

Clinical Features and Outcomes Based on Liver Injury Patterns in Liver Injury Caused by Immune Checkpoint Inhibitors

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Abstract

Background

The clinical course of liver injury induced by immune checkpoint inhibitors (ICIs) varies among individuals, and there were few reports on the therapeutic effects of corticosteroids based on the patterns of liver injury.

Methods

We evaluated the characteristics and clinical course of immune-related liver injury in 1087 patients treated with ICIs for advanced malignancies between August 2014 and December 2020.

Results

During the follow-up period (median, 270 days), 56 patients (5.2%) had immune-related liver injury (\geq Grade 3). The liver-injury patterns were hepatocellular ($n = 25$, 44.6%), mixed ($n = 10$, 17.9%), or cholestatic ($n = 21$, 37.5%), and the median time to onset of liver injury was 36, 85, and 53 days, respectively; the hepatocellular pattern occurred earlier than the other types ($P = 0.036$). Corticosteroids were administered to 29 (51.8%) patients. While liver injury was improved in almost all patients with the hepatocellular pattern ($n = 13/14$, 92.9%), that failed to show improvement in over half of the patients with the non-hepatocellular patterns (mixed, $n = 8$; cholestatic, $n = 7$), and three patients with mixed patterns needed secondary immunosuppression with mycophenolate mofetil. Liver biopsies performed in 13 patients mainly showed lobular injury, endothelialitis, and spotty necrosis with infiltration of T cells positive for CD3 and CD8, but not CD4 or CD20.

Conclusion

The incidence pattern and therapeutic response to corticosteroids in immune-related liver injury differs according to the injury type. Although corticosteroids were effective for the hepatocellular pattern, an additional strategy for refractory non-hepatocellular patterns is needed.

Introduction

The number of patients exposed to immune checkpoint inhibitors (ICIs), including anti-programmed cell death 1 (PD-1), programmed cell death ligand 1 (PD-L1), and anti-cytotoxic T-lymphocyte antigen 4 (CTLA-4) antibodies, for advanced malignancies has increased over the past few years ^{1–3}. Although ICIs are generally well tolerated, they have the ability to disrupt the immune system, which can generate unique side effects that mimic autoimmune conditions, termed “immune-related adverse events” (irAEs) ⁴. IrAEs can affect various organs, but are most often found in the skin, gastrointestinal tract, and endocrine system ⁵. Immune-related liver injury caused by ICIs is a relatively rare irAE; however, it can lead to patient mortality ⁶. The appearance of severe immune-related liver injury should be followed by prompt

withdrawal of ICIs. Systemic administration of corticosteroids is used as the first-line therapy for immune-related liver injury, while second-line immunosuppressants are necessary in steroid-refractory cases ⁷.

Recently, we reported that severe immune-related liver injury (\geq Grade 3) occurred in 29 patients (5.3%) in an analysis of 546 patients treated with ICIs, and the median period between the initial administration of ICIs and the incidence of irAEs was 52 days ⁸. In addition, more than half of the patients with immune-related liver injury showed a cholestatic and mixed pattern, not hepatocellular. Some patients were resistant to steroid therapy, but the detailed clinical courses of immune-related liver injury are not well known.

The aim of this retrospective study was to examine the clinical features and response to immunosuppressive treatment based on liver injury patterns in patients with liver injury caused by ICIs.

Methods

Study population

We retrospectively collected clinical data from 1087 patients with advanced malignancies who were treated between August 2014 and December 2020 with PD-1, PD-L1, or anti-CTLA-4 agents as monotherapy or in combination with an anti-CTLA-4 agent at Nagoya University Hospital and Ogaki Municipal Hospital (Nagoya University Hospital, n = 660; Ogaki Municipal Hospital, n = 427). We reviewed the detailed clinical course of patients with immune-related liver injury, which was defined as liver injury induced by ICIs (liver-irAE) in this article. The study protocol was carried out in accordance with the Declaration of Helsinki and was approved by the Institutional Review Boards of both Nagoya University Hospital and Ogaki Municipal Hospital (no. 2018 – 0438, 15006).

Diagnosis of immune-related liver injury

We evaluated the patients' general condition carefully with blood tests at intervals of least 3 weeks after the start of ICI administration to assess the incidence of side effects, including immune-related liver injury. The severity of irAEs was assessed using the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) version 5.0. We checked no evidence of hepatitis A, B, or C virus infection, and no other potential causes of liver disease (i.e., massive alcohol consumption [> 80 g/day], other hepatotoxic drug use, autoimmune hepatitis, primary biliary cirrhosis, or hemochromatosis). In addition, we performed ultrasonography, contrast-enhanced computed tomography, or magnetic resonance imaging to exclude liver injury induced by liver metastasis and bile duct obstruction. The patterns of liver injuries were defined as follows on the basis of previous reports ^{9,10}: (i) hepatocellular pattern, alanine aminotransferase (ALT) level alone is elevated ≥ 5 -fold above the upper limit of normal (ULN) or the ratio of serum activities (expressed as a multiple of ULN) of ALT and alkaline phosphatase (ALP) is ≥ 5 ; (ii) cholestatic pattern, ALP level alone is elevated ≥ 2 -fold above the ULN or the ratio of serum activities of ALT and ALP is ≤ 2 ; (iii) mixed pattern, the ratio of the serum activities of ALT and ALP was > 2 and < 5 .

We generally followed the Society's guidelines for the management of immune-related liver injury^{5,7}. These guidelines suggest that the immediate start of corticosteroids should be considered for severe liver-irAEs (Grades 3 and 4). However, some cases showed spontaneous improvement without the introduction of treatment. A recent review also suggested corticosteroid treatment if there was no improvement after a few days of follow-up after the withdrawal of ICIs¹¹. Therefore, for patients with Grade 3 liver injury, we performed careful follow-up for one week after the appearance of the injury and started oral prednisone 0.5-1.0 mg/kg/day if there was no improvement. For Grade 4 liver injury, steroid pulse with methylprednisolone was started immediately, followed by treatment with prednisone 1.0–2.0 mg/kg/day. The use of ursodeoxycholic acid (UDCA) was considered if obstructive jaundice could be ruled out in cases of cholestatic or mixed-type disease. We defined "improvement" as recovery to within normal levels or baseline levels before the start of ICI treatment. A part of patients who could not achieve "improvement" included best supportive care (BSC) for original disease progression after the occurrence of immune-related liver injury.

Assessment of pathological findings

Percutaneous liver biopsy was performed with a 16- to 17-gauge needle under ultrasonographic guidance, and liver biopsy specimens were immediately fixed in 10% formalin and embedded in paraffin. An adequate liver biopsy sample was defined as a specimen with a length of > 1.5 cm. Pathological slides were reviewed by a single pathologist without knowledge of the clinical data.

Statistical analysis

Values are expressed as median (first-third interquartile) and number (%). We used the chi-square test to compare categorical variables, and the Wilcoxon rank-sum test or Kruskal–Wallis test to compare continuous variables between two or three groups (hepatocellular, mixed, and cholestatic liver injury groups). For all tests, statistical significance was defined as $p < 0.05$. We used the lower or upper limits of the reference values at Nagoya University Hospital and Ogaki Municipal Hospital as the cut-off values for laboratory data. Statistical analyses were performed using EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria)¹².

Results

Clinical characteristics of patients with severe immune-related liver injury based on the type of liver injury

Table 1 shows the clinical characteristics of patients with immune-related liver injury (\geq Grade 3), which was noted in 56 patients during the follow-up period (median, 270 days). The median age was 64 years, and the study population had a predominance of men (55.4%). The patterns of liver injuries were hepatocellular ($n = 25$, 44.6%), mixed ($n = 10$, 17.9%), and cholestatic ($n = 21$, 37.5%). Age, sex, tumor type, ICI type, previous history of chemotherapy, and incidence of irAEs other than liver injury did not vary

significantly among the three groups. The median number of doses from the first ICI administration to liver injury was 2, 4, and 3 ($P = 0.045$), and the median time to onset of liver injury was 36, 85, and 53 days ($P = 0.036$) in the hepatocellular, mixed, and cholestatic types, respectively. Thus, the hepatocellular pattern occurred earlier than the other types. Notably, baseline liver enzyme levels did not affect the incidence of each type of liver injury, and almost all patients showed normal immunoglobulin G (IgG) levels and negative results for anti-nuclear antibodies (86.0%) and anti-mitochondrial M2 antibodies (95.3%).

Table 1
Patient characteristics of the study based on liver injury type (\geq Grade 3) induced by immune checkpoint therapy

Factors	Total	Live injury types			P value
	n = 56	hepatocellular	mixed	cholestatic	
		25 (44.6)	10 (17.9)	21 (37.5)	
Age (years) *	64 [55, 70]	66 [55, 72]	60 [51, 69]	63 [58, 67]	0.651
Sex, n (%)					0.417
Female	25 (44.6)	13 (52.0)	5 (50.0)	7 (33.3)	
Male	31 (55.4)	12 (48.0)	5 (50.0)	14 (66.7)	
Tumor type					0.513
Lung cancer	21 (37.5)	6 (24.0)	5 (50.0)	10 (47.6)	
Malignant melanoma	13 (23.2)	6 (24.0)	3 (30.0)	4 (19.0)	
Renal cell carcinoma	12 (21.4)	7 (28.0)	1 (10.0)	4 (19.0)	
Head and neck carcinoma	3 (5.4)	1 (4.0)	0 (0.0)	2 (9.5)	
Others	7 (12.5)	5 (20.0)	1 (10.0)	1 (4.8)	
ICIs type					0.210
anti-PD-1 agents	33 (58.9)	11 (44.0)	6 (60.0)	16 (76.2)	
anti-PD-L1 agents	9 (16.1)	5 (20.0)	2 (20.0)	2 (9.5)	

P values are for comparison among liver injury types only.

*Values are expressed as * median (first-third interquartiles) or ** median (range), and number (%).*

ICI, immune checkpoint inhibitor ; PD-1, anti-programmed death receptor 1; CTLA-4, cytotoxic T-lymphocyte-associated antigen 4; irAE, immune-related adverse events; AST, aspartate aminotransferase; ALT, alanine transaminase; GGT, gamma glutamyl transferase; ALP, alkaline phosphatase; T-BIL, total bilirubin; IgG, immunoglobulin G; PSL, prednisolone; UDCA, ursodeoxycholic acid; MMF, Mycophenolate mofetil.

Factors	Total	Live injury types			<i>P</i> value
anti-CTLA-4 agents	7 (12.5)	3 (12.0)	2 (20.0)	2 (9.5)	0.724
anti-PD-1 + CTLA-4 agents	7 (12.5)	6 (24.0)	0 (0.0)	1 (4.8)	
ICI treatment					
1st. line	48 (85.7)	22 (88.0)	9 (90.0)	17 (81.0)	0.045
≥ 2nd. line	8 (14.3)	3 (12.0)	1 (10.0)	4 (19.0)	
The number of dose from first ICI administration to liver injury ** (times)	2 [1–15]	2 [1–6]	4 [1–6]	3 [1–15]	
Days between ICIs and liver injury (≥ Grade 3) ** (days)	49 [1–473]	36 [1–158]	85 [29–473]	53 [4–414]	0.036
irAE other than liver injury					0.580
present	20 (35.7)	8 (32.0)	5 (50.0)	7 (33.3)	0.868
absent	36 (64.3)	17 (68.0)	5 (50.0)	14 (66.7)	
AST (baseline) * (U/L)	20 [16, 24]	20 [16, 24]	20 [16, 21]	20 [16, 25]	
ALT (baseline) * (U/L)	15 [11, 22]	15 [13, 23]	16 [11, 21]	15 [9, 22]	0.837
GGT (baseline) * (U/L)	34 [22, 49]	28 [19, 45]	28 [20, 47]	38 [31, 54]	0.133
ALP (baseline) * (U/L)	252 [213, 300]	223 [197, 264]	251 [225, 302]	292 [231, 344]	0.051

P values are for comparison among liver injury types only.

*Values are expressed as * median (first-third interquartiles) or ** median (range), and number (%).*

ICI, immune checkpoint inhibitor ; PD-1, anti-programmed death receptor 1; CTLA-4, cytotoxic T-lymphocyte-associated antigen 4; irAE, immune-related adverse events; AST, aspartate aminotransferase; ALT, alanine transaminase; GGT, gamma glutamyl transferase; ALP, alkaline phosphatase; T-BIL, total bilirubin; IgG, immunoglobulin G; PSL, prednisolone; UDCA, ursodeoxycholic acid; MMF, Mycophenolate mofetil.

Factors	Total	Live injury types			P value
T-bil (baseline) * (mg/dL)	0.5 [0.4, 0.6]	0.5 [0.4, 0.7]	0.5 [0.4, 0.6]	0.4 [0.3, 0.6]	0.492
AST (at the time of liver injury) * (U/L)	227 [113, 336]	273 [169, 385]	280 [228, 362]	77 [51, 244]	0.002
ALT (at the time of liver injury) * (U/L)	248 [141, 470]	349 [223, 488]	330 [242, 497]	148 [52, 258]	0.006
GGT (at the time of liver injury) * (U/L)	244 [101, 580]	122 [75, 163]	433 [116, 968]	558 [391, 1069]	< 0.001
ALP (at the time of liver injury) * (U/L)	566 [361, 1351]	361 [322, 396]	1027 [644, 1412]	1840 [860, 2582]	< 0.001
T-bil (at the time of liver injury) * (mg/dL)	0.9 [0.6, 1.2]	0.7 [0.5, 1.0]	1.1 [0.6, 1.6]	1.0 [0.7, 1.3]	0.221
Eosinocytes (at the time of liver injury) * (%)	0.7 [0.00, 2.5]	0.9 [0.0, 2.4]	0.4 [0.0, 3.2]	1.0 [0.1, 4.0]	0.820
Anti-nuclear antibodies (n = 43)					0.380
negative	37 (86.0)	17 (81.0)	9 (100.0)	11 (84.6)	
positive	6 (14.0)	4 (19.0)	0 (0.0)	2 (15.4)	
Anti-mitochondrial M2 antibody (n = 43)					0.333
negative	41 (95.3)	19 (90.5)	7 (0.0)	15 (100.0)	
positive	2 (4.7)	2 (9.5)	0 (0.0)	0 (0.0)	

P values are for comparison among liver injury types only.

*Values are expressed as * median (first-third interquartiles) or ** median (range), and number (%).*

ICI, immune checkpoint inhibitor ; PD-1, anti-programmed death receptor 1; CTLA-4, cytotoxic T-lymphocyte-associated antigen 4; irAE, immune-related adverse events; AST, aspartate aminotransferase; ALT, alanine transaminase; GGT, gamma glutamyl transferase; ALP, alkaline phosphatase; T-BIL, total bilirubin; IgG, immunoglobulin G; PSL, prednisolone; UDCA, ursodeoxycholic acid; MMF, Mycophenolate mofetil.

Factors	Total	Live injury types			<i>P</i> value
Serum IgG level (n = 44) (at the time of liver injury) (g/L)	1219 [1058, 1424]	1331 [1130, 1458]	941 [740, 1411]	1116 [1049, 1293]	0.151
Treatment for liver-irAE					
PSL use					0.044
present	29 (51.8)	14 (56.0)	8 (80.0)	7 (33.3)	
absent	27 (48.2)	11 (44.0)	2 (20.0)	14 (66.7)	
UDCA use					0.007
present	12 (21.4)	1 (4.0)	5 (50.0)	6 (28.6)	
absent	44 (78.6)	24 (96.0)	5 (50.0)	15 (71.4)	
MMF use					0.001
present	3 (5.4)	0 (0.0)	3 (30.0)	0 (0.0)	
absent	53 (94.6)	25 (100.0)	7 (70.0)	21 (100.0)	
Follow-up period ** (days)	270 [20–1838]	278 [29–931]	190 [77–880]	301 [20–1838]	0.831
<i>P</i> values are for comparison among liver injury types only.					
<i>Values are expressed as * median (first-third interquartiles) or ** median (range), and number (%).</i>					
ICI, immune checkpoint inhibitor ; PD-1, anti-programmed death receptor 1; CTLA-4, cytotoxic T-lymphocyte-associated antigen 4; irAE, immune-related adverse events; AST, aspartate aminotransferase; ALT, alanine transaminase; GGT, gamma glutamyl transferase; ALP, alkaline phosphatase; T-BIL, total bilirubin; IgG, immunoglobulin G; PSL, prednisolone; UDCA, ursodeoxycholic acid; MMF, Mycophenolate mofetil.					

Sub-analysis of the incidence of immune-related liver injury

A recent report showed that irAEs involving multiple organ systems, called multisystem irAEs, differ from single irAEs in terms of the pattern of incidence and prognosis ¹³. Therefore, we evaluated the characteristics of immune-related liver injury coexisting with other irAEs. During follow-up, 20 patients (35.7%) developed multisystem irAEs, and 36 (64.3%) developed only immune-related liver injury

(Table 1). The most common irAEs other than liver irAEs in patients with multisystem irAEs were endocrine irAEs (n = 8: thyroid, n = 4; type 1 diabetes, n = 2; pituitary, n = 1; and adrenal gland; n = 1), gastrointestinal (GI) irAEs (n = 7: intestine, n = 5; pancreas, n = 2) (Table 2). The median number of days until the incidence of immune-related liver injury in patients with multisystem irAEs was significantly longer than that in those with only liver irAEs. However, the presence of multisystem irAEs did not affect the degree of liver injury (Supplementary Table 1).

Table 2
The other type of developed immune-related adverse events accompanied by liver injury

Type of developed irAE	N
Endocrine-irAEs	8
(Thyroid)	4
(Type1 diabetes)	2
(Pituitary)	1
(Adrenal gland)	1
Gasrtrointestinal-irAEs	7
(Intestine)	5
(Pancreas)	2
Lung-irAEs	3
Skin-irAEs	3
Arthro-irAE	1
Nerve-irAE	1
Total	23*
* Three cases had three irAEs	
irAE, immune-related adverse events;	

Next, we investigated the differences in characteristics in relation to the use of the anti-CTLA-4 antibody because administration of this antibody is known to be a risk factor for immune-related liver injury ^{8,14} (Supplementary Table 2). This analysis showed a significant difference in tumor types between the two groups because anti-CTLA-4 antibody treatment for malignant melanoma and renal cell carcinoma is covered by insurance in Japan. The incidence rate of multisystem irAEs in the group treated with the anti-CTLA-4 antibody was significantly higher than that in the group that was not treated with this antibody (*P*

= 0.022). Anti-CTLA-4 antibody use was also not associated with the degree of liver injury as well as the presence of multisystem irAEs.

Clinical course in patients with immune-related liver injury

We treated 29 patients (51.8%) with immune-related liver injury with corticosteroids (Supplemental Figure); we mainly treated the cases with hepatocellular- and mixed-type injuries, and only 30% of the cases with cholestatic-type injuries were treated with steroids (Table 1). Figure 1 shows the clinical course of patients with immune-related liver injury based on injury patterns. Importantly, almost all patients with hepatocellular-type injuries who received corticosteroids improved ($n = 13/14$, 92.9%); however, less than half of the patients with the non-hepatocellular patterns (mixed, $n = 4/8$ [50.0%]; cholestatic, $n = 3/7$ [28.6%]) who received corticosteroid treatment improved regardless of UDCA use. Three patients with mixed patterns needed secondary immunosuppression with mycophenolate mofetil (MMF) because of steroid resistance. They all once improved with initial corticosteroid, but underwent MMF therapy since they deteriorated during steroid tapering. Among them, only one patient subsequently showed improvement using MMF. Additionally, 18 patients (32.1%; hepatocellular, $n = 9$; mixed, $n = 2$; and cholestatic, $n = 7$) improved without treatment, including corticosteroids, and 3 patients with cholestatic type improved with UDCA alone (Fig. 1 and Supplementary Figure).

Liver pathological findings in patients with immune-related liver injury

In the present study, we performed a liver biopsy in 13 patients to confirm the diagnosis of immune-related liver injury induced by ICIs (hepatocellular, $n = 11$; mixed, $n = 1$; and cholestatic, $n = 1$). Table 3 shows a summary of the histologic features, and the detailed information is listed in Supplemental Table 3. The common histologic findings were lobular injury ($n = 11$, 84.6%), endothelialitis ($n = 10$, 76.9%), and spotty necrosis ($n = 13$, 100%) with infiltration of T cells positive for cluster of differentiation (CD) 3 and CD8, but not CD4 or CD20. Some cases showed the findings of granulomas, as well as the characteristics reported in previous studies^{15,16} (Fig. 2). On the other hand, infiltration of plasma cells, which is observed in autoimmune hepatitis (AIH), was rare ($n = 2$, 15.4%). Vascular endothelial damage and spotty necrosis with CD3- and CD8-positive T-cell infiltration were also observed in two cases with non-hepatocellular types, indicating that they may be common findings in liver-irAE independent of liver injury types (Supplementary Table 4).

Table 3
Pathological features in patients with immune-related liver injury (n = 13)

Findings				
Lobular injury	non to mild	2 (15.4%)	moderate to severe	11 (84.6%)
Portal inflammation	non to mild	5 (38.4%)	moderate	8 (61.5%)
Interface hepatitis	non to mild	9 (69.2%)	moderate	4 (30.7%)
Endothelialitis	non to mild	3 (23.1%)	moderate to severe	10 (76.9%)
Cholangitis	non to mild	6 (46.2%)	moderate	7 (53.8%)
Granulomas	absence	9 (69.2%)	presence	4 (30.7%)
Spotty necrosis	absence	0 (0%)	presence	13 (100%)
Fatty liver change	absence	11 (84.6%)	presence	2 (15.4%)
infiltrating inflammatory cells				
Neutrophil	absence	10 (76.9%)	presence	3 (23.1%)
Eosinophil	absence	8 (61.5%)	presence	5 (38.5%)
Plasma cells	absence	11 (84.6%)	presence	2 (15.4%)
immunostaining				
CD3 *	negative	0 (0%)	positive	11 (100%)
CD4 **	negative	8 (66.6%)	positive	4 (33.3%)
CD8 **	negative	0 (0%)	positive	12 (100%)
CD20 *	negative	8 (72.7%)	positive	3 (27.3%)
* n = 11, ** n = 12				
CD, cluster of differentiation, <i>Values are expressed as number (%)</i> .				

Discussion

ICIs are an essential breakthrough for the treatment of advanced malignancies, and the incidence of irAEs will continue to rise with the increasing use of ICIs for various types of cancers ^{4,17}. Immune-related liver injury is a type of irAE, along with an excessive immune response to one's own normal organs by ICIs. However, the detailed mechanism underlying immune-related liver injury is unclear, and the development process and severity show substantial heterogeneity. Few reports have focused on the outcomes of immune-related liver injury caused by immunosuppression therapy, including corticosteroids.

In this study, we estimated the characteristics and clinical course of immune-related liver injuries based on the patterns of liver injury. Our study showed that the hepatocellular pattern was the most common (44.6%), and it occurred earlier than the other types (mixed or cholestatic). The hepatocellular pattern showed good response to corticosteroids; however, some cases with non-hepatocellular patterns showed corticosteroid resistance. In particular, 3 cases with mixed patterns required additional immunosuppression with mycophenolate mofetil. These results indicate that the response to corticosteroids in patients with liver injuries marked by an increase in biliary enzyme (i.e., ALP and γ -glutamyl transpeptidase [GGT]) levels was poor. ICIs are reported to rarely induce immune-related cholangitis^{8,18,19}, which is characterized by predominant elevation of biliary enzymes, while liver transaminase levels are only moderately increased. A recent case report showed the utility of UDCA administration in patients with immune-mediated cholangitis induced by anti-PD-1 antibody that showed resistance to high-dose corticosteroids¹⁹. The pathogenesis of immune-related liver injury is different from that of classical autoimmune liver diseases such as AIH or primary biliary cholangitis (PBC)²⁰. However, the results of our study and previous reports indicate the need to plan the treatment strategy for each type of liver injury, such as the use of steroids for AIH with elevated aspartate aminotransferase (AST) and ALT levels, and UDCA for PBC with elevated ALP and GGT levels.

In the society's guidelines, ICIs should be immediately withdrawn on the appearance of severe immune-related liver injury (\geq Grade 3), and steroid administration should be initiated^{5,7}. However, De Martin et al. reported that 6 patients (38%) with 16 biopsy-proven immune-related liver injuries did not receive any corticosteroid therapy and experienced spontaneous improvement¹⁶. Similarly, our study also demonstrated that 21 patients (38%) who did not receive any treatment ($n = 18$) or received only UDCA ($n = 3$) improved without requiring corticosteroids. A previous report indicated that corticosteroid use is associated with an increased risk of serious infections in patients receiving ICIs²¹. Thus, recovery from immune-related liver injury without corticosteroid administration can contribute to an improved prognosis. Therefore, we followed up Grade 3 liver injuries with stable general conditions without corticosteroid therapy for approximately one week. On the other hand, since ICIs can rarely lead to fulminant hepatitis²², an immediate start of corticosteroid therapy should be considered for Grade 4 or acute liver failure.

In our study, one-third of all cases of immune-related liver injury were accompanied by other irAEs. Few studies have assessed the differences in clinical characteristics of immune-related liver injury between multisystem and single irAEs. We found that immune-related liver injuries in multisystem irAEs occurred later than those in cases with liver irAEs alone, and the use of the anti-CTLA-4 antibody was associated with the incidence of multisystem irAEs, consistent with previous reports^{23,24}. Our study demonstrated that the common types of irAEs accompanying liver injury were endocrine and GI injuries. Regarding the mechanism of these irAEs, the expression of CTLA-4 in normal pituitary cells for hypophysitis, preexisting autoantibodies for thyroiditis, and cytokines (e.g., interleukin-17) and specific gut microbiota for colitis have been reported to be key factors for onset^{4,25-27}. However, the detailed pathogenic mechanisms

underlying hepatic irAEs remains unclear. In multisystem irAEs, the mechanism of liver irAEs may differ from that of the other irAEs, even though they occur in a consecutive clinical course.

Liver biopsies can assist not only in differential diagnosis but also in assessing the severity of histological liver damage in immune-related liver injury ^{11,16}. Recent research emphasized that the corticosteroid dose should be determined based on the pathological inflammatory state to avoid unnecessary systemic corticosteroid treatment ^{11,16}. In addition, it is useful to exclude the possibility of classical AIH or drug-induced liver injury caused by other drugs ²⁰. The pathological findings of typical ICI-induced liver injury have been described as lobular hepatitis with numerous histiocytes, sometimes forming loose, well-formed, or fibrin ring granulomas, endothelialitis, and varying portal inflammation ^{15,20}. Consistent with these previous reports, the main histologic features in the 13 patients with biopsy-proven immune-related liver injury in our study were lobular injury, endothelialitis, and spotty necrosis with infiltration of CD3-and CD8 cells. It is unclear why these findings appear in immune-related liver injury, but we hypothesize that this may be related to the mechanism underlying the injury. PD-L1, the ligand for PD-1, is expressed on vascular endothelial cells in peripheral tissues. Hepatic non-parenchymal cells, including sinusoidal endothelial cells and Kupffer cells, express PD-L1 and inhibit cell division of activated T cells expressing PD-1 ^{28,29}. ICIs can activate CD8-positive T-cells and induce the destruction of sinusoidal endothelium by inhibiting the PD-L1 pathway, as a mechanism of ICI-induced cardiac injury ^{4,30}. However, further studies focusing on gene expression and infiltrating immune cells are needed to support this hypothesis.

Although more than half of all immune-related liver injuries were of the non-hepatocellular type according to laboratory data, 11 of 13 patients who underwent liver biopsies (85%) had hepatocellular-type injuries. This is presumably because liver biopsies were performed in patients scheduled to undergo steroid use. The pathological cholangitis was observed in both mixed or cholestatic pattern, however, these data were limited as there were only two non-hepatocellular patterns in this study.

We also recognize several limitations in our study. First, since this study was conducted retrospectively, treatment for immune-related liver injuries was not completely consistent. Treatment is performed according to the guidelines, but it depends on the judgment of the attending physician. Second, we could not assess the characteristics and outcomes of patients with grade 1 and 2 injuries. In fact, it is difficult to confirm the diagnosis of immune-related liver injury in these patients, especially Grade 1 injury, because patients with advanced malignancies often have a history of multiple medications. Additionally, disease progression, such as liver metastasis, can induce liver injury. It is not practical to perform liver biopsy in all patients with Grade 1–2 injuries because of the risk of bleeding. However, we strictly excluded the other liver diseases in the included patients with \geq grade 3 injuries in our study. We targeted only patients with immune-related liver injury (grade \geq 3), which is the most clinically important because they require therapeutic intervention such as steroids and immunosuppression.

In conclusion, our study found differences in the response to immunosuppression therapy in patients with immune-related liver injury due to liver injury. The clinical course of the hepatocellular pattern is

generally favorable; however, the efficacy of steroid therapy is limited in some cases with mixed patterns. Liver biopsy is helpful for diagnosing immune-related liver injury and evaluating its severity. Because patients with immune-related liver injury have advanced malignancies, liver transplantation is generally not a treatment option, even in fulminant hepatitis. Clinicians following malignancies treated by ICIs need to diagnose immune-related liver injury early and select adequate treatment based on the severity and pattern of the injury.

Abbreviations

irAE, immune-related adverse event; ICIs, immune checkpoint inhibitors; PD-1, programmed cell death 1; PD-L1, programmed cell death ligand 1, CTLA-4 cytotoxic T-lymphocyte antigen 4; CTCAE, Common Terminology Criteria for Adverse Events; ULN, upper limit of normal; AST, aspartate aminotransferase; GGT, gamma-glutamyl transpeptidase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; GGT, γ -glutamyl transpeptidase; T-BIL, total bilirubin; PSL, prednisolone; UDCA, ursodeoxycholic acid; MMF, mycophenolate mofetil, IgG; immunoglobulin G; GI, gastrointestinal; CD, cluster of differentiation; AIH, autoimmune hepatitis; PBC, primary biliary cholangitis; BSC, best supportive care.

Declarations

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Author contribution: Concept and study design: Takanori Ito, acquisition of data: Takanori Ito, Takafumi Yamamoto, Kazuyuki Mizuno, Yoshie Shimoyama, Drafting of the manuscript: Takanori Ito, Critical revision of the manuscript for important intellectual content: Masatoshi Ishigami, Kenta Yamamoto, Norihiro Imai, Yoji Ishizu, Takashi Honda, Hiroki Kawashima, Satoshi Yasuda, Hidenori Toyoda, Kenji Yokota, Tetsunari Hase, Naoki Nishio, Osamu Maeda, Masashi Kato, Naozumi Hashimoto, Hideharu Hibi, Yasuhiro Kodera, Yuichi Ando, Masashi Akiyama, Yoshie Shimoyama, Mitsuhiro Fujishiro; Statistical analysis: Takanori Ito. All authors have approved the final version of the article.

Ethical Statement:

1. **Compliance with Ethical Standards:** The study was performed according to the 1964 Declaration of Helsinki
2. **Funding:** No external funding to disclose
3. **Conflict of Interest:** H.: reports fees from AstraZeneca, Chugai Pharmaceutical Co. Ltd., Ono Pharmaceutical Co. Ltd., and Bristol-Myers Squibb Co., Y. A.: reports fees for lectures from Chugai, Pharmaceutical Co., Ltd., and commercial research funding from Chugai Pharmaceutical Co., Ltd. and Ono Pharmaceutical Co., Ltd. The remaining authors have nothing to disclose.
4. **Ethical approval:** The study protocol was approved by the Institutional Review Board at Nagoya University Hospital and Ogaki Municipal Hospital.

5. **Informed consent:** Informed consent in the present study was obtained in the form of opt-out on the website.
6. **Clinical Trials Registration:** Not applicable.
7. **Data sharing:** We agree with the policy in the journal.

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Figures

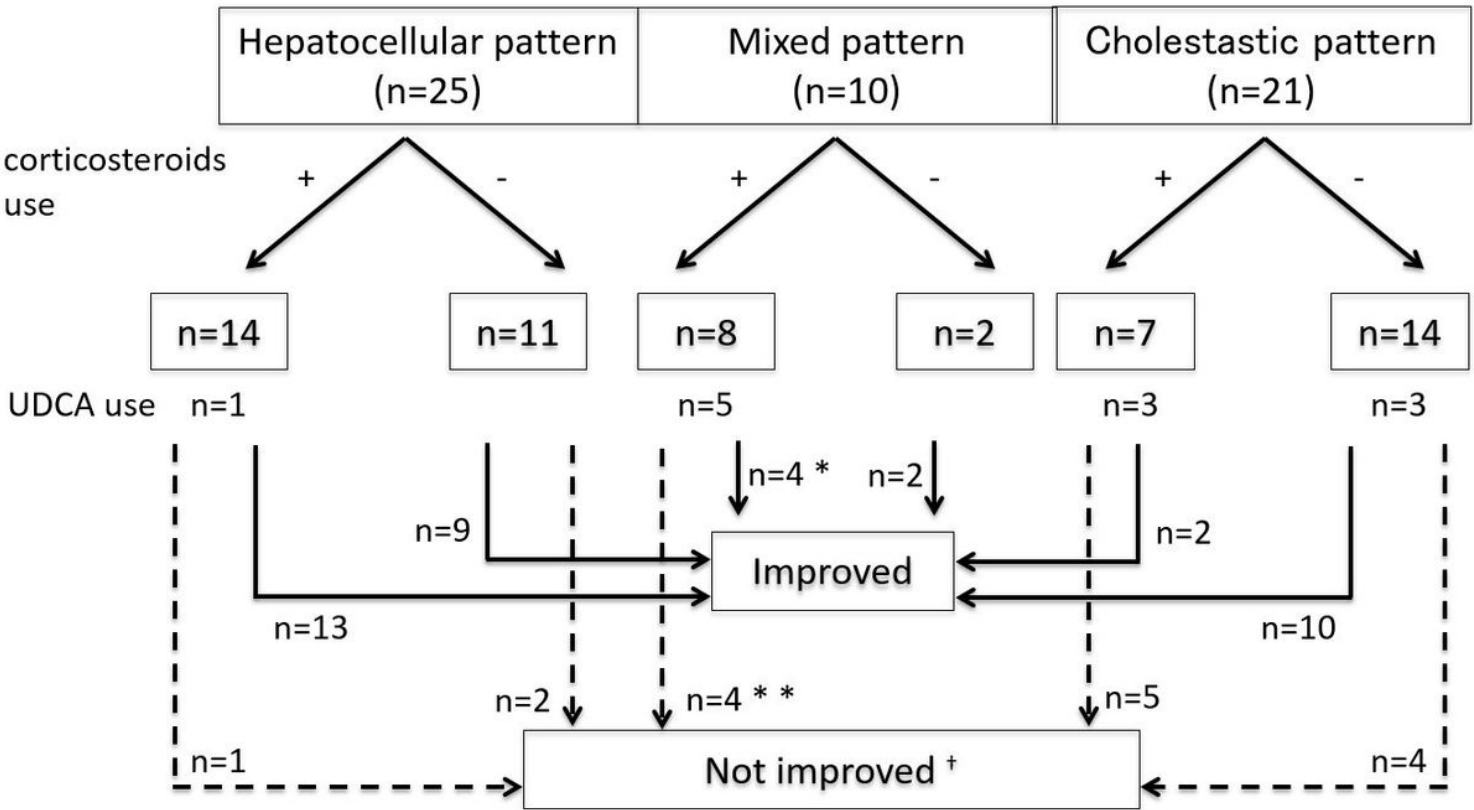


Figure 1

Clinical course of immune-related liver injury ≥Grade3 by injury pattern. UDCA, ursodeoxycholic acid; MMF, mycophenolate mofetil. MMF use * n=1, ** n=2. † including best supportive care for the progression

of malignancies.

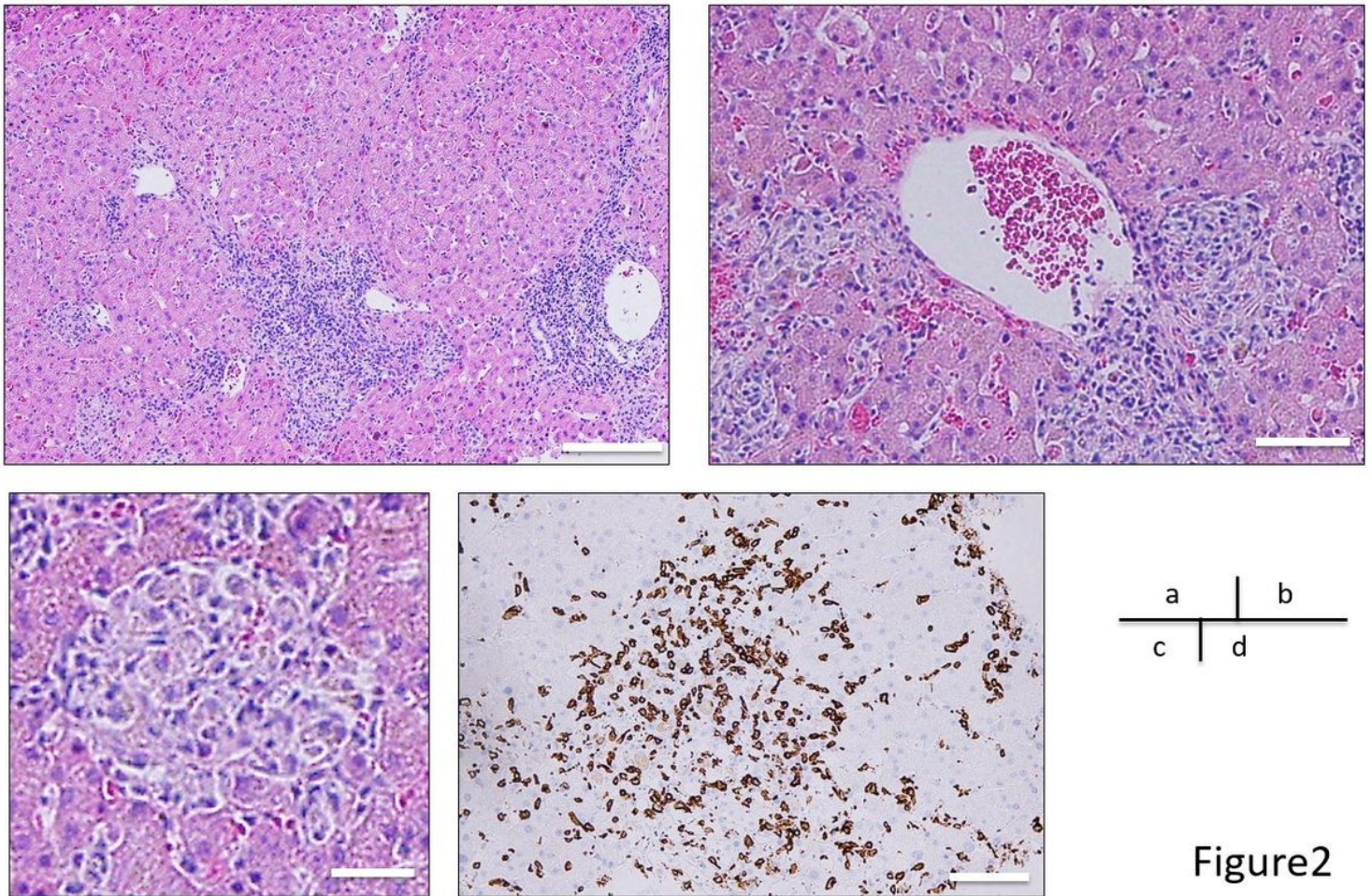


Figure 2

Representative hepatic pathological images in immune-related liver injury (hepatocellular injury pattern)
a) portal inflammation with interface hepatitis and spotty necrosis, Scale bar 200µm, b) endothelialitis, Scale bar 100µm, c) Granuloma, Scale bar 50µm, d) infiltrating CD8 positive T cells, Scale bar 100µm.

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