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Diagnosing autoimmune hepatitis in nonalcoholic fatty liver disease: is the International Autoimmune Hepatitis Group scoring system useful?

Satoru Yatsuji, Etsuko Hashimoto, Hiroyuki Kaneda, Makiko Taniai, Katsutoshi Tokushige, and Keiko Shiratori

Department of Internal Medicine and Gastroenterology, Tokyo Women's Medical University, 8-1 Kawada-cho, Shinjuku-ku, Tokyo 162-8666, Japan

Background. There are no surrogate serum markers for autoimmune hepatitis (AIH) and nonalcoholic fatty liver disease (NAFLD). An AIH scoring system was reported by the International Autoimmune Hepatitis Group; however, the criteria did not focus on making the distinction between AIH and NAFLD. We examined the effectiveness of using the AIH score for diagnosing AIH in NAFLD patients. We also identified the prevalence of autoimmune phenomena, in terms of various auto-antibodies, including antinuclear antibodies (ANA), to determine whether these markers had any clinicopathological significance, and whether they were related to the patients' clinical courses. ***Methods.*** We studied 212 patients (103 males and 109 females) with biopsy-proven NAFLD. The AIH score of each patient was calculated without including the liver biopsy results. The patients were divided into three groups based on their clinicopathological features: the overlap group (those with clinical and histological features of both NAFLD and AIH), the systemic group (those with systemic antoimmune disease other than AIH), and the "other" group (patients with no antoimmune disease). To evaluate the clinicopathological significance of ANA in NAFLD patients, those without autoimmune diseases (the "others" group) were classified according to their ANA positivity and ANA titer. ***Results.*** Seventy patients (33.0%) were positive for ANA. Among the female patients, 106 patients (97.2%) had an AIH score of 10 or more. Of the 103 male patients, 21 (20.4%) had an AIH score of 10 or more. However, after liver biopsy, only 1 patient (0.5%) could be classified as "definite AIH." In the NAFLD patients without autoimmune diseases ("other" group), multivariate logistic regression analysis found that female sex was an independent predictor of the presence of ANA ($P = 0.029$). In contrast, multivariate logistic regression analysis found that severe obesity (body mass index [BMI], $\geq 30\,kg/m^2$) was the only independent predictor of the presence of an ANA titer of 1:80 or more ($P = 0.026$). ***Conclusions.*** The AIH score without liver biopsy findings was not useful for diagnosing AIH in NAFLD patients. In patients with elevated ANA titers and risk factors for NAFLD, it is very important to perform a liver biopsy to make a definitive diagnosis before treatment.

Key words: nonalcoholic steatohepatitis, nonalcoholic fatty liver disease, autoimmune hepatitis, autoimmune hepatitis scoring system, antinuclear antibody

Introduction

Over the past two decades, lifestyle changes have resulted in a dramatic increase in the prevalence of obesity in developed countries. This rising incidence of obesity has been paralleled by a dramatic increase in fatty liver in these countries. In fact, nonalcoholic fatty liver disease (NAFLD) is now emerging as the most common liver disease.[1–4] Most NAFLD patients have benign simple fatty liver, though some patients develop nonalcoholic steatohepatitis (NASH), which can progress to cirrhosis and hepatocellular carcinoma.[5–7] The diagnosis of NAFLD requires the exclusion of alcohol abuse and other causes of liver diseases such as viral hepatitis, autoimmune liver diseases, and metabolic or hereditary liver diseases. Because there are no surrogate serum markers for NAFLD, a definitive diagnosis requires a liver biopsy. Although liver biopsy is generally a safe procedure, it does carry a small risk of complications.[8] Thus, because most NAFLD patients have simple fatty livers, and therefore have a benign prognosis, a liver biopsy is not routinely performed.

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Reprint requests to: E. Hashimoto

Autoimmune hepatitis (AIH) has been recognized for more than 40 years, and its characteristic features have been extensively reviewed. Similar to patients with NAFLD, patients with AIH have no particular signs, symptoms, or liver test abnormalities that are of sufficient specificity to be considered part of the diagnostic criteria. Thus, a diagnosis of AIH was needed to exclude other liver diseases. Accordingly, in 1999, criteria for the diagnosis of AIH (AIH score) were reported by the International Autoimmune Hepatitis Group.[9] At that time, the criteria did not focus on making the distinction between AIH and NAFLD. Although AIH and NAFLD show completely different histological features, their clinical features can be similar in some patients, except for the presence of autoimmune phenomena primarily in AIH patients and the complication of metabolic syndrome plus steatosis that may be detected on noninvasive imaging primarily in NAFLD patients. Some patients with NAFLD may have autoimmune phenomena. As well, NAFLD patients with advanced fibrosis decrease steatosis, and imaging does not detect steatosis involving less than 30% of the liver.[10] Therefore, if a liver biopsy is not performed in NAFLD patients with mild steatosis and autoimmune phenomena, they may be misdiagnosed as having AIH based on their AIH score.

There have been several reports dealing with NAFLD and autoimmunity in western countries.[11–16] However, in Japan to date there has been only one case report of this phenomenon.[17] Epidemiological studies of patients with AIH and NAFLD have shown that ethnicity can be predictive of disease characteristics and complications.[18,19] Thus, it is important to clarify the prevalence and clinical significance of autoimmune phenomena in Japanese patients with NAFLD.

In this study, we examined the effectiveness of using the AIH score for diagnosing AIH in NAFLD patients. Also, we identified the prevalence of antinuclear antibodies (ANA), anti-smooth muscle antibody (ASMA), antimitochondrial antibody (AMA), anti-liver/kidney microsome type 1 antibody (LKM-1), and the elevation of immunoglobulins among patients with NAFLD, to determine whether these markers had any clinical, biochemical, or histological significance, and whether they were related to the patients' clinical courses.

Patients and methods

Two hundred and eighty-eight patients were diagnosed as having biopsy-proven NAFLD at the Tokyo Women's Medical University and affiliated hospitals from 1993 to January 2005. Their clinical data were collected prospectively. Informed consent was obtained from each patient, and the study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki. We excluded 76 patients due to missing data or a lack of informed consent. Therefore, 212 patients (103 males and 109 females) were studied.

The diagnosis of NAFLD was based on the following criteria: (1) presence of steatosis (>30%) or steatohepatitis on liver biopsy (steatohepatitis was defined as the detection of steatosis [>10%], inflammatory infiltrates, ballooning degeneration with or without Mallory bodies, or pericellular/perivenular fibrosis); (2) intake of less than 100 g of ethanol per week, as confirmed by the attending physician and family members who were in close contact with the patient; and (3) appropriate exclusion of liver diseases other than AIH. Alcoholic liver disease, viral hepatitis, biliary obstruction, and metabolic liver diseases were ruled out based on standard clinical, biochemical, and histological criteria. The medical history and physical status, including body mass index (BMI), and the presence of the metabolic syndrome at the time of liver biopsy were noted. None of the patients had received drug treatment for NASH prior to liver biopsy. Hepatomegaly was diagnosed by physical examination and ultrasonography. All patients had the following laboratory parameters measured: liver function tests (aspartate aminotransferase [AST], alanine aminotransferase [ALT], total bilirubin, alkaline phosphatase [ALP], gamma-glutamyltranspeptidase [GGTP]), total protein, albumin, triglycerides, total cholesterol, platelet count, prothrombin time, hepaplastin test, type 4 collagen 7S, and hyaluronic acid. At the time of the liver biopsy, all patients underwent ultrasonography.

To analyze autoimmune phenomena, autoantibodies (ANA, ASMA, AMA, LKM-1), and immunoglobulins (IgG, IgA, IgM) were examined. ANA, ASMA, and AMA were measured by the fluorescent antibody (FA) method, and LKM-1 was measured by enzyme-linked immunosorbent assay (ELISA). Titers of 1:40 and above were considered to be positive. The AIH score of each patient was calculated without including the liver biopsy results. The AIH score includes the following criteria: sex (female; +2), ALP/AST (or ALT) ratio (<1.5; +2), serum globulins or IgG above normal, positive for autoantibodies, hepatitis viral markers (negative; +3), drug history (negative; +1), alcohol intake (negative; +2), liver histology, and the presence of other autoimmune diseases. Probable AIH is defined as a score of 10 or more, and definite AIH is defined as a score of more than 15.[9]

Based on their clinicopathological features, we divided the patients into three groups: the overlap group included patients who had clinical and histological features of both NAFLD and AIH, either separately or concurrently; the systemic group included patients with systemic autoimmune disease other than AIH; and the

"other" group included patients with no autoimmune disease.

To evaluate the clinicopathological significance of ANA in NALFD patients, patients without autoimmune diseases ("other" group) were classified according to their ANA titer. First, these patients were classified based on ANA positivity, and then they were classified based on their ANA titers (more than 1:40, 1:40, or negative).

Eighteen patients (8 patients who were ANA-positive and 10 patients who were ANA-negative) who had received diet and exercise therapy had second liver biopsies 5–59 months after the first biopsy (mean, 49.4 months). Their clinical, biochemical, and liver biopsy histology results were compared.

Liver biopsy

All liver biopsy specimens were examined using the following stains: hematoxylin-eosin, Mallory, silver reticulin, Victoria blue stain for copper binding protein, and Perls iron stain for hemosiderosis. Assessment was done by one reviewer (E.H.) blinded to the clinical and biochemical data of the patients. For the evaluation of NAFLD, fibrosis was scored using a five-grade scale: F0, normal connective tissue; F1, foci of perivenular or pericellular fibrosis in zone 3; F2, perivenular or pericellular fibrosis confined to zones 3 and 2, with or without portal/periportal fibrosis; F3, bridging fibrosis; and F4, cirrhosis.[1,3,20] Steatosis was graded as mild to severe. Necroinflammation was graded as mild, moderate, or severe based on the reviewer's overall impression after evaluating the specimens for ballooning degeneration, Mallory bodies, giant mitochondria, disarray of hepatocytes, lobular and portal inflammation, focal necrosis, Councilman bodies, lipogranulomas, and pigmented macrophages. Features suggestive of AIH were defined as follows: prominent interface hepatitis; moderate to severe lymphoplasmacytic infiltrate in portal and periportal areas; prominent bridging necrosis or confluent necrosis with severe lymphoplasmacytic inflammatory changes; and the formation of liver-cell rosettes.

Statistical analysis

The χ^2 test was used to compare frequency data. Univariate and multivatiate analyses were conducted using a logistic regression model to identify independent risk factors associated with ANA titers.

Results

The median age of this study population was 53 years (range, 10–89 years). One hundred and nine patients (51.4%) were women. The median BMI was 26.5 kg/m^2. One hundred and forty patients (66.0%) were obese (BMI, ≥25 kg/m^2). Eighty-seven patients (41.0%) had type 2 diabetes mellitus, 122 patients (57.5%) had hyperlipidemia, and 51 patients (24.1%) had hypertension. The median AST level was 52 IU/l, and the median ALT was 72 IU/l. Seventy patients (33.0%) were positive for ANA; 51% of the ANA-positive patients showed a homogeneous ANA pattern, 18% showed a speckled pattern, and 16% had a discrete speckled ANA pattern. Three patients who were positive for ANA were also ASMA-positive, with a titer of 1:40. Three patients had weakly positive AMA titers (1:40); however, these 3 patients did not show any clinicopathological features of primary biliary cirrhosis. None of the patients were positive for LKM-1. Four patients were diagnosed as having AIH at the time of the histological diagnosis of NAFLD, or they had previously been so diagnosed. However, there were no patients diagnosed as having primary biliary cirrhosis or primary sclerosing cholangitis. Eight patients had other autoimmune diseases: rheumatoid arthritis (RA) in 3 patients; systemic lupus erythematosus (SLE) in 2 patients; and Sjogren's syndrome and other diseases in 3 patients. Histologically, 41 patients had F3 (bridging fibrosis) and 46 had F4 (cirrhosis). Necroinflammatory activity was mild in 68 patients, moderate in 114, and severe in 30 patients. Fifty patients had mild steatosis, 72 had moderate steatosis, and 90 had severe steatosis.

AIH score without histological evaluation

Among the 212 NAFLD patients, 1 patient (0.5%) had definite AIH (score, >15), and 126 patients (59.4%) had probable AIH (score, 10 to 15). The AIH score, not including the liver biopsy results of NAFLD patients, stratified by sex, is shown in Table 1.

Female sex

Among the 109 female patients, 106 patients (97.2%) had a score of 10 or more, including the 1 patient with a score of 16 (definite AIH). Based on the pattern of their accompanying autoimmune phenomena, there were 2 patients in the overlap group, 7 in the systemic group, and 100 in the "other" group.

In the overlap group, case 1 (a 57-year-old woman) had a score of 16 and case 2 (a 57-year-old woman) had a score of 12. Case 1 had been obese (BMI, 36 kg/m^2) for 10 years prior to the liver biopsy, and was diagnosed as having concurrent AIH and NAFLD. The patient's liver biopsy showed typical steatohepatitis (with moderate steatosis, ballooning degeneration, Mallory bodies, and perivenular and pericellular fibrosis) and AIH (severe portal lymphoplasmacytic infiltrate with interface

Table 1. Score for diagnosis of autoimmune hepatitis, without features of histology, in NAFLD patients stratified by sex ($n = 212$)

		Female ($n = 109$)			Male ($n = 103$)		
AIIH score	Total	Overlap[a]	Systemic[b]	"Other"[c]	Overlap[a]	Systemic[b]	"Other"[c]
	212	2	7	100	2	1	100
16	1	1	0	0	0	0	0
15	1	0	0	1	0	0	0
14	6	0	4	2	0	0	0
13	9	0	1	8	0	0	0
12	16	1	2	10	1	1	1
11	41	0	0	30	0	0	11
10	53	0	0	46	1	0	6
<10	85	0	0	3	0	0	82

[a] Overlap, patients who had NAFLD and autoimmune hepatitis clinicopathologically
[b] Systemic, patients who had systemic autoimmune disease other than autoimmune hepatitis
[c] "Other", patients who had no autoimmune disease

Table 2. Scoring parameters for diagnosis of autoimmune hepatitis in NAFLD patients without autoimmune disease ("other" group) stratified by sex ($n = 200$)

	AIH score	Female ($n = 100$)	Male ($n = 100$)	Total
Serum globulins or IgG above normal				
>2.0	+3	1	0	1
1.5–2.0	+2	1	1	2
1.0–1.5	+1	23	17	40
<1.0	0	75	82	157
ANA titer				
>1:80	+3	10	12	22
1:80	+2	7	3	10
1:40	+1	20	8	28
<1:40	0	63	77	140

hepatitis and rosette formation). She was treated with unsodeoxycholic acid (UDCA) and weight control. Her transaminases (AST, 138 IU/l; ALT, 120 IU/l) normalized after a weight reduction of 10 kg. However, her ANA titer remained at 1:640 and her IgG at 3670 mg/dl. Case 2 had been previously diagnosed clinicopathologically as having AIH, with no steatohepatitis shown on liver biopsy at that time. After prednisolone treatment, her transaminases returned to normal, and she developed type 2 diabetes mellitus. Five years later, her transmainases became gradually elevated, and her liver biopsy specimen showed steatohepatitis without features of AIH.

In the systemic group, 7 patients had high titers of ANA, or their immunoglobulin levels were high due to the presence of systemic autoimmune disease. Thus, their AIH scores were high. However, none of these patients had histological features of AIH. In the "other" group, 97 patients had scores showing probable AIH. Among them, 3 patients had relatively high scores; 1 patient had a score of 15, and 2 patients had a score of 14. These 3 patients had high AIH scores due to high titers of IgG in 1 patient and high titers of ANA plus high titers of IgG in 2 patients. Histologically, these 3 patients all had advanced fibrosis with moderate to severe steatosis and ballooning degeneration, without features of AIH.

Male sex

Of the 103 male patients, 21 (20.4%) had an AIH score of 10 or more. The highest score was 12 (3 patients): 1 patient was in the overlap group; 1 patient with SLE was in the systemic group; and 1 patient was in the "other" group. The histological findings of these 3 patients showed advanced fibrosis (1, F3; 2, F4), as well as moderate to severe steatosis with moderate to severe inflammatory changes, without AIH features.

Scoring parameters for diagnosis of AIH in NAFLD patients without autoimmune disease ("other" group) stratified by sex

To evaluate the scoring parameters for the diagnosis of AIH, we evaluated the AIH scores for titers of serum globulin or IgG, and the ANA titers, as summarized in Table 2. Forty-three patients had positive scores for titers of serum globulin or IgG (1, score 3; 2, score 2; 40,

score 1). Only 3 patients (2 females, 1 male) had an AIH score of more than 1. All 3 of these patients had advanced fibrosis (1, F3; 2, F4). With respect to ANA titers, more females had a titer of 1:40 (20 females, 8 males), while the prevalence of a titer of 1:80 or greater was similar in females and males (17 females, 15 males).

Clinicopathological features of NAFLD patients without autoimmune disease ("other" group) stratified by ANA positivity

Two hundred patients (100 females, 100 males) without any autoimmune disease ("other" group) were classified based on their ANA titers. Table 3 shows the univariate comparison of the clinicopathological features of the ANA-positive and -negative patients. There were more females in the ANA-positive group (61.7%) than in the ANA-negative group (45%; $P = 0.032$). Age, prevalence of obesity (BMI, ≥25 kg/m^2), diabetes, hyperlipidemia, and hypertension were similar in the ANA-positive and ANA-negative groups. The prevalence of severely obese patients (BMI, ≥30 kg/m^2) was higher in the ANA-positive group (30.0%) than in the ANA-negative group (19.3%), but the difference was not statistically significant. Laboratory data, including ALP, GGTP, and liver function tests and fibrosis markers, were similar in the ANA-positive group and the -negative group. Patients positive for ANA had higher levels of IgG (the median level of IgG was 1420 mg/dl in ANA-positive patients vs 1259 mg/dl in ANA-negative patients), AST (the median level of AST was 59 IU/l in ANA-positive patients vs 47 IU/l in ANA-negative patients), and ALT (the median level of ALT was 81 IU/l in ANA-positive patients vs 65 IU/l in ANA-negative patients), but the differences were not statistically significant. Histologically, the stage of fibrosis, the grade of necroinflammation, and the degree of steatosis were not significantly different between the two groups.

Multivariate logistic regression analysis found that female sex was an independent predictor of the presence of ANA ($P = 0.029$). The odds ratio for female sex was 2.222, with 95% confidence intervals of 1.085–4.553.

Clinicopathological features in NAFLD patients classified into two groups by ANA titers are presented in Table 4. The prevalence of females was the same in the two groups. However, the prevalence of severely obese patients (BMI, ≥30 kg/m^2) was significantly higher in the group with an ANA titer of 1:80 or more (40.6%) than in the group with an ANA titer of 1:40 or less (19%; $P = 0.009$). The prevalence of metabolic syndrome and the laboratory data were similar in the two groups. However, the prevalence of a necroinflammation grade showing severe activity (grade 3) was higher in the group with an ANA titer of 1:80 or more (25%) compared to the group with an ANA titer of 1:40 or less (11.3%; $P = 0.037$). Multivariate logistic regression analysis showed that severe obesity (BMI, ≥30 kg/m^2) was the only independent predictor of the presence of an ANA titer of 1:80 or more ($P = 0.026$). The odds ratio for severe obesity was 2.538, with 95% confidence intervals of 1.117 to 5.768.

Fibrotic changes, necroinflammation, and steatosis in the period between biopsies

During the follow-up period, no patients lost more than 2 kg in weight. The liver biopsy results (fibrosis, necroinflammation, and steatosis) of eight patients who were initially ANA-positive and ten patients who were initially ANA-negative are shown in Fig. 1. Fibrosis progressed in three patients (two positive for ANA, one negative for ANA). Inflammation progressed in five patients (two positive for ANA, three negative for ANA) and improved in five patients (one positive for ANA, four negative for ANA). Steatosis decreased in three patients positive for ANA but increased in seven patients (three positive for ANA, four negative for ANA). There was no correlation between the changes in histology and positivity for ANA. Furthermore, there

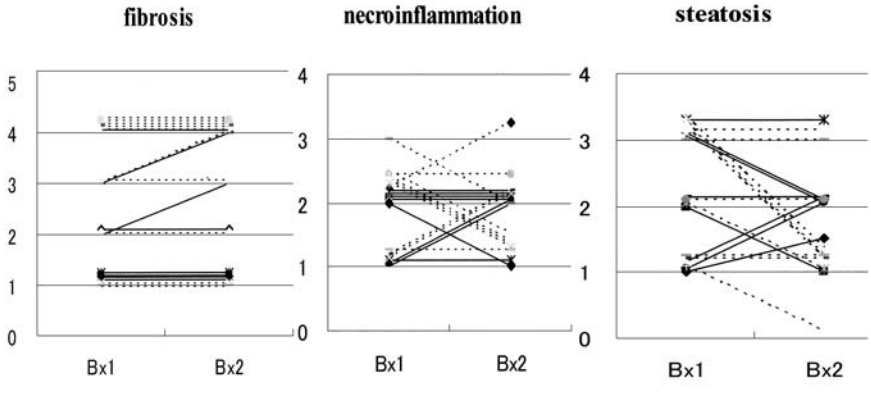

Fig. 1. Histological changes of fibrosis, necroinflammation, and steatosis occurring in the period between two biopsies (*Bx*); $n = 18$ (antinuclear antibodies [ANA]-positive, $n = 8$; *continuous lines*; ANA-negative, $n = 10$; *dashed lines*)

Table 3. Univariate comparison of clinicopathological features in NAFLD patients without autoimmune disease ("other" group) stratified by ANA positivity ($n = 200$)

	Parameter	Total; $n = 200$ (100%)	ANA-positive; $n = 60$ (30.0%)	ANA-negative; $n = 140$ (70.0%)	P value
	Female (%)	100 (50.0%)	37 (61.7%)	63 (45.0%)	$P = 0.032$
	Age, years (range)	53 (10–89)	61 (20–77)	53 (10–89)	NS
Metabolic syndrome	Obesity (%)	133 (66.5%)	39 (65.0%)	94 (67.1%)	NS
	BMI $\geqq 30\,kg/m^2$	45 (22.5%)	18 (30.0%)	27 (19.3%)	NS
	Diabetes (%)	82 (41.0%)	28 (46.7%)	54 (38.6%)	NS
	Hyperlipidemia (%)	119 (59.5%)	37 (61.7%)	82 (58.6%)	NS
	Hypertension (%)	48 (24.0%)	16 (26.7%)	32 (22.9%)	NS
Laboratory data	Albumin, g/dl (range)	4.3 (1.8–5.4)	4.3 (2.7–5.0)	4.3 (1.8–5.4)	NS
	Total bilirubin, mg/dl (range)	0.6 (0.2–3.1)	0.6 (0.2–2.5)	0.6 (0.2–3.1)	NS
	AST, IU/l (range)	51 (9–392)	59 (20–225)	47 (9–392)	NS
	ALT, IU/l (range)	68 (5–740)	81 (9–339)	65 (5–740)	NS
	Alkaline phosphatase, IU/l (range)	225 (53–1407)	225 (72–935)	224 (53–1407)	NS
	γ GTP, IU/ml (range)	70 (10–1543)	64 (17–487)	72 (10–1543)	NS
	Platelet count, $\times 10^4$ (range)	21.6 (3.7–45.1)	20.9 (3.7–37.5)	21.9 (4.5–45.1)	NS
	Prothrombin time, % (range)	93.7 (35.7–107)	93.0 (61–107)	93.9 (35.7–103)	NS
	Hepaplastin test, % (range)	101 (39–150)	106.4 (42.3–150)	100 (39–150)	NS
	Hyaluronic acid, ng/ml (range)	31 (9–1060)	37 (9–346)	31 (9–1060)	NS
	Type IV collagen 7S domain ng/dl (range)	4.2 (1.8–13)	4.5 (1.8–9.8)	4.2 (1.9–13)	NS
	Immunoglobulin G, mg/dl (range)	1291 (461–4240)	1420 (531–2564)	1259 (461–3860)	NS
Pathology	Fibrosis score (0/1/2/3/4)	(9/71/40/37/43)	(0/17/20/10/13)	(9/54/20/27/30)	NS
	Necroinflammation grade (1/2/3)	(64/109/27)	(16/33/11)	(48/76/16)	NS
	Steatosis grade (1/2/3)	(44/70/86)	(15/19/26)	(29/51/60)	NS

Table 4. Univariate comparison of clinicopathological features in NAFLD patients without autoimmune disease ("other" group) stratified by ANA titer ($n = 200$)

	Parameter	Total $n = 200$ (100%)	ANA ≧ 1:80; $n = 32$ (16.0%)	ANA ≦ 1:40; $n = 167$ (84.0%)	P value
	Female (%)	100 (50.0%)	17 (53.1%)	83 (49.4%)	NS
	Age, years (range)	53 (10–89)	59 (20–77)	53 (10–89)	NS
Metabolic syndrome	Obesity (%)	133 (66.5%)	23 (71.9%)	110 (65.4%)	NS
	Severe obesity, BMI ≧ 30 kg/m^2 (%)	45 (22.5%)	13 (40.6%)	32 (19.0%)	$P = 0.009$
	Diabetes (%)	82 (41.0%)	14 (43.8%)	68 (40.5%)	NS
	Hyperlipidemia (%)	119 (59.5%)	19 (59.3%)	100 (59.5%)	NS
	Hypertension (%)	48 (24.0%)	10 (31.3%)	38 (22.6%)	NS
Laboratory data	Albumin, g/dl (range)	4.3 (1.8–5.4)	4.1 (3.2–5.0)	4.3 (1.8–5.4)	NS
	Total bilirubin, mg/dl (range)	0.6 (0.2–3.1)	0.6 (0.3–2.5)	0.6 (0.2–3.1)	NS
	AST, IU/l (range)	51 (9–392)	61 (20–179)	46 (9–392)	NS
	ALT, IU/l (range)	68 (5–740)	95 (9–339)	65 (5–740)	NS
	Alkaline phosphatase, IU/l (range)	225 (53–1407)	278 (157–935)	222 (53–1407)	NS
	γ GTP, IU/ml (range)	70 (10–1543)	61 (17–381)	72 (10–1543)	NS
	Platelet count, $\times 10^4$ (range)	21.6 (3.7–45.1)	20.5 (4.9–37.5)	21.9 (3.7–45.1)	NS
	Prothrombin time, % (range)	93.7 (35.7–107)	93.7 (61–107)	93.9 (35.7–107)	NS
	Hepaplastin test, % (range)	101 (39–150)	107.0 (46–150)	101.0 (39–150)	NS
	Hyaluronic acid, ng/ml (range)	31 (9–1060)	20 (9–346)	34 (9–1060)	NS
	Type IV collagen 7S domain, ng/dl (range)	4.2 (1.8–13)	4.1 (2.8–6.8)	4.2 (1.8–13)	NS
	Immunoglobulin G, mg/dl (range)	1291 (461–4240)	1450 (900–2564)	1225 (461–3830)	NS
Pathology	Fibrosis score (0/1/2/3/4)	(9/71/40/37/43)	(0/6/14/7/5)	(9/65/26/30/38)	NS
	Necroinflammation grade (1/2/3)	(64/109/27)	(7/17/8)	(57/92/19)	$P = 0.037$
	Steatosis grade (1/2/3)	(44/70/86)	(7/9/16)	(37/61/70)	NS

were no significant differences between ANA-positivity and ANA-negativity with respect to physical features, clinical course, and laboratory data.

Discussion

All our patients with biopsy-proven NAFLD were negative for hepatitis viral markers (AIH score, +3), drug history (+1), and alcohol intake (+2), and in almost all patients the ALP/AST (or ALT) ratio was less than 1.5 (+2). Before the liver biopsy, almost all the female patients (97.2%) and 21 (20.4%) male patients had a probable or definite AIH score. Distinguishing patients with NAFLD who had a probable or definite AIH score (but who did not have AIH) from patients with coexisting NAFLD and AIH was only possible by liver biopsy. After liver biopsy, only 1 patient (0.5%) could be classified as "definite AIH". This result demonstrated that the AIH score without liver biopsy findings was not useful for diagnosing AIH in patients with NAFLD, and reiterated the importance of a liver biopsy in making an accurate diagnosis and thus avoiding unnecessary AIH treatment. Previous reports from western countries have shown that 20%–30% of NAFLD patients have autoantibodies, but that only about 10% of NAFLD patients who showed autoantibody -positive NAFLD (about 2%–3% of all NAFLD patients) were diagnosed as having both AIH and NAFLD. In our study, the NAFLD and AIH overlap was much less than that documented in reports from western countries, and this is probably due to differences in the ethnicity.

In patients with concurrent NAFLD and AIH, it is difficult to decide whether to institute corticosteroid treatment. Steroids are likely to worsen the steatosis but improve the AIH-related inflammation. We found that, in patients with histological evidence of concurrent NASH and AIH, both weight loss and UDCA therapy were able to normalize the transaminase levels. This would suggest that NASH and autoimmunity are both important in the pathogenesis of patients' liver inflammation and possible in disease progression.

In our study, a high AIH score was mainly due to ANA positivity. ANA is the most important nonspecific marker of AIH and can also be found in many other inflammatory systemic and liver diseases. According to previous reports, 7%–52% of patients with chronic liver disease of various causes are positive for ANA.[21,22] However, the significance of ANA in chronic liver disease is uncertain, because the presence of ANA often does not signify the presence of AIH. The presence of ANA therefore might be a nonspecific reactive epiphenomenon in genetically predisposed individuals.[22,23]

With regard to our patients, the coexistence of AIH and NAFLD was rare. However, the prevalence of ANA was significantly greater than would have been expected from findings in Japanese patients with other chronic liver diseases.[21] ANA-positive patients were significantly more likely to be female (61.7% of females and 45.0% of males; $P = 0.029$), although there was no significant sex difference among patients with high ANA titers (≥1:80). The number of severely obese patients (BMI, ≥30 kg/m^2) was significantly higher among the patients with high ANA titers. Loria et al.[12] suggested that ANA positivity was an accompanying feature of insulin resistance and was not an index of primary autoimmune disease. It has been speculated that the host's or dietary triglycerides enhance adipose tissue or monocyte production of proinflammatory cytokines, such as tumor necrosis factor (TNF) alpha and interleukin (IL)-6,[13,24] and this might promote liver injury and/or trigger the production of autoantibodies.

Moreover, essential fatty acid deficiency is known to have a beneficial effect in the development of systemic lupus erythematosus (SLE) in mice.[25] Unfortunately, in our study we did not assess insulin resistance, though it is very important to note that female sex was significantly associated with a low ANA titer, while severe obesity was significantly associated with high ANA titers. Thus, we propose that low ANA titers represent nonspecific changes occurring in patients with chronic liver disease, while high ANA titers are specific features of severe obesity, which also cause NAFLD. It is presently unknown whether therapeutic interventions aimed at improving insulin sensitivity can reverse ANA positivity in patients with NAFLD. Obviously, further studies are required to clarify this issue.

The prevalence of ANA positivity in NASH has been reported to range from 12% to 35%.[11–16] Adams et al.[13] found that the presence of autoantibodies was associated with a higher fibrosis stage and a higher necroinflammatory grade, as well as higher serum levels of gammaglobulins and a higher frequency of hypergammaglobulinemia. Our data documented that high ANA titers (≥1:80) were weakly associated with necroinflammatory changes on univariate analysis, but ANA positivity was not associated with the fibrosis stage. Furthermore, ANA positivity had no prognostic clinicopathological significance in our follow-up study. Loria et al.[12] also have reported that ANA is not associated with any histologic injury. Therefore, we would suggest that ANA positivity frequently represents a nonspecific antibody response in NAFLD patients. However, the clinical significance of ANA in NAFLD has not yet been determined and requires further study.[1]

The main findings of our study included that: (1) the AIH score without liver biopsy results was not useful for diagnosing AIH in NAFLD patients; (2) patients with NAFLD had a higher prevalence of ANA posi-

tivity than patients with other chronic liver disease; (3) ANA positivity was significantly higher in female patients than in male patients, while obesity was significantly associated with a high ANA titer (≥1:80) in NAFLD patients; and (4) the presence of ANA did not have prognostic clinicopathological significance. In patients who have both elevated ANA titers and risk factors for NAFLD, these findings underscore the importance of performing a liver biopsy to make a definitive diagnosis before starting corticosteroid therapy.

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