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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Clinicopathological features of severe and fulminant forms of autoimmune hepatitis

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Abstract
Background and aims Diagnosis of the acute presentation of autoimmune hepatitis (AIH) is difficult because patients do not always show typical clinicopathological features of AIH. Although some of them progress to fulminant hepatitis, the survival rate of which is <20% without liver transplantation, their clinicopathological features have remained uncertain. We examined them for a better understanding and improvement of the prognosis of "life-threatening" severe and fulminant AIH.
Methods Clinical, biochemical and pathological features of 28 patients with severe or fulminant AIH and treatment responses were examined retrospectively.
Results At the time of admission, mean immunoglobulin G was 2479 ± 1170 mg/dl, with 7 (25%) patients showing normal levels. Anti-nuclear antibody was ≤1:40 in 8 (29%). Liver histology showed severe activity in 95% and acute hepatitis in 86% of the patients. Centrilobular necrosis including submassive and massive necrosis was characteristic. Of the 25 patients treated with corticosteroids, 17 responded and 8 did not. Responders to corticosteroids showed younger age and higher prothrombin time (PT) activity than non-responders at the time of corticosteroid administration. The improvement of PT activity during 2 weeks and 4 weeks and total bilirubin level during 4 weeks was statistically significant in responders, but not in non-responders.
Conclusions We should diagnose and treat acute onset AIH patients before they develop into severe and fulminant disease. Performing liver biopsy at the early stage of acute onset AIH, evaluating the biopsy specimens precisely and initiating corticosteroid therapy may be essential for improving the prognosis without liver transplantation.

Keywords Autoimmune hepatitis · Severe hepatitis · Fulminant hepatitis · Immunosuppressive therapy · Liver histology

Abbreviations
AIH Autoimmune hepatitis
FH Fulminant hepatitis
CN Centrilobular necrosis

Introduction

Autoimmune hepatitis (AIH) is regarded as a chronic hepatitis, characterized by the presence of interface hepatitis and plasma cell infiltration on histological examination, hypergammaglobulinemia and autoantibodies [1, 2]. An acute presentation of AIH is common [3–6], and severe and fulminant hepatitis is possible [7].

An AIH scoring system based on the clinicopathological features has been proposed by the international AIH group

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[8]. There have been some patients who do not show typical features of AIH. AIH with clinical features of acute, severe and fulminant hepatitis (acute onset AIH) is one of these conditions. Patients with acute onset AIH are at risk of losing the timing for the initiation of immunosuppressive therapy, and it is sometimes resistant to immunosuppressive therapy and has a poor prognosis. A nationwide survey of patients with fulminant hepatitis and late onset hepatic failure between 1998 and 2003 in Japan revealed that the prognosis was especially poor in AIH patients, whose survival rate was 17.1% without liver transplantation [9]. This is recognized everywhere around the world [10, 11].

Lefkowitch et al. [12] first reported AIH patients presenting with histologically acute hepatitis. In a Japanese nationwide survey study, 5.6% of patients with AIH were found to have a feature of acute hepatitis upon histological examination [13]. In fact, the actual number of acute onset AIH patients may possibly have been underestimated, as its diagnosis is sometimes very difficult using the AIH scoring system and because exact understanding of the pathological features of these patients is sometimes lacking. A major problem is that there is no gold standard for making the diagnosis of acute onset AIH.

Recently, we reported that histological examination was useful for an early diagnosis of acute onset AIH and that prognosis might indeed be improved by getting a head start on corticosteroid therapy in clinically non-severe cases [14]. In the present study, we examined the clinicopathological features and treatment responses of severe and fulminant forms of AIH patients, and attempted to determine the exact requirements for a more precise diagnosis and the correct time point for switching to liver transplantation after the administration of immunosuppressive therapy in order to improve their very poor prognoses.

Patients and methods

Selection criteria of patients

Patients with severe and fulminant AIH were enrolled between 1990 and 2009. A diagnosis of AIH was made based on the presence of anti-nuclear antibody (ANA) and/or anti-smooth muscle antibody (ASMA), as well as on the criteria defined by the International Autoimmune Hepatitis Group reaching the score for probable or definite AIH [8].

Eligibility criteria of clinically "acute onset" AIH were as follows, in addition to the AIH criteria described above: (1) acute onset liver injury, (2) no history of chronic liver injury, (3) negativity of active viral markers such as hepatitis A, B, C and E viruses, Epstein–Barr virus (EBV), cytomegalovirus (CMV) and herpes simplex virus (HSV), drug-induced liver injury, toxic and metabolic disorders, and (4) no signs of chronicity on the basis of physical examination, laboratory data and abdominal ultrasound findings.

Eligibility criteria of severe and fulminant AIH, in addition to the criteria described above, were as follows: patients with prothrombin time (PT) activity <50% of control or total bilirubin level more than 20 mg/dl during the disease course were defined as having severe AIH, and patients with PT activity <40% of control and hepatic encephalopathy were defined as having fulminant AIH. Informed consent was obtained from all patients or appropriate family members.

Clinical, biochemical and immunoserologic analysis

Data obtained from patients were as follows: sex; age at diagnosis; time of onset, severe disease and fulminant disease; complications; serum levels of alanine aminotransferase (ALT), total bilirubin (T-Bil), alkaline phosphatase (ALP), PT activity, immunoglobulin G (IgG), immunoglobulin M (IgM), ANA, anti-smooth muscle antibody (ASMA), liver kidney microsomal antibody-1 (LKM-1) and anti-mitochondrial antibody (AMA); human leukocyte antigen (HLA); types of therapy; and response to therapy. They were also examined for any history of recent exposure to drugs and chemical agents as well as heavy alcohol consumption (>50 g/day). ANA and ASMA were examined by a fluorescent antibody method, and AMA was examined by a fluorescent antibody method or an enzyme linked immunosorbent assay (ELISA), and LKM-1 was examined by ELISA.

In acute onset AIH, early symptoms including fever, general malaise, fatigue, nausea, vomiting and right upper quadrant discomfort are frequently observed, so we defined the beginning of these symptoms as clinical onset.

Virological analysis

Patients were examined for viral markers such as IgM anti-hepatitis A virus antibody (IgM-HA), IgM anti-HBc antibody (IgM-HBc), HBsAg, anti-HCV antibody, HCV RNA, HEV RNA, IgM anti-Epstein-Barr virus (EBV) antibody (IgM-EBV), IgM anti-herpes simplex virus (HSV) antibody (IgM-HSV) and IgM anti-cytomegalovirus (CMV) antibody (IgM-CMV). None of the patients had clinical or laboratory evidence of acquired immune deficiency syndrome.

Histological analysis

Histological examination was performed by a percutaneous or transjugular approach, explanted liver or post mortem.

Twelve were percutaneous needle biopsy, 2 transjugular needle biopsy, 2 explanted liver, and 7 post mortem. Three specialists (M.N., K.F. and O.Y.) independently reviewed the histopathological changes by evaluating the degrees of portal and lobular changes and plasma cell infiltrations on hematoxylin-eosin stained sections. Staging and grading were evaluated based on the classification of Desmet et al. [15]. (−), (±), (+), (++) and (+++) represent absent, very mild, mild, moderate and severe in interface hepatitis, zonal necrosis, plasma cell infiltration and collapse. (−), (±), (+) and (++) represent absent, slightly present, present and prominent in rosette formation and cobblestone appearance. The scores were averaged and presented in Table 3.

Treatment response

In this study, we defined responders and non-responders according to the recovery of liver function (regeneration), not to the control of liver inflammation, and we judged them at 2 weeks after the starting of corticosteroid therapy. We used PT as a marker of liver regeneration, which is generally used in acute liver failure. We also defined recovery of liver regeneration and normalization of liver inflammation as complete response (CR) and non-recovery of liver regeneration as no response (NR).

Statistical analysis

Differences in proportions among the groups were compared by Fisher's exact probability test, Student's t test and Welch's t test ($p < 0.05$ was considered significant).

Results

Clinical and biochemical features

Twenty-eight patients, 7 men and 21 women, were enrolled in the study. Fourteen were cases of fulminant hepatitis and 14 severe hepatitis. The clinical and biochemical features of all patients at admission are provided in Tables 1 and 2. Mean age at the time of diagnosis was 46.9 ± 15.8 years. Mean ALT was 527 ± 458 IU/l, mean highest T-Bil 23.3 ± 11.6 mg/dl and mean lowest PT activity 28 ± 15%.

Mean IgG was 2479 ± 1170 mg/dl. The IgG level was normal (<1.0 × upper normal value: UNV) in 7 of 28 (25%), 1.0–1.5 × UNV in 12 (43%), 1.5–2.0 × UNV in 5 (18%) and >2.0 × UNV in 4 (14%). ANA was positive (≥1:40) in 25 of 28 (89%) patients, <1:40 in 3 (11%), 1:40 in 5 (18%), 1:80 in 6 (21%) and >1:80 in 14 (50%). ASMA was positive (≥1:40) in 8 of 27 (30%). One patient was positive for LKM-1.

The duration from initial symptoms to the admission to our unit was 48.4 ± 39.9 days (11–176 days). Twelve patients (43%) had primary complications and histories of medications, five with hypertension, three with Hashimoto disease, one with hyperuricemia, one with ischemic heart disease, one with Sjögren syndrome and one with neurosis.

No patients were positive for HBs Ag, and one patient was positive for HCV Ab. Although 43% of the patients had primary complications and histories of medications as described above, suspected hepatotoxic drugs were excluded according to the drug-induced liver injury diagnostic scale of Maria and Victorino [16] in this study.

In AIH, there are two forms according to HLA-DR differences. In Japan, almost all AIH patients do not have HLA-DR 3. This suggests the possible benefit of examining the HLA-DR backgrounds, although we could perform this analysis in only 13 of the patients because this procedure is not covered by the Japanese national health insurance plan. None of the 13 had HLA-DR 3, but 6 had HLA-DR 4.

Histological features

The pathological characteristics of the patients are summarized in Table 3. Histological examination was performed in 23 of 28 patients, and 22 were appropriate for evaluation. Nineteen (86%) of 22 showed acute hepatitis, exhibiting zonal, submassive and massive necrosis with or without plasma cell accumulation in portal and centrilobular areas. Three (14%) showed chronic hepatitis.

Twenty-one of 22 (95%) patients showed severe activity, 10 with massive necrosis, 2 with submassive necrosis, 7 with severe acute hepatitis and 2 with severe activity with fibrosis stage 2–3. Only one showed moderate activity with fibrosis stage 2.

The duration from onset to histological examination was 85.6 ± 47.6 days. That in massive necrosis, submassive necrosis, severe acute hepatitis and chronic hepatitis was 61.0 ± 31.4, 135.0 ± 79.2, 85.7 ± 17.6 and 134.3 ± 79.2, respectively. The difference between the acute and chronic form was not statistically significant ($p = 0.35$).

AIH scoring system

The provisional scoring system (AIH score) proposed by the International Autoimmune Hepatitis Group [8] was used to score all patients (Table 4). The AIH score ranged from 12 to 22 (16.3 ± 2.8) before the treatment. Fourteen of 28 patients (50%) were diagnosed as having 'definite' AIH and 14 (50%) as having 'probable.'

Table 1 Clinical characteristics of 28 patients

Patients	Diagnosis	Onset (year)	Age	Sex	ALT (IU/l)	ALP (IU/l)	Highest T-Bil (mg/dl)	PT activity on admission (%)	Lowest PT activity (%)	IgG (mg/dl)
1	FH	1989	26	F	189	415	20.5	25	19	2630
2	FH	1990	59	F	916	304	33.7	10	10	3325
3	AHs	1991	45	F	812	261	49.9	31	31	2057
4	FH	1993	27	F	427	285	18.3	35	5	1654
5	AHs	1993	31	F	317	362	32.1	42	42	1193
6	AHs	1994	30	F	673	172	22.2	53	53	2192
7	AHs	1994	40	F	900	185	32.5	48	42	2155
8	AHs	1995	66	M	244	163	15.8	58	41	2676
9	AHs	2000	44	F	122	568	20.4	49	48	1320
10	AHs	2000	51	M	513	578	3.6	48	46	1870
11	FH	2002	17	F	496	802	14.8	29	29	2400
12	FH	2003	56	M	49	280	38.4	15	15	3053
13	FH	2004	64	M	1998	513	22.9	40	33	2377
14	AHs	2005	37	F	498	499	11.2	49	49	6424
15	AHs	2006	61	M	333	341	12.0	36	35	2662
16	AHs	2007	39	F	424	543	19.8	49	44	1249
17	AHs	2007	72	F	964	651	22.8	45	43	2295
18	FH	2007	58	F	230	367	32.0	16	15	1957
19	FH	2007	56	M	355	427	26.3	28	23	2123
20	AHs	2007	26	F	183	377	5.8	48	35	1233
21	FH	2007	52	M	329	989	33.8	34	8	1274
22	AHs	2007	23	F	185	646	7.9	52	33	4127
23	FH	2007	70	F	148	396	45.4	25	9	4178
24	FH	2008	71	F	607	445	33.8	21	9	1990
25	FH	2008	55	F	395	472	28.3	18	7	4322
26	FH	2009	49	F	1789	379	23.6	31	29	2868
27	FH	2009	38	F	321	469	12.9	16	16	2546
28	AHs	2009	49	F	349	369	12.3	33	26	1272

FH fulminant hepatitis, *AHs* acute hepatitis severe type, *ALT* alanine aminotransferase, *ALP* alkaline phosphatase, *T-Bil* total bilirubin, *PT* prothrombin time, *IgG* immunoglobulin G

Treatment response and outcome

In 25 of 28 (89%) patients, an initial dose of 40–60 mg prednisolone or 1000 mg methylprednisolone daily was administered. Patients with marked elevation of ALT and prolongation of PT were treated with 1000 mg of methylprednisolone pulse therapy followed by prednisolone therapy. Seventeen (61%) survived without liver transplantation, one (4%) survived with liver transplantation and seven (25%) died without liver transplantation because of liver failure. One (4%) received a liver transplantation and died. Two patients (7%) were already in terminal stage liver failure and died (Table 4).

Changes in PT activities, ALT levels and T-Bil levels during the initial 4 weeks after the introduction of corticosteroid therapy are shown in Fig. 1. Improvement of PT activities was found in all of the responders. In non-responders, PT activities did not improve, although some of them showed a transient rise in the first 2 weeks by infusions of fresh frozen plasma. The elevations of PT activities during 2 weeks and 4 weeks were statistically significant ($p = 0.001$ and $p < 0.001$, respectively) in responders, but not in non-responders. The ALT levels fell during the course, with the declines in the first 2 weeks and 4 weeks being statistically significant in responders ($p = 0.003$ and $p < 0.001$, respectively) and non-responders ($p = 0.004$ and $p = 0.033$, respectively). T-Bil levels fell during the course, with the declines in the first 4 weeks being statistically significant ($p = 0.031$) in responders, but they did not fall in non-responders.

Patients were analyzed according to their responses to corticosteroid therapies—responders or non-responders

Table 2 Immunoserological and virological analysis of 28 patients

Patient	ANA (fold)	ASMA (fold)	AMA	LKM-1	HLA-DR	IgM-HA	HBsAg/ IgM-HBc	HCV-Ab/ RNA	HEV-RNA	IgM-EBV/ HSV/CMV
1	320	<40	–	ND	ND	–	–/–	–/–	–	–/–/–
2	<40	40	–	ND	ND	–	–/–	–/–	–	–/–/–
3	40	80	–	ND	4	–	–/–	–/–	–	–/–/–
4	1280	<40	–	ND	ND	–	–/–	–/–	–	–/–/–
5	<40	40	–	–	8, 9	–	–/–	–/–	–	–/–/–
6	160	<40	–	–	ND	–	–/–	–/–	–	–/–/–
7	<40	40	–	–	ND	–	–/–	–/–	–	–/–/–
8	40	320	–	–	8, 9	–	–/–	–/–	–	–/–/–
9	40	<40	–	ND	ND	–	–/–	–/–	–	–/–/–
10	80	40	–	ND	4, 8	–	–/–	–/–	–	–/–/–
11	320	<40	–	–	ND	–	–/–	–/–	–	–/–/–
12	>1280	80	–	–	ND	–	–/–	–/–	–	–/–/–
13	640	ND	–	+	ND	–	–/–	–/–	–	–/–/–
14	1280	<40	–	–	ND	–	–/–	–/–	–	–/–/–
15	640	<40	–	–	ND	–	–/–	–/–	–	–/–/–
16	40	<40	–	–	4, 12	–	–/–	–/–	–	–/–/–
17	80	<40	–	ND	ND	–	–/–	–/–	–	–/–/–
18	80	<40	–	ND	ND	–	–/–	–/–	–	–/–/–
19	80	<40	–	–	ND	–	–/–	–/–	–	–/–/–
20	80	<40	–	ND	14, 15	–	–/–	–/–	–	–/–/–
21	80	<40	–	ND	12, 13	–	–/–	–/–	–	–/–/–
22	1280	<40	–	–	9, 13	–	–/–	–/–	–	–/–/–
23	>1280	<40	–	ND	ND	–	–/–	–/–	–	–/–/–
24	40	<40	–	–	4, 8	–	–/–	–/–	–	–/–/–
25	640	<40	–	–	8, 12	–	–/–	–/–	–	–/–/–
26	640	40	–	–	13, 15	–	–/–	–/–	–	–/–/–
27	320	<40	–	–	4	–	–/–	–/–	–	–/–/–
28	160	<40	–	–	4, 12	–	–/–	–/–	–	–/–/–

ANA anti-nuclear antibody, *ASMA* anti-smooth muscle antibody, *AMA* anti-mitochondrial antibody, *LKM-1* liver kidney microsomal antibody-1, *HLA* human leukocyte antigen, *IgM-HA* IgM anti-hepatitis A virus antibody, *IgM-HBc* IgM anti-hepatitis B virus core antibody, *IgM-EBV* IgM anti-Epstein–Barr virus antibody, *IgM-HSV* IgM anti-herpes simplex virus antibody, *IgM-CMV* IgM anti-cytomegalovirus antibody, *ND* not done

(Table 4). The differences in sex, mean ALT, mean T-Bil, mean IgG, ANA titer and AIH score were not statistically significant. Mean age was higher in non-responders than in responders ($p < 0.05$). PT activity was lower in non-responders than in responders ($p < 0.001$). All responders survived, 7 of non-responders died ($p < 0.001$) and one non-responder survived with liver transplantation. Interestingly, the duration from initial symptoms to the administration of corticosteroids was not different between responders and non-responders (Table 5; Fig. 2).

Long-term changes in ALT levels after the start of treatment in all the patients are shown in Fig. 3. ALT levels remained normal except for two patients with some transient exacerbations.

Discussion

After the establishment of the criteria of the International Autoimmune Hepatitis Group [8] and the recognition of acute onset AIH [6], the diagnosis of severe and fulminant type of AIH came to be made, and most of those thusly diagnosed would have been diagnosed as cryptogenic hepatitis. But some patients have no autoantibodies and/or no hypergammaglobulinemia, and at present they are being diagnosed with cryptogenic hepatitis. Kaymakoglu et al. [17] reported that severe cryptogenic chronic hepatitis was similar to AIH in clinical, biochemical and histological features as well as responsiveness to immunosuppressive therapy, and severe cryptogenic chronic hepatitis patients might have an autoimmune liver disease with no identified

Table 3 Pathological characteristics of patients

Patient	Histological diagnosis	Interface hepatitis	Zonal necrosis	Plasma cell infiltration	Rosette formation	Collapse	Cobblestone appearance	Duration from onset to exam. (days)	Method
1	ND	ND	ND	ND	ND	ND	ND	ND	ND
2	Massive necrosis	+++	+++	+	−	−	−	45	Post mortem
3	ND	ND	ND	ND	ND	ND	ND	ND	ND
4	Inappropriate for evaluation	×	×	×	×	×	×	40	Percutaneous
5	Severe acute hepatitis	+	++	+	−−	±	−	95	Percutaneous
6	CH (F3, severe)	+++	+++	++	+	+	+	43	Percutaneous
7	Submassive necrosis	+	+++	++	±	+	±	79	Percutaneous
8	CH (F3, severe)	+++	++	++	+	+	+	176	Percutaneous
9	Severe acute hepatitis	+	+	++	+	−	+	107	Percutaneous
10	ND	ND	ND	ND	ND	ND	ND	ND	ND
11	Massive necrosis	+++	+++	−	×	×	×	25	Explanted
12	ND	ND	ND	ND	ND	ND	ND	ND	ND
13	CH (F2, moderate)	+++	+	−	−	+	±	184	Percutaneous
14	Severe acute hepatitis	++	++	+	−	+	−	80	Percutaneous
15	ND	ND	ND	ND	ND	ND	ND	ND	ND
16	Massive necrosis	+++	+	−	−	−	−	36	Transjugular
17	Severe acute hepatitis	+	+	−	−	+	+	107	Percutaneous
18	Massive necrosis	+++	+++	++	−	−	−	52	Post mortem
19	Submassive necrosis	++	++	++	−	−	−	191	Post mortem
20	Massive necrosis	+++	+++	±	×	×	×	51	Transjugular
21	Massive necrosis	+++	++	−	×	×	×	68	Post mortem
22	Severe acute hepatitis	++	+	+	+	−	++	71	Percutaneous
23	Massive necrosis	+++	+++	+++	−	−	−	121	Post mortem
24	Massive necrosis	+++	+++	+++	×	×	×	39	Post mortem
25	Massive necrosis	+++	+++	+	−	−	−	110	Post mortem
26	Severe acute hepatitis	++	++	+	+	++	+	78	Percutaneous
27	Massive necrosis	+++	+++	++	−	−	−	63	Explanted
28	Severe acute hepatitis	++	++	+	−	++	−	62	Percutaneous

CH chronic hepatitis, *ND* not done, × inappropriate for evaluation, *percutaneous* percutaneous needle biopsy, *transjugular* transjugular needle biopsy

immunoserologic marker. Potthoff et al. [18] suggested that steroids have to be considered in the therapy for severe acute cryptogenic hepatitis, and the response to steroid treatment may indicate an autoimmune genesis of the disease. On the other hand, Bernal et al. reported that autoantibodies were present in 30% of patients with acute liver failure and that significantly higher international AIH scores were found in patients with cryptogenic disease as compared to other etiological ones. They suggested it is difficult to evaluate whether primary autoimmune processes are responsible for the condition, although cryptogenic cases have features of autoimmune pathogenesis [19].

The duration from onset to admission to our unit was 48.4 (11–176) days in the present study. In most patients, the severity of hepatitis was not severe at onset, but they advanced to severe diseases without precise diagnosis and treatment, and were referred to our unit. This means that the diagnosis of acute onset AIH is still difficult in Japan (Figs. 4, 5, 6, 7).

In our study, ANA was $\leq$1:40 in 29% of the patients, ASMA was negative (<1:40) in 68%, and none was negative for both. In the Italian study of acute onset AIH, 27% of the patients were negative for ANA, 18% were negative for ASMA, and 9% were negative for both [20]. In the US study of acute onset AIH, 31% were negative for ANA, 15% were negative for ASMA, and 4% were negative for both [21]. Thus, the negativity of ANA was about the same as other reports of acute onset AIH, but the negativity of ASMA was higher in our patients. In another Japanese study of acute AIH, 56% were negative for ASMA [22].

It was reported that the period of initial symptoms to the diagnosis of fulminant or severe acute hepatitis is occasionally longer than that of acute hepatitis based on their

Table 4 Treatment and outcome of all patients

Patients	Treatment	Loading dose of corticosteroid (mg/day)	Days from onset to corticosteroid therapy	Response to corticosteroid	Outcome	AIH score	Simplified AIH score
1	PSL	60	43	CR	Recovery	15	6
2	Dex	10	19	NR	Death	17	6
3	mPSL	1000	124	CR	Recovery	14	6
4	SNMC				Death	14	5
5	PSL	60	22	CR	Recovery	14	4
6	PSL	60	24	CR	Recovery	18	8
7	PSL	60	18	CR	Recovery	16	6
8	PSL	30	161	CR	Recovery	17	8
9	PSL	60	31	CR	Recovery	15	5
10	PSL	60	96	CR	Recovery	12	6
11	LT				Death after LT	17	7
12	PSL	60	62	NR	Death	13	6
13	PSL	50	134	CR	Recovery	15	7
14	PSL	40	36	CR	Recovery	22	7
15	PSL	60	86	CR	Recovery	12	6
16	PSL	60	30	CR	Recovery	15	4
17	mPSL	1000	54	CR	Recovery	16	7
18	SNMC				Death	17	7
19	mPSL	1000	134	NR	Death	15	7
20	PSL	40	45	CR	Recovery	15	5
21	mPSL	1000	25	NR	Death	13	5
22	mPSL	1000	24	CR	Recovery	22	8
23	mPSL	500	45	NR	Death	20	7
24	mPSL	1000	17	NR	Death	19	5
25	mPSL	1000	85	NR	Death	20	6
26	mPSL	1000	28	CR	Recovery	20	8
27	mPSL	1000	46	NR	Recovery after LT	15	5
28	mPSL	1000	10	CR	Recovery	18	5

PSL prednisolone, *Dex* dexamethasone, *mPSL* methylprednisolone, *SNMC* stronger neominophagen C, *LT* liver transplantation, *CR* complete response, *NR* no response

observation that severe or fulminant patients had a higher titer of ANA and higher levels of IgG than non-severe ones [23, 24]. In our present study, the IgG level was higher than normal in 75% of the patients and was significantly higher than that of our non-severe acute onset AIH patients [14] ($p = 0.002$), indicating that our severe and fulminant patients might also have longer clinical courses than non-severe patients.

The mean AIH score was the same as in non-severe patients, but all patients were diagnosed as 'probable' or 'definite,' although 77% of non-severe patients were diagnosed as 'probable' or 'definite' in our previous study [14].

In our previous study of non-severe acute onset AIH [14], liver histology showed severe activity with zonal necrosis in 95% of the patients, despite PT activity being maintained, suggesting that AIH with low PT activity must have very severe and advanced histology (submassive to massive necrosis) and present impaired hepatocellular regeneration, a condition that may be associated with resistance to immunosuppressive therapy. This was confirmed in the present study.

Liver histology showed acute hepatitis (massive necrosis, submassive necrosis and severe acute hepatitis) in 86% of our patients and chronic hepatitis in 14%. The duration from onset to histological examination was not different between acute hepatitis and chronic hepatitis ($p = 0.35$). The differences were not statistically significant among the four histological forms described above, which means that the difference in histological findings did not depend on the timing of histological examination. That in non-severe acute onset AIH patients was 32.4 ± 24.0 days in our previous study [14], and the difference was significant between non-severe and severe/fulminant ones ($p < 0.001$). This also

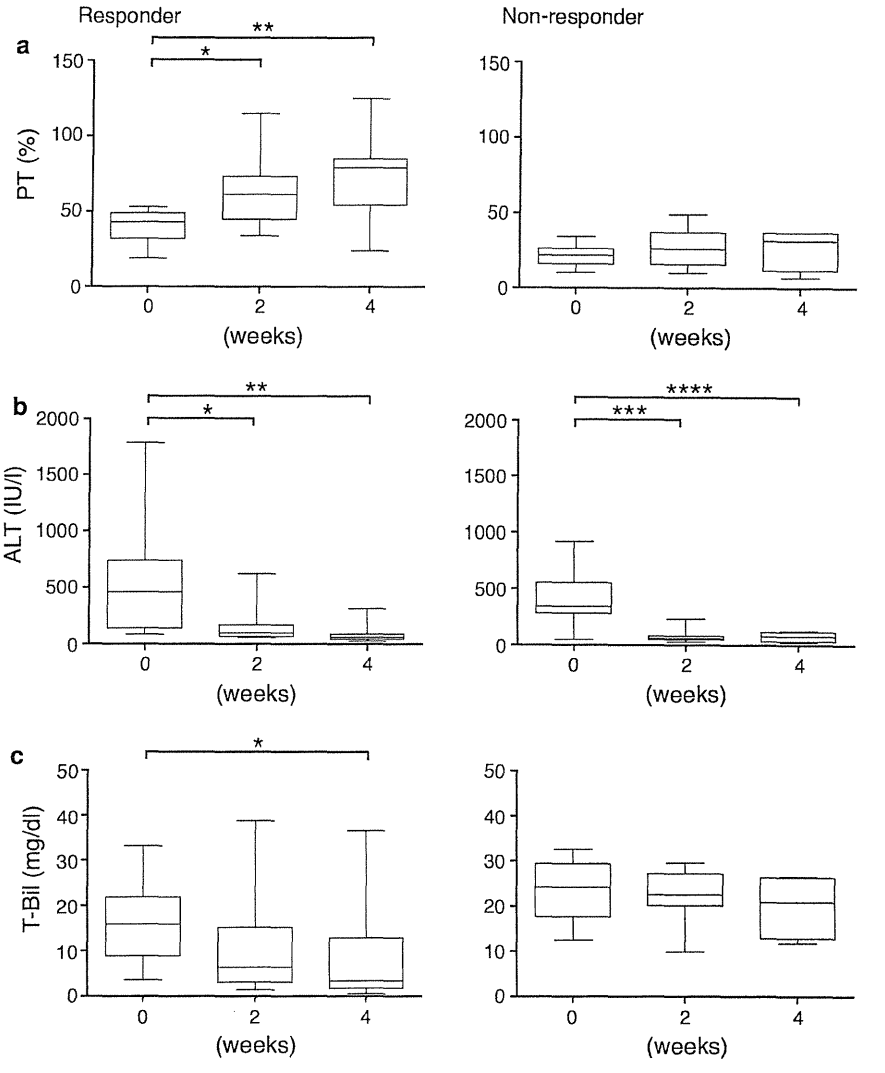

Fig. 1 Prothrombin time (PT) activities, alanine transaminase (ALT) levels and total bilirubin (T-Bil) levels before, 2 weeks after and 4 weeks after the administration of corticosteroid in 17 responders and 8 non-responders. **a** The mean PT activity at each point was 40 ± 10, 62 ± 22 and 74 ± 26% in responders and 21 ± 7, 27 ± 13 and 27 ± 14% in non-responders, respectively (*$p = 0.001$, **$p < 0.001$). **b** The mean ALT level at each point was 552 ± 489, 152 ± 142 and 81 ± 66 IU/l in responders and 404 ± 257, 79 ± 65 and 78 ± 43 IU/l in non-responders, respectively (*$p = 0.003$, **$p < 0.001$, ***$p = 0.004$, ****$p = 0.033$). **c** The mean T-Bil level at each point was 16.1 ± 8.9, 10.7 ± 10.6 and 8.5 ± 10.7 mg/dl in responders and 23.4 ± 6.8, 22.3 ± 6.0 and 20.1 ± 7.3 mg/dl in non-responders, respectively (*$p = 0.031$)

means that our severe and fulminant patients have longer clinical courses than non-severe patients.

Regarding three patients showing histologically chronic hepatitis, the fibrosis stage of patient 6 was F3, and the duration from clinical onset to histological examination was 43 days. Therefore, we speculate that this patient might have had mild-moderate fibrosis before the severe exacerbation and progressed during the relatively short period. In contrast, the fibrosis stage of patient 8 and 13 was F3 and F2, and the duration was 176 and 184 days, respectively. We speculate that they might have less than mild-moderate fibrosis before the exacerbation and progressed during the long period.

In the majority of patients, severe centrizonal necrosis with or without plasma cell accumulation was found. There are no morphological features pathognomonic of AIH, but the characteristic histological picture is that of an interface hepatitis with predominantly lymphoplasmacytic necroinflammatory infiltrates, with or without lobular involvement and bridging necrosis, often with the formation of liver cell rosettes [8]. Moreover, there are only a few reports on the histological features of acute onset AIH. Abe et al. [22] reported that the histological findings are very useful for differentiating between acute AIH and acute hepatitis resulting from other causes, because the former showed plasma cell infiltration, zonal necrosis and early cell infiltration into portal areas, features absent in the latter, and that early histological diagnosis and treatment might be important for patients with acute AIH. Centrilobular necrosis (CN) is associated with an acute clinical presentation and might reflect an early lesion preceding portal involvement, although CN with sparing of the portal areas represents a rare histological pattern in AIH. Recognition of this particular histological appearance enables an early diagnosis of AIH and a timely initiation of immunosuppressive therapy [6, 24–27].

Table 5 Comparison of responders and non-responders

	Responder	Non-responder	*p* value
N	17	8	
Age (years)[a]	44.3 ± 15.0	57.1 ± 10.4	0.004*
Sex (M/F)	4/13	3/5	0.640**
ALT (IU/l)[a,b]	552 ± 489	404 ± 257	0.435*
T-Bil (mg/dl)[a,b]	16.1 ± 8.9	23.4 ± 6.8	0.054*
PT activity (%)[a,b]	40.4 ± 9.9	21.4 ± 7.3	<0.001*
IgG (mg/dl)[a]	2388 ± 1291	2851 ± 1072	0.388*
ANA ≤×40	6	2	1.000**
ANA ≥×80	11	6	
AIH score[a]	16.2 ± 2.7	16.5 ± 2.9	0.823*
Simplified AIH score[a]	6.3 ± 1.4	6.6 ± 1.0	0.654*
Days from onset to corticosteroid therapy (days)[a]	56.8 ± 45.9	54.1 ± 39.7	0.888*
Survivor/non-survivor	17/0	1/7	<0.001**

$p < 0.05$ was considered significant

[a] Mean ± SD

[b] Day 1, the day when corticosteroid was administered

* Unpaired *t* test

** Fisher's exact test

Days from onset to corticosteroid therapy (days)	
0-9	
10-19	○○▲▲
20-29	○○○△▲
30-39	○○○
40-49	○△▲▲
50-59	○
60-69	▲
70-79	
80-89	○▲
90-99	○
100-109	
110-119	
120-129	○
130-139	△▲
140-149	
150-159	
160-169	○

○ : Severe hepatitis responder,
△ : Fulminant hepatitis responder,
▲ : Fulminant hepatitis non-responder

Fig. 2 The duration from initial symptoms to the administration of corticosteroids and outcome

One of the pathological characteristics of severe and fulminant AIH is its heterogeneity. Unenhanced CT scan shows both hypoattenuation and hyperattenuation areas. The former reflect massive hepatic necrosis and the latter regenerative islands [28–30]. Ultrasound also shows the same heterogeneity. These findings would be of help for the diagnosis of severe and fulminant AIH.

In our study, non-responders to corticosteroid treatment, with significance, showed higher age than non-responders. They also showed lower PT activity at the time of corticosteroid administration than responders. This might be due to the impaired hepatocyte regeneration of non-responders, because considerably large numbers of hepatocytes would already have been destroyed, and inhibition of inflammatory reaction might not be effective enough to allow regeneration. The T-Bil level has been considered a critical prognostic factor in fulminant hepatic failure [31] and also in fulminant AIH [32]. Miyake et al. [32] reported that patients whose T-Bil levels worsen during days 8–15 after the diagnosis of fulminant hepatic failure should be considered for liver transplantation. Czaja et al. [33] reported that mortality has uniformly occurred in those whose histology showed multilobular collapse and whose laboratory data did not improve within 2 weeks of corticosteroid treatment, and that such patients are in need of urgent transplantation. In our present study, the improvement of PT activity, a marker of liver regeneration, during the first 2 weeks after the corticosteroid treatment was statistically significant in responders, but not in non-responders. Even in the responders, the improvement of liver function, especially T-Bil, was slow in our patients. This means that the time limit for switching to liver transplantation to avoid infectious complications is 2 weeks after the administration of corticosteroids, and we agree with Czaja's report. Czaja et al. [34] also reported that treatment failure in AIH tends to develop in patients with early age onset, HLA DRB1*03 or variant syndromes, especially in those with primary sclerosing cholangitis, but these were not found in our patients.

As described above, the disease severity of non-responders at the time of starting corticosteroid therapy was more advanced than in responders. The duration from initial symptoms to the administration of corticosteroids was not different between responders and non-responders. This means that response to corticosteroid therapy is not associated with the time duration from clinical onset but with the disease severity. Therefore, we should diagnose

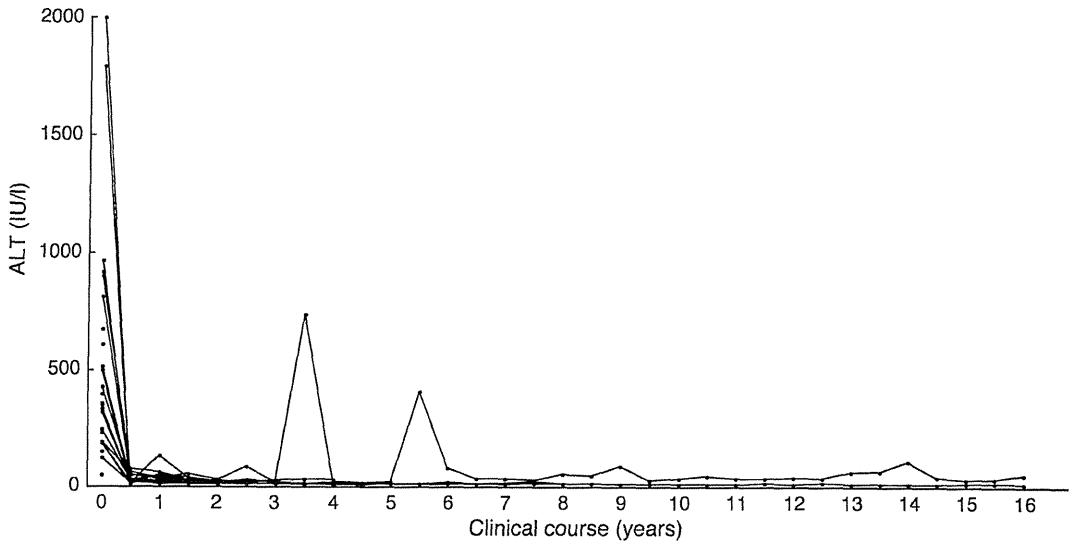

Fig. 3 Long-term changes in ALT levels after the start of treatment in all patients

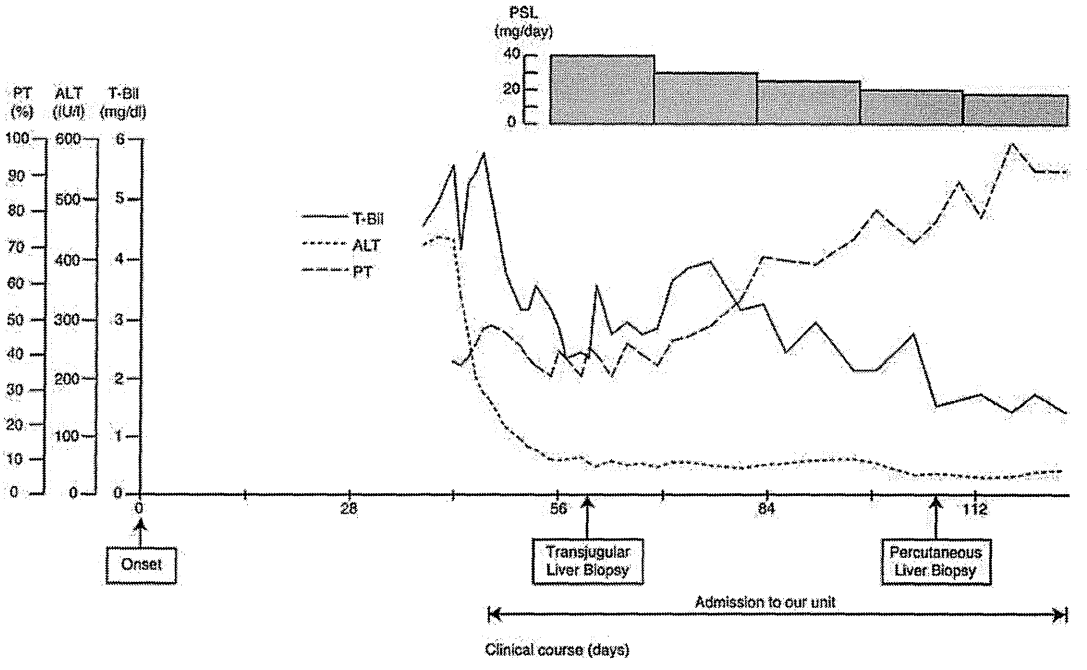

Fig. 4 Clinical course of a 26-year-old female patient (patient 20, responder). She suffered from cryptogenic acute hepatitis showing 35% of PT activity after a month of treatment and was admitted to our unit. Corticosteroid was administered, and her liver function tests gradually improved

and treat acute onset AIH patients before they develop severe and fulminant disease. AIH patients with low PT activity have very severe and advanced histology and present impaired hepatocellular regeneration, which is associated with resistance to immunosuppressive therapy.

In conclusion, we should be aware of the possibility that acute onset AIH patients exist among those with cryptogenic hepatitis, and that this condition can cause severe hepatitis and fulminant hepatic failure with a poor prognosis if the diagnosis was delayed and sufficient immunosuppressive therapy could not be introduced at an early stage. However, making the diagnosis of acute onset AIH is very difficult because there is no gold standard for it. It is most important to exclude other causes systematically, remember acute onset AIH in the differential diagnosis and then apply the scoring system, and comprehensive evaluations of clinical,

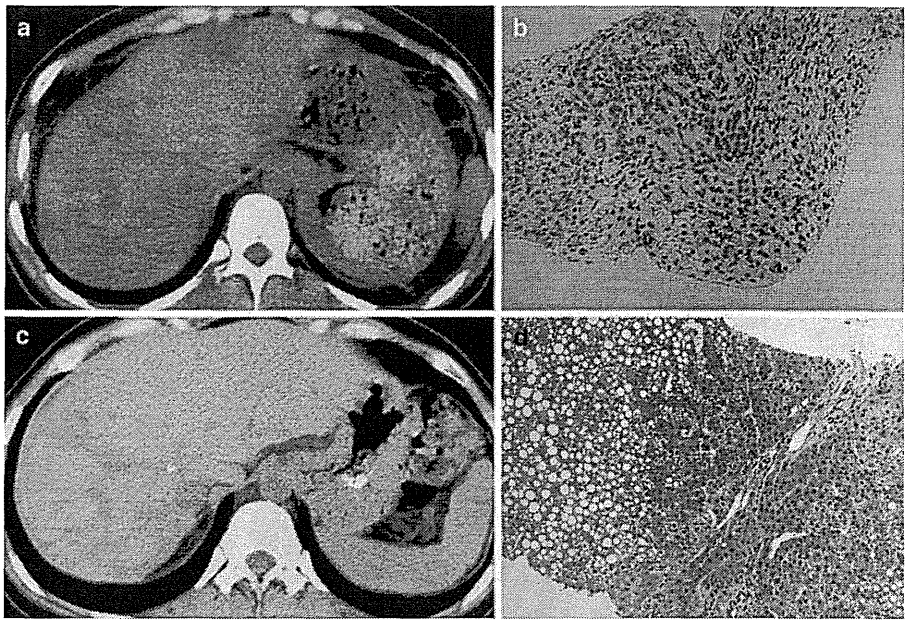

Fig. 5 CT and histological findings of a responder (patient 20). At admission to our unit, CT scan showed marked liver atrophy with heterogeneous hypoattenuation areas (**a**), suggesting massive hepatic necrosis, and liver histology showed massive necrosis without plasma cell accumulation, compatible with acute autoimmune hepatitis (**b**). Two months after the administration of corticosteroid, CT scan showed enlargement of the left lobe and a decrease in the hypoattenuation areas (**c**), and liver histology showed simple steatosis with minimal necroinflammatory change (**d**)

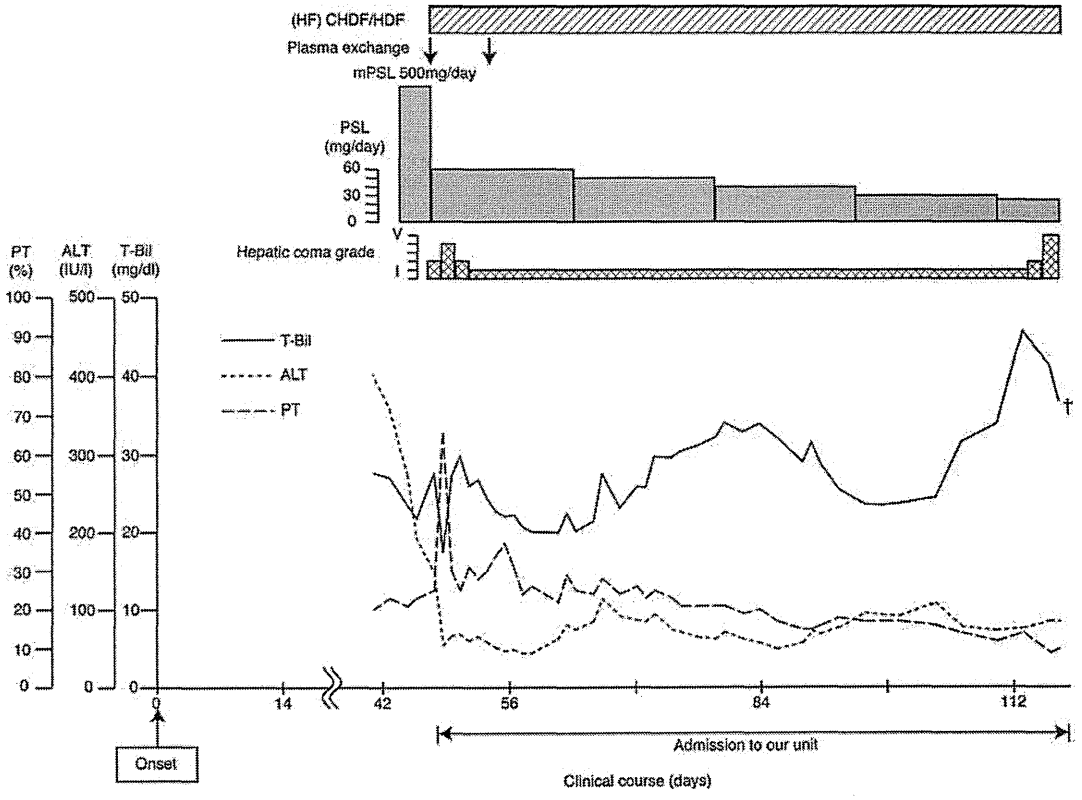

Fig. 6 Clinical course of a 70-year-old female patient (patient 23, non-responder). She suffered from cryptogenic acute hepatitis showing 20% of PT activity and 28 mg/dl of T-Bil after 50 days of treatment and was admitted to our unit. Corticosteroid was administered in combination with artificial liver support: high flow (HF)-continuous hemodiafiltration (CHDF) and plasma exchange. Her hepatic encephalopathy improved to grade I, but liver function tests did not improve. She died 3 months after admission because of hepatic failure

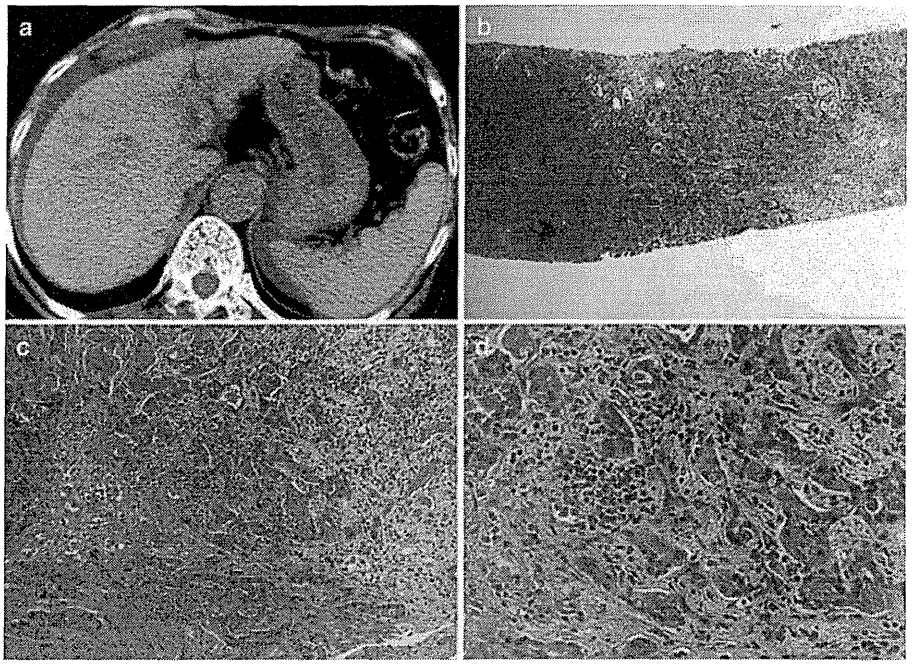

Fig. 7 CT and histological findings of non-responder (patient 23). At admission she presented grade III hepatic encephalopathy, and CT scan showed mild liver atrophy (**a**). Post mortem liver histology showed massive necrosis with plasma cell accumulation, compatible with acute autoimmune hepatitis (**b–d**)

biochemical, radiological and histological features are necessary for early diagnosis. Especially precise pathological evaluation plays an important role in the differential diagnosis: recognition of severe centrizonal necrosis with or without plasma cell accumulation. We should study and recognize the pathological characteristics of acute onset AIH. The prognosis might indeed be improved without liver transplantation by the introduction of sufficient immunosuppressive therapy at an early stage. Multicenter studies are also needed to clarify the features of severe and fulminant AIH and define the treatment strategies.

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Conflict of interest No conflicts of interest exist.

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tic analyses should be regarded with caution. They simultaneously used high-correlated variables to conduct this analysis, such as four obesity indices. As a result (Table 3 presented in ref. [1]), waist circumference was not selected as a significant independent variable to predict ALT elevation in subjects with and without fatty liver. In contrast, BMI was selected as a significant independent variable to predict ALT elevation in fatty liver subjects. Although they quoted references in which BMI and waist circumference were significant predictors for ALT elevation, they concluded that visceral adipose tissue (VAT) alone was identified as a predictor of ALT elevation in subjects with and without fatty liver.

VAT is logically supposed to be a better predictor of ALT elevation than the other variables, and it is a difficult to predict VAT by simple anthropometric measurements [2]. It is partly explained by race/ethnicity and aging [3,4]. However prediction of ALT elevation by VAT itself has a risk of over or underestimation by other obesity-related variables from the viewpoint of multicollinearity [5]. The same logic applies to systolic and diastolic blood pressure. Multicollinearity cannot be solved by selecting a stepwise method as statistical procedure. Selection of independent variables should be made before statistical analysis. Explanatory variables should bring separate information and a sensible choice of candidates is a pre-requisite for a good final model. When evaluating the effects of variables that are closely related, it is recommended to use ridge estimators [6].

The author proposes three points of view; (1) to avoid multicollinearity of independent variables on obesity and blood pressure, simultaneous use of obesity indices and blood pressure should be carefully handled. (2) As different criteria of metabolic syndrome were adopted excluding subjects with medical treatment for hypertension, dyslipidaemia, or diabetes mellitus, data including subjects with medical treatments should also be considered. (3) Appropriate transformation of skewed variables such as triglyceride levels is needed, and binary data according to the criteria on each component of metabolic syndrome is recommended, although information loss cannot be ignored.

Finally, information on viral antibody/antigen status would be useful to clarify the association between ALT and alcohol consumption.

Conflict of interest statement

There is no conflict of interest in this study.

Acknowledgment

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Autoimmune fulminant hepatic failure in chronic hepatitis C during Peg-interferon-alpha 2b plus ribavirin treatment showing histological heterogeneity

Sir,

Interferon has been reported to induce autoimmune hepatitis (AIH), but the report of fulminant hepatic failure (FHF) is rare [1,2]. Here, we report a case of FHF due to AIH during the treatment of chronic hepatitis C with pegylated interferon (Peg-IFN) and ribavirin (RBV).

A 38-year-old woman was chronically infected with hepatitis C virus (HCV) genotype Ib and high viral load. Her liver histology showed mild fibrosis and moderate necro-inflammatory activity compatible with chronic hepatitis C. Combination therapy with Peg-IFN-alpha 2b 80 μg weekly and RBV 600 mg daily was begun. Biochemical and histological autoimmune parameters were negative before treatment. HCV RNA became negative at week 4 and continued to be negative. At week 20, she developed Hashimoto's thyroiditis and was treated with thyroid hormone replacement. During the course, RBV was reduced to 200 mg per day because of anemia, and antiviral therapy was prolonged beyond week 48. At week 52, a rise of alanine aminotransaminase (ALT) of 167 IU/l was noted. At week 56, jaundice and prothrombin time (PT) prolongation ensued; interferon therapy was stopped and the patient was admitted to the hospital. Two weeks later, her liver function tests worsened and she developed hepatic encephalopathy, and was referred to our unit.

On admission, the patient presented with grade II hepatic encephalopathy and laboratory tests revealed ALT 321 IU/l, total bilirubin 12.2 mg/dl and PT activity 16%. HCV RNA, hepatitis A virus, hepatitis B virus, herpes simplex, cytomegalovirus and Epstein-Barr virus were negative. Anti-nuclear antibody was 1:320 and immunoglobulin G was 2546 mg/dl. Anti-smooth muscle antibody, anti-mitochondrial antibody and liver kidney microsomal antibody-1 were negative; HLA-DR was DR4. There was no family history of autoimmune disease. AIH score was 17 according to the revised original scoring system by the international AIH group, thus making AIH very likely.

Corticosteroids (methylprednisolone) were administered in combination with artificial liver support. Hepatic encephalopathy improved to grade I, but signs of liver regeneration were not observed. The patient received a living donor liver transplantation on the 18th hospital day (Fig. 1a). Abdominal CT showed liver atrophy and heterogeneous hypoattenuating areas (Fig. 1b). Section of extracted liver showed massive haemorrhagic necrosis in the right lobe corresponding to heterogeneous hypoattenuating areas on CT, and nodular regeneration or preserved parenchyma in the left lobe corresponding to hyperattenuating areas on CT (Fig. 1c). Liver histology showed massive haemorrhagic necrosis with plasma cell accumulation in the right lobe (Fig. 1d, arrow in Fig. 1c), and submassive necrosis with impaired regeneration in the left lobe (Fig. 1e, arrow head in Fig. 1c). After transplantation, her liver function test improved gradually and she was discharged on the 65th hospital day.

Combination of Peg-IFN and RBV is well established and the standard treatment for HCV infection worldwide. In chronic hepatitis C patients with genetically predisposed underlying autoimmune

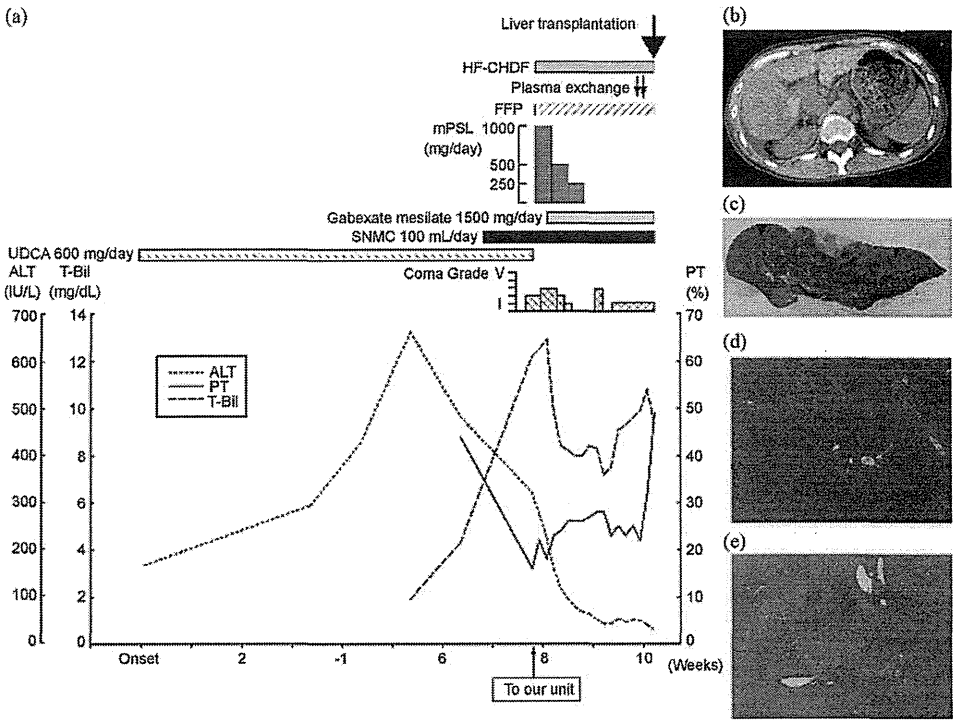

Fig. 1. Clinical course, CT image and histological findings of the patient. UDCA, ursodeoxycholic acid; SNMC, Stronger Neo-Minophagen C; mPSL, methylprednisolone; FFP, fresh frozen plasma; HF-CHDF, high flow-continuous haemodiafiltration.

diathesis, an elevation of serum ALT without the recent use of hepatotoxic drugs and the reappearance of HCV RNA, and with elevated levels of IgG and titers of autoantibodies during IFN treatment strongly suggest the diagnosis of AIH, triggered by the immunostimulating effects of IFN. Non-severe AIH patients show improvements in liver functions after stopping IFN and initiating immunosuppressive therapy, but patients who had already developed into autoimmune FHF usually show resistance to immunosuppressive therapy and require liver transplantation. We should recognize *de novo* acute onset AIH triggered by IFN during the treatment and possibility of developing into FHF. Early diagnosis and treatment before the development of FHF could improve their prognoses without liver transplantation. Histological heterogeneity and centrilobular necrosis/collapse are characteristic and useful for the early diagnosis of acute onset AIH [3–5].

Conflicts of interest statement

None declared.

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CLINICAL STUDIES

Diagnostic value and utility of the simplified International Autoimmune Hepatitis Group criteria in acute-onset autoimmune hepatitis

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Keywords
autoimmune hepatitis – fulminant hepatitis – liver histology – scoring system – severe hepatitis

Abbreviations
AIH, autoimmune hepatitis.

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Abstract

Background: The diagnosis of autoimmune hepatitis (AIH) is already difficult, and that of acute-onset AIH with atypical features is even more challenging, even though the revised original diagnostic criteria created by an international AIH group were widely accepted and incorporated into clinical practice. *Aims:* Recently, simplified diagnostic criteria were proposed. We compared the performance parameters of the simplified scoring system in patients with acute-onset AIH and examined its usefulness and limitations. *Methods:* Fifty-five patients with acute-onset AIH (29 non-severe, 14 severe and 12 fulminant) were assessed according to the simplified scoring system and compared with the revised original one. *Results:* Of the 55 patients, 22 (40%) were diagnosed as 'definite' AIH, 28 (51%) as 'probable' and five (9%) as 'non-diagnostic' based on the revised original scoring system. By the simplified scoring system, six (11%) were diagnosed as 'definite' AIH, 16 (29%) as 'probable' and 33 (60%) as 'non-diagnostic'. Anti-nuclear antibody titres did not differ among the three groups. The immunoglobulin G level was higher in fulminant than in non-severe patients ($P = 0.01$). Sixty-five per cent showed acute hepatitis (massive necrosis, submassive necrosis and severe acute hepatitis) and 35% showed chronic hepatitis. *Conclusions:* The revised original scoring system performed better in patients with acute-onset AIH than the simplified scoring system.

Autoimmune hepatitis (AIH) is generally regarded as a clinically and histologically 'chronic' hepatitis, characterized by the presence of autoantibodies, hypergammaglobulinaemia and interface hepatitis and plasma cell infiltration on histological examination (1, 2). As AIH patients with clinical features of acute, severe and fulminant hepatitis (acute-onset AIH) do not show such typical features of AIH, they are at a risk of losing the timing for the initiation of immunosuppressive therapy and are sometimes resistant to the therapy in liver regeneration and have a poor prognosis. The survival rate of fulminant AIH has been $< 20\%$ without liver transplantation, and this is recognized everywhere around the world as well as in our unit (3–6).

Diagnostic criteria for AIH based on the clinicopathological features were created by an international AIH group in 1993 (7) and revised in 1999 (8), and were widely accepted and incorporated into clinical practice. Nevertheless, the diagnosis of AIH is still a challenging task, and especially in patients with atypical features. There is no gold standard for making the diagnosis, and the diagnosis of acute-onset AIH is the most challenging.

The revised original criteria from 1999 provided clinical guidelines for the diagnosis of AIH, but they were complex and intended purely for scientific purposes. To resolve these difficulties, a simplified scoring system for routine clinical practice was proposed in 2008 (9). This new scoring system can be easily put to use in daily clinical practice for chronic AIH with a high sensitivity and specificity, but this does not apply to the evaluation of acute-onset AIH.

Recently, we examined the clinical and histological features of acute-onset AIH and reported that centrilobular necrosis/collapse was characteristic and that the disease severity was associated with advanced histology (6, 10–12).

In the present study, we compared the performance parameters of the revised original scoring system and the simplified one in patients with acute-onset AIH (non-severe, severe and fulminant) and examined the usefulness and limitations of each system.

Patients and methods

Selection criteria of patients

Patients with acute-onset AIH were enrolled between 2000 and 2009. A diagnosis of AIH was made based on the criteria of the International AIH Group defining the score for probable or definite AIH (8) and/or on liver histological

findings compatible with AIH, consisting of interface hepatitis, centrilobular necrosis and plasma cell infiltration.

The eligibility criteria of clinically 'acute-onset' AIH were as follows in addition to the AIH criteria described above: (i) acute-onset liver injury, (ii) no histories of chronic liver injury, (iii) negativity of active viral markers such as hepatitis A, B, C and E viruses, Epstein–Barr virus (EBV), cytomegalovirus (CMV) and herpes simplex virus (HSV), drug-induced liver injury, toxic and metabolic disorders and (iv) no signs of chronicity on the basis of physical examination, laboratory data and abdominal ultrasound findings.

The eligibility criteria of severe and fulminant AIH, in addition to the criteria described above, were as follows: patients with prothrombin time (PT) activity $< 50\%$ of the control or the total bilirubin (T-Bil) level > 20 mg/dl during the disease course were defined as severe AIH, and patients with PT activity $< 40\%$ of control and hepatic encephalopathy were defined as fulminant AIH. Informed consent was obtained from all patients or appropriate family members. The work described in this manuscript has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki).

Clinical, biochemical and immunoserological analysis

Data obtained from patients were as follows: sex; age at diagnosis; time of onset; non-severe, severe and fulminant disease; serum levels of alanine aminotransferase (ALT), T-Bil, alkaline phosphatase, PT activity, immunoglobulin G (IgG), anti-nuclear antibody (ANA), anti-smooth muscle antibody (ASMA), liver kidney microsomal antibody-1 (LKM-1) and anti-mitochondrial antibody (AMA); and human leucocyte antigen. They were also examined for any histories of recent exposure to drugs and chemical agents as well as heavy alcohol consumption (> 50 g/day). ANA and ASMA were examined by a fluorescent antibody method, and AMA was examined by a fluorescent antibody method or an enzyme-linked immunosorbent assay (ELISA), and LKM-1 was examined by ELISA.

The ANA assay should be performed using rodent frozen tissues or HEp2 cells according to the original articles of revised original criteria (8) and simplified criteria (9). In almost all Japanese hospitals including university hospitals, it has been performed using HEp2 cells. Therefore, we halved the values according to the original article in the application of the simplified scoring system.

In acute-onset AIH, early symptoms including fever, general malaise, fatigue, nausea, vomiting and right upper quadrant discomfort are frequently observed; hence, we defined the beginning of these symptoms as clinical onset.

Virological analysis

Patients were examined for viral markers such as IgM anti-hepatitis A virus antibody (IgM-HA), IgM anti-HBc antibody (IgM-HBc), HBsAg, anti-HCV antibody, HCV RNA, HEV RNA (for severe and fulminant patients), IgM anti-EBV antibody (IgM-EBV), IgM anti-HSV antibody (IgM-HSV) and IgM anti-CMV antibody (IgM-CMV). None of the patients had clinical or laboratory evidence of acquired immune deficiency syndrome.

Histological analysis

Histological examination was performed in 49 patients by the percutaneous approach, transjugular approach, explanted liver or post-mortem. Thirty-nine were percutaneous needle biopsy, two transjugular needle biopsy, two explanted liver and six post-mortem. Three specialists (M. N., K. F. and O. Y.) independently reviewed the histopathological changes by evaluating the degrees of portal and lobular changes and plasma cell infiltrations on haematoxylin–eosin-stained sections. Staging and grading were evaluated based on the classification of Desmet *et al.* (13).

Scoring systems

The revised original scoring system of the international AIH group (8) and the simplified system of the same group (9) were applied to all patients before treatment.

Model of end-stage liver disease scores

Scores by the model of end-stage liver disease (MELD) (14) at admission were calculated for severe and fulminant patients.

Statistical analysis

Differences in proportions among the groups were compared by Fisher's exact probability test, Student's t-test and Welch's t-test ($P < 0.05$ was considered significant).

Results

Clinical and biochemical features

Fifty-five patients, 12 men and 43 women, were enrolled in the study. Twenty-nine has non-severe hepatitis, 14 had severe hepatitis and 12 had fulminant hepatitis. The clinical and biochemical features of all patients at admission are provided in Table 1. The mean age at the time of diagnosis was 51.6 ± 14.3 years. The mean ALT was 612 ± 478 IU/l, the mean T-Bil was 9.5 ± 9.4 mg/dl and the mean PT activity was $65 \pm 31\%$.

The mean IgG was 2192 ± 960 mg/dl. The IgG level was normal [$< 1.0 \times$ upper normal value (UNV)] in 17 of 55 (31%), 1.0–1.5 $\times$ UNV in 24 (44%), 1.5–2.0 $\times$ UNV in nine (16%) and $> 2.0 \times$ UNV in five (9%). It was 1.0–1.1 $\times$ UNV in six (11%) and $> 1.10 \times$ UNV in 32 (58%).

Anti-nuclear antibody was positive ($\geq$ 1:40) in 49 of 55 (89%) patients, $<$ 1:40 in six (11%), 1:40 in eight (15%), 1:80 in 16 (29%) and $>$ 1:80 in 25 (46%). ASMA was positive ($\geq$ 1:40) in 15 of 50 (30%). One patient was positive for LKM-1.

Table 1. Clinical features of patients

	Non-severe type	Severe type	Fulminant type
n	29	14	12
Sex (male/female)[1]	4/25	4/10	4/8
Age (years)[2]	51.7 ± 14.0	48.7 ± 14.4	54.8 ± 15.8
PT (%)[3]	90 ± 17	46 ± 8	25 ± 8
ALT (IU/l)[4]	626 ± 392	600 ± 534	597 ± 625
T-Bil (mg/dl)[5]	3.3 ± 3.6	12.9 ± 8.3	20.8 ± 8.1
ANA ≥40 (fold)[6]	20	10	11
IgG (mg/dl)[7]	1874 ± 571	2448 ± 1400	2662 ± 885
Revised original score before treatment[8]	13.6 ± 3.4	14.8 ± 3.1	16.5 ± 3.1
Simplified score before treatment[9]	4.6 ± 1.6	4.6 ± 1.6	5.5 ± 1.1

Values are mean ± SD or number.

[1], [2], [4], [6], [9]No statistical significance among the three groups.

[3]Significant difference ($P < 0.001$) between non-severe and severe by Welch's *t*-test, between non-severe and fulminant by Welch's *t*-test, between non-severe and fulminant by Student's *t*-test.

[5]Significant difference between non-severe and severe by Welch's *t*-test ($P < 0.001$), between non-severe and fulminant by Welch's *t*-test ($P = 0.02$), between non-severe and fulminant by Student's *t*-test ($P < 0.001$).

[7]Significant difference between non-severe and fulminant by Welch's *t*-test ($P = 0.01$).

[8]Significant difference between non-severe and fulminant by Student's *t*-test ($P = 0.02$).

ALT, alanine aminotransferase; ANA, anti-nuclear antibody; PT, prothrombin time; SD, standard deviation; T-Bil, total bilirubin.

No patients were positive for HBs Ag. Two patients were positive for HCV Ab. One is a non-severe patient with HCV RNA, and the other is a patient who developed fulminant disease during peg-interferon plus ribavirin treatment without HCV RNA. In two patients with non-severe and severe disease, AIH was triggered by hepatitis A.

The duration from the onset to admission to our unit was 43.2 ± 33.8 days for all patients, consisting of 42.6 ± 33.1 days for non-severe, 49.1 ± 34.9 for severe and 37.7 ± 35.9 for fulminant. The differences were not significant among the three groups.

Histological features

The pathological characteristics of the patients are shown in Tables 2–4 and are summarized in Figure 1. Histological examination was performed in 49 of 55 patients, with 32 (65%) showing acute hepatitis, exhibiting zonal, submassive and massive necrosis with or without plasma cell accumulation in portal and centrilobular areas. Seventeen (35%) showed chronic hepatitis.

Forty-six of the 49 (94%) patients showed severe activity, nine with massive necrosis, three with submassive necrosis, 20 with severe acute hepatitis and three with moderate activity with fibrosis stage 1–3.

The proportion of acute hepatitis increased with disease severity, and the difference was significant between non-severe and fulminant patients ($P = 0.04$).

In eight severe and fulminant patients, histological examinations were performed in explanted livers and post-mortem. We could not perform a histological examination before the start of the treatment because of the complicated coagulopathy and ascites in these patients. It is possible that this might influence histological appearance and therefore influence the results of the scoring, and that the histological findings in these patients might be misleading as a result of changes because of critical illness during ICU treatment.

We could find emperipolesis (15, 16) frequently in periportal and lobular areas in patients with high activity, although there was a limitation in terms of its evaluation by light microscopic examination.

Comparison of scoring systems for autoimmune hepatitis

The revised original diagnostic criteria (revised original criteria) proposed by the International AIH Group in 1999 (8) and the simplified diagnostic criteria (simplified criteria) by the same group in 2008 (9) were used to score all patients (Tables 2–4, Figs 2 and 3).

In the revised original criteria, the AIH score ranged from 7 to 22 (14.5 ± 3.4) before treatment. Twenty-two of 55 patients (40%) were diagnosed as 'definite' AIH (score > 15), 28 (51%) as 'probable' (score 10–15) and five (9%) as 'non-diagnostic' (score < 10). Five non-diagnostic patients were all non-severe (Fig. 2).

In the simplified criteria, the AIH score ranged from 2 to 7 (4.8 ± 1.5) before treatment. Six of 55 patients (11%) were diagnosed as 'definite' AIH (score ≥7), 16 (29%) as 'probable' (score ≥6) and 33 (60%) as 'non-diagnostic' (score < 6). Only nine (31%) were diagnostic in non-severe, five (36%) in severe and eight (67%) in fulminant patients (Fig. 3). Regarding the points of each variable, the titre of autoantibodies was slightly higher in fulminant than in non-severe and severe patients, although there was no statistical significance ($P = 0.10$ and $P = 0.18$ respectively). The IgG level was higher in fulminant patients than that in non-severe ones ($P = 0.002$). The point of liver histology was higher in non-severe than that in severe and fulminant patients ($P = 0.04$ and $P = 0.003$ respectively).

Table 2. Histological features and scores of non-severe patients

Patient	Liver histology	Revised original score	Simplified score	Variables of simplified criteria			Absence of viral hepatitis
				Autoantibody	IgG	Liver histology	
1	CH (F1A3)	16	7	2	2	1	2
2	sAH	15	2	0	0	0	2
3	sAH	16	3	1	0	0	2
4	sAH	9	4	2	2	0	0
5	ND	14	6	2	2	0	2
6	CH (F2A2)	19	7	2	2	1	2
7	sAH	11	4	2	0	0	2
8	sAH	14	6	2	2	0	2
9	SMN	15	3	1	0	0	2
10	ND	13	5	1	2	0	2
11	sAH	16	3	1	0	0	2
12	CH (F3A3)	15	4	0	1	1	2
13	CH (F3A3)	9	6	1	2	1	2
14	sAH	9	4	1	1	0	2
15	CH (F1A3)	18	7	2	2	1	2
16	CH (F2A3)	16	7	2	2	1	2
17	sAH	7	2	0	2	0	2
18	CH (F3A3)	10	5	2	0	1	0
19	CH (F2A3)	15	4	0	0	2	2
20	CH (F2A3)	14	5	2	2	1	2
21	CH (F1A1)	18	5	1	0	2	0
22	sAH	13	4	1	1	0	2
23	CH (F3A3)	15	3	0	0	1	2
24	CH (F2A3)	18	6	2	1	1	2
25	CH (F2A3)	11	5	1	0	2	2
26	sAH	14	6	2	2	0	2
27	sAH	7	2	0	0	0	2
28	sAH	18	5	2	1	0	2
29	sAH	10	2	0	0	0	2

CH, chronic hepatitis; ND, not done; sAH, severe acute hepatitis; SMN, submassive necrosis.

Table 3. Histological features and scores of severe and fulminant patients

Patient	Liver histology	Revised original score	Simplified score	Variables of simplified criteria			Absence of viral hepatitis
				Autoantibody	IgG	Liver histology	
S1	CH (F1A3)	15	7	2	2	1	2
S2	sAH	17	2	0	0	0	2
S3	CH (F4A3)	12	6	1	2	1	2
S4	ND	12	5	1	2	0	2
S5	CH (F3A3)	10	5	0	2	1	2
S6	sAH	16	3	1	2	0	0
S7	sAH	16	6	2	2	0	2
S8	ND	13	6	2	2	0	2
S9	MN	15	2	0	0	0	2
S10	sAH	16	5	1	2	0	2
S11	MN	15	3	1	0	0	2
S12	sAH	22	6	2	2	0	2
S13	SMN	11	4	0	2	0	2
S14	sAH	17	4	2	0	0	2
F1	MN	14	6	2	2	0	2
F2	ND	11	6	2	2	0	2
F3	CH (F2A2)	14	7	2	2	1	2
F4	MN	17	5	1	2	0	2
F5	SMN	15	5	1	2	0	2
F6	MN	13	3	1	0	0	2
F7	MN	20	6	2	2	0	2
F8	MN	19	4	0	2	0	2
F9	ND	16	6	2	2	0	2
F10	MN	20	6	2	2	0	2
F11	sAH	20	6	2	2	0	2
F12	MN	19	6	2	2	0	2

CH, chronic hepatitis; F, fulminant; MN, massive necrosis; ND, not done; S, severe; sAH, severe acute hepatitis; SMN, submassive necrosis.