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Clinical and Pathological Features of Immune Checkpoint Inhibitor-induced Liver Injury in Comparison with Drug-induced Liver Injury and Autoimmune Hepatitis

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ABSTRACT

Background & Aims: Immune checkpoint inhibitors may cause various types of organ damage as immune-related adverse events, of which, liver damage is the most common. Herein, we evaluated the clinicopathological features of immune checkpoint inhibitor-related liver injury and investigated the differences between immune checkpoint inhibitor-related liver injury and drug-induced liver injury or autoimmune hepatitis.

Methods: We selected patients with ≥ grade 3 liver injury who were diagnosed with immune checkpoint inhibitor-related liver injury (n=15). Liver biopsies were performed in 10 of the 15 cases. We also selected cases in which a liver biopsy was performed and drug-induced liver injury (n=7) or autoimmune hepatitis [n=21: acute exacerbation (n=13) was diagnosed and cases of acute onset (n=8), in which liver function test results corresponded to ≥ grade 3].

Results: Portal fibrosis and periportal activity scores were significantly higher in the acute exacerbation autoimmune hepatitis group than in the other groups. Portal and lobular activity were not different between the groups. Plasma cell infiltration showed a higher trend in the autoimmune hepatitis group than in the other groups. Granuloma formations were seen in 90% of immune checkpoint inhibitor-related liver injury cases. The CD4/8 ratio was significantly lower in the immune checkpoint inhibitor-related liver injury group than in the other groups. Patients with bile duct injury had poorer response to corticosteroid therapy than those without.

Conclusions: There are some obvious differences among immune checkpoint inhibitor-related liver injury, drug-induced liver injury, and autoimmune hepatitis in liver histology. Liver biopsy is helpful for the diagnosis and severity evaluation of liver injury.

Key words: immune checkpoint inhibitors – immune-related adverse events – liver damage – drug-induced liver injury – autoimmune hepatitis.

Abbreviations: AHI: autoimmune hepatitis; ALT: alanine aminotransferase; AST: aspartate aminotransferase; CTCAE: Common Terminology Criteria for Adverse Events; CTLA-4: cytotoxic T-lymphocyte antigen-4; DILI: drug-induced liver injury; ICI: immune checkpoint inhibitor; irAE: immune-related adverse event; PD-1: programmed death-1; PD-L1: programmed death-ligand 1; PSL: Prednisolone; RUCAM: Roussel Uclaf Causality Assessment Method; T.bil: total bilirubin.

INTRODUCTION

Since the approval of nivolumab for the treatment of unresectable malignant melanoma, immune checkpoint inhibitors (ICIs) have been added for the treatment of various malignancies. Immune checkpoint inhibitors have improved the survival of patients with unresectable malignancies [1]. However, ICIs may cause various types of organ damage as immune-related adverse events (irAEs). One of the major irAEs caused by ICIs is liver damage [2-4]. There are two main types of ICIs: programmed death-1 (PD-1)/programmed death-ligand 1 (PD-L1) antibodies and cytotoxic T-lymphocyte antigen-4 (CTLA-4) antibodies. The incidences of hepatotoxicity of these drugs have been reported to be 1–3% and 3–9%, respectively [5]. The incidence of hepatotoxicity with the combination of PD-1/PD-L1 and CTLA-4 antibodies is 18%, and the incidence of hepatotoxicity is higher with combined agents than with single agents [6, 7]. Risk factors for ICI-related liver injury, such as the ICI class or baseline absolute lymphocyte number and fever >38 °C within 24 hours of initial ICI administration,

have been reported [3, 6, 8, 9]; however, the specific immune mechanisms responsible for the development of ICI-related liver injury remain unclear [10].

In addition, a diagnosis of ICI-related liver injury only accounts for a fraction (< 20–30%) of all patients who were reported to have erratic liver function test results as an adverse event in clinical trials [11, 12]. Therefore, ICI-related liver injury is based on a diagnosis of exclusion, and it is important to conduct a thorough assessment to exclude other differential diagnoses. A liver biopsy is useful to exclude differential diagnoses and assess the degree of liver inflammation.

Herein, we evaluated the clinical and pathological features of ICI-related liver injury and discussed the pathogenesis by investigating the differences between ICI-related liver injury and drug-induced liver injury (DILI) or autoimmune hepatitis (AIH). In our study, we aimed to characterize histological features and to explore potential diagnostic markers for ICI-related liver injury.

METHODS

Study Design and Ethics Statements

We retrospectively extracted diagnosed patients with DILI, AIH, and ICI-related liver injury based on liver biopsy findings at a university hospital between January 2011 and June 2021. All participants gave their written informed consent to participate in the study. The study was approved by the local Ethics Committee and conformed to the ethical guidelines of the Declaration of Helsinki.

Assessment of ICI-related Liver injury, AIH, and DILI

Patients with ≥ grade 3 liver injury according to the Common Terminology Criteria for Adverse Events, version 5.0 (CTCAE v5.0) [13] and diagnosed with ICI-related liver injury were included in this study. Patients with CTCAE v5.0 ≤ grade 2 were excluded because most of these patients had not been investigated for the cause of liver damage in detail. The type of liver injury was classified according to the diagnostic scale of the Roussel Uclaf Causality Assessment Method (RUCAM) criteria [14]. The RUCAM criteria classify liver injury as "hepatocellular," "mixed," or "cholestatic," which are determined by alanine aminotransferase (ALT) and alkaline phosphatase levels at onset.

Patients with DILI were included if they had serum aspartate aminotransferase (AST) or ALT levels > 5 × upper limit of normal (ULN) and/or total bilirubin (T.bil) levels > 5 mg/dL (which is equivalent to CTCAE v5.0 [3] grade 3) and with hepatocellular injury type according to the diagnostic scale of the RUCAM criteria [14]. The characteristics of DILI in this study are shown in Supplemental Table I.

The diagnosis of AIH was performed according to the revised scoring system of the international AIH group [15]. We divided patients with AIH into either acute exacerbation or acute onset cases [16]. Acute exacerbation AIH is a clinicopathologic concept of an acute hepatitis-like exacerbation during chronic hepatitis. Acute onset AIH is an acute onset case in the absence of preceding chronic hepatitis. In each group, we selected patients with serum AST or ALT levels > 5 × ULN and/or T.bil levels > 5 mg/dL.

Table I. Characteristics of the patients who had CTCAE v5.0 ≥ grade 3 liver damage after receiving immune checkpoint inhibitors

	n = 15
Patients with a previous history, n	5 (33.3%)
Time between immunotherapy and hepatitis, days, median (range)	51 (2–273)
Number of injections, median (range)	2 (1–15)
Aspartate aminotransferase level, U/L, median (range)	449 (127–1,717)
Alanine aminotransferase level, U/L, median (range)	489 (247–1,594)
Total bilirubin level, mg/dL, median (range)	1.1 (0.5–4)
Alkaline phosphatase level, U/L, median (range)	418 (244–2,698)
Gamma-glutamyl transferase level, U/L, median (range)	317 (53–988)
Antinuclear antibody ≥ 1:80	1 (6.7%)
Immunoglobulin G level, g/L, median (range)	12.1 (8.3–18.8)
Liver biopsy, n	10

CTCAE: Common Terminology Criteria for Adverse Events;

Histochemical Analysis of the Liver

Liver biopsies were performed using 14-gauge or 16-gauge needles. The biopsy specimens were reviewed by two hepatic expert pathologists who focused on the following features: portal fibrosis (0–4) and portal/periportal/lobular inflammation (0–3) according to the METAVIR score [17], and the presence and degree of granuloma, centrilobular necrosis, or cholangiopathy. We also counted the number of eosinophil and plasma cell infiltrations in the portal and lobular regions.

Liver samples were stained with either hematoxylin-eosin or immunostained with anti-CD3, anti-CD4, anti-CD8, and anti-CD20 antibodies (Roche Diagnostics, Tokyo, Japan). The number of positive cells was counted in high power fields in the portal and lobular regions, respectively.

Statistical Analysis

Data are reported as mean ± standard deviation or median (range) for continuous variables and proportion/frequency for dichotomous variables. Categorical variables were assessed using the chi-square test or the Kruskal–Wallis test. Continuous variables are expressed as medians and were assessed using the nonparametric Mann–Whitney U test. The incidence of ICI-related liver injury was calculated using the Kaplan–Meier method, and differences between the groups were assessed by the log rank test. Statistical significance was set as $p < .05$. We used SPSS statistical software version 28.0.0 (IBM Corp., Armonk, NY) to perform all statistical analyses. If there were missing data, the analysis was performed with available data.

RESULTS

Patient Characteristics

The number of patients who received ICIs at our hospital during the study period was 463. If multiple treatments were performed at separate times on one patient, each treatment was counted as a separate case. Of 463 patients, the median

(range) period after the initiation of ICIs was 200 (3–1568) days. Patients had lung cancer (n=166, 35.9%), urothelial cancer (n=89, 19.2%), malignant melanoma (n=80, 17.3%), gastrointestinal cancer (n=61, 13.2%), head-and-neck carcinoma (n=49, 10.5%), and other malignancies (n=18, 3.9%). Patients received an anti-PD-1 agent (n=348, 75.2%), an anti-PD-L1 agent (n=83, 17.9%), an anti-CTLA-4 agent (n = 15, 3.2%), or a combination of anti-PD-1 and CTLA-4 agents (n=17, 3.7%) for a median (range) of 5 (1–99) cycles.

Of 463 patients, 47 patients had AST or ALT levels > 5 × ULN and/or T.bil levels > 5 mg/dL (≥ grade 3 according to CTCAE v5.0). Among them, 15 patients were diagnosed as having ICI-related liver injury, excluding those clearly suspected of having liver injury due to factors other than ICIs, such as obstructive jaundice, bacterial infection, and congestive heart failure (Fig. 1).

Among 15 patients with clinically diagnosed ICI-related liver injury (Tables I and II), the median (range) period after the start of ICIs was 285 (31–933) days. Patients had malignant melanoma (n=7, 46.7%), urothelial cancer (n=4, 26.7%), lung cancer (n=3, 20.0%), and head-and-neck carcinoma (n=1, 6.6%). Patients received an anti-PD-1 agent (n=8, 2.3%), an anti-CTLA-4 agent (n=3, 20.0%), or a combination of anti-PD-1 and CTLA-4 agents (n=4, 23.5%) for a median (range) of 2 (1–15) cycles. No patients received an anti-PD-L1 agent in this category.

CTCAE v5.0 ≥ grade 3 cases were more common among CTLA-4-treated patients, including those treated with a combination therapy (hazard ratio: 6.69, 95% confidence interval: 1.87–23.92; p<0.01). In most cases, liver test result abnormalities were characterized by disproportionately high AST and ALT levels in sera. There were no cases in which antinuclear antibodies (ANA) in sera turned positive (1:80 or more) or the serum immunoglobulin (Ig) G level increased after ICI administration. However, there was one case each of ANA positivity and elevation of the IgG level before ICI administration.

Computed tomography (CT) was performed in 14 patients. Abnormalities in the gall bladder and both intra- and extrahepatic bile ducts were not observed in any patient. Hepatomegaly, gallbladder wall thickening and periportal edema were observed in four, eight and seven patients, respectively. There was no association between these findings and the degree of liver injury.

Prednisolone (PSL) treatment was initiated in 13 out of the 15 patients, and serum transaminase levels were normalized in all 14 patients who we were able to follow-up with. Two patients showed an insufficient response to PSL, so mycophenolate mofetil was added to their treatment regimen. Both patients received PD-1 and CTLA-4 combination therapy.

Clinical Presentation of DILI and AIH

The times from the initiation of suspected ICI use to the appearance of liver injury for patients with ICI-related liver injury and DILI were 91.8 ± 93.9 days and 43.7 ± 25.3 days, respectively, with a non-significant trend toward longer periods in ICI-related liver injury cases than in DILI cases (Supplemental Table II). In all cases, drug administration was

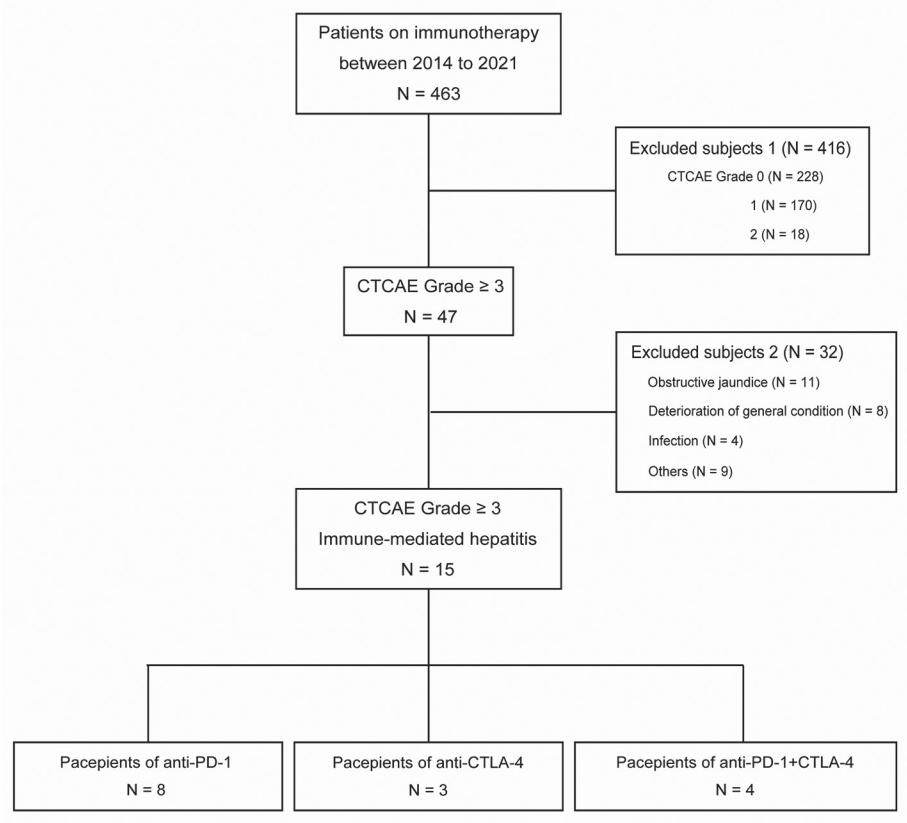

Fig. 1. Flowchart of the study population. CTCEA v5.0, Common Terminology Criteria for Adverse Events, version 5.0; CTLA-4, cytotoxic T-lymphocyte antigen-4; PD-1, programmed death-1.

Table II. Clinical course of the patients with CTCAE v5.0 ≥ grade 3 liver damage who received immune checkpoint inhibitors

Patient	Immunotherapy	Type of liver injury	AST level (U/L)	ALT level (U/L)	Total bilirubin level (mg/dL)	ALP level (U/L)	GGT level (U/L)	ANA	IgG (g/L)
1	Anti-PD-1	Cholestatic	138	260	0.5	2,698	745	negative	12.9
2	Anti-PD-1	Cholestatic	127	267	2.4	1,272	817	negative	11.1
3	Anti-CTLA-4	Hepatocellular	599	648	0.9	622	101	negative	9.4
4	Anti-CTLA-4	Hepatocellular	333	440	0.5	341	64	1:640	10.0
5	Anti-PD-1	Hepatocellular	456	554	1	567	103	negative	15.7
6	Anti-PD-1	Cholestatic	282	399	0.9	2,516	376	negative	10.6
7	Anti-PD-1 + CTLA-4	Hepatocellular	434	822	1.5	377	53	negative	14.1
8	Anti-PD-1	Mixed	392	537	2.2	1,045	546	negative	16.3
9	Anti-PD-1	Hepatocellular	1,286	673	2.5	427	258	negative	18.8
10	Anti-PD-1	Hepatocellular	596	903	1.2	398	517	negative	14.4
11	Anti-PD-1 + CTLA-4	Hepatocellular	1,717	1,348	2.1	363	494	negative	10.0
12	Anti-CTLA-4	Mixed	506	247	1	409	152	negative	15.9
13	Anti-PD-1 + CTLA-4	Mixed	442	351	0.9	546	684	negative	10.1
14	Anti-PD-1	Hepatocellular	567	328	1	244	233	negative	8.3
15	Anti-PD-1 + CTLA-4	Hepatocellular	523	1,594	2.8	381	247	negative	12.2

Patient	Time between immunotherapy and hepatitis, (days)	Prednisolone, dose	Mycophenolate mofetil, dose	Outcome	Time between therapy and recovery, (days)
1	14	-	-	Drop out*	-
2	87	0.1 mg/kg	-	Recovery	14
3	7	0.5 mg/kg	-	Recovery	27
4	91	0.5 mg/kg	-	Recovery	6
5	20	1 mg/kg	-	Recovery	11
6	63	1 mg/kg	-	Recovery	21
7	55	0.5 mg/kg	-	Recovery	5
8	14	1 mg/kg	-	Recovery	15
9	66	1 mg/kg	-	Recovery	14
10	246	1 mg/kg	-	Recovery	8
11	46	1000 mg	-	Recovery	19
12	83	1 mg/kg	-	Recovery	18
13	26	1mg/kg	2,000 mg	Recovery	38
14	11	-	-	Recovery	8
15	10	1mg/kg	2,000 mg	Recovery	30

* He refused treatment during chemotherapy. AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase; GGT: gamma-glutamyl transferase; PD-1: programmed death-1; CTLA-4: cytotoxic T-lymphocyte antigen-4.

discontinued when CTCAE ≥ grade 3 liver injury was observed, and all patients showed improvement in liver injury either spontaneously, or after PSL treatment.

The antinuclear antibody was detected in sera in one out of ten (10%) ICI-related liver injury cases, and in ten out of thirteen (77%) and four out of eight (50%) AIH patients with acute exacerbation and acute hepatitis phase, respectively ($p<0.05$). Serum levels of IgG were elevated in one out of ten (10%) ICI-related liver injury cases, and in twelve out of thirteen (92%) and in one out of eight (13%) cases of AIH patients with acute exacerbation and acute hepatitis, respectively ($p<0.01$) (Supplemental Table III).

Histological Findings

Liver biopsies were performed on 10 out of 15 patients diagnosed with ICI-related liver injuries (Fig. 2 and Table III). Hepatocyte necrosis and inflammation were observed in the parenchymal and central venous regions, while the degree of necrosis and inflammation in the portal area were mild (Fig. 2A–C). Centrilobular necrosis was seen in six cases (60%). Notably, granuloma was observed in nine cases (90%) in our cohort (Fig. 2 D, E). Fibrosis was not observed. There was no correlation between the degree of parenchymatous inflammation or the presence of centrilobular necrosis and serum transaminase levels. There was also no relationship

between the size or number of granulomas and the degree of inflammation (data not shown). Steatosis was observed in two patients.

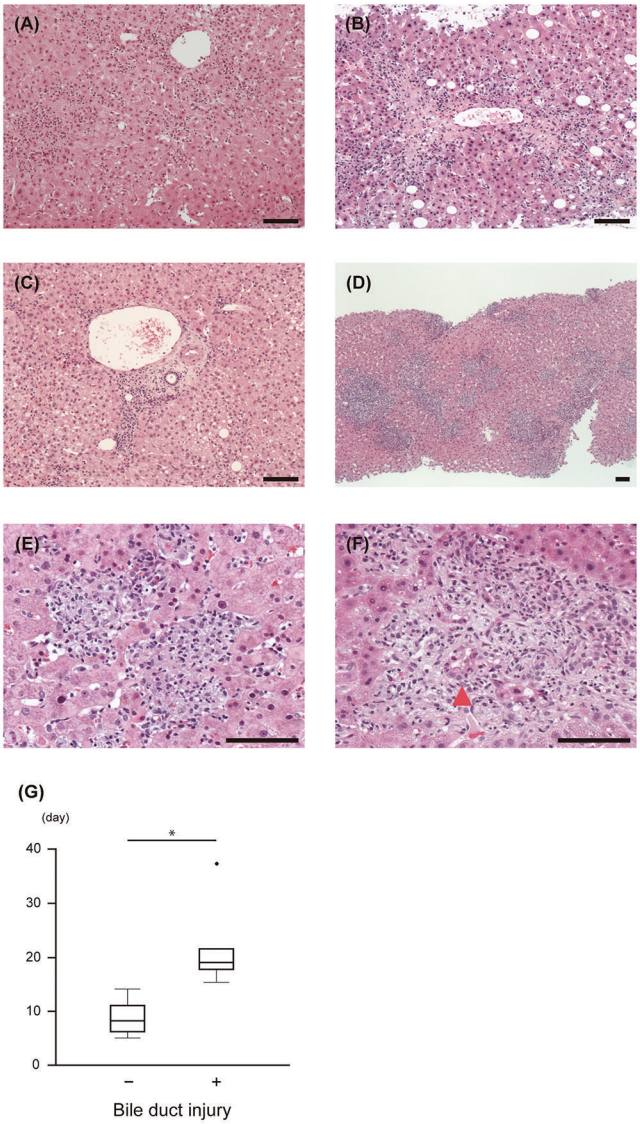

Fig. 2. Histopathology of immune checkpoint inhibitor-related liver injury. (A) The liver parenchyma is damaged. (B) Some cases show centrilobular perivenular zonal cell loss. (C) There is little infiltration of inflammatory cells in the portal vein area. (D, E) Granulomas are found in the liver parenchyma in most cases. (F) Some cases show bile duct injury. Arrowheads indicate the bile ducts. Scale bars, 100 μm. (G) The time from corticosteroid initiation to normalization of transaminases with and without bile duct injury. The results are shown in box-and-whisker diagrams. *p < .05.

Although neither bile duct loss nor ductopenia was observed, bile duct injury was present in five cases (50%) (Fig. 2F). The median times (ranges) from the initiation of PSL to normalization of transaminases were 19 (18–22) days in patients with bile duct injury and 8 (6–11) days in patients without bile duct injury ($p<0.05$) (Fig. 2G). The degree of inflammatory cell infiltration of the portal was positively correlated with the time from the start of treatment with PSL to normalization of transaminases ($r=0.76$, $p<0.05$). This observation suggests that the greater the inflammatory cell infiltration in the portal area, the longer the time to normalization of transaminases.

Next, we compared the pathological findings between AIH, DILI, and ICI-related liver injury (Fig. 3). Portal fibrosis and periportal inflammatory activity scores were significantly higher in the acute exacerbation AIH group than in the other groups (Fig. 3A). Portal and lobular activity scores were not different between each group (Fig. 3B–D). Plasma cell infiltration was more prevalent in the AIH group than in the other groups (Fig. 3E and F), whereas eosinophil infiltration showed no obvious differences between the groups (Fig. 3 G and H). Granulomas were observed in nine out of ten (90%) liver tissues with ICI-related liver injury, and they were identified in two cases (28.5%) and one case (4.8%) of DILI and AIH, respectively ($p<0.01$) (Fig. 3I). Macrogranulomas were not observed in DILI or AIH cases.

We performed immunostaining to establish the types of immune cells that accumulated in the lobular area and portal area of patients with AIH, DILI, and ICI-related liver injury (Fig. 4). The CD4/8 ratios in the portal and lobular areas were significantly lower in the ICI-related liver injury cases than in the AIH and DILI cases (Fig. 4E and F).

DISCUSSION

We observed an overall ICI-related liver injury of ≥ grade 3 in 3.5% of the 463 patients who received ICIs at our hospital. The incidence was similar in previous reports [18]. In addition, a previous report showed that 16% of patients experienced ≥ grade 3 liver injury with combination therapy with ICIs [19], which is also consistent with our current findings.

The recommended treatment for ≥ grade 3 liver injury is discontinuation of ICIs and commencement of corticosteroid therapy at doses of 1.0–2.0 mg/(kg/day) [20-22]. A retrospective cohort study of patients with ≥ grade 3 ICI-related liver injury reported a favorable course of ICI liver injury due to corticosteroid therapy [23]. Additionally, the effectiveness of corticosteroid therapy was confirmed in our cohort. Patients in whom corticosteroid therapy was able to be introduced earlier after the appearance of liver damage tended to respond well to the treatment, suggesting that close collaboration with hepatologists is necessary.

In addition to the exclusion of liver damage due to other etiologies, pathological findings are helpful for the diagnosis of ICI-related liver injury. The most common patterns are acute hepatitis with spotty or confluent lobular inflammation and centrilobular necrosis [24]. Sawada et al. [25] reported that fatty liver disease was a risk factor for ICI-related liver injury; however, only two patients had liver steatosis in this study. Interestingly, granuloma formation was observed in most of the patients who underwent a liver biopsy in this current study. Previous studies have also demonstrated that the development of granulomatous hepatitis is one of the characteristics of ICI-related liver injury [10, 26]. Similarly, granulomas were found in biopsied tissues from cases of ICI-related pneumonia and renal failure [27, 28]. However, the cause of the development of granulomas is unknown. Previous studies regarding granuloma formation in the liver [29-31] indicated that some

Table III. Histological characteristics of patients with immune checkpoint inhibitor-related liver injury who underwent liver biopsy

Patient	Immunotherapy	Portal fibrosis (METAVIR score, 0–4)	Portal activity (METAVIR score, 0–3)	Periportal activity (METAVIR score, 0–3)	Eosinophils in the portal area (>5 cells/HPF)	Plasmacytosis in the portal area (>10 cells/HPF)	Periportal hepatic rosette formation	Bile duct injury
2	Anti-PD-1	0	1	0	+	–	–	–
4	Anti-PD-1	0	2	1	–	–	–	–
5	Anti-PD-1	0	1	0	–	–	–	+
6	Anti-PD-1	0	2	1	–	+	–	–
7	Anti-PD-1	1	2	2	–	–	–	+
9	Anti-CTLA-4	0	1	0	–	–	–	–
10	Anti-CTLA-4	0	2	1	–	–	–	+
11	Anti-PD-1 + CTLA-4	0	1	0	–	–	–	–
12	Anti-PD-1 + CTLA-4	0	1	0	+	–	–	+
13	Anti-PD-1 + CTLA-4	0	3	2	+	–	–	+

Patient	Lobular activity (METAVIR score, 0–3)	Centrilobular necrosis	Eosinophils in the lobular area (>5 cells/HPF)	Plasmacytosis in the lobular area (>10 cells/HPF)	Granulomas
2	2	–	–	–	–
4	3	–	–	–	+
5	2	+	–	–	+
6	3	+	–	–	+
7	2	+	–	–	+
9	2	–	–	–	+
10	3	+	–	+	+
11	3	+	–	–	+
12	3	+	+	–	+
13	2	–	–	–	+

PD-1: programmed death-1; CTLA-4: cytotoxic T-lymphocyte antigen-4; HPF: high power field.

inflammatory mediators, including cytokines and chemokines, may be involved in the mobilization of mononuclear cells in ICI-related liver injury, inferring that further studies in this regard are necessary.

Here, half of the patients showed degeneration of the intralobular bile ducts among those with ICI-related liver injury. Liver injury recovery was delayed in patients with bile duct degeneration when compared to those without. The recovery time for DILI is longer for the cholestasis type than for the hepatocellular injury type [32, 33]. Additionally, ICI-related sclerosing cholangitis, which shows diffuse bile duct inflammation similar to primary sclerosing cholangitis, has been reported to show a moderate to poor response to corticosteroid therapy [34, 35]. Although no patients showed radiographical evidence of sclerosing cholangitis in extrahepatic bile ducts in this study, the possibility of intrahepatic bile damage was not ruled out. Therefore, a liver biopsy was considered key, not only for the diagnosis of ICI-related liver injury but also for predicting the patients' treatment course.

The increased presence of CD8+ T-cells with a lesser proportion of CD4+ T-cells in ICI-related liver injury can assist in differentiating it from AIH [24, 36]. Kido et al. [37] reported that after a neonatal thymectomy, PD-1-/- mice developed fatal hepatitis and that CD8+ T-cells played an important role in the progression of liver damage [37]. Moreover, several observations have linked CD8+ T-cells and the development of ICI-related liver injury [38, 39]. Furthermore, excessive infiltration of CD8+ T-cells may be a factor in granuloma formation [40]. However, the exact mechanisms of how activated CD8+ T-cells infiltrate during the development of ICI-related liver injury remain unknown. One of the possible explanations for the infiltration of CD8+ T-cells into hepatocytes is chemokine alterations. Cathrin et al. [37] found that CCR2 expression was significantly increased, while CCR7 expression was decreased with ICI-related liver injury. We are currently conducting studies to investigate the mechanisms of infiltration of immune cells in the liver in cases of ICI-related liver injury.

There are some limitations to this study. First, clinical data were lacking, including baseline laboratory data and liver histology findings. Second, this study was a single-center, retrospective analysis and included various tumor types and different ICI treatment regimens. Lastly, the number of patients with ≥ grade 3 liver injury and/or liver biopsy results was small. Therefore, prospective studies using a large number of patients are necessary to help clarify the clinical features and pathogenesis for ICI-related liver injury.

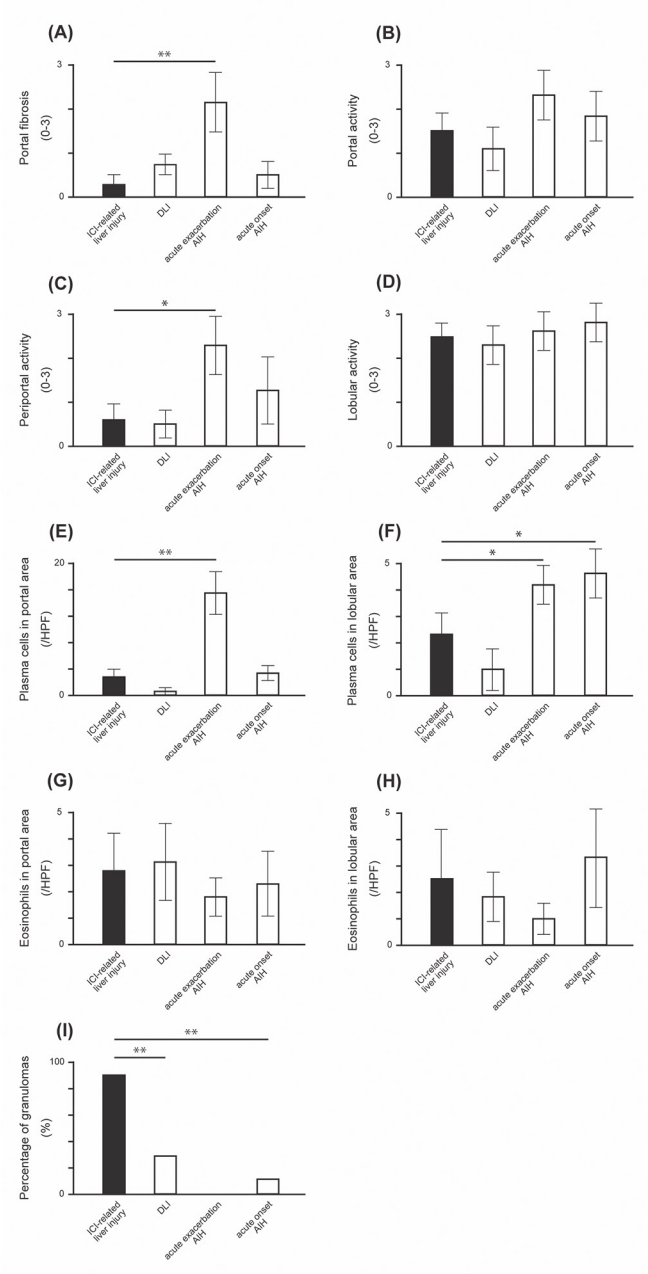

Fig. 3. Pathological findings of ICI-related liver injury, DILI, and AIH. The degrees of portal fibrosis (A), portal activity (B), periportal activity (C), and lobular activity (D) were scored according to the METAVIR score [18]. The number of plasma cell (E and F) and eosinophil (G and H) infiltrates in the lobular and portal regions in high power field (HPF), respectively, were counted. (I) The percentage of the presence of granulomas. The results are presented as mean ± standard error of the mean. *p < .05, **p < .01. ICI: immune checkpoint inhibitor; DILI: drug-induced liver injury; AIH: autoimmune hepatitis.

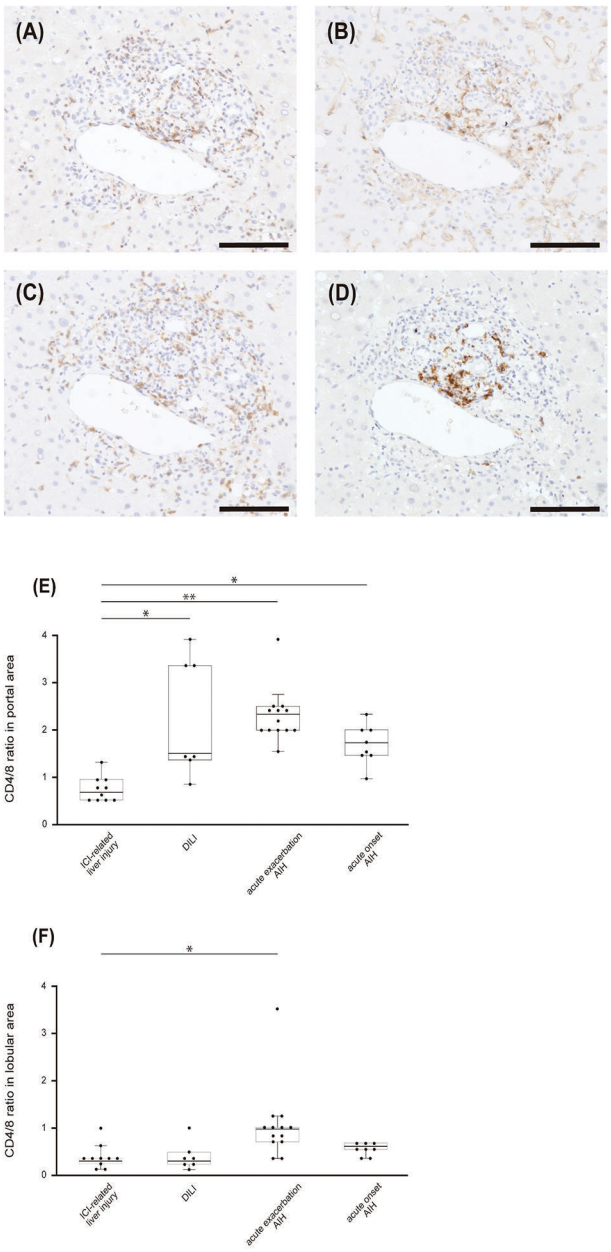

Fig. 4. Immunohistochemical analysis of immune checkpoint inhibitor-related liver injury. (A) CD3, (B) CD4, (C) CD8, (D) CD20. Scale bars, 100 μm. (E,F) Comparison of immune cell infiltration using the CD4/8 ratio of ICI-related liver injury, DILI, and AIH. The number of positive cells was counted in high power fields in the portal (E) and lobular regions (F), respectively. The results are presented as mean ± standard error of the mean. *p < .05, **p < .01. ICI: immune checkpoint inhibitor; DILI: drug-induced liver injury; AIH: autoimmune hepatitis; CD: cluster of differentiation.

CONCLUSIONS

We conclude that ICI-related liver injury of ≥ grade 3 occurred in 3.5% of the treated patients. Early diagnosis of ICI-related liver injury is desirable, as therapeutic intervention with corticosteroids can rapidly improve liver damage. Liver histology is paramount for the diagnosis and severity evaluation of liver damage. Further studies are necessary to elucidate the mechanisms for identifying liver injury and establishing management strategies.

Conflicts of interest: None to declare.

Authors' contributions: K.S. and M.A. designed and conceived the study. K.S., M.A., O.Y., T.W., Y.N., Y.I., K.Y., M.H. and Y.T. collected the data. K.S., M.A., O.Y., T.W. and Y.T. interpreted the results. K.S.

and M.A. analyzed the data and drafted the manuscript. M.A. and Y.H. were the overall study supervisors and edited the manuscript. All authors critically revised the manuscript, approved the final version to be published, and agreed to be accountable for all aspects of the work.

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Supplementary material: To access the supplementary material visit the online version of the *J Gastrointestin Liver Dis* at http://dx.doi.org/10.15403/jgld-5045

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