Centrilobular zonal necrosis is a unique subtype of autoimmune hepatitis A cohort study

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Abstract
BACKGROUND Centrilobular zonal necrosis (CZN) is advocated as a histological hallmark present in a small number of patients with autoimmune hepatitis (CZN-AIH). Moreover, CZN has been detected in the absence of significant interface hepatitis, one of the most important histological findings of AIH. The concept of CZN-AIH as a distinctive subtype of AIH remains controversial, due to the rarity of CZN-AIH and the ambiguous definition of CZN. To elucidate the clinicopathological and immunogenetic features of CZN-AIH, and to evaluate the significance of co-existent interface hepatitis in CZN-AIH. METHODS A total of 102 biopsy samples of AIH, obtained at The Jikei University Katsushika Medical Center and Jikei University Hospital, were reviewed. The 32 patients whose biopsies showed CZN were selected as the CZN-AIH group and the remaining 70 were grouped as the non-CZN-AIH controls. In the CZN-AIH group, interface hepatitis was histologically present in 37.5% (n=12; mixed-type) and absent in 62.5% (n=20; pure-type). Data on clinical, histopathological and immunogenetic features were statistically compared between the CZN-AIH group and the non-CZN-AIH controls. Additionally, significance of interface hepatitis in CZN-AIH was determined by comparative analysis of the mixed-type and pure-type subgroups. RESULTS Cases of CZN-AIH were more frequently of acute-onset hepatitis (56.2% vs chronic: 32.9%, P=0.031), lower immunoglobulin G level (P<0.001), lower antinuclear antibodies titer (P<0.001), and lower AIH score (P<0.001). Compared to the non-CZN-AIH cases, the CZN-AIH cases also tended to lack the typical histological characteristics of AIH and of the immunogenetic disproportionate distribution of HLA-DR genotypes in AIH (increased HLA-DR4 and decreased HLA-DR9), and responded more favorably to first-line therapy (P=0.054). For the acute-onset CZN-AIH cases, the clinical and histological features were indistinguishable from the non-acute cases. In contrast, the acute-onset non-CZN-AIH cases were distinguishable from the non-acute cases by lower antinuclear antibodies titer, lower immunoglobulin G level, and less advanced histological stage. The presence of interface hepatitis generally did not influence the morbidity of CZN-AIH, except for comorbid autoimmune diseases, higher gamma-glutamyltranspeptidase level, and increased immunoglobulin M level. CONCLUSION CZN-AIH is clinicopathologically and immunogenetically distinguishable. CZN can characterize a distinct AIH
subtype, regardless of onset-pattern or co-existent interface hepatitis.

Background
Autoimmune hepatitis (AIH) is one of the organ-specific autoimmune diseases, distinguished by its destruction of hepatocytes. The clinicopathological spectrum of AIH is wide. Typical AIH, a chronic progressive disease, is characterized by presence of antinuclear antibodies (ANA), increased serum immunoglobin (Ig)G, excellent response to immunosuppressive therapy, and the histological feature of moderate to severe interface hepatitis[1, 2]. In contrast, acute AIH often lacks both detectable ANA and the increased IgG[3, 4].

A minor proportion of AIH cases, regardless of acute or chronic state or extent of interface hepatitis, present the histological feature of centrilobular zonal necrosis (CZN)[5, 6]. AIH with the CZN feature (known as CZN-type AIH, and referred to herein as CZN-AIH) may have clinical features and immunogenetic backgrounds different from the typical AIH. Considering this new information for such a well-defined disease, a novel AIH classification system including the existence of CZN has been proposed by us and other groups[7–9]. However, the validity and value of this novel classification approach remains controversial, due at least in part to the rarity of CZN-AIH and the continuing ambiguity of the definition of CZN in the literature[8–13].

In the present study, we sought to make this classification system more robust. We first collected a large number of CZN-AIH cases according to the application of a strict pathological diagnostic criterion for CZN. The identified CZN-AIH cases were then examined in detail to identify the pathogenic profiles of clinical and immunogenetic features. The identified CZN-AIH cases were also examined for the co-existence or absence of apparent interface hepatitis, in order to evaluate the potential of a further sub-classification system for ‘mixed’-type or ‘pure’-type, respectively. Finally, these sub-types were examined in detail for their particular pathogenic profiles of clinical and immunogenetic features, allowing for determination of the significance of interface hepatitis in CZN-AIH.

Methods
Patients
Patients who were serially diagnosed as AIH and received liver biopsy at the time of diagnosis between the years of 2000 to 2018, at The Jikei University Katsushika Medical Center, was selected for study inclusion. Among the 88 patients identified and subsequently enrolled, 18 showed the histological feature of CZN (CZN-AIH group) and the remaining 70 did not show CZN (non-CZN-AIH group) (Fig. 1). In addition, 14 patients who were diagnosed as AIH with CZN at The Jikei University Hospital in the same time frame were included in the CZN-AIH group, for a total number of 32 cases. All enrolled patients were immunosuppressive therapy-naïve.

This study was conducted with the approval of the ethics committee of The Jikei University School of Medicine (Approval No. 29–305 (8921)).

**Diagnosis of AIH**

For all patients, the diagnosis of AIH was made by empirical judgment of experienced hepatologists after referring to the classification of definitive or probable AIH according to the diagnostic criteria of the International Autoimmune Hepatitis Group\[14\].

**Evaluation of liver histology**

Percutaneous liver biopsy was performed using 18-G or 16-G needle. The length of each biopsy sample exceeded 10 mm. Pathological examination was carried out by hematoxylin-eosin staining and Masson’s trichrome staining.

CZN was identified according to the definition given in our antecedent manuscript[8]. CZN status was verified by careful examination of necrosis presence in hepatocytes in zone 3 (presence shown in Fig. 2; absence shown in Fig. 3). Briefly, typical CZN (i.e. presence) showed a broad necro-inflammatory region arising from the central zone, which occasionally extended to the portal area or adjacent central zone. In general, the CZN was found to be spread nearly throughout the biopsied liver tissue and significant necro-inflammatory change other than CZN was rarely seen in the hepatic parenchyma (Fig. 2). In contrast, for non-CZN-AIH, broad necrotic change in the central zone was not found (Fig. 3).

Histological activity and fibrosis stage of the liver tissue were classified based on the METAVIR scoring system\[15\]. Activity was evaluated by the severity of interface hepatitis, expressed as A0 (no
interface hepatitis), A1 (mild interface hepatitis), A2 (moderate interface hepatitis) or A3 (severe interface hepatitis). Fibrotic stage was expressed as F0 (no fibrosis), F1 (portal expansion), F2 (portal to portal or central to central connection), F3 (portal to central connection) or F4 (cirrhosis). The pure-type CZN-AIH (Fig. 2A) was considered as typical CZN without significant interface hepatitis (A0 or A1). The mixed-type CZN-AIH (Fig. 2B) was considered as typical CZN with significant activity (A2 or A3) that co-existed in the same biopsy tissue.

Histological characteristics of AIH (e.g., lymphoplasmacytic infiltration, rosetting) were also evaluated for each biopsy sample. These histopathological investigations were carried out with blinding of the clinical information and under the supervision of a hepatic pathologist (Toru Harada).

**Classification of onset pattern**

Based on the study by Miyake et al[4], we classified AIH cases as acute or chronic onset. Acute clinical presentation (acute onset) was defined by: acute elevation of serum alanine aminotransferase (ALT) or aspartate aminotransferase (AST) levels to greater than 10 times the upper normal limit; or acute development of liver-related symptoms (e.g., fatigue, jaundice, appetite loss) without evidence of previous (> 6 mo before the time of diagnosis) liver dysfunction. Any other clinical presentations were defined as chronic.

**Data collection**

Data on patient background (i.e. age, sex, presence of complication with other autoimmune disease), blood parameters, and clinical course after immunosuppressive treatment were retrieved from the medical records. We selected the data for blood parameters from the date of the highest ALT level. Laboratory data that was extracted for analysis included levels of AST, ALT, gamma-glutamyltranspeptidase (GGT) alkaline phosphatase (ALP), total bilirubin, albumin, IgG and IgM, as well as platelet count, prothrombin time, and ANA (detected by indirect immunofluorescence using the human epithelial HEp-2 cells). ANA titers of equal to or greater than 40X were defined as positive, and equal to or greater than 160X as highly positive. Data on the HLA-DR antigens were available for 29 of the 32 CZN-AIH cases and 67 of the 70 non-CZN-AIH patients; in all these cases, the HLA-DR data had been obtained by a reverse sequence-specific oligonucleotide method.

**Efficacy of immunosuppressive therapy**
First-line immunosuppressive therapy involved prednisolone, or prednisolone + azathioprine. The initial dose of prednisolone/azathioprine and the reduction of prednisolone dosage after the initiation of therapy were decided by the attending physician, based upon the proposal by Czaja et al.[16]. Efficacy of the first-line therapy was classified by good response or poor response; the latter was defined as ALT not normalizing with administration of the first-line therapy. Efficacy of the first-line therapy was able to be evaluated in all patients, except for one in the CZN-AIH group. This patient (female) stopped attending medical consultation soon after the diagnosis of AIH was confirmed; however, 7 years and 1 mo later, she returned to our hospital in a state of advanced cirrhosis.

**Statistical analysis**
Continuous variables were expressed as median (Q1-3), and categorical data were expressed as n (%). Between-group comparisons were performed using the Mann-Whitney U-test for continuous and ordinal variables, and the Fisher`s exact test for categorical variables. P-values were expressed to three decimal places. Two-tailed P-values equal to or less than 0.05 were defined as statistically significant and those 0.05 < P ≤ 0.1 were considered as signifying a trend difference. All statistical analyses were carried out using Minitab version 18 for Windows.

**Results**

**Classification of the study population**
Among the total 32 CZN-AIH patients, 18 (56.3%) were sub-classified into the acute-onset group, while only 23 (32.9%) of the total 70 non-CZN-AIH patients were sub-classified into the acute-onset group.

Histologically, 20 (62.5%) of the total 32 CZN-AIH patients were sub-classified into the pure-type CZN-AIH group, while only 12 (37.5%) of the total 32 were sub-classified into the mixed-type CZN-AIH group. The full study population and classifications are illustrated in Fig. 1.

**Clinical, histological and immunogenetic features distinguishing the CZN-AIH group from the non-CZN-AIH group**
The clinicopathological and immunogenetic characteristics are summarized in Table 1 and Table 2.
Table 1
Comparison of clinicopathological data between CZN-AIH and non-CZN-AIH groups

|                          | CZN-AIH, n = 32 | non-CZN-AIH, n = 70 | P-value |
|--------------------------|-----------------|---------------------|---------|
| Age in yr                | 65 (49–74)      | 60 (45–72)          | 0.421   |
| Female sex               | 28 (88)         | 59 (84.3)           | 0.771   |
| Acute onset              | 18 (56.3)       | 23 (32.9)           | 0.031   |
| Other autoimmune disease | 7 (21.9)        | 5 (7.1)             | 0.046   |
| ANA titer                | 60 (10–80)      | 160 (80–560)        | <0.001  |
| Negative for ANA         | 8 (25)          | 9 (12.9)            | 0.155   |
| ANA high titer positive  | 6 (18.8)        | 34 (48.6)           | 0.046   |
| AST, IU/L                | 327 (168–627)   | 301 (115–547)       | 0.487   |
| ALT, IU/L                | 422 (220–730)   | 373 (133–668)       | 0.318   |
| ALP, IU/L                | 415 (281–464)   | 507 (339–648)       | 0.010   |
| GGT, IU/L                | 139 (82–210)    | 201 (88–303)        | 0.207   |
| ALAT/ALT                 | 3.33 (1.22–5.65)| 1.97 (0.99–3.65)    | 0.049   |
| Albumin, g/dL            | 4 (3.9–4.2)     | 3.8 (3.5–4.2)       | 0.056   |
| Serum IgG, mg/dL         | 1590 (1343–2036)| 2354 (1767–3052)    | <0.001  |
| Serum IgM, mg/dL         | 110 (77–152)    | 316 (233–412)       | <0.001  |
| PT, 10^4/µL              | 19.5 (16.1–21.2)| 17.9 (12.6–23.2)    | <0.001  |
| Histology                |                 |                     | 0.419   |
| F 0–1                    | 17 (53.1)       | 12 (17.1)           | <0.001  |
| F 2–4                    | 15 (46.9)       | 58 (82.9)           |         |
| A 0–1                    | 20 (62.5)       | 9 (12.9)            | <0.001  |
| A 2–3                    | 12 (37.5)       | 61 (87.1)           |         |
| Lymphoplasmacytic infiltrate | 25 (71.4)     | 54 (77.1)           | 1.000   |
| Rosetting                | 26 (81.3)       | 53 (75.7)           | 0.617   |
| Pathological score       | 2 (2–4)         | 5 (4–5)             | <0.001  |
| Pretreatment AIH score   | 12 (9–13)       | 13 (11–14)          | 0.001   |
| Pretreatment AIH score including pathological score | 14 (12–16) | 18 (15–19) | <0.001 |
| Poor response to 1st -line therapy | 0 (0)      | 8 (11.4)            | 0.054   |

Data are presented as n (range) or n (mean). A: Histological activity; AIH: Autoimmune hepatitis; ALP: Alkaline phosphatase; ALT: Alanine aminotransferase; ANA: Antinuclear antibodies; AST: Aspartate aminotransferase; CZN: Centrilobular zonal necrosis; F: Fibrosis; GGT: Gamma-glutamyltranspeptidase; IgG: Immunoglobulin G; IgM: Immunoglobulin M; Plt: Platelet count; PT: Prothrombin time; T-Bil: Total bilirubin.

Table 2
Distribution of HLA-DR phenotype in CZN-AIH and non-CZN-AIH groups

| HLA-DR | CZN-AIH, n = 29 | non-CZN-AIH, n = 67 | P-value | Healthy Japanese |
|--------|-----------------|---------------------|---------|------------------|
| 1      | 3 (10.3)        | 9 (13.4)            | 1.000   | 11.4%            |
| 4      | 10 (34.5)       | 41 (61.2)           | 0.025   | 41.8%            |
| 7      | 0 (0)           | 1 (1.5)             | 1.000   | 0.7%             |
| 8      | 5 (17.2)        | 19 (28.4)           | 0.311   | 23.5%            |
| 9      | 11 (37.9)       | 7 (10.4)            | 0.033   | 26.6%            |
| 10     | 0 (0)           | 1 (1.5)             | 1.000   | 1.0%             |
| 11     | 2 (6.9)         | 2 (3.0)             | 0.582   | 5.1%             |
| 12     | 2 (6.9)         | 1 (1.5)             | 0.216   | 10.9%            |
| 13     | 4 (13.8)        | 7 (10.4)            | 0.730   | 12.6%            |
| 14     | 5 (17.2)        | 16 (23.9)           | 0.595   | 13.6%            |
| 15     | 12 (41.4)       | 25 (37.3)           | 0.820   | 33.3%            |
| 16     | 0 (0)           | 2 (3.0)             | 1.000   | 1.8%             |

Data are presented as n (%). AIH: Autoimmune hepatitis; CZN: Centrilobular zonal necrosis.

There was no difference between the two groups for age at onset or sex. Acute onset was more frequent in the CZN-AIH group (56.2% vs 32.9%; P = 0.031). Co-existence of other autoimmune
diseases was also more frequent in the CZN-AIH group (P = 0.046). Although there was no difference in levels of AST, ALT, GGT, and total bilirubin between the two groups, the CZN-AIH group had lower levels of ALP (P = 0.010), ANA titer (P < 0.001), IgG (P < 0.001) and IgM (P < 0.001), as well as higher platelet count (P < 0.001) and higher ALT/GGT (P 0.049). The CZN-AIH group also showed a trend towards higher albumin (P 0.066).

Although there was no difference in frequency of negative status of ANA, the CZN-AIH group had a lower frequency of ANA-high titer positive status (P = 0.046); the maximum ANA titer was 320 in the CZN-AIH group and 2560 in the non-CZN-AIH group. Histologically, the severity of interface hepatitis (i.e. activity) was greater (P < 0.001) and fibrosis stage was more advanced (P < 0.001) in the non-CZN-AIH group. Pretreatment AIH score (P = 0.002) and AIH score including pathological score was lower (P < 0.001) in the CZN-AIH group. Poor response to first-line therapy tended to be less frequent in the CZN-AIH group (P = 0.054). There was no refractory case in the CZN-AIH group but 8 cases of such in the non-CZN-AIH group.

Concerning the distribution of HLA-DR, frequency of the HLA-DR4 phenotype was higher (P = 0.003) and that of the HLA-DR9 phenotype was lower (P = 0.003) in the non-CZN-AIH group than in the CZN-AIH group.

Clinical, histological and immunogenetic features distinguishing the acute-onset CZN-AIH subgroup from the acute-onset non-CZN-AIH subgroup

The clinicopathological and immunogenetic characteristics of the acute-onset CZN-AIH subgroup and the acute-onset non-CZN-AIH subgroup are summarized in Table 3.
The age at onset tended to be higher in the acute-onset CZN-AIH subgroup than in acute-onset non-CZN subgroup (P = 0.058). The acute-onset CZN-AIH subgroup had significantly lower levels of ALP (P = 0.003), GGT (P = 0.034), IgG (P = 0.032) and IgM (P = 0.044) and of histological activity (P = 0.006) and fibrosis stage (P = 0.097) with marginal significance. In contrast, no significant difference was found for frequency of positive ANA status nor level of ANA titer. Although there was no between-subgroup difference for the pretreatment AIH score (P = 0.456), the AIH score including pathological score was lower (P = 0.020) in the acute-onset CZN-AIH subgroup.

The distribution of HLA-DR was similar in the acute-onset CZN-AIH subgroup as in the entire CZN-AIH group; a similar finding was obtained for the non-CZN-AIH sub- and entire groups. However, there was
no statistically significant difference in the frequency of HLA-DR4 and HLA-DR9 between the acute-onset CZN-AIH subgroup and the acute-onset non-CZN-AIH subgroup.

Clinical and immunogenetic features distinguishing the pure-type CZN-AIH subgroup and the mixed-type CZN-AIH subgroup

In order to investigate the significance of interface hepatitis in CZN-AIH, we compared the pure-type CZN-AIH subgroup (Fig. 2A) and the mixed-type CZN-AIH subgroup (Fig. 2B).

In the mixed-type CZN-AIH subgroup, co-existence of other autoimmune diseases tended to be more frequent than in the pure-type CZN-AIH subgroup ($P = 0.074$). The mixed-type CZN-AIH subgroup had higher level of IgM ($P = 0.047$), pretreatment AIH score ($P = 0.004$) and AIH score including pathological score ($P < 0.001$), and showed a trend towards higher level of GGT ($P = 0.008$) (Table 4).

Positive ANA status, level of ANA titer did not show a dominant difference between the two subgroups. In addition, there was no statistically significant difference in the distribution of HLA-DR phenotypes between the two subgroups.
Table 4
Comparison of clinicopathological data between pure-type CZN-AIH and mixed-type non-CZN-AIH groups

|                                      | Acute onset CZN-AIH (18) | Acute onset non-CZN-AIH (23) | p-value |
|--------------------------------------|--------------------------|-------------------------------|---------|
| Age (year)                           | 66 (55–74)               | 50 (39–67)                    | 0.058   |
| Female sex                           | 16 (88.9)                | 20 (87.0)                     | 1.000   |
| Other autoimmune disease             | 4 (22.2)                 | 1 (4.3)                       | 0.150   |
| ANA titer                            | 60 (40–80)               | 40 (0–320)                    | 0.914   |
| negativity of ANA                    | 3 (16.7)                 | 7 (30.4)                      | 0.467   |
| ANA-high titer positive ≥ 160        | 3 (16.7)                 | 7 (30.4)                      | 0.467   |
| AST (IU/l)                           | 506 (307–659)            | 521 (375–1084)                | 0.351   |
| ALT (IU/l)                           | 640 (414–842)            | 668 (547–1034)                | 0.590   |
| ALP (IU/l)                           | 430 (317–477)            | 608 (460–791)                 | 0.003   |
| GGT (IU/l)                           | 155 (90–238)             | 216 (143–358)                 | 0.034   |
| ALT/GGT                             | 4.37 (2.69–5.70)         | 2.85 (1.79–4.83)              | 0.168   |
| T-Bil (mg/dl)                        | 1.1 (1.0–2.5)            | 2.8 (0.9–6.8)                 | 0.292   |
| Alb (g/dl)                           | 4 (3.9–4.1)              | 4.0 (3.6–4.5)                 | 0.792   |
| Serum IgG (mg/dl)                    | 1638 (1476–2052)         | 2154 (1663–3561)              | 0.032   |
| Serum IgM (mg/dl)                    | 124 (86–162)             | 166 (108–294)                 | 0.044   |
| Plt (104/µl)                         | 20.1 (18.3–27.3)         | 19.4 (14.3–23.6)              | 0.423   |
| PT (%)                               | 85 (73–97)               | 93 (78–99)                    | 0.355   |
| **Histology**                        |                          |                               |         |
| F 0–1                                | 9 (50.0)                 | 5 (21.7)                      | 0.097   |
| F 2–4                                | 9 (50.0)                 | 18 (78.3)                     |         |
| A 0–1                                | 10 (55.6)                | 3 (13.0)                      | 0.006   |
| A 2–3                                | 8 (44.4)                 | 20 (87.0)                     |         |
| Lymphoplasmacytic infiltrate         | 13 (72.2)                | 19 (82.6)                     | 0.471   |
| Rosetting                            | 14 (77.8)                | 17 (73.9)                     | 1.000   |
| Pathological score                   | 2 (2–4)                  | 5 (4–5)                       | 0.006   |
| pre treatment AIH score              | 12 (10–13)               | 12 (11–14)                    | 0.417   |
| pre treatment AIH score including pathological score | 14 (12–16) | 17 (14–18) | 0.024 |
| poor response to 1st line therapy    | 0 (0)                    | 1 (4.3)                       | 1.000   |
| HLA-DR 4                             | 8 (47.1)                 | 14 (60.9)                     | 0.553   |
| HLA-DR 9                             | 5 (29.4)                 | 2 (8.7)                       | 0.113   |

Data are presented as n (range) or n (mean). A: Histological activity; AIH: Autoimmune hepatitis; ALP: Alkaline phosphatase; ALT: Alanine aminotransferase; ANA: Antinuclear antibodies; AST: Aspartate aminotransferase; CZN: Centrilobular zonal necrosis; F: Fibrosis; GGT: Glutamyltranspeptidase; IgG: Immunoglobulin G; IgM: Immunoglobulin M; Plt: Platelet count; PT: Prothrombin time; T-Bil: Total bilirubin.

Discussion

Although the histological finding of prominent necro-inflammation in zone 3 of the liver parenchyma is considered not to be completely specific for AIH, this pathological change has been indicated to be a unique feature of some AIH patients[5, 6]. This change, called ‘CZN’, has tended to be given a broad interpretation because necro-inflammatory change of the liver parenchyma in AIH cases has shown greater intensity in zone 3 than in the other zones. Actually, small- to medium-size necrosis has been reported as not infrequent in zone 3 of typical AIH cases[12].

Therefore, the inarticulate definition of CZN may obscure the significance of CZN in medical care of AIH. In the present study, we used a strict definition of CZN — that being, broad necro-inflammatory...
lesions systematically spreading through the hepatic parenchymal tissue. Although one research

group has speculated that CZN is a histological feature of early-stage AIH\textsuperscript{[17]}, we previously found

that CZN-AIH is clinically and immunogenetically different from the majority of AIH (non-CZN-AIH)
cases. However, consensus on the significance of CZN-AIH remains elusive, presumably due at least

in part to the rarity of this type of AIH. To address this controversy, we collected 32 cases of CZN-AIH,

and the clinicopathological features of these patients were studied in comparison to cases of non-

CZN-AIH.

Distinguishing features of CZN-AIH from non-CZN-AIH have been studied previously by us and

others\textsuperscript{[8, 9, 12]}\textsuperscript{1}. The findings from the current study that CZN-AIH tended to develop similar to acute

hepatitis and had low IgG, ANA titer and AIH score (Table 1) are in good agreement with findings from

our previous study\textsuperscript{[8]}\textsuperscript{1} but different from those of Miyake et al\textsuperscript{[12]}\textsuperscript{1}. This discrepancy is probably due
to the difference in histological definition of CZN between the studies. Our current findings also

strengthen the previous findings\textsuperscript{[8, 9]}\textsuperscript{1} that the characteristics of typical AIH were poor in CZN-AIH. In

addition, we have newly determined that ALT/GGT is higher in CZN-AIH; this finding may correspond
to relatively mild portal inflammation in CZN-AIH that may affect elevation of biliary enzymes.

Another novel finding from the current study was the higher co-existent rate of other autoimmune
diseases in CZN-AIH (Table 1). We also showed that response to first-line immunosuppressive therapy

was better for the CZN-AIH cases. Immunogenetically, in comparison with non-CZN-AIH, we found low

frequency of HLA-DR4 and high frequency of HLA-DR9 (Table 2). While the observed frequencies of

HLA-DR4 and HLA-DR9 in CZN-AIH are not distinct from those in the healthy Japanese population\textsuperscript{[18]},

the increase of HLA-DR4 and decrease of HLA-DR9 in non-CZN-AIH is distinct from healthy Japanese.

These findings strongly suggest that disease susceptibility for CZN-AIH is not defined by HLA-DR

phenotype.

It has been reported that some AIH cases develop similar to acute hepatitis, a situation prompting the

name of ‘acute-onset AIH’\textsuperscript{[4]}\textsuperscript{1}. We found in this study that CZN is frequently associated with acute-
onset AIH, though a small portion of non-CZN-AIH cases also developed similar to acute hepatitis as
well (Table 1). When the clinical presentations of acute-onset CZN-AIH and acute-onset non-CZN-AIH were compared, the results were partially different from those obtained by the comparison between the entire CZN-AIH group and the entire non-CZN-AIH group (Table 1-3). In acute-onset AIH, ANA titer was not different between the CZN-AIH group and the non-CZN-AIH group; thus, the AIH score excluding the pathological score is similar between the two groups (Table 3). In the acute-onset non-CZN-AIH group, low ANA titer, low amount of IgG, and less advanced stage were the distinctive features of acute onset compared to entire CZN-AIH. However, no distinctive features of acute-onset CZN-AIH were found in comparison to the entire CZN-AIH group.

Although Miyake et al.[4] have previously described acute AIH in comparison with chronic AIH, they did not sub-classify the acute AIH according to CZN and non-CZN. In a previous study by Abe et al.[7], acute AIH cases were divided into two groups based on the presence or absence of central necrosis, for investigation of the clinicopathological differences among them. Those findings are partially discordant with ours, especially regarding the frequency of ANA negativity, serum ALP level, and presence of interface hepatitis. It is important to note that the other study included both a considerable number of cases that showed exacerbation in the chronic phase of AIH and patients with inconspicuous ALT elevation (5 X < maximum ALT < 10 X upper normal limit). Our comparative analysis of acute-onset CZN and acute-onset non-CZN, performed under the strict definition of both acute onset and CZN, was novel. Our findings of differences in laboratory data between acute-onset CZN-AIH and acute-onset non-CZN-AIH were serum levels of ALP, GGT, IgG and IgM. Tendency of elderly onset in acute CZN-AIH was noted. Histologically, acute-onset non-CZN-AIH had lower activity and tendency of less advanced fibrosis stage. Moreover, our data suggested that the difference between CZN-AIH and non-CZN-AIH could become more apparent as they evolve to a chronic state. In our previous study, we pointed out that the clinical feature of early fibrotic stage CZN-AIH was clearly different from that of early fibrotic stage non-CZN-AIH[8]. When that finding is taken into consideration, our current findings strongly suggest that the manner of clinical and histological progression in CZN-AIH is distinctive from that of typical non-CZN-AIH.
Finally, we examined significance of interface hepatitis in CZN-AIH (Table 4), because interface hepatitis has been considered as the most important pathological feature of AIH\cite{1}. Although the severity of interface hepatitis is generally slight in CZN-AIH, we found significant interface hepatitis in 12 of our 32 patients. However, we did not find any distinctive clinical feature of these mixed-type CZN-AIH cases, except for increase of IgM, compared to pure-type CZN-AIH. Immunogenetically, distribution of the HLA-DR phenotype was similar between the pure-type CZN-AIH and mixed-type CZN-AIH. These findings suggest that existence of interface hepatitis in CZN-AIH is a part of the pathogenic evolution of CZN-AIH, manifesting at any time during the clinical course of CZN-AIH. The fate of untreated CZN-AIH may be acute hepatic failure or decompensated liver cirrhosis. We experienced one patient who died by chronic hepatic failure. However, the course of progression in CZN-AIH is not clearly understood, because liver architecture is severely distorted in advanced stage of disease that made it difficult to distinguish CZN-AIH from non-CZN-AIH.

The current study has some limitations that must be considered when interpreting or seeking to generalize our findings. First, this was not a prospective study; however, as CZN is a rare histological finding, it is extremely difficult to conduct a prospective study. Second, there was a small number of cases in the CZN-AIH group, which may have affected our sub-analyses; in order to verify our results, a larger group of CZN-AIH cases, identified based on the same strict definition of CZN we used, is needed. We will collect and examine more cases of CZN-AIH in the future.

Conclusion
CZN-AIH had less of the clinicopathological characteristics of typical AIH, with a tendency towards favorable response to first-line therapy. Even in acute-onset AIH, the CZN-AIH cases had lower serum IgG and lower pretreatment AIH score including pathological score, as compared with the non-CZN-AIH cases. The CZN-AIH cases also did not exhibit the HLA-DR phenotype disproportion characteristic of non-CZN-AIH cases, suggesting that the two types are immunogenetically different. Moreover, existence of significant interface hepatitis in the CZN-AIH cases was not affected on the clinical characteristics in CZN-AIH. Therefore, this combination may be a part of the disease evolution that occur during the clinical course of CZN-AIH.
Declarations

**Ethics approval and consent to participate**

This study was conducted in accordance with the Declaration of Helsinki and ethical guidelines issued from administrative departments, and was approved by the Local Ethics Committee of The Jikei University School of Medicine (No.29-305 [8921]) and carried out by the opt-out consent process. Written informed consent was waived by the ethics committee.

**Consent for publication**

Not applicable

**Availability of data and materials**

**Competing interests**

The authors declare that they have no competing interests

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**Authors' contributions**

All authors helped to perform the research; Kaoru U and Yoshio A designed the study, interpreted and analyzed the data, reviewed the pathological tissues, and wrote the manuscript; Kaoru U, Chika K, Tomohisa N, Atsushi H, Yoshio A, Jinya I, Chisato S, Tsunekazu O and Masayuki S collected the data; Toru H, Kaoru U and Yoshio A evaluated HE and Masson staining; All authors read and approved the final manuscript.

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**Authors' information (optional)**

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Figures
Figure 1

Study population. AIH: Autoimmune hepatitis; CZN: Centrilobular zonal necrosis.
Figure 2

Liver biopsy pathology. A: Pathology of pure-type CZN-AIH has CZN without interface hepatitis; B: Pathology of mixed-type CZN-AIH has CZN with interface hepatitis. Masson’s trichrome staining. AIH: Autoimmune hepatitis; C: Centrilobular; CZN: Centrilobular zonal necrosis; P: Portal area.
Figure 3

Liver biopsy pathology. Non-CZN-AIH has interface hepatitis without CZN. Masson’s trichrome staining. AIH: Autoimmune hepatitis; C: Centrilobular; CZN: Centrilobular zonal necrosis; P: Portal area.