNA and hepatitis C virus (HCV) antibody were negative. Antinuclear antibody (ANA) was positive (1:40) with a speckled pattern and slightly elevated IgG. On abdominal ultrasonography (US)

Table. Characteristics of This Patient on Admission.

Hematology		Serology	
WBC	7.0×10 ³ /μL	CRP	3.5 mg/dL
Neut	55.9 %		
Eos	4.3 %	Coagulation	
RBC	3.4×10 ⁶ /μL	PT	14.1 sec
Hb	9.4 g/dL	PT-%	75 %
Hct	29.3 %	PT-INR	1.12
Plt	33.3×10 ³ /μL		
Biochemistry		Immunology	
TP	7.1 g/dL	ANA	80×
Alb	3.2 g/dL	Homogeneous	(-)
T-Bil	0.6 mg/dL	Speckled	(+)
D-Bil	0.2 mg/dL	Centromere	(-)
AST	313 U/L	Nucleolar	(-)
ALT	208 U/L	Peripheral	(-)
LDH	295 U/L	Granular	(-)
ALP	1,075 U/L	Anti-LKM1	<5 index
γ-GTP	285 U/L	AMA	<20×
BUN	11 mg/dL	AMA M2	1.4
Cre	0.57 mg/dL	IgG	1,870 mg/dL
Na	140 mmol/L	IgM	111 mg/dL
K	4.1 mmol/L	IgG4	82.1 mg/dL
Cl	102 mmol/L	Tumor markers	
ChE	236 U/L	AFP	4.6 ng/mL
CPK	36 U/L	CEA	2.4 ng/mL
Fe	30 μg/dL	SLX	32.3 U/mL
Ferritin	701 ng/mL		
TSH	1.77 μIU/mL	Virus markers	
FT3	2.5 pg/mL	HBs Ag	(-)
FT4	1.19 ng/dL	HBs Ab	(+)
FPG	78 mg/dL	HBc Ab	(+)
HbA1c	5.6 %	HBV DNA	(-)
Hyaluronic acid	62 ng/mL	HCV Ab	(-)
Type IV collagen	134 ng/mL		
M2BPGi	2.29 COI	Urinalysis	
Autotaxin	0.727 mg/L	Protein	(±)

TP: total protein, Alb: albumin, T-Bil: total bilirubin, D-Bil: direct bilirubin, Ig: immunoglobulin, AST: aspartate aminotransferase, ALT: alanine aminotransferase, LDH: lactate dehydrogenase, ALP: alkaline phosphatase, γ-GTP: gamma-glutamyl transpeptidase, BUN: blood urea nitrogen, Cre: creatinine, ChE: cholinesterase, CPK: creatine phosphokinase, TSH: thyroid-stimulating hormone, FPG: fast plasma glucose, M2BPGi: Mac-2-binding protein glycosylation isomer, CRP: C-reactive protein, PT: prothrombin time, ANA: antinuclear antibody, LKM: liver kidney microsome, AMA: antimitochondrial antibody, Ig: immunoglobulin, AFP: alfa fetoprotein, CEA: carcinoembryonic antigen, SLX: Sialyl Lewis X, HBV: hepatitis B virus, HCV: hepatitis C virus

(Fig. 1C, D) and contrast-enhanced computed tomography (CT) (Fig. 1F) performed at admission, no liver metastasis was evident. CT demonstrated a swollen liver (Fig. 1F) compared to the findings before chemotherapy (Fig. 1E). Periportal edema or periportal color sign were not evident. The histopathological findings of the first liver biopsy revealed moderately acute inflammatory infiltrate and spotty necrosis within hepatic lobules and few plasma cells in the portal area with disrupted limiting plates (Fig. 2A), whereas liver fibrosis was limited (Fig. 2B). Immunostaining for im-

mune cell infiltration showed that CD3⁺, CD8⁺ and CD4⁺ T lymphocytes were predominantly observed (Fig. 3A-C), while CD20⁺ B lymphocytes were rare (Fig. 3D). We diagnosed her with immune-related acute hepatitis due to atezolizumab.

Although we recommended starting corticosteroid therapy immediately, she rejected the idea due to adverse effects. One month later, however, she accepted our proposal, and we started the oral administration of prednisolone 45 mg/day (1 mg/kg/day) and increased dose of UDCA (600 mg/

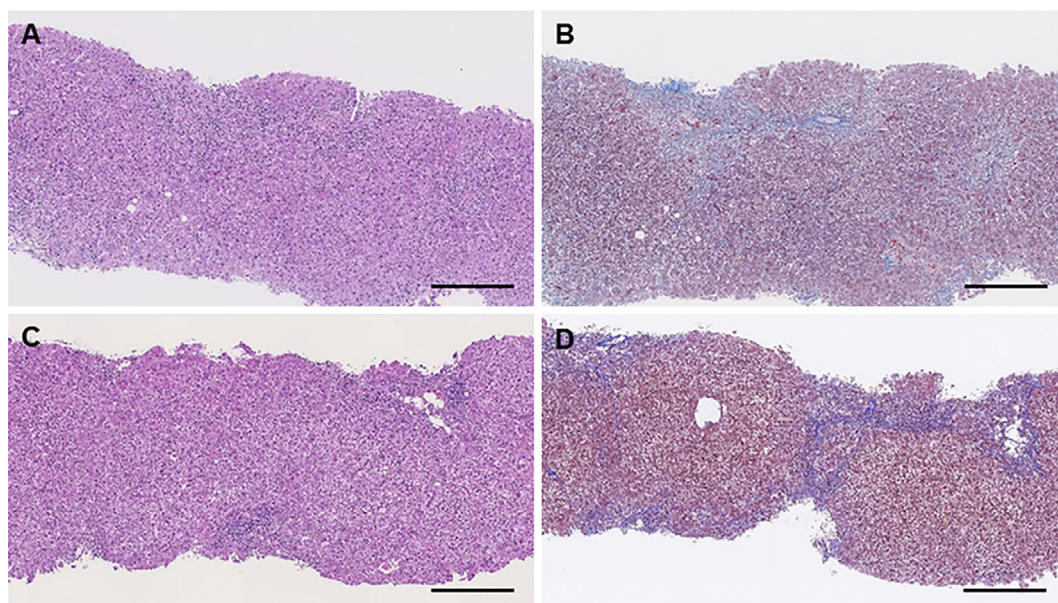


Figure 2. Histopathological findings of the liver biopsy specimen. The first liver biopsy (A, B) showed active pan-lobular hepatitis with moderate inflammatory infiltrate consisting of lymphocytes, macrophages and a few plasma cells in the portal area with disrupted limiting plates and spotty necroses within hepatic lobules. Scattered eosinophils were also seen. Liver fibrosis was limited. The second liver biopsy (C, D) demonstrated mild to moderate inflammatory infiltrate and fibrosis with bridging fibrosis associated with scattered intralobular spotty or focal necrosis. (A), (C) Hematoxylin and Eosin staining ($\times 100$). (B), (D) Masson-trichrome staining ($\times 100$). Scale bars: 500 μm .

day). Subsequently, the elevation of both serum aminotransferases and IgG immediately improved. ALP and γ -GTP were also improved after the administration of prednisolone (Fig. 4). The total bilirubin level increased to 3.2 mg/dL, and the prothrombin time (PT) decreased to 60.7% after the initiation of the chemotherapy. Corticosteroid therapy immediately improved both the total bilirubin and prothrombin time. Because no recurrence in the serum aminotransferase elevation was observed, we tapered the prednisolone dose.

One month after the initiation of corticosteroid therapy, we performed a second liver biopsy. This liver biopsy showed that the inflammatory infiltrate in hematoxylin and eosin staining (Fig. 2C) and immunostaining of CD3⁺, CD4⁺ and CD8⁺ T lymphocytes (Fig. 3E-G) had improved. Infiltrate of CD20⁺ was rare (Fig. 3H). In contrast, the liver fibrosis rapidly progressed, resulting in bridging fibrosis (Fig. 2D). Serum liver fibrosis markers, including hyaluronic acid (62 vs. 1,260 ng/mL), type 4 collagen (134 vs. 186 ng/mL), Mac-2-binding protein glycosylation isomer (M2BPGi) (2.29 vs. 3.15 COI) and autotaxin (0.72 vs. 0.94 mg/L), were higher at the second liver biopsy than at the first liver biopsy.

Discussion

Immune-related hepatitis induced by ICIs seems to be an infrequent but well-known irAE. It is often seen between 6 and 14 weeks following the initiation of ICIs but may occur at any time (4). The incidence of any-grade (grade 3-4) liver

injury was reported to be 1-10% (1-2%) in patients receiving an approved dose of anti-PD-1 and anti-CTLA-4 monotherapy (5, 6). In some cases, ICIs can lead to acute liver failure (7, 8).

Atezolizumab (Tecentriq[®]) is an anti-PD-L1 antibody initially approved for non-small-cell lung cancer and urothelial carcinoma by the Food and Drug Administration (FDA) in 2016. Liver injury was shown to occur in 8-10% (grade 3-4 in 1-2%) of patients in the clinical trials of atezolizumab in combination with chemotherapy in advanced lung cancer (9, 10). The safety profile of atezolizumab in combination with anti-cancer agents was consistent with the known adverse events related to single-agent atezolizumab, and no new adverse events were observed (9). A phase 3 clinical study revealed benefit to the overall and progression-free survival rates by the combination of atezolizumab and bevacizumab in unresectable HCC (11). Following the results of this study, atezolizumab became the first clinically approved ICI in the treatment of advanced HCC. The incidence of any-grade (grade 3-4) increases in AST and ALT in this clinical trial was 19.0% and 14.0% (7.0% and 3.6%), respectively (11). The incidence of liver injury in HCC therefore seems to be more frequent than in other cancers. Treatment of bevacizumab in combination with anti-cancer agents except for ICIs has not been considered to be associated with liver injury (12). The incidence of elevation of aminotransferases in bevacizumab therapy for advanced HCC was shown to be 38% (grade 1-2) and 13% (grade 3-4) (13). VEGF has been reported to have immune-

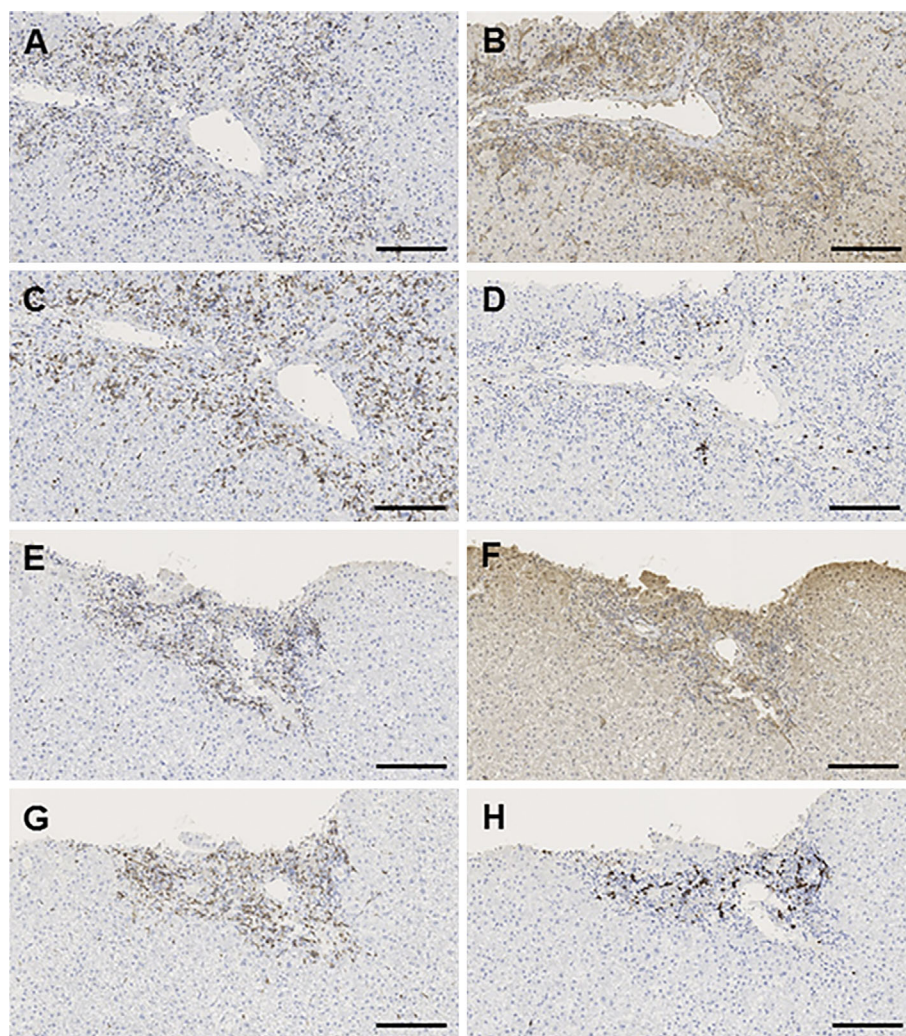


Figure 3. Immunostaining of CD3⁺ (A), CD4⁺ (B), CD8⁺ (C) and CD20⁺ (D) of the first liver biopsy ($\times 200$) and CD3⁺ (E), CD4⁺ (F), CD8⁺ (G) and CD20⁺ (H) of the second liver biopsy ($\times 200$). Scale bars: 200 μ m.

suppressive effects, such as downregulating T cell activation and increasing myeloid-derived suppressor cells and regulatory T cells in the tumor microenvironment (14). Because anti-VEGF treatment is considered to enhance the immunomodulating effects of anti-PD-L1 therapy, the combination of atezolizumab plus bevacizumab may increase irAEs, including immune-related hepatic injury.

AIH is an immune-mediated inflammatory liver disease characterized by autoantibodies and hypergammaglobulinemia. In contrast, immune-related hepatitis due to ICIs apparently has immune-mediated mechanisms, although most patients do not develop conventional autoantibodies. The ANA titer and extent of IgG elevation in immune-related hepatitis was reported to be lower than in classical AIH (15). In the present case, ANA was mildly positive with a low titer (1:40-1:80), and serum IgG was slightly elevated.

The histopathological features of immune-related hepatitis have been reported, with anti-CTLA-4 inducing active panlobular hepatitis with inflammatory infiltrate consisting predominantly of lymphocytes and anti-PD-1 exhibiting lobular hepatitis with mild portal inflammation and scattered necro-

sis (16). In the present patient, the histopathological findings revealed acute inflammatory infiltrate within the hepatic lobules and interface hepatitis, but plasma cells were few. These findings were compatible with the previously reported immune-related hepatitis (17). We also evaluated the immune cell infiltration by immunostaining including T lymphocytes (CD3⁺), cytotoxic T lymphocytes (CD8⁺), helper T lymphocytes (CD4⁺) and B lymphocytes (CD20⁺). CD3⁺, CD4⁺ and CD8⁺ were predominantly observed, as previously reported (18). Serum bilirubin and the biliary enzymes ALP and γ -GTP were also elevated in this case. We previously reported secondary sclerosing cholangitis due to an irAE by pembrolizumab (19). However, neither bile duct dilation on abdominal US or CT nor histological cholestatic changes on a liver biopsy were detected.

Immune-related hepatitis usually resolves within four to six weeks after stopping ICIs but is occasionally prolonged. The effectiveness of corticosteroid therapy has been demonstrated (20). Corticosteroid therapy can allow the continuation of treatment with ICIs, but rare cases have been reported to be refractory to steroids (21). In our case, corti-

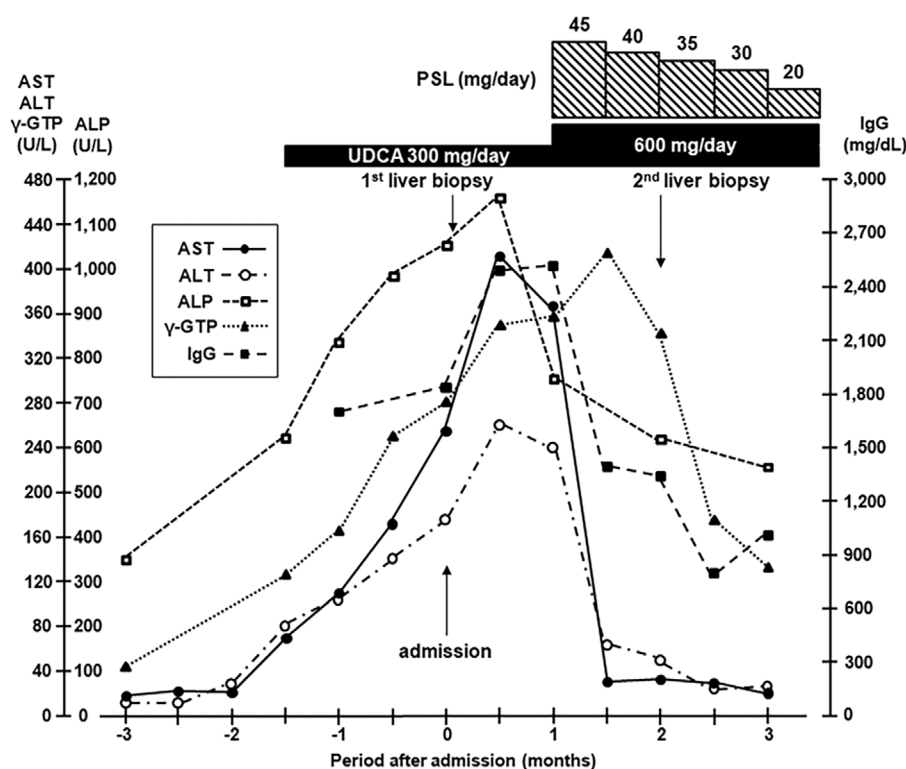


Figure 4. Clinical course of the present case. Treatment with 300 mg/day ursodeoxycholic acid (UDCA) was started at 1.5 months before the first liver biopsy. Because the liver injury worsened, the first liver biopsy was performed for the histopathological diagnosis. One month later, oral prednisolone (PSL) 45 mg/day (1 mg/kg/day) was started, and the dose of UDCA was increased (600 mg/day). After starting PSL, the elevation of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and immunoglobulin (Ig) G immediately improved. Serum alkaline phosphatase (ALP) and gamma-glutamyl transpeptidase (γ -GTP) levels also gradually decreased. Subsequently, oral PSL was tapered, and no recurrence in the elevation of serum AST, ALT, ALP or γ -GTP levels was observed. A second liver biopsy was performed one month after starting treatment with PSL.

costeroid therapy successfully improved the elevation of serum aminotransferases and IgG. Furthermore, the improvement in the histopathological findings of CD3⁺, CD4⁺ and CD8⁺ lymphocyte infiltration was demonstrated at the second liver biopsy performed one month after the initiation of corticosteroid therapy. Because atezolizumab and bevacizumab controlled the lung cancer in this patient well, we considered continuation of this combination with corticosteroid therapy favorable for this patient. Surprisingly, the second liver biopsy performed two months after the first one revealed the rapid progression of the liver fibrosis during this short term. Rapid progression to acute liver failure or hepatic decompensation has been demonstrated in some cases with AIH (22). However, a previous report suggested that immune-related hepatitis could induce liver fibrosis (17, 23). To our knowledge, few reports have described the progression of liver fibrosis due to immune-related acute hepatitis by sequential paired liver biopsies, except for two case reports involving treatment with nivolumab (23) and pembrolizumab (17). Because immune-related hepatitis can start off as asymptomatic or with minimal symptoms (most commonly elevation of AST and ALT with mild elevation of total bilirubin), a histopathological assessment by a liver bi-

opsy seems necessary in addition to careful monitoring of liver enzymes. Serum liver fibrosis markers, such as hyaluronic acid, type 4 collagen, M2BPGi and autotaxin are also helpful for assessing the progression of liver fibrosis.

In conclusion, immune-related hepatitis due to atezolizumab can induce rapid liver fibrosis progression despite improvements in the liver injury by corticosteroid therapy. Even in cases of immune-related acute hepatitis, a liver biopsy should be considered for evaluating the severity of liver fibrosis. HCC occurs most often in advanced chronic liver diseases, such as liver cirrhosis. We should therefore pay closer attention to immune-related hepatic injury during the treatment of HCC using ICI than during treatment of other malignancies.

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

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