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Centrilobular zonal necrosis is a unique subtype of autoimmune hepatitis

A cohort study

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Abstract

Backgrounds: Centrilobular zonal necrosis (CZN) is described as a histological feature present in a small number of autoimmune hepatitis (CZN-AIH) patients. CZN may be detected in the absence of significant interface hepatitis, which is the most important histological finding of AIH. The clinical and histopathological spectra of CZN-AIH were not homogeneous, and 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.

Methods: To elucidate the clinical and immunogenetic features of CZN-AIH, a total of 102 biopsy samples of AIH, obtained at The Jikei University Katsushika Medical Center and Jikei University Hospital from 2000 to 2018, 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 (control AIH). Data on clinical, histopathologic, and immunogenetic features were statistically compared between the CZN-AIH and the control AIH group. Additionally, the impact of the onset pattern (acute or chronic) and coexistent significant interface hepatitis in CZN-AIH was determined.

Results: In CZN-AIH, the frequency of acute-onset cases was significantly higher than that in control AIH (56.2% vs 32.9%; $P < .05$), and the number of cases with moderate-to-severe interface hepatitis in liver histology was significantly lower (37.5% vs 87.1%; $P < .001$). Compared to the control AIH, cases of CZN-AIH had lower immunoglobulin G level ($P < .001$), lower antinuclear antibodies titer ($P < .001$), and lower AIH score ($P < .001$). The immunogenetic disproportionate distribution of HLA-DR phenotypes in control AIH (increased HLA-DR4 and decreased HLA-DR9) was not found in CZN-AIH. Moreover, CZN-AIH was less frequently relapsed ($P < .05$). For the acute-onset CZN-AIH cases, the clinical features were hardly indistinguishable from the chronic CZN-AIH cases. Similarly, the existence of interface hepatitis did not influence on the pathophysiology of CZN-AIH. Moreover, the acute-onset CZN-AIH cases is clinically distinguishable from acute-onset control AIH

Conclusion: CZN can characterize as a distinct AIH subtype, regardless of onset-pattern or coexistence of significant interface hepatitis. To further strengthen this hypothesis, collection of more CZN-AIH cases is needed.

Abbreviations: AIH = autoimmune hepatitis, ALP = alkaline phosphatase, ALT = alanine aminotransferase, ANA = antinuclear antibodies, AST = aspartate aminotransferase, CZN = centrilobular zonal necrosis, GGT = gamma-glutamyl transferase, IgG = immunoglobulin G, IgM = immunoglobulin M, Plt = platelet count, PT = prothrombin time, T-Bil = total bilirubin

Keywords: acute onset, autoimmune hepatitis, centrilobular zonal necrosis, HLA, interface hepatitis

1. Introduction

Autoimmune hepatitis (AIH) is 1 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 immunoglobulin G (IgG), excellent response to immunosuppressive therapy, and the histological feature of moderate to severe (significant) interface hepatitis.[1–3] In contrast,

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The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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.

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acute-onset AIH often lacks both detectable ANA and the increased IgG.[4,5]

A minor proportion of AIH cases (17.5%–29%), regardless of acute or chronic state or extent of interface hepatitis, present the histological feature of centrilobular zonal necrosis (CZN).[6–10] AIH with the CZN feature (known as CZN-type AIH, and referred to herein as CZN-AIH) may have unique 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.[9–11] However, the validity and value of this novel classification approach remain controversial, due at least in part to the rarity of CZN-AIH and the continuing ambiguity of the definition of CZN in the literature.[8–16]

In the present study, in order to clarify the significance of CZN-AIH, we first collected a larger number of CZN-AIH cases according to the application of a strict pathological diagnostic criterion for CZN, and the clinicopathological and immunogenetic features were compared to non CZN (control) AIH. The patients who collected this study were added to the previous study population.[10]

In addition, considering the heterogeneity of clinical and histological scope of CZN-AIH cases, we subdivided CZN-AIH cases according to the onset pattern (acute or chronic) or the existence/absence of significant interface hepatitis. Then, in order to evaluate the potential of a further subclassification for CZN-AIH, we examined the difference in the clinical and immunogenetic features of subdivided groups.

Finally, in order to clarify whether acute type CZN-AIH could be distinguished from acute type control AIH, we compared the feature of acute CZN-AIH and acute control AIH because CZN-AIH has been thought to be 1 of the histological phenotype of acute AIH.[4,9,11,16–19]

2. Methods

2.1. Patients

Patients who were diagnosed as AIH and received percutaneous liver biopsy at the time of diagnosis between the years of 2000 to 2018, at The Jikei University Katsushika Medical Center, were 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 (control 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]).

2.2. 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.[20] We strictly ruled out the possibility of drug induced liver damage by more than twice of medical interviews as detailed as possible. We also extendedly examine the use of dietary supplements. Patients using supplements that may cause liver damage were excluded from this study. Moreover, possibility of other liver diseases that may induce centrilobular necrosis was carefully excluded. We did not measure liver kidney microsomal antibody because type 2 AIH is extremely rare in Japan. Hepatitis due to hepatitis A, B, C, and E virus, Epstein-Barr virus, and cytomegalovirus were excluded by serological test.

2.3. Evaluation of liver histology

After confirming the diagnosis of AIH, we evaluated disease severity according to Japanese criteria.[21–24] Due to bleeding tendency, percutaneous liver biopsy was not performed in the patients with severe AIH. Thus, severe AIH were excluded from this study.

We used 18-G or 16-G needle for liver biopsy. The length of each biopsy sample exceeded 10 mm. Pathological examination was carried out by hematoxylin-eosin staining and Masson trichrome staining.

CZN was identified according to the definition given in our antecedent manuscript.[10] CZN 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

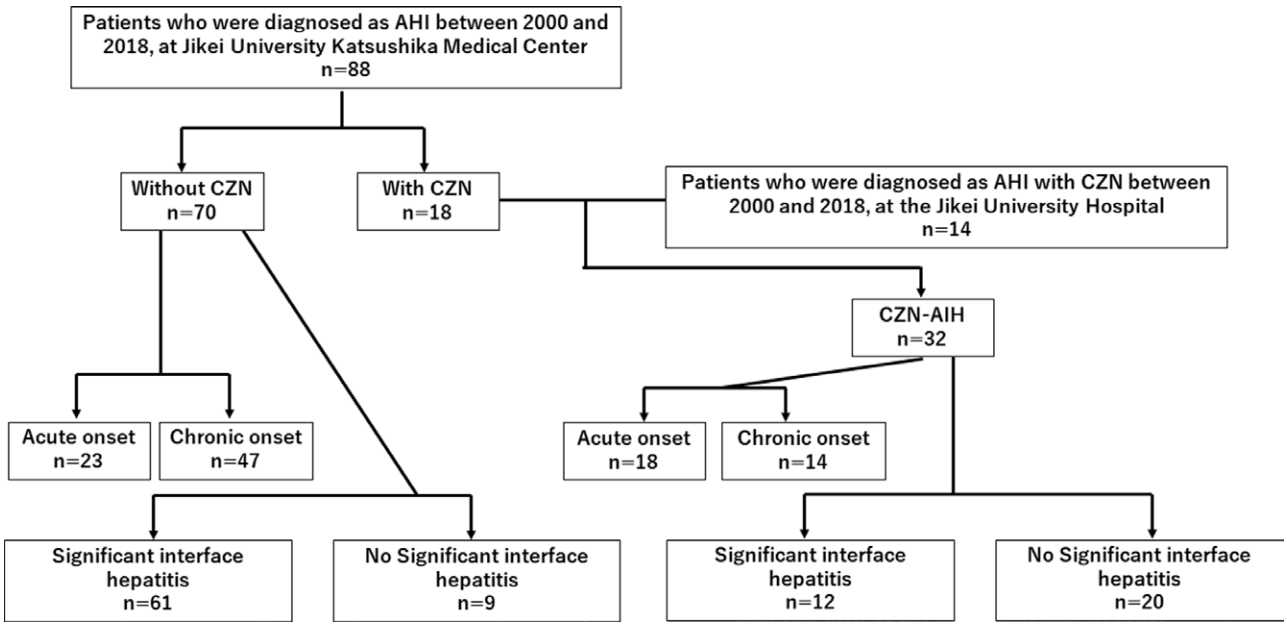

Figure 1. Study population. AIH = autoimmune hepatitis, CZN = centrilobular zonal necrosis.

(i.e., presence) showed a broad necroinflammatory 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 necroinflammatory 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.[25] 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 CZN-AIH without coexistence of significant interface hepatitis (Fig. 2A) was considered as typical CZN (A0 or A1). The CZN-AIH coexistent with significant interface hepatitis (A2 or A3) (Fig. 2B) was considered as CZN-AIH with significant activity.

Histological characteristics of AIH (e.g., lymphoplasmacytic infiltration and 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).

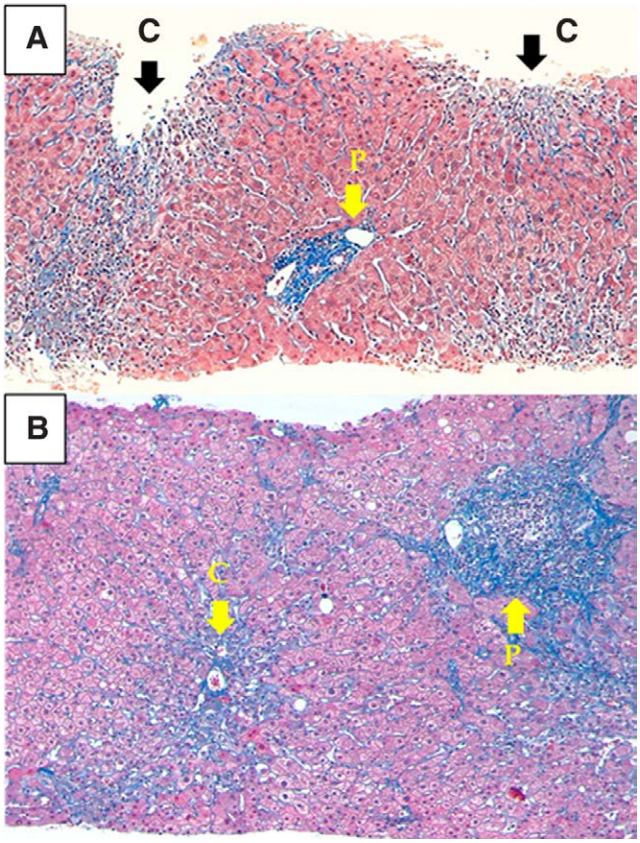

Figure 2. Liver biopsy pathology. (A) Pathology of CZN-AIH without having significant interface hepatitis. (B) Pathology of CZN-AIH having significant interface hepatitis. Masson's trichrome staining. AIH = autoimmune hepatitis, C = centrilobular, CZN = centrilobular zonal necrosis, P = portal area.

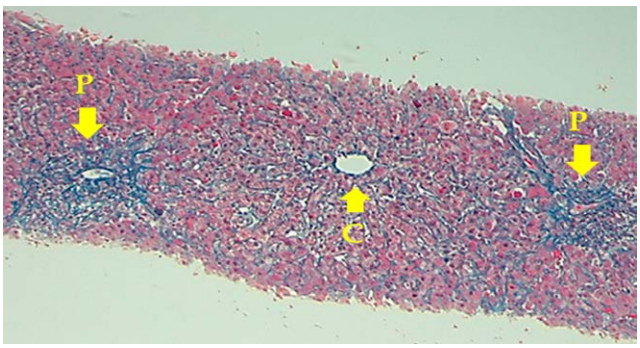

Figure 3. Liver biopsy pathology. Control AIH has interface hepatitis without CZN. Masson trichrome staining. AIH = autoimmune hepatitis, C = centrilobular, CZN = centrilobular zonal necrosis, P = portal area.

2.4. Classification of onset pattern

Based on the study by Miyake et al,[5,8] 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, and appetite loss) without evidence of previous (> 6 mo before the time of diagnosis) liver dysfunction. Any other clinical presentations were defined as chronic.

2.5. Data collection

Data on patient background (i.e., age, sex, and presence of complication with other autoimmune disease), blood parameters, clinical course after immunosuppressive treatment, relapse rate, and period until relapse were retrieved from the medical records. We selected the data for blood parameters at the date when ALT level was examined just before start of immunosuppressive therapy. Laboratory data that were extracted for analysis included levels of AST, ALT, gamma-glutamyl transpeptidase (GGT) alkaline phosphatase (ALP), total bilirubin, albumin, IgG, and immunoglobulin M (IgM), as well as platelet count (Plt), prothrombin time, and ANA (detected by indirect immunofluorescence using the human epithelial 2 cells).[21] ANA titers of equal to or greater than 40× were defined as positive, and equal to or greater than 160× as highly positive. Data on the HLA-DR antigens were available for 29 of the 32 CZN-AIH cases and 67 of the 70 control AIH patients; in all these cases, the HLA-DR antigens were determined by a corresponding HLA-B1 genotype obtained by a reverse sequence-specific oligonucleotide method.

2.6. 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 on the proposal by Czaja and Freese.[26] 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 1 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 month later, she returned to our hospital in a state of advanced cirrhosis.

We followed up these patients after administration of immunosuppressive drugs for evaluating the efficacy of therapy. Relapse is defined as at least twice or more than twice the upper limit of normal after ALT normalizes.

2.7. Statistical analysis

Continuous variables were expressed as median (Q1–Q3), and categorical data were expressed as n (%). Between-group

Table 1

Comparison of clinicopathological data between CZN-AIH and control AIH groups.

	CZN-AIH (N = 32)	Control AIH (N = 70)	*P* value
Age (yr)	65 (49–74)	60 (45–72)	NS
Female sex	28 (88)	59 (84.3)	NS
Acute onset	18 (56.3)	23 (32.9)	<.05
Other autoimmune disease	7 (21.9)	4 (5.7)	<.05
ANA titer	60 (10–80)	160 (80–560)	.001
Negativity of ANA	8 (25)	10 (14.3)	NS
ANA-high titer positive ≥ 160	6 (18.8)	34 (48.6)	<.05
AST (IU/l)	327 (168–627)	301 (115–547)	NS
ALT (IU/l)	422 (220–730)	373 (133–668)	NS
ALP (IU/l)	415 (281–464)	507 (339–648)	<.01
GGT (IU/l)	139 (82–210)	201 (88–303)	NS
ALT/GGT	3.33 (1.22–5.65)	1.97 (0.99–3.65)	<.05
T-Bil (mg/dl)	1.1 (0.8–1.7)	1.1 (0.8–4.1)	NS
Albumin (g/dl)	4 (3.9–4.2)	3.8 (3.5–4.2)	NS
Serum IgG (mg/dl)	1590 (1343–2036)	2354 (1767–3052)	<.001
Serum IgM (mg/dl)	110 (77–152)	161 (100-–73)	<.001
Plt (104/μl)	19.5 (16.1–21.2)	17.9 (12.6–23.2)	<.001
PT (%)	85 (73–93)	88 (76–99)	NS
Histology			
F 0–1	17 (53.1)	12 (17.1)	<.001
F 2–3	15 (46.9)	49 (70.0)	
F 4	0 (0)	9 (12.9)	
A 0–1	20 (62.5)	9 (12.9)	<.001
A 2–3	12 (37.5)	61 (87.1)	
Lymphoplasmacytic infiltrate	25 (71.4)	61 (87.1)	NS
Rosetting	26 (81.3)	56 (80.0)	NS
Pathological score	2 (2–4)	5 (4–5)	<.001
Pretreatment AIH score	12 (9–13)	13 (11–13)	<.01
Pretreatment AIH score including pathological score	14 (12–16)	17 (15–19)	<.001
Poor response to first-line therapy	0 (0)	8 (11.4)	NS
Relapse	5 (15.6)	29 (41.4)	<.05
Period until relapse*	506 (194–1000)	772 (333–1651)	NS

*Data missing in 9 of 29 cases

Data are presented as *n* (range) or *n* (mean).

AIH = autoimmune hepatitis, ALP = alkaline phosphatise, ALT = alanine aminotransferase, ANA = antinuclear antibodies, AST = aspartate aminotransferase, CZN = centrilobular zonal necrosis, GGT = gamma-glutamyl transpeptidase, IgG = immunoglobulin G, IgM = immunoglobulin M, IU = international unit, NS = not statistically significant, 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)	Control AIH (N = 67)	*P* value	Healthy Japanese
1	3 (10.3)	9 (13.4)	NS	11.4%
4	10 (34.5)	41 (61.2)	<.05	41.8%
7	0 (0)	1 (1.5)	NS	0.7%
8	5 (17.2)	19 (28.4)	NS	23.5%
9	11 (37.9)	7 (10.4)	<.05	26.6%
10	0 (0)	1 (1.5)	NS	1.0%
11	2 (6.9)	2 (3.0)	NS	5.1%
12	2 (6.9)	1 (1.5)	NS	10.9%
13	4 (13.8)	7 (10.4)	NS	12.6%
14	5 (17.2)	16 (23.9)	NS	13.6%
15	12 (41.4)	25 (37.3)	NS	33.3%
16	0 (0)	2 (3.0)	NS	1.8%
17	0 (0)	0 (0)	NS	0.28%

Data are presented as *n* (%).

AIH = autoimmune hepatitis, CZN = centrilobular zonal necrosis, NS = not statistically significant.

comparisons were performed using the Mann-Whitney *U* test for continuous and ordinal variables, and the Fisher exact test for categorical variables. *P* values were expressed to 3 decimal places. Two-tailed *P* values less than .05 were defined as statistically significant. *P* values less than .05 were expressed as $P < .05$, values less than .01 were expressed as $P < .01$, values less than .001 were expressed as $P < .001$. All statistical analyses were carried out using Minitab version 18 (Kozo Keikaku Engineering, Tokyo, Japan) for Windows.

3. Results

3.1. Classification of the study population

Among the total 32 CZN-AIH patients, 18 were subclassified into the acute-onset group, whereas 23 of the total 70 control AIH patients were subclassified into the acute-onset group. Histologically, 20 of the total 32 CZN-AIH patients were subgrouped into CZN-AIH without significant interface hepatitis, and coexistent significant interface hepatitis was found 12

patients. Whereas significant interface hepatitis were found in 61 of 70 cases in the control AIH. The full study population and classifications are illustrated in Figure 1.

3.2. Clinical, histological, and immunogenetic features distinguishing the CZN-AIH group from the control AIH group

The clinicopathological and immunogenetic characteristics are summarized in Tables 1 and 2. There was no difference between the 2 groups for age at onset or sex. Acute onset was more frequent in the CZN-AIH group (56.3% vs 32.9%; $P < .05$). Seven cases of coexistent other autoimmune diseases in CZN-AIH were: 1 of multiple sclerosis, 2 of chronic thyroiditis, 1 of Graves' disease, 1 of type 1 diabetes mellitus, 1 of Still disease, and 1 of rheumatoid arthritis. Four cases of coexistent other autoimmune diseases in control AIH was: 1 of chronic thyroiditis, 1 of Graves' disease, 1 of Sjogren syndrome, and 1 of psoriasis vulgaris. There was no difference in levels of AST, ALT, GGT, and total bilirubin between the 2 groups, the CZN-AIH group had lower levels of ALP ($P < .01$), IgG ($P < .001$), IgM ($P < .001$), and ANA titer ($P < .001$) as well as higher Plt ($P < .001$).

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 ($P < .05$); the maximum ANA titer was 320 in the CZN-AIH group and 2560 in the control AIH group. Histologically, the severity of interface hepatitis (i.e., activity) was mild ($P < .001$), and fibrosis stage was less advanced ($P < .001$) in the CZN-AIH group. Pretreatment AIH score ($P < .01$) and AIH score including pathological score were lower ($P < .001$) in the CZN-AIH group. The relapse rate was significantly lower in the CZN-AIH group (15.6% in 5 cases) than in control group (41.4% in 29 cases) ($P < .05$). The period until relapse was not different between in the CZN group (506 days [194–1000]) and control group (772 days [333–1651]).

Concerning the distribution of HLA-DR, frequency of the HLA-DR4 phenotype was higher ($P < .05$) and that of the HLA-DR9 phenotype was lower ($P < .05$) in the control AIH group than in the CZN-AIH group (Table 2).

3.3. Comparison of characteristics among the acute-onset CZN-AIH subgroup and the chronic CZN-AIH subgroup

Clinical features and laboratory data were similar among acute-onset CZN-AIH and chronic CZN-AIH except that acute-onset CZN-AIH had higher levels of AST ($P < .05$), ALT ($P < .01$), and ALT/GGT ($P < .05$) than chronic CZN-AIH (Table 3).

3.4. Significance of the coexisting interface hepatitis on the characteristics of CZN-AIH

In order to investigate the significance of interface hepatitis in CZN-AIH, we examined the difference in the features of

Table 3

Comparison of clinicopathological data between acute-onset CZN-AIH and chronic CZN-AIH.

	Acute onset CZN-AIH (n = 18)	Chronic onset CZN-AIH (n = 14)	*P* value
Age (yr)	66 (55–74)	62 (43–73)	NS
Female sex	16 (88.9)	12 (85.7)	NS
Other autoimmune disease	4 (22.2)	3 (21.4)	NS
ANA titer	60 (40–80)	60 (0–100)	NS
Negativity of ANA	3 (16.7)	5 (35.7)	NS
ANA-high titer positive ≥160	3 (16.7)	3 (21.4)	NS
AST (IU/l)	506 (307–659)	172 (120–344)	<.05
ALT (IU/l)	640 (414–842)	213 (92–335)	<.001
ALP (IU/l)	430 (317–477)	400 (255–449)	NS
GGT (IU/l)	155 (90–238)	99 (71–224)	NS
ALT/GGT	4.37 (2.69–5.70)	1.42 (0.62–4.56)	<.05
T-Bil (mg/dl)	1.1 (1.0–2.5)	1.1 (0.8–1.6)	NS
Albumin (g/dl)	4 (3.9–4.1)	4.0 (3.9–4.2)	NS
Serum IgG (mg/dl)	1638 (1476–2052)	1389 (1179–2053)	NS
Serum IgM (mg/dl)	124 (86–162)	98 (58–114)	NS
Plt (104/μl)	20.1 (18.3–27.3)	17.8 (14.9–20.0)	NS
PT (%)	85 (73–97)	86 (72–91)	NS
PT-INR>1.5	0	0	NS
Histology			
F 0–1	9 (50.0)	8 (57.1)	NS
F 2–3	9 (50.0)	6 (42.9)	
F 4	0 (0)	0 (0)	
A 0–1	10 (55.6)	4 (28.6)	NS
A 2–3	8 (44.4)	10 (71.4)	
Lymphoplasmacytic infiltrate	13 (72.2)	13 (92.9)	NS
Rosetting	14 (77.8)	12 (85.7)	NS
Pathological score	2 (2–4)	2 (2–4)	NS
Pretreatment AIH score	12 (10–13)	11 (7–13)	NS
Pretreatment AIH score including pathological score	14 (13–16)	14 (9–16)	NS
Poor response to first-line therapy	0 (0)	0 (0)	NS
Relapse	3 (16.7)	2 (14.3)	NS
Period until relapse	644 (217–1204)	483 (170–795)	NS
HLA-DR 4	8 (47.1)	2 (16.7)	NS
HLA-DR 9	5 (29.4)	6 (50.0)	NS

Data are presented as *n* (range) or *n* (mean).

AIH = autoimmune hepatitis, ALP = alkaline phosphatise, ALT = alanine aminotransferase, ANA = antinuclear antibodies, AST = aspartate aminotransferase, CZN = centrilobular zonal necrosis, GGT = gamma-glutamyl transpeptidase, IgG = immunoglobulin G, IgM = immunoglobulin M, INR = international normalized ratio, IU = international unit, NS = not statistically significant, Plt = platelet count, PT = prothrombin time, T-Bil = total bilirubin.

CZN-AIH depending on existence of the significant interface hepatitis (Table 4). The CZN-AIH patients having significant interface hepatitis had higher level of IgM ($P < .05$), pretreatment AIH score including histology ($P < .01$), and higher level of GGT ($P < .01$). However, serum IgG level, positive ANA status, and level of ANA titer did not show a dominant difference. In addition, there was no statistically significant difference in the distribution of HLA-DR phenotypes.

3.5. Clinical, histological, and immunogenetic features distinguishing the acute-onset CZN-AIH from the acute-onset control AIH

The clinicopathological and immunogenetic characteristics of the acute-onset CZN-AIH subgroup and the acute-onset control AIH subgroup are summarized in Table 5.

The age at onset is slightly higher in the acute-onset CZN-AIH subgroup, but the difference was not significant. The acute-onset CZN-AIH subgroup had significantly lower levels of ALP ($P < .01$), GGT ($P < .05$), IgG ($P < .05$), IgM ($P < 0.05$), histological activity ($P < .01$), and AIH score including histology ($P < .05$). No significant difference was found for fibrosis stage, frequency of positive ANA status, nor level of ANA titer. Relapse rate was significantly lower in the acute-onset CZN-AIH group (16.7% in 3 cases) than in the acute-onset control group (52.2% in 12 cases) ($P < .05$). The period until relapse was not different between in the acute-onset CZN group (644 days [217–1204]) and control group (597 days [333–1282]).

No statistically significant difference in the frequency of HLA-DR4 or -DR9 was obtained.

3.6. Clinical, histological, and immunogenetic features distinguishing the chronic-onset CZN-AIH from the chronic-onset control AIH

The clinicopathological and immunogenetic characteristics of the chronic-onset CZN-AIH subgroup and the chronic-onset control AIH subgroup are summarized in Table 6.

The chronic-onset CZN-AIH subgroup had significantly lower levels of IgG ($P < .05$), ANA titer ($P < .01$), frequency of high titer, AIH score ($P < .01$), and AIH score including pathology ($P < .001$). No significant difference was found for activity score and relapse rate. In chronic-onset CZN-AIH, there was no significant difference of frequency of HLA-DR4, but frequency of HLA-DR 9 was significantly higher than that in chronic-onset control AIH.

4. Discussion

In the present study, we found higher frequency of acute-onset cases and lower inflammation activity in CZN-AIH compared with control AIH. Lower IgG level, lower ANA titer, and lower

Table 4

Comparison of clinicopathological data between CZN-AIH having histological finding of significant hepatitis and without having significant interface hepatitis.

	CZN-AIH having significant interface hepatitis (N = 12)	CZN-AIH without having interface hepatitis (N = 20)	*P* value
Age (yr)	67 (52–74)	63 (41–74)	NS
Female sex	10 (83.3)	18 (90.0)	NS
Acute onset	8 (66.7)	10 (50.0)	NS
Other autoimmune disease	5 (41.7)	2 (10.0)	NS
ANA titer	80 (10–140)	40 (10–80)	NS
Negativity of ANA	3 (25.0)	4 (20.0)	NS
ANA-high titer positive ≥160	3 (25.0)	3 (15.0)	NS
AST (IU/l)	322 (225–516)	361 (154–750)	NS
ALT (IU/l)	409 (289–664)	495 (212–1026)	NS
ALP (IU/l)	372 (275–453)	439 (281–543)	NS
GGT (IU/l)	178 (100–396)	100 (75–184)	<.01
ALT/GGT	2.20 (1.01–4.55)	4.02 (1.35–6.25)	<.01
T-Bil (mg/dl)	1.0 (0.8–1.2)	1.3 (0.9–1.9)	NS
Alb (g/dl)	4.0 (3.9–4.1)	4.0 (3.8–4.2)	NS
Serum IgG (mg/dl)	1879 (1498–2036)	1481 (1303–2027)	NS
Serum IgM (mg/dl)	144 (91–201)	101 (57–122)	<.05
Plt (104/μl)	20.1 (16.4–26.0)	18.8 (16.1–21.0)	NS
PT (%)	84 (68–91)	86 (79–95)	<.01
Histology			
F 0–1	5 (41.7)	12 (60.0)	NS
F 2–3	7 (58.3)	8 (40.0)	
F 4	0 (0)	0 (0)	
A 0–1	0 (0)	20 (100)	<.001
A 2–3	12 (100)	0 (0.0)	
Lymphoplasmacytic infiltrate	7 (58.3)	18 (90.0)	NS
Rosetting	10 (83.3)	16 (80.0)	NS
Pathological score	5 (4–5)	2 (1–2)	<.001
Pretreatment AIH score	13 (10–13)	11 (8–13)	NS
Pretreatment AIH score including pathological score	17 (15–18)	13 (9–14)	<.01
Relapse	3 (25.0)	2 (10.0)	NS
Period until relapse	644 (217–1204)	483 (170–795)	NS
HLA-DR 4	3 (27.3)	7 (38.9)	NS
HLA-DR 9	4 (36.4)	7 (38.9)	NS

Data are presented as *n* (range) or *n* (mean).

AIH = autoimmune hepatitis, Alb = XXX, ALP = alkaline phosphatise, ALT = alanine aminotransferase, ANA = antinuclear antibodies, AST = aspartate aminotransferase, CZN = centrilobular zonal necrosis, GGT = gamma-glutamyl transpeptidase, IgG = immunoglobulin G, IgM = immunoglobulin M, IU = international unit, NS = not statistically significant, Plt = platelet count, PT = prothrombin time, T-Bil = total bilirubin.

Table 5

Comparison of clinicopathological data between acute-onset CZN-AIH and acute-onset control CZN-AIH.

	Acute onset CZN-AIH (n = 18)	Acute onset control AIH (n = 23)	*P* value
Age (yr)	66 (55–74)	50 (39–67)	NS
Female sex	16 (88.9)	20 (87.0)	NS
Other autoimmune disease	4 (22.2)	1 (4.3)	NS
ANA titer	60 (40–80)	40 (0–320)	NS
Negativity of ANA	3 (16.7)	7 (30.4)	NS
ANA-high titer positive ≥160	3 (16.7)	7 (30.4)	NS
AST (IU/l)	506 (307–659)	521 (375–1084)	NS
ALT (IU/l)	640 (414–842)	668 (547–1034)	NS
ALP (IU/l)	430 (317–477)	608 (460–791)	<.01
GGT (IU/l)	155 (90–238)	216 (143–358)	<.05
ALT/GGT	4.37 (2.69–5.70)	2.85 (1.79–4.83)	NS
T-Bil (mg/dl)	1.1 (1.0–2.5)	2.8 (0.9–6.8)	NS
Albumin (g/dl)	4 (3.9–4.1)	4.0 (3.6–4.5)	NS
Serum IgG (mg/dl)	1638 (1476–2052)	2154 (1663–3561)	<.05
Serum IgM (mg/dl)	124 (86–162)	166 (108–294)	<.05
Plt (104/μl)	20.1 (18.3–27.3)	19.4 (14.3–23.6)	NS
PT (%)	85 (73–97)	93 (78–99)	NS
PT-INR>1.5	0 (0)	2 (8.7)	NS
Histology			
F 0–1	9 (50.0)	5 (21.7)	NS
F 2–3	9 (50.0)	17 (73.9)	
F 4	0 (0)	1 (4.3)	
A 0–1	10 (55.6)	3 (13.0)	<.01
A 2–3	8 (44.4)	20 (87.0)	
Lymphoplasmacytic infiltrate	13 (72.2)	19 (82.6)	NS
Rosetting	14 (77.8)	17 (73.9)	NS
Pathological score	2 (2–4)	5 (4–5)	<.01
Pretreatment AIH score	12 (10–13)	12 (11–14)	NS
Pretreatment AIH score including pathological score	14 (13–16)	17 (14–18)	<.05
Poor response to first-line therapy	0 (0)	1 (4.3)	NS
Relapse	3 (16.7)	12 (52.2)	<.05
Period until relapse	644 (217–1204)	597 (333–1282)*	NS
HLA-DR 4	8 (47.1)	14 (60.9)	NS
HLA-DR 9	5 (29.4)	2 (8.7)	NS

Data are presented as *n* (range) or *n* (mean).

AIH = autoimmune hepatitis, ALP = alkaline phosphatise, ALT = alanine aminotransferase, ANA = antinuclear antibodies, AST = aspartate aminotransferase, CZN = centrilobular zonal necrosis, GGT = gamma-glutamyl transpeptidase, IgG = immunoglobulin G, IgM = immunoglobulin M, INR = international normalized ratio, IU = international unit, NS = not statistically significant, Plt = platelet count, PT = prothrombin time, T-Bil = total bilirubin.

*Data missing in 4 of 12 cases.

AIH score were clinical features of CZN-AIH. The immunogenetic disproportionate distribution of HLA-DR phenotypes in control AIH (increased HLA-DR4 and decreased HLA-DR9) was not found in CZN-AIH. After immunosuppressive therapy, CZN-AIH was less frequently relapsed. These characteristics of CNZ-AIH were observed regardless of the difference in the onset pattern and the presence or absence of interface hepatitis.

At first, in our study, we diagnosed CZN-AIH under a strict definition by liver biopsy and evaluated the significance of CZN on the clinical features of AIH. Then, we sought to prove that CZN-AIH is a unique subtype of AIH that is clinically distinguishable from typical AIH.

Distinguishing features of CZN-AIH from control AIH have been studied previously by us and others.[8–10] 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[10] and strengthen the previous findings[9,10] that the characteristic laboratory findings of typical AIH were poor in CZN-AIH. In addition, we have newly determined that ALP is lower, and Plt is higher in CZN-AIH; these findings may correspond to mild portal inflammation and less advanced fibrosis in CZN-AIH, respectively.

The features in CZN-AIH obtained in our study are quite different from those of Miyake et al.[8] They did not find specific laboratory features in CZN-AIH. This discrepancy is probably due to the difference in definition of CZN. Miyake et al[8] defined inflammation in the central vein region as CZN. However, inflammatory cells tended to accumulate to the central zone in severe lobular inflammation without significant central necrosis. Thus, their inclusion criteria of CZN were thought to be too lenient.

Another novel finding in our study is lower relapse rate in CZN-AIH. This point is very important in a treatment of AIH. However, this finding may be not definitely determined because observation period in CZN-AIH cases is shorter than control AIH cases. In order to confirm this evidence, longer observation period in larger CZN-AIH patients is required.

Immunogenetically, we found the distribution of HLA-DR phenotype of CZN-AIH was similar to healthy Japanese and different from control AIHs (Table 2). Elevating frequency of HLA-DR4 and HLA-DR9 in control AIH was not observed in CZN-AIH.[27] This finding strongly suggest that disease susceptibility for CZN-AIH is not defined by HLA-DR phenotype. This finding is in concordant with our previous small number study and strengthen our idea that CZN-AIH may be a novel distinct phenotype of AIH.

CZN-AIH has been supposed 1 of the histological subtype of early stage AIH that may lack of laboratory findings specific for typical AIH. Certainly, 18 of 32 cases of CZN-AIH develop similar to acute hepatitis; "acute-onset AIH" [4] and the majority of CZN-AIH (20 of our 32) do not show histological hallmark of chronic active hepatitis known as significant interface hepatitis. Alternatively, this finding suggests that not a few number of CZN-AIH patients had the feature of chronic disease.

Table 6

Comparison of clinicopathological data between chronic onset CZN-AIH and chronic onset control CZN-AIH.

	Chronic onset CZN-AIH (n = 14)	Chronic onset control AIH (n = 47)	*P* value
Age (yr)	62 (43–73)	64 (51–72)	NS
Female sex	12 (85.7)	39 (83.0)	NS
Other autoimmune disease	3 (21.4)	3 (6.4)	NS
ANA titer	60 (0–100)	160 (80–560)	<.01
Negativity of ANA	5 (35.7)	3 (6.4)	NS
ANA-high titer positive ≥160	3 (21.4)	30 (63.8)	<.01
AST (IU/l)	172 (120–344)	196 (89–439)	NS
ALT (IU/l)	213 (92–335)	172 (88–442)	NS
ALP (IU/l)	400 (255–449)	451 (323–619)	NS
GGT (IU/l)	99 (71–224)	169 (67–262)	NS
ALT/GGT	1.42 (0.62–4.56)	1.61 (0.86–2.75)	NS
T-Bil (mg/dl)	1.1 (0.8–1.6)	0.9 (0.7–3.1)	NS
Albumin (g/dl)	4.0 (3.9–4.2)	3.8 (3.4–4.0)	<.05
Serum IgG (mg/dl)	1389 (1179–2053)	2388 (1770–3014)	<.001
Serum IgM (mg/dl)	98 (58–114)	159 (96–269)	NS
Plt (104/μl)	17.8 (14.9–20.0)	17.6 (12.2–23.1)	NS
PT (%)	86 (72–91)	86 (74–99)	NS
PT-INR>1.5	0	0	NS
Histology			
F 0–1	8 (57.1)	7 (14.9)	<.01
F 2–3	6 (42.9)	32 (68.1)	
F 4	0 (0)	8 (17.0)	
A 0–1	4 (28.6)	6 (12.8)	NS
A 2–3	10 (71.4)	41 (87.2)	
Lymphoplasmacytic infiltrate	13 (92.9)	42 (89.4)	NS
Rosetting	12 (85.7)	39 (83.0)	NS
Pathological score	2 (2–4)	5 (4–5)	<.001
Pretreatment AIH score	11 (7–13)	13 (12–15)	<.005
Pretreatment AIH score including pathological score	14 (9–16)	18 (15–19)	<.001
Poor response to first-line therapy	0 (0)	4 (8.5)	NS
Relapse	2 (14.3)	17 (36.2)	NS
Period until relapse	483 (170–795)	816 (258–1846)*	NS
HLA-DR 4	2 (16.7)	17 (61.4)	NS
HLA-DR 9	6 (50.0)	5 (11.4)	<.01

AIH = autoimmune hepatitis, ALP = alkaline phosphatise, ALT = alanine aminotransferase, ANA = antinuclear antibodies, AST = aspartate aminotransferase, CZN = centrilobular zonal necrosis, GGT = gamma-glutamyl transpeptidase, IgG = immunoglobulin G, IgM = immunoglobulin M, INR = international normalized ratio, IU = international unit, NS = not statistically significant, Plt = platelet count, PT = prothrombin time, T-Bil = total bilirubin.

*Data missing in 5 of 17 cases.

In this way, the clinical and histological statuses of CZN-AIH are not homogenous. Therefore, we subdivided CZN-AIH according to the pattern of onset (acute or chronic) or coexistence of significant interface hepatitis, and examined the difference in clinicopathological and immunogenetic features. As a result, regardless of the onset pattern or presence/absence of significant interface hepatitis, we found a minor few differences in clinical and laboratory findings, though we did not find a definitive differences on serum IgG level, positive ANA status, and ANA titer. Immunogenetically, difference on the distribution of HLA-DR was not found. These findings strongly suggested that CZN-AIH is not a 1 of histological phenotypes of early stage AIH but a distinctive subtype of AIH including wide clinical and histological status. Our results suggest that significant interface hepatitis in CZN-AIH may indicate the extent of inflammation but not the association with acute-onset and overlap manner.

Finally, we examine the differential features between CZN-AIH and control AIH in the case of acute onset and chronic onset. We found that lower levels of IgG, IgM, ALP, and relapse rate were observed in acute-onset CZN-AIH compared with those in acute-onset control AIH (Table 5). No difference in distribution of HLA-DR between acute CZN-AIH and acute control AIH was presumably due too small number to detect the difference. Similarly, in the case of chronic-onset CZN-AIH, we found lower levels of IgG, ANA titer, AIH score, and higher frequency of HLA-DR-9 (Table 6). These differences were partially in concordant with the difference between whole CZN-AIH and whole control AIH, regardless of onset pattern (acute or chronic). Thus, CZN is a unique subtype of AIH, and we speculate that acute-onset CZN-AIH may develop into chronic CZN-AIH, whereas acute control AIH can progress to chronic control AIH (Fig. 4). Further analyses are needed to address the precise role of CZN, and performing serial liver biopsies is 1 of the options.

In the previous study by Miyake et al,[5] clinical features of acute AIH were compared to chronic AIH, though they did not subclassify the AIH according to the existence of CZN. In another previous study by Abe et al,[11] acute AIH cases were divided into 2 groups based on the presence or absence of central necrosis. Their findings are partially discordant with ours, especially the frequency of ANA negativity, serum ALP level, GGT level, and the frequency of interface hepatitis. It is important to note that their study included both a considerable number of cases that showed exacerbation in the chronic phase of AIH and patients

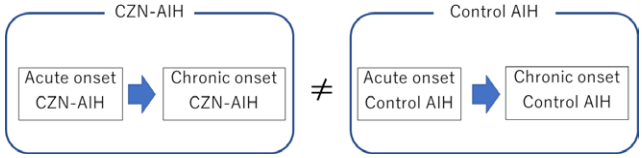

Figure 4. Schema from acute to chronic AIH. Acute onset CZN-AIH may develop into chronic CZN-AIH, whereas acute control AIH can progress to chronic control AIH. AIH = autoimmune hepatitis, CZN = centrilobular zonal necrosis.

with inconspicuous ALT elevation (5× < maximum ALT < 10× upper normal limit). Therefore, our comparative analysis of acute-onset CZN and acute-onset control AIH, performed under the strict definition of both acute onset and CZN, was novel. Our data clearly indicated that the feature of acute-onset CZN-AIH is distinctive even if it is compared to acute-onset control AIH.

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.[10] Moreover, in the present study, we found that some of the features that characterize CZN-AIH were commonly found regardless of the clinical phase or the presence/absence of significant interface hepatitis. Taken into consideration of these findings, we strongly suggested that CZN-AIH is distinctive with lack of laboratory and immunogenetic findings characteristic for typical AIH.

The fate of untreated CZN-AIH may be acute hepatic failure or decompensated hepatic cirrhosis, the same as control AIH. Virtually, we experienced 1 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 or terminal stage of acute hepatic failure. This made it difficult to distinguish CZN-AIH from control-AIH.

Although various mechanisms are said to be the immune mechanism of AIH, it is not completely understood at present and self-antigens have not yet been identified. ZN-AIH and normal AIH have different inflammation heads, and further research is needed on the cause.[3,28]

In this study, the significance of CZN-AIH compared with control AIH was more strongly clarified by subdividing the patients in onset pattern, presence of significant interface hepatitis. Moreover, this is the first report to mention relapse rate of CZN-AIH.

However, our 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 were a small number of cases in the CZN-AIH group, which may have affected our subanalyses; 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.

5. Conclusion

CZN-AIH characterized by low relapse rate after immunosuppressive therapy had unique clinicopathological characteristics (lower serum IgG and lower pretreatment AIH score including pathological score) compared with typical AIH. The CZN-AIH cases did not exhibit the HLA-DR phenotype disproportion characteristic of control AIH cases, suggesting that the 2 types are immunogenetically different.

Author contributions

All authors helped to perform the research; Kaoru Ueda and Yoshio Aizawa designed the study, interpreted and analyzed the data, reviewed the pathological tissues, and wrote the article; Kaoru Ueda, Yoshio Aizawa, Chika Kinoshita, Tomohisa Nagano, Jinya Ishida, Chisato Saeki, Tsunekazu Oikawa, Atsushi Hokari, and Masayuki Saruta collected the data; Kaoru Ueda, Yoshio Aizawa, and Toru Harada evaluated HE and Masson staining; all authors read and approved the final article.

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