

## Case and Review

# Intrahepatic Bile Duct Injury as a Hepatic Immune-Related Adverse Event after Immune-Checkpoint Inhibitor Treatment

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## Keywords

Immune-checkpoint inhibitors · Immune-related adverse events · Cholangitis · Cytokeratin-7 · Mycophenolate mofetil

## Abstract

The increased use of immune-checkpoint inhibitors to treat various types of cancer has increased the incidence of immune-related adverse events (irAEs). Hepatic irAEs are frequent and can lead to serious conditions. Among the various types of hepatic irAEs reported to date, bile duct injury has been shown refractory to steroid treatment. This study describes 2 patients with hepatic irAEs manifesting as intrahepatic bile duct injury. Immunostaining with antibodies to both CD8 and cytokeratin-7 was useful for the diagnosis, and both patients were refractory to steroid treatment. Prompt diagnosis and active immunosuppressive therapies are required in such cases.

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## Introduction

Immune-checkpoint inhibitors (ICIs) have been approved for the treatment of various malignant tumors [1]. Although ICIs inhibit tumor growth by blocking the transmission of immunosuppressive signals, such as the binding of program death-1 (PD-1) and program

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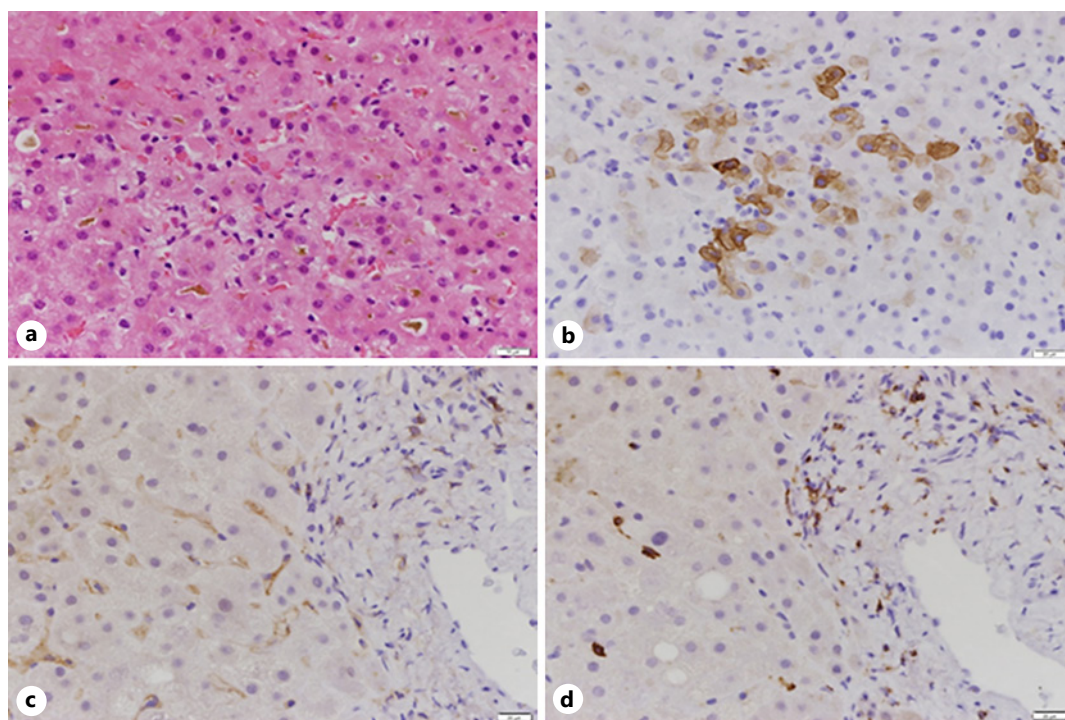
death-ligand 1 (PD-L1) or the binding of cytotoxic T-lymphocyte antigen-4 (CTLA-4) with CD28-ligands (CD28L), these agents induce various immune-related adverse events (irAEs) in various organs [2]. For example, hepatic irAEs have been reported in 1–16% of patients administered ICIs and can lead to serious conditions [3, 4]. Depending on the degree of liver damage, hepatic irAEs can be treated with immunosuppressive agents such as prednisolone (PSL) and other immunosuppressive [5]. The present study describes 2 patients who experienced severe cholestatic liver injury after treatment with ICIs. Both patients showed injury to intrahepatic small bile ducts, with these injuries being refractory to steroid administration. One patient recovered after treatment with mycophenolate mofetil (MMF). The literature of patients who experienced hepatic irAE manifesting as bile duct damage is discussed.

## Case Reports

### *Patient 1*

The patient was an 83-year-old man who had undergone laparoscopic left nephrectomy for advanced left renal cell carcinoma. He was found to have pleural metastasis 1 month after the operation and was treated with 4 cycles of the anti-PD-1 antibody nivolumab and the anti-CTLA-4 antibody ipilimumab for 12 weeks. Disease progression was observed, and the patient was started on second-line treatment with pazopanib. Blood tests 4 weeks later showed a marked elevation of liver enzymes, including an aspartate aminotransferase (AST) concentration of 980 IU/L (normal range 12–31 IU/L), an alanine transaminase (ALT) concentration of 714 IU/L (normal range 8–40 IU/L), an alkaline-phosphatase (ALP) concentration of 2004 IU/L (normal range 100–310 IU/L), and a total bilirubin concentration of 4.02 mg/dL. He was treated with ursodeoxycholic acid (300 mg/day) and PSL (1.0 mg/kg/day), but his liver function and jaundice did not improve. One week later, he was referred to our hospital. At admission, he showed prolonged severe cholestatic liver injury, including AST 903 IU/L, ALT 1045 IU/L, ALP 3083 IU/L, total bilirubin 20.0 mg/dL, and direct bilirubin 15.6 mg/dL and elongation of prothrombin time (PT) (PT-%, 45%, PT-INR 1.60). Serological examination showed no evidence of hepatitis A, B, C, or E virus infection and no evidence of infection with other viruses, such as Epstein-Barr virus and cytomegalovirus (CMV). His serum immunoglobulin G concentration was within the normal range, and he was negative for antinuclear and anti-mitochondrial antibodies. Computed tomography and ultrasonography showed no evidence of abnormalities in his biliary tracts. He was started on steroid-pulse therapy with methylprednisolone (1,000 mg/day for 3 days) and vitamin K supplementation. Although his PT activity improved to 100%, his cholestatic liver injury showed no improvements, with AST 358 IU/L, ALT 646 IU/L, ALP 2187 IU/L, total bilirubin 23.7 mg/dL, and direct bilirubin 18.0 mg/dL. A percutaneous liver biopsy was performed to evaluate his liver injury.

Liver biopsy showed infiltration of inflammatory cells, mainly lymphocytes, into the portal area, as well as interface hepatitis. Advanced fibrosis was not observed (shown in Fig. 1a). Cytokeratin (CK)-7 staining showed partial destruction of the intrahepatic bile duct and cholestasis around such destroyed bile duct, mimicking chronic nonsuppurative destructive cholangitis (shown in Fig. 1b). Immunostaining showed that most of the infiltrating lymphocytes were positive for CD8, with few positive for CD4 (shown in Fig. 1c, d). These findings suggested that an autoimmune mechanism induced by ICIs caused intrahepatic bile duct injury, leading to severe cholestasis. Although pazopanib-induced liver injury could not be completely ruled out, those histological findings and the timing of liver injury support a diagnosis of hepatic irAE. Because he was considered steroid refractory, a decision



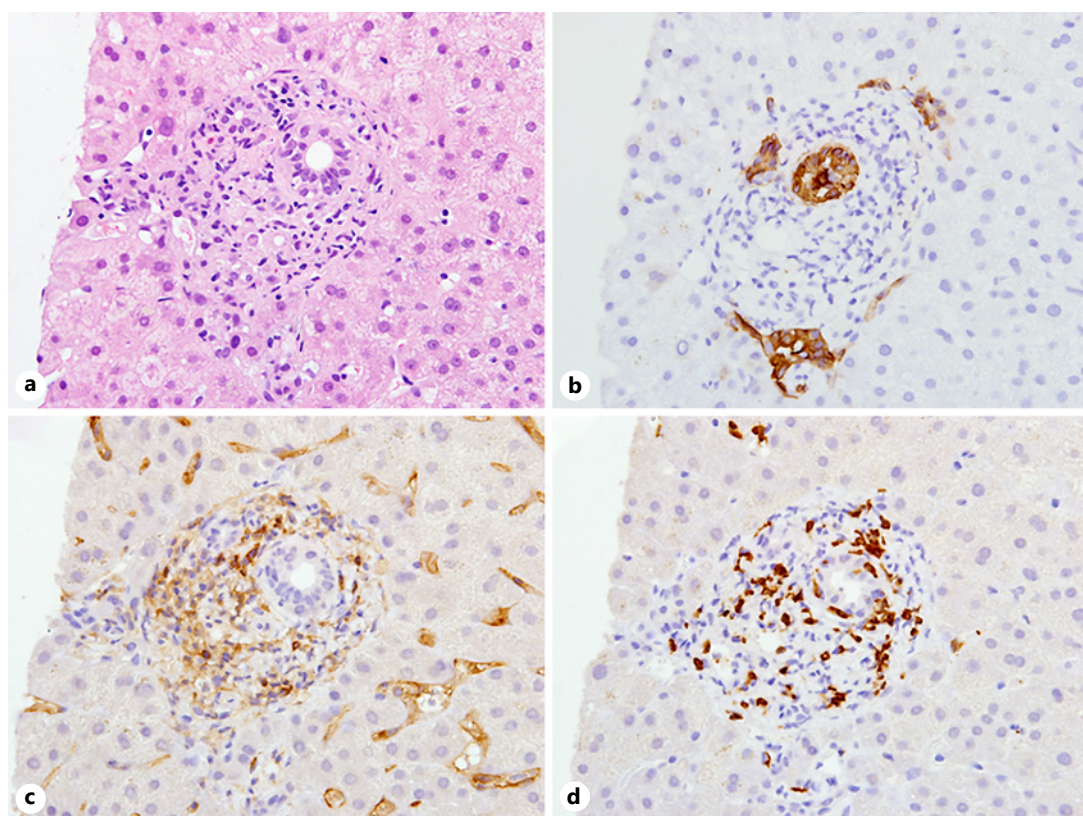
**Fig. 1.** Liver biopsy findings in Patient 1. **a** Hematoxylin-eosin staining, portal area.  $\times 200$ . **b** CK-7 staining.  $\times 200$ . **c** Azan staining.  $\times 40$ . **d** CD4 staining, nonspecific staining was found.  $\times 200$ . **e** CD8 staining.  $\times 200$ . CK, cytokeratin.

was made to treat him with MMF, and approval from the institutional review board of the Ethics Committee of our center was obtained. However, he developed CMV pneumonia, requiring treatment with ganciclovir for 2 weeks to control the CMV infection. After the control of CMV, he was started on MMF (500 mg/bid). However, his general condition already worsened markedly and his organ function deteriorated. Two days after starting MMF treatment, the patient died of multiple organ failures.

#### *Patient 2*

The patient was a 77-year-old man who had undergone resection of a malignant melanoma on his left foot 3 years earlier but had developed multiple lymph node recurrences. He was, therefore, treated with the anti-PD-L1 antibody pembrolizumab, which had shown good antitumor activity. Eleven months after pembrolizumab treatment, a blood examination revealed liver injury with jaundice, including AST 147 IU/L, ALT 263 IU/L, ALP 739 IU/L, and total bilirubin 3.8 mg/dL. He was serologically negative for viral hepatitis including hepatitis A, B, C, and E viruses and for other viruses such as Epstein-Barr virus and CMV. Administration of new drugs was not recognized during these several months. He was also negative for antinuclear and anti-mitochondrial antibodies. Abdominal ultrasonography showed no evidence of biliary obstructive changes. He was thereafter referred to our hospital and was tentatively diagnosed with hepatic irAE, for which he was treated with high-dose PSL (1.5 mg/kg/day). Five days after the steroid therapy, he showed prolonged liver injury, including AST 188 IU/L, ALT 483 IU/L, ALP 1265 IU/L, and total bilirubin 3.0 mg/dL. A US-guided percutaneous liver biopsy was performed to evaluate his liver injury, and after approval by the institutional review board of the Ethics Committee of our institution, he was started on MMF (500 mg/bid).





**Fig. 2.** Liver biopsy findings in Patient 2. **a** Hematoxylin-eosin staining.  $\times 100$ . **b** CK-7 staining.  $\times 100$ . **c** CD4 staining, nonspecific staining was found.  $\times 100$ . **d, e** CD8 staining.  $\times 100$ . CK, cytokeratin.

Liver biopsy showed the infiltration of inflammatory cells, mainly lymphocytes, into the portal area, along with interface hepatitis, but advanced fibrosis was not observed (shown in Fig. 2a). CK-7 staining showed that inflammation occurred mainly in the portal areas, and partial destruction of intrahepatic bile duct was also observed (shown in Fig. 2b). Immunostaining showed that most of the lymphocytes accumulating around the intrahepatic bile ducts were CD8-positive, not CD4-positive (shown in Fig. 2c, d). These findings suggested that an autoimmune mechanism induced by ICI caused intrahepatic bile ductal damage, leading to cholestatic liver injury. Two weeks after starting MMF, his liver injury had improved, with AST 33 IU/L, ALT 124 IU/L, ALP 456 IU/L, and total bilirubin 0.9 mg/dL. He was subsequently discharged, and PSL was gradually reduced. Eight weeks after MMF administration, his liver injury had normalized and MMF followed PSL were discontinued. His condition remained good, with no evidence of recurrence of liver injury.

## Discussion

ICIs that suppress interactions between PD-1 and PD-L1 or between CTLA-4 and CD28L have become widely used to treat cancer, increasing the incidence of irAEs associated with ICIs [1]. Hepatic irAE have been reported to occur 4–12 weeks after ICIs administration [4]. Rates of liver dysfunction with a single ICI were found to be 6.4% for nivolumab and 7.1% for ipilimumab. However, the incidence of all grade liver dysfunction in patients treated with a combination of nivolumab and ipilimumab combination therapy was 30% and rates of severe

liver injury (Grade 3 or higher) was as high as 18.8% [2, 3], indicating that stronger immunosuppression could lead to more and more severe irAEs.

The patterns of liver damage from hepatic irAEs vary from hepatocellular type to cholangitis type, with ICIs recently reported to induce various types of cholangitis. A review of patients with hepatic irAEs, including cholangitis, showed the occurrence of cholangitis at various levels of the bile ducts, from the extrahepatic to the intrahepatic bile ducts (Table 1) [6–11]. Histopathological findings include the infiltration of mostly CD8-positive T cells around Glisson's capsule [7, 10, 11]. Liver biopsy from both of our patients showed interface hepatitis with intrahepatic bile duct injury and infiltration predominantly by CD8 positive cells. Although the intrahepatic bile duct could not be clearly found in Patient 1 by hematoxylin-eosin staining, however, CK-7 staining showed damage to the intrahepatic bile duct. CK-7 staining was shown effective in evaluating intrahepatic bile ductal damage in patients with severe damage, such as patients with vanishing bile duct syndrome [9]. Both CD8 and CK-7 staining should be considered in evaluating patients with hepatic irAEs showing cholestatic liver injury.

Guidelines regarding treatment for hepatic irAEs have suggested that patients with Grade 4 liver dysfunction be treated with a high-dose steroid such as PSL 2 mg/kg. Patients who do not improve after 3 days of high-dose PSL should be switched to treatment with MMF [12]. ICI-induced cholangitis is a serious adverse event with an inadequate response to steroid treatment [6, 9]. Moreover, liver injury with cholangitis due to ICIs is regarded as a hepatic irAE [12], suggesting that hepatic irAEs with cholestasis be managed more cautiously. Imaging methods are required to evaluate extrahepatic bile ductal changes and liver biopsy should also be considered.

Steroid treatment is not recommended for patients with chronic cholestatic liver diseases, such as primary biliary cholangitis (PBC) and primary sclerosing cholangitis due to adverse events. In contrast, budesonide, a steroid almost completely metabolized during its first pass in the liver, may be effective in patients with cholestatic liver disease [13, 14]. Furthermore, immunosuppressants such as MMF, cyclosporine, and azathioprine are not recommended for patients with PBC or primary sclerosing cholangitis [13]. Budesonide has been shown effective in patients with early stage PBC but not in cirrhotic patients [14]. Nonspecific immunosuppressive treatment may be effective only in patients with early stage cholestatic liver diseases but may be harmful in patients with later stage diseases due to adverse events. Previous studies and the present report suggest that patients with severe damage to the bile ducts who develop organic changes such as vanishing bile duct are refractory to immunosuppressive treatment and that this condition could be fatal. Earlier MMF administration may have improved the clinical outcome in Patient 1 of the present study. Early diagnosis and positive interventions are important for improving the prognosis of patients with hepatic irAE and cholangitis, preventing organic damage to the bile ducts.

In conclusion, this study described 2 patients with hepatic irAE with cholestasis. Immunostaining with antibodies to both CD8 and CK-7 may be useful for the diagnosis of this condition. Prompt and active immunosuppressive treatment should be considered for these patients.

### Statement of Ethics

Written informed consent for publication was obtained from the patient's wife and son in Patient 1 and from the patient in Patient 2. Our work was approved by our institutional clinical Ethics Committee (Clinical Ethics Committee of Toyama University Hospital, Approved number: MKTY2019001).

**Table 1.** Treatment and the response in reported cases with hepatic irAE showing cholangitis

Case	Age	Sex	Pattern of cholangitis	Steroid (/day), mg/kg	Additional treatment	Biliary drainage	Improvement	Ref
1	81	F	EHD, IHD	mPSL 2	MMF 1 g/day	None	Yes	[6]
2	75	M	NE	mPSL 2	None	Yes	No	[6]
3	55	M	EHD, IHD	mPSL 2	MMF 1 g/day	Yes (EST)	Yes	[6]
4	82	F	EHD, IHD	mPSL 2	None	None	Yes	[6]
5	64	M	EHD	PSL 0.5	None	None	Yes	[7]
6	73	F	EHD	PSL 0.5	None	Yes	Yes	[7]
7	82	F	EHD	None	None	Yes	Yes	[7]
8	63	M	EHD	PSL 2	None	Yes	Yes	[8]
9	49	F	IHD (VBDS)	PSL 1	MMF 1 g/day	None	No	[9]
10	59	F	IHD	PSL 1	None	None	Yes	[9]
11	76	M	IHD (partial VBDS)	PSL 1	MMF 500 mg	None	Partially improved	[9]
12	79	M	IHD	mPSL 1	None	None	Yes	[10]
13	76	M	EHD, IHD	PSL 0.5	None	None	Yes	[11]
14	83	M	IHD	mPSL 20	MMF 1 g/day	None	No	Our case
15	77	M	IHD	PSL 1.5	MMF 1 g/day	None	Yes	Our case

irAE, immune-related adverse event; F, female; M, male; MMF, mycophenolate mofetil; F, female; M, male; EHD, extrahepatic duct; IHD, intrahepatic duct; NE, not evaluated; VBDS, vanishing bile duct syndrome; mPSL, methyl prednisolone; BD, biliary drainage; EST, endoscopic sphincterotomy; PSL, prednisolone.

## Conflict of Interest Statement

The authors declare that they have no conflicts of interest.

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## Author Contributions

M.A. and T.K. wrote this paper, and all the authors contributed to the patient's medical treatment.

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