Clinicopathological characteristics of autoimmune-like hepatitis after drug-induced liver injury

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Abstract. Autoimmune hepatitis (AIH) can be induced by drugs, but the underlying mechanisms remain unclear. The present study attempted to elucidate the clinicopathological characteristics of autoimmune-like hepatitis following drug-induced liver injury (DILI-ALH). The medical records of 57 patients diagnosed with AIH at Mie General Medical Center (Yokkaichi, Japan) were retrospectively reviewed, paying particular attention to the history of drug administration. Patients were classified into three groups: *De novo* AIH, drug-induced ALH (DI-ALH) and DILI-ALH. DILI-ALH was newly defined as cases in which the patient had a history of DILI and where the liver injury initially improved after drug discontinuation, but later worsened and was diagnosed as AIH. Of the 57 patients diagnosed with AIH, 42 patients were included in this study. *De novo* AIH was diagnosed in 29 patients, DI-ALH in 10 patients and DILI-ALH in 3 patients. Suspected causative drugs for drug-related pathologies were variable, including statins, health foods and supplements. No significant differences in sex or mean age were observed for DI-ALH and DILI-ALH compared with those for AIH. Distinguishing DI-ALH or DILI-ALH from AIH serologically and pathologically is difficult. No significant differences in the number of steroids used or the recurrence rate were observed between any groups. These findings suggest that drugs may present a more diverse cause of ALH than generally predicted. In particular, some AIH cases clearly present with DILI-ALH. Clarifying the involvement of drugs in the pathogenesis of AIH and establishing guidelines for diagnosis and treatment represent important issues for the future.

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Abbreviations: DILI, drug-induced liver injury; AIH, autoimmune hepatitis; DI-ALH, drug-induced autoimmune-like hepatitis; DILI-ALH, autoimmune-like hepatitis following drug-induced liver injury; T-Bil, total bilirubin; ALT, alanine aminotransferase; IgG, immunoglobulin G; ANA, antinuclear antibody

Key words: AIH, DI-ALH, DILI

Introduction

Autoimmune hepatitis (AIH) is an inflammatory disease of the liver resulting from a loss of immune tolerance to hepatocyte antigens, which is triggered by unknown factors. Although the mechanisms underlying autoimmune responses in AIH are not well understood, a combination of genetic and external factors, such as infection, drugs or herbal medicines, are likely to be involved. In fact, drugs can cause a variety of phenotypes of liver injury that demonstrate similar clinical features of AIH and liver dysfunction, with positive results for autoantibodies (1-5).

In fact, as overlap is often observed between the clinical and pathological features of idiosyncratic drug-induced liver injury (DILI) and AIH, distinguishing AIH from DILI based on the diagnostic criteria commonly used in clinical settings can be difficult (6-9). Such cases are often referred to as drug-induced AIH, and have also been termed drug-induced autoimmune-like hepatitis (DI-ALH), immune-mediated DILI or DILI with autoimmune features (10-14). However, the terminology remains poorly defined and controversial. Recently, the term DI-ALH has been used more frequently to describe these conditions (15,16).

In terms of pathogenesis, DILI and AIH share molecular mechanisms, and genetic associations with human leukocyte antigen variants point to relevant roles of hepatic inflammation through antigen presentation (4,6,17). In addition, intrahepatic immunocompetent cells related to pro-inflammatory conditions and immune regulation are present in both DILI and AIH (4). Such findings suggest the existence of common pathways in the molecular biological mechanisms underlying DILI and AIH pathogeneses. DI-ALH may thus involve a complex interplay of both mechanisms and clear separation of these entities is often difficult.

The most commonly reported causative agents in DI-ALH are interferons, statins, methylprednisolone, adalimumab, imatinib and diclofenac, but an even wider variety of drugs

may be capable of involvement (14-16,18-21). The clinical picture also seems to differ depending on the type of offending agent. In particular, statins are considered to be associated with a clinical picture more similar to that of AIH (15).

Contrasting with AIH, which requires long-term immunosuppressive therapy, DI-ALH often resolves or improves upon discontinuation of the immunosuppressive drug (1,15,16). However, the clinical features seem to depend on the type of drug and the susceptibility of the individual to the drug. An accurate diagnosis of DI-ALH is therefore necessary to avoid overmedication.

These unpredictable idiosyncratic or hypersensitive drug reactions may be involved in a proportion of classical AIH cases. However, details of the incidence and spectrum of DI-ALH and other related diseases have yet to be elucidated in detail.

We have already reported several cases of AIH with unusual clinical courses (22). These cases were initially diagnosed as DILI, and the liver dysfunction improved after discontinuation of the causal drugs, only to relapse later despite no further administration of the drug and with significantly elevated levels of antinuclear antibody (ANA) and immunoglobulin (Ig)G. Histological findings were consistent with AIH. These courses differed from DILI with subclinical AIH or a second episode of DILI or conventional DI-ALH, suggesting different patterns of etiology and molecular pathogeneses. We named this condition DILI-ALH.

Little is known about the frequencies of DILI-ALH and DI-ALH due to the uncertain clinical definition. Therefore, in the present study, a detailed retrospective survey was conducted of medication history at and before the diagnosis of AIH to detect DI-ALH and DILI-ALH with particular reference to recently proposed criteria (16). In addition, the clinicopathological features of DI-ALH and DILI-ALH were compared to distinguish these entities from idiopathic AIH.

Patients and methods

Patients. The medical records of 57 patients diagnosed with AIH at Mie Prefectural General Medical Center (Yokkaichi, Japan) between January 2011 and December 2022 were retrospectively reviewed. Patients with a history of other liver diseases such as viral hepatitis (e.g., hepatitis B or C) were excluded. A liver biopsy was not performed in some patients due to advanced age, poor general condition, bleeding tendency or a lack of consent, and these patients were also excluded.

Overall, 42 patients were included in the analysis. Data on age, sex, body mass index, laboratory data, pathological data, complications and causative drugs were retrospectively collected. This study was approved by the Medical Ethics Committee of Mie Prefectural General Medical Center (approval no. O-0094).

Evaluation of clinicopathological findings. The cases of patients diagnosed with AIH were examined with particular attention paid to the medical history. AIH was defined using the criteria developed by the International Autoimmune Hepatitis Group (23). The Revised AIH Score was used in this study as a commonly used index both in Japan and worldwide. In patients where drugs are involved, such as drug-related AIH, the revised AIH score will be 4 points lower, resulting in an apparent lower AIH score (23). A cut-off value of 10 points, as the cutoff for probable AIH, was therefore used for the pretreatment AIH score in this analysis.

Although several scoring methods are available for the diagnosis of DILI, the Roussel Uclaf Causality Assessment Method (RUCAM) score, which is widely used internationally, was used in this study (24). The RUCAM score is a means of assigning points to clinical, biochemical, serological and radiological features of liver injury, resulting in an overall assessment score that reflects the likelihood that liver injury is due to a particular drug.

For the present study, patients with AIH were classified into three groups: i) *De novo* AIH; ii) DI-ALH; and iii) DILI-ALH. *De novo* AIH was defined as patients with no history of DILI, a pretreatment AIH score of ≥10 and clinical or pathological suspicion of AIH. DI-ALH was defined as AIH directly induced by drugs. To study the influence of drugs in greater detail, a stricter definition was used with reference to the definition provided by Björnsson *et al* (15), as follows: i) Patients without liver dysfunction before drug administration; ii) no underlying other liver disease; iii) a temporal association between the start of drug administration and the onset of AIH; iv) a clinical and pathological diagnosis of AIH, with a pretreatment AIH score of ≥10; and v) clinically, drugs are involved in the onset of AIH, with a RUCAM score of ≥6.

DILI-ALH, which was newly proposed in the present study, was defined as a case in which the patient had a history of DILI and showed initial improvement of liver injury, indicated by ALT returning to normal or falling to less than one-half of its peak level, after discontinuation of the suspected causal drug, followed by exacerbation leading to the diagnosis of AIH. DILI-ALH was considered to differ from DI-ALH in the lack of a direct trigger for the onset of AIH. DILI-ALH was defined as follows: i) Patients who were clinically diagnosed with DILI showing a RUCAM score of ≥6 and an AIH score of ≤9 at the time of initial liver injury, with no apparent cause of liver injury other than medication; ii) improvement of initial liver injury with drug discontinuation; and iii) recurrence of liver injury with no apparent drug-induced cause. AIH was clinically diagnosed when the RUCAM score was ≤5 and the AIH score was ≥10.

For *de novo* AIH and DI-ALH, blood test data were collected at the first visit. For DILI-ALH, data were collected at the time of the DILI diagnosis and at subsequent visits due to worsening liver injury. The following variables were obtained by chart review: Total bilirubin (T-Bil), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels, alkaline phosphatase (ALP) ratio (ALP real value/upper normal limit) (adopted due to multiple different methods of measuring ALP), γ-glutamyltransferase level, prothrombin time (PT), peripheral blood eosinophil count, IgG titer, ANA-positive rate and anti-mitochondrial antibody (AMA)-positive rate. Pre-treatment AIH score was used in this study.

Liver biopsy specimens and the report for each individual were reviewed anonymously by the pathologist and the following data were collected: Inflammation grade (A0-A3) and fibrosis grade (F0-F4) based on the new Inuyama classification (25), lobular inflammation, interface hepatitis, rosette

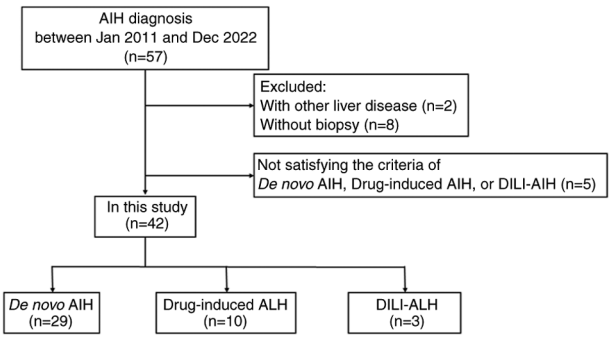

Figure 1. Flowchart of patient selection. AIH, autoimmune hepatitis; DI-ALH, drug-induced ALH; DILI-ALH, autoimmune-like hepatitis following drug-induced liver injury.

formation and infiltrating cells, steatosis, cholestasis and bile duct injury. Inflammation grades were as follows: A0, no necro-inflammatory reaction; A1, mild necro-inflammatory reaction; A2, moderate inflammatory reaction; and A3, severe necro-inflammatory reaction. Fibrosis grades were as follows: F0, no fibrosis; F1, fibrous portal expansion; F2, bridging fibrosis (mild fibrosis); F3, bridging fibrosis with lobular distortion (severe fibrosis); and F4, cirrhosis (25).

Statistical analysis. Categorical variables are presented as numbers and percentages, while continuous variables are presented as medians and interquartile ranges. Differences between groups were determined using Fisher's exact test for categorical variables and using the Kruskal-Wallis rank-sum test with the Steel-Dwass non-parametric post hoc test for continuous variables. $P<0.05$ was considered to indicate a statistically significant difference. All statistical analyses were performed using EZR version 1.52 (Jichi Medical University), a graphical user interface for R version 4.02 (The R Foundation for Statistical Computing; R Core Team). More specifically, EZR is a modified version of R Commander designed to provide statistical functions commonly used in biostatistics (26).

Results

Patient characteristics of DILI-ALH. Of the 57 patients diagnosed with AIH at Mie General Medical Center, 2 patients were excluded due to hepatitis B. A total of 42 patients who met the selection criteria were included in the final analysis, including 29 patients with *de novo* ALH, 10 patients with DI-ALH and 3 patients with DILI-ALH (Fig. 1).

The suspected causative drugs for the DI-ALH and DILI-ALH groups are shown in Table I. The most common causative drugs for DI-ALH were statins in 3 cases and dietary supplements in 2 cases. The most common causative drugs for DILI-ALH were health foods/supplements in 2 cases and Tiaramide in 1 case. Of these 3 patients, patient 1 took a dietary supplement consisting primarily of docosahexaenoic acid, eicosapentaenoic acid, docosapentaenoic acid and nattokinase during a 3-month period. Patient 2 had been taking tiaramide hydrochloroide for ~1 year, but stopped after being diagnosed with DILI. Patient 3 was diagnosed with DILI in November after taking a supplement containing zinc, maca, arginine and suppon powder as the main ingredients. Although statins and health foods/supplements, including herbs, were the most common causative drugs in this study, a variety of drugs were suspected to be involved in DI-ALH and DILI-ALH.

Table I. Drugs suspected to be responsible for DI-ALH and DILI-ALH in this study.

Drug	n
DI-AIH	
Statin	3
Cefcapene pivoxil	1
Benzbromarone	1
Febuxostat	1
Esomeprazole magnesium	1
Herbal medicine (Hochu-ekkito)	1
Health foods/supplements	2
DILI-AIH	
Tiaramide hydrochloride	1
Health foods/supplements	2

DI-ALH, drug-induced autoimmune-like hepatitis; DILI-ALH, ALH following drug-induced liver injury.

DILI-ALH was newly defined as a case in which the patient had a history of DILI and initially showed improvement of liver injury after discontinuing the drug in question, but later worsened and was diagnosed with AIH. The present study included 3 such cases. The characteristics of these cases are shown in Table II and the clinical course of each case is shown in Fig. 2. A clear precipitating drug was identified in the first episode of each case and DILI was suspected based on scoring, but the diagnosis of AIH was not met at that time. With the second episode, liver injury occurred without any obvious drug trigger and AIH was diagnosed.

In all 3 patients in this study, AIH score at the time of the first DILI was ≤9, indicating that AIH was unlikely. However, when liver injury occurred again, the score increased by ≥6, indicating that the diagnosis of AIH was either possible or probable.

Patient 1 was diagnosed with liver injury after taking a dietary supplement and showing a RUCAM score of 6 (probable DILI) and an AIH score of 5 (unlikely AIH). The patient was initially diagnosed with DILI, which only improved with discontinuation of the supplement. During the follow-up, the patient was no longer on medication, but 5 months later, a recurrence of the liver injury occurred. AIH was diagnosed based on a RUCAM score of 4 (possible DILI) and an AIH score of 16 (definite AIH). Patients 1 and 2 also showed DILI that improved only with drug discontinuation, while patient 3 had previously received steroids as treatment for DILI. All patients showed exacerbation of liver injury, during steroid tapering in patient 3 and during follow-up in the other two patients, without resumption of discontinued medications. Although none of the patients showed any increase in DILI score for the recurrent liver injury, all displayed an increase in AIH score of ≥6 points, indicating a possible or probable diagnosis of AIH. Relapse of liver damage was observed in only one case, and liver damage worsened during steroid tapering.

Table II. RUCAM scores and revised AIH scores in patients with DILI-ALH.

Characteristic	Patient 1	Patient 2	Patient 3
Age, years	66	68	40
Sex	Male	Female	Male
BMI, kg/m^2	20.6	14.5	27.8
History of autoimmune disease	None	None	None
On first episode			
T-Bil, g/ml	0.4	1.2	12.9
ALT, U/l	238	274	2031
IgG, mg/dl	1,819	NA	NA
ANA	+ (discrete)	x40 (nucleolar)	NA
Drug suspected for DILI	Supplement	Tiaramide hydrochloride	Supplement
Treatment	Discontinued drug	Discontinued drug	Discontinued drug and PSL 0.8 mg/kg/day orally
Duration between first episode and latter episode, months	6	10	3
On latter episode			
T-Bil, g/ml	1.43	0.78	0.5
ALT, U/l	213	273	1,095
IgG, mg/dl	2,292	2805	1,687
ANA	^{3}x1,280	x80	Negative
Treatment	PSL0.8 mg/kg/day orally	PSL 0.6 mg/kg/day orally	PSL 0.6 mg/kg/day orally
Outcome	No relapse for 40 months	No relapse for 100 months	Relapse during steroid tapering
Scoring			
First episode			
RUCAM score	6 (probable)	7 (probable)	6 (probable)
Revised AIH score	5 (unlikely)	9 (unlikely)	3 (unlikely)
Latter episode			
RUCAM score	4 (possible)	3 (possible)	4 (possible)
Revised AIH score	16 (probable)	15 (possible)	11 (possible)

AIH, AIH, autoimmune hepatitis; DILI, drug-induced liver injury; BMI, body mass index; T-Bil, total bilirubin; ALT, alanine aminotransferase; IgG, immunoglobulin G; ANA, antinuclear antibody; PSL, prednisolone; RUCAM, Roussel Uclaf Causality Assessment Method.

Clinical characteristics of AIH, DI-ALH and DILI-ALH. The baseline characteristics and laboratory findings of the patients are shown in Table III. A total of 29 patients had *de novo* AIH, 10 patients had DI-ALH and 3 patients had DILI-ALH. In the *de novo* AIH group, patients had significantly fewer subjective symptoms (*de novo* AIH vs. DI-ALH, P=0.01; *de novo* AIH vs. DILI-ALH, P>0.99; DI-ALH vs. DILI-ALH, P=0.22; Steel-Dwass test). The *de novo* AIH group included a greater proportion of women, but the difference was not significant (*de novo* AIH vs. DI-ALH, P=0.33; *de novo* AIH vs. DILI-ALH, P=0.25; DI-ALH vs. DILI-ALH, P=0.56; Steel-Dwass test). The proportion of older patients tended to be higher in the DILI-ALH group than in the other groups (*de novo* AIH vs. DI-ALH, P>0.99; *de novo* AIH vs. DILI-ALH, P>0.99; DI-ALH vs. DILI-ALH, P>0.99; Steel-Dwass test). No significant differences in relapse or complication rates of autoimmune diseases were evident among the three groups (relapse; *de novo* AIH vs. DI-ALH, P>0.99; *de novo* AIH vs. DILI-ALH, P>0.99; DI-ALH vs. DILI-ALH, P>0.99, and complication rates of autoimmune diseases; P=0.80, P>0.99 and P>0.99; Steel-Dwass test).

Blood tests showed that ALT levels were significantly higher in the DI-ALH group compared with those in the *de novo* AIH group (*de novo* AIH vs. DI-ALH, P=0.039; *de novo* AIH vs. DILI-ALH, P=0.58; DI-ALH vs. DILI-ALH, P=0.78; Steel-Dwass test). No significant differences in T-Bil (P=0.15, P=0.74 and P=0.15), AST (P=0.17, P=0.53 and P=0.78), ALP ratio (P=0.94, P=0.66 and P=0.78), γ-glutamyl transferase (P=0.22, P=0.91 and P=0.94), PT (P=0.92, P=0.89 and P=0.98), ANA-positive rate (P>0.99, P>0.99 and P=0.69) or AMA-positive rate (P>0.99, P>0.99 and P>0.99) were observed among the three groups. Peripheral blood eosinophil percentage (P=0.93, P=0.17 and P=0.18) tended to be higher in the DILI-ALH group, and IgG titer (P=0.18,

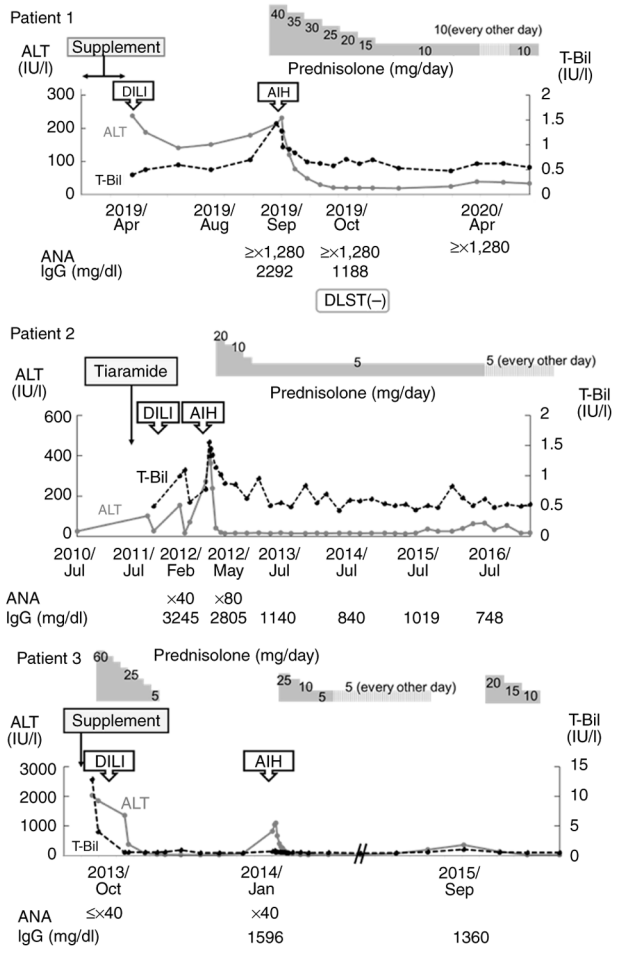

Figure 2. Short-term clinical courses of 3 patients with DILI-ALH. Changes to ALT and T-Bil in each patient along with ANA, IgG, diagnoses of DILI and AIH, and suspected causative drugs. DILI-ALH, autoimmune-like hepatitis following drug-induced liver injury; AIH, autoimmune hepatitis; T-Bil, total bilirubin; ALT, alanine aminotransferase; IgG, immunoglobulin G; ANA, antinuclear antibody; DLST, drug-induced lymphocyte stimulation.

P=0.81 and P=0.87) tended to be higher in the *de novo* AIH group, although these results were not statistically significant. Distinguishing these types of AIH by blood tests was difficult. Revised AIH scores were lower in DI-ALH, possibly due to drug subtraction in AIH scoring. Steroids were used for treatment in 82.8% of the AIH group, 100.0% of the DI-ALH group and 100.0% of the DILI-ALH group, and the frequencies of recurrent liver injury were 34.5, 40.0 and 33.3%, respectively. No significant differences in steroid use or recurrence rates were observed between the groups (P=0.91, P>0.99 and P>0.99).

Pathological features of AIH, DI-ALH and DILI-ALH. Table IV shows the pathological characteristics of AIH, DI-ALH and DILI-ALH. Interface hepatitis was observed in three groups, and there is no significant difference in rosettes (*de novo* AIH vs. DI-ALH, P=0.26; *de novo* AIH vs. DILI-ALH, P>0.99; DI-ALH vs. DILI-ALH, P>0.99; Steel-Dwass test). Lymphocyte infiltration was seen in all three groups. There are no significant differences in plasma cells (*de novo* AIH vs. DI-ALH, P>0.99; *de novo* AIH vs. DILI-ALH, P>0.99; DI-ALH vs. DILI-ALH, P>0.99; Steel-Dwass test), eosinophils (P>0.99; P>0.99 and P>0.99) and neutrophils infiltration (P>0.99, P>0.99 and P>0.99). Also, there are no significant differences in steatosis (P>0.99 P>0.99 and P>0.99), hepatocellular cholestasis (P>0.99 P>0.99 and P>0.99), cholangiolar cholestasis (P>0.99 P>0.99 and P>0.99) and bile duct injury (P>0.99 P>0.99 and P>0.99). In the present study, no significant differences were found among the three groups, but a higher percentage of cases in the DI-ALH group tended to show lobular inflammation (*de novo* AIH vs. DI-ALH, P=0.19; *de novo* AIH vs. DILI-ALH, P>0.99; DI-ALH vs. DILI-ALH, P=0.33; Steel-Dwass test). By contrast, all three cases in the DILI-ALH group showed inflammatory findings, mainly in the portal area. No significant difference in the progression of fibrosis was observed among the three groups.

Bile duct injury and eosinophilic infiltration, which are characteristics of DILI, tended to be higher in the DI-ALH group, but no significant differences were evident.

Overall, pathological findings suggestive of DILI were not prominent in the DILI-ALH group, and distinguishing DILI from *de novo* ALH based on pathological findings alone was difficult.

Discussion

DILI is known to be able to cause all forms of liver injury (17). A condition that is clinically, biochemically, immunologically and histologically similar to AIH is referred to as DI-ALH. However, distinguishing DI-ALH from idiopathic AIH is important (2,3,15,16). This is due to the fact that DI-ALH does not require long-term immunosuppressive treatment, and some cases have been reported to resolve spontaneously after discontinuation of the drugs involved. However, the number of cases of DI-ALH that are drug-related and which drugs are involved is not well understood.

The present study reviewed in detail the premorbid drug history of our previous AIH cases (22), identifying three cases with notable clinical courses. When liver injury occurred, these cases were diagnosed as DILI based on the scoring systems of AIH and RECUM, and did not meet the diagnostic criteria for AIH. Liver injury improved after discontinuing the drugs suspected as triggers. Liver injury then flared within 3-10 months. At that time, ANA titer and IgG levels were elevated, and the liver pathology was compatible with AIH. AIH was therefore considered as the most likely form of disease in the second episode. This notable clinical course of AIH after DILI has rarely been reported, although we have previously reported a case with a similar course (22). The present study showed that similar cases certainly exist for a certain percentage of cases with AIH.

A previous study suggested that a second episode of DILI is more characteristic of AIH (18). However, the second liver injury in the present cases were considered unlikely to represent DILI, as the patients had already stopped taking the drug that was considered to be the trigger. The possibility that AIH was present at baseline cannot be excluded, but at least at the time of the first episode, clear demonstration of the presence of AIH using the AIH scoring system was difficult. The pathological form that follows such a course and develops AIH was therefore categorized as DILI-ALH.

Table III. Characteristics and laboratory findings for *de novo* AIH, DI-ALH and DILI-ALH.

Characteristics	*De novo* AIH (n=29)	DI-ALH (n=10)	DILI-ALH (n=3)	P-value
Female, n (%)	25 (86.2)	6 (60.0)	1 (33.3)	P=0.33[a], P=0.25[b], P=0.56[c]
Age, years	67.0 [54.0, 73.0]	57.5 [48.5, 67.8]	66.0 [53.0, 67.0]	P=0.77[a], P=0.70[b], P=0.96[c]
≥65 years old	15 (51.7)	4 (40.0)	2 (66.6)	P>0.99[a], P>0.99[b], P>0.99[c]
BMI, kg/m^2	23.6 [22.2, 26.2]	25.4 [20.9, 28.7]	20.6 [17.5, 24.2]	P=0.88[a], P=0.56[b], P=0.57[c]
Symptoms	10 (34.5)	9 (90.0)	1 (33.3)	P=0.01[a,d], P>0.99[b], P=0.22[c]
Follow-up period, months	39.0 [16.0, 58.0]	23.5 [18.8, 44.5]	100.0 [68.5, 104]	P=0.95[a], P=0.35[b], P=0.15[c]
Autoimmune disease, n (%)	2 (6.9)	2 (20.0)	0 (0.0)	P=0.80[a], P>0.99[b], P>0.99[c]
T-Bil, g/ml	0.91 [0.65, 1.99]	2.80 [1.33, 7.96]	0.78 [0.64, 1.43]	P=0.15[a], P=0.74[b], P=0.15[c]
AST, U/l	138 [91.0, 514]	531 [312, 714]	474 [409, 482]	P=0.17[a], P=0.53[b], P=0.78[c]
ALT, U/l	216 [93.0, 537]	663 [401, 1076]	273 [243, 684]	P=0.039[a,d], P=0.58[b], P=0.78[c]
ALP ratio	0.96 [0.69, 1.22]	1.11 [0.78, 1.25]	1.30 [1.22, 1.39]	P=0.94[a], P=0.66[b], P=0.78[c]
γ-glutamyl transpeptidase, U/l	162 [114, 282]	233 [188, 333]	208 [155, 285]	P=0.22[a], P=0.91[b], P=0.94[c]
PT, %	84 [75, 101]	87 [75, 103]	87 [79, 105]	P=0.92[a], P=0.89[b], P=0.98[c]
Peripheral blood eosinophil, %	2.4 [1.6, 4.1]	3.2 [1.1, 3.5]	5.1 [4.2, 7.2]	P=0.93[a], P=0.17[b], P=0.18[c]
IgG, mg/dl	2,458 [1863, 2972]	2,059 [1773, 2326]	2,292 [1990, 2459]	P=0.18[a], P=0.81[b], P=0.87[c]
ANA positivity, n (%)	26 (89.7)	10 (100.0)	2 (66.6)	P>0.99[a], P>0.99[b], P=0.69[c]
AMA positivity, n (%)	7 (24.1)	1 (10.0)	0 (0.0)	P>0.99[a], P>0.99[b], P>0.99[c]
Revised AIH score before treatment	15 [14, 17]	13 [11, 14]	12 [11.5, 13.5]	P=0.040[a,d], P=0.16[b], P=0.99[c]
Steroid therapy, n (%)	24 (82.8)	10 (100.0)	3 (100.0)	P=0.91[a]. P>0.99[b], P>0.99[c]
Relapse after steroid tapering, n (%)	10 (34.5)	4 (40.0)	1 (33.3)	P>0.99[a], P>0.99[b], P>0.99[c]

[a]*De novo* AIH vs. DI-ALH; [b]*de novo* AIH vs. DILI-ALH; [c]DI-ALH vs. DILI-ALH; [d]P<0.05. Data are presented as median [range] unless otherwise indicated. AIH, AIH, autoimmune hepatitis; DILI, drug-induced liver injury; BMI, body mass index; T-Bil, total bilirubin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP ratio: alkaline phosphatase/upper normal limit; PT, prothrombin time; IgG, immunoglobulin G; ANA, antinuclear antibody; AMA, anti-mitochondrial antibodies.

The pathogenesis of DILI was considered to mainly involve an immune response to drugs, their metabolites or their metabolites when bound to self-proteins (4,8,17). No major antigens have been identified for AIH, but the possibility of long-term exposure to self-proteins or environmental, dietary or bacterial antigens has been suggested (4,9). In the present case of DILI-ALH, exposure to drug antigens had already ended after the initial liver injury. We speculate that an autoimmune response is evoked after discontinuation of the drug administration in patients or induced in response to some antigen produced after the liver injury in patients with DILI-ALH.

Previous studies have reported AIH induced by hepatitis A, hepatitis B or Epstein-Barr virus (27-31). In some cases, AIH was diagnosed from the liver injury flare several months after the onset of hepatitis. In those cases, genetic predispositions and immune responses to asialoglycoprotein receptors were suggested (27-31). Taking such factors into account, DILI-ALH could also be caused by immune induction due to liver damage associated with DILI, rather than by the drug or its metabolites acting directly as antigens. A number of suspected drugs were identified in the present study, including statins and herbal medicines known to cause liver damage similar to that with AIH. This suggests that certain drug classes may be involved, rather than specific drugs.

The next question is with regard to how many cases of AIH are suspected to be drug-related. Previous reports have shown that DI-ALH is present in 9-17% of cases, representing a relatively wide range (1,11,14,16,19,32,33). This may be due in part to the difficulty of making these diagnoses. Once a diagnosis of AIH is made clinically, immunosuppressive drugs are administered, generally without consideration of drug involvement as a trigger. On the other hand, once DILI is diagnosed, the diagnosis of AIH tends to be delayed with respect to subsequent liver injury. In the present study, drug involvement (as DI-ALH) or DILI involvement (as DILI-ALH) was suspected in 31.0% of cases diagnosed with AIH. This high rate may be due to the brief, retrospective review of the medication history for the patient prior to the diagnosis of AIH. In numerous cases, drug involvement was not considered at the time AIH was actually diagnosed. This may have been due to the fact that a number of the patients were receiving ongoing treatment for other conditions and were therefore receiving pharmacotherapy. However, further studies are

Table IV. Results of liver biopsy for patients with *de novo* AIH, DI-ALH, DILI-ALH.

Parameter	*De novo* AIH (n=29)	DI-ALH (n=10)	DILI-ALH (n=3)	P-value
Fibrosis[a], n				
F0	0	0	0	
F1	14	6	1	
F2	7	2	1	
F3	6	2	1	
F4	2	0	0	
Inflammation[a], n				
A0	1	0	0	
A1	8	1	0	
A2	14	4	2	
A3	6	5	1	
Lobular inflammation, n (%)	16 (55.2)	9 (90.0)	1 (33.3)	P=0.19[b], P>0.99[c], P=0.33[d]
Interface hepatitis, n (%)	29 (100.0)	10 (100.0)	3 (100.0)	-
Rosette, n (%)	5 (17.2)	5 (50.0)	1 (33.3)	P=0.26[b], P>0.99[c], P>0.99[d]
Lymphocyte infiltration, n (%)	29 (100.0)	10 (100.0)	3 (100.0)	-
Plasma cell infiltration, n (%)	28 (96.6)	9 (90.0)	3 (100.0)	P>0.99[b], P>0.99[c], P>0.99[d]
Eosinophil infiltration, n (%)	9 (31.0)	5 (50.0)	1 (33.3)	P>0.99[b], P>0.99[c], P>0.99[d]
Neutrophil infiltration, n (%)	5 (17.2)	2 (20.0)	0 (0.0)	P>0.99[b], P>0.99[c], P>0.99[d]
Steatosis, n (%)	8 (27.6)	3 (30.0)	1 (33.3)	P>0.99[b], P>0.99[c], P>0.99[d]
Hepatocellular cholestasis, n (%)	5 (17.2)	3 (30.0)	1 (33.3)	P>0.99[b], P>0.99[c], P>0.99[d]
Cholangiolar cholestasis, n (%)	2 (6.9)	0 (0.0)	0 (0.0)	P>0.99[b], P>0.99[c], P=1.00[d]
Bile duct injury, n (%)	11 (37.9)	4 (40.0)	0 (0.0)	P>0.99[b], P>0.99[c], P>0.99[d]

[a]Each case was reviewed as follows based on the new Inuyama classification. Inflammation grade: A0, no necro-inflammatory reaction; A1, mild necro-inflammatory reaction; A2, moderate inflammatory reaction; A3, severe necro-inflammatory reaction. Fibrosis: F0, no fibrosis; F1, fibrous portal expansion; F2, bridging fibrosis (mild fibrosis); F3, bridging fibrosis with lobular distortion (severe fibrosis); F4, cirrhosis. [b]*De novo* AIH vs. DI-ALH; [c]*de novo* AIH vs. DILI-ALH; [d]DI-ALH vs. DILI-ALH. AIH, AIH, autoimmune hepatitis; DI-ALH, drug-induced autoimmune-like hepatitis; DILI-ALH, ALH following drug-induced liver injury.

needed to clarify the exact rates of DI-ALH and DILI-ALH using established diagnostic criteria.

The clinical presentations of AIH, DI-ALH and DILI-ALH were next compared. Female patients made up 86.2% of the AIH cases, which is compatible with the Japanese national survey of AIH, whereas the proportions of female patients in the DI-ALH and DILI-ALH groups were 60.0 and 33.3%, suggesting some differences in the pathogeneses of these clinical entities (34). In terms of blood biochemistry, ALT levels were significantly higher in DI-ALH than in *de novo* AIH. However, AMA positivity was observed in only one case of DI-ALH and not at all in the DILI-ALH group. DI-ALH reportedly tends to have an acute onset and higher liver function test results compared with AIH (19,35). However, IgG and ANA are not reported to show significant differences (13,14,32). Such findings are consistent with the present results, in which no significant difference with regard to these factors was found in the DILI-ALH and AIH groups, suggesting that DILI-ALH presents a clinical picture close to that of AIH.

Histological examination also revealed no significant differences between the three groups in this study. However, the DI-ALH group tended to show more cases with an acute hepatitis pattern and eosinophil infiltration. No previous reports have described significant histological differences between AIH and DI-ALH (19,35). However, advanced fibrosis is reportedly not observed in DI-ALH (1,16,31). By contrast, in the present study, 3 patients with DI-ALH (27.3%) displayed a liver fibrosis grade of ≥F3. Although the etiology of this was not clear, the possibility that AIH was potentially present cannot be ruled out.

Another factor distinguishing DI-ALH from AIH is that DI-ALH has been reported to respond to treatment earlier than AIH and is characterized by the absence of relapse when immunosuppressive agents are discontinued (16). In the present study, the majority of patients with AIH and DILI-ALH were treated with steroids, and all 3 patients in the DILI-ALH group required steroids to achieve recovery. However, the results also showed 17.2% of the AIH group did not require immunosuppressive drugs. In fact, a Japanese national study also found that ~20% of patients with AIH did not receive immunosuppressive therapies such as steroids (34). Distinguishing between the three groups based on differences in treatment methods or response to treatment might thus be difficult.

In addition, no significant difference in relapse rate during steroid tapering was evident between the three groups. According to previous reports, the absence of

recurrence after long-term follow-up without immunosuppressive therapy is an important feature of DI-ALH (15,16). However, in the present case, 40.0% of patients in the DI-ALH group experienced recurrence. As some drugs, including statins, may induce a more classical form of AIH, differentiating AIH by the presence of recurrence may be difficult.

The present study identified a new phenotype after DILI in which liver damage with the characteristics of AIH appears. There are some limitations to the study. First, differentiating this diagnosis from AIH based on clinical, laboratory and histological examinations in addition to response to steroid therapy was considered difficult. Second, the total number of cases in this study was relatively small in order to obtain definite results. In addition, the cases were considered from a clinical perspective, so the immunological mechanisms of disease formation could not be fully elucidated. It will be necessary to accumulate more cases and further elucidate the pathogenesis.

In conclusion, the present study suggested that drugs may play a greater role in the pathogenesis of AIH than currently recognized, and the associations between DILI immunophenotypes and AIH should be properly classified and investigated in detail to clarify the exact epidemiologies of drug-related AIH. The present study revealed a new phenotype, DILI-ALH, as one phenotype of drug-related ALH. Specific biomarkers that allow differentiation of phenotypes are also important, and further studies will be needed to develop guidelines for the management of these patients.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

KI, SF, MK, YS, YN, HM, YN, DS, IM, YY, HI and KS designed the research and performed data curation. KI, SF, KF and KS confirm the authenticity of all the raw data, and were responsible for data collection and statistical analyses. KI and KS were major contributors to writing the original draft of the manuscript. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

This study was approved by the Medical Ethics Committee of Mie Prefectural General Medical Center (Yokkaichi, Japan; no. O-0094). Informed consent was considered to have been obtained from all patients included in the study via an opt-out protocol.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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