Prevalence and Significance of Autoantibodies in Patients With Non-Alcoholic Steatohepatitis

Scott J. Cotler, MD, Kiran Kanji, MD,† Ali Keshavarzian, MD,† Donald M. Jensen, MD,† and Shriram Jakate, MD†*

Goals: The aim of this study is to evaluate the prevalence and the clinical and histologic correlates of autoantibodies in patients with nonalcoholic steatohepatitis (NASH).

Background: Antinuclear antibodies (ANA) and anti-smooth muscle antibodies (ASMA) have been identified in patients with NASH. The significance of autoantibodies in NASH is uncertain.

Study: Clinical data from patients with a histologic diagnosis of NASH at a university hospital in Chicago, Illinois between January 1999 and April 2003 were reviewed retrospectively. Seventy-four patients who were tested for autoantibodies and had no history of alcohol abuse or a systemic autoimmune disease were included. Demographic information and laboratory data were collected. Autoantibody titers $\geq$1:40 were considered positive. A single pathologist reviewed all liver biopsies and scored features of NASH and identified characteristics of autoimmune hepatitis.

Results: Thirty-four percent of patients with NASH had positive ANA titers and 6% were ASMA positive. Demographic and laboratory parameters did not differ by ANA status, except that women were more frequently ANA positive then men ($P = 0.01$). The severity of steatosis, inflammation, and fibrosis on liver biopsy were similar in the ANA positive and negative groups. Only 15% of ANA positive patients with NASH had a plasma cell infiltrate on liver biopsy and there was no difference in the frequency of histologic features of autoimmune hepatitis between ANA positive and negative patients.

Conclusions: Antinuclear antibodies are common in patients with NASH and most frequently represent a nonspecific antibody response that is not associated with the pattern or severity of injury on liver biopsy.

Key Words: non-alcoholic steatohepatitis, anti-nuclear antibody, liver histology

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From the *University of Illinois at Chicago and †RUSH-Presbyterian, St. Luke's Medical Center, Chicago, Illinois.

Reprints: Scott Cotler, MD, Section of Hepatology (MC 787), 840 South Wood Street, Chicago, IL 60612 (e-mail: scotler@uic.edu).

Non-alcoholic fatty liver disease (NAFLD) is a histologic diagnosis consisting of hepatic steatosis with or without steatohepatitis. The pathogenesis of steatosis is related to insulin resistance and NAFLD is associated with the metabolic syndrome.[1–3] Some patients with steatosis develop NASH with hepatic inflammation and fibrosis. Oxidative stress, proinflammatory cytokines, and mitochondrial dysfunction seem to be integral to the development of steatohepatitis.[4]

Autoantibodies including ANA and ASMA have been observed in patients with NAFLD. The prevalence of autoantibodies in 15 patients with hepatic steatosis and 46 patients with steatohepatitis was reported in abstract form.[5] Fourteen percent of patients with steatosis and 35% of those with steatohepatitis had elevated ANA titers. Anti-smooth muscle antibody titers were increased in 17% of patients with steatosis and 5% with steatohepatitis. A subsequent case series from Japan reported 2 patients with biopsy proven NASH who had elevated ANA levels in a homogenous pattern and hyperglobulinemia.[6]

The proceedings of a recent AASLD single topic conference on NASH noted the uncertain significance of immunologic markers in patients with NASH and recommended further research on this topic.[4] In this study, we identified patients who had measurement of autoantibodies as part of their assessment for abnormal liver enzymes and had a liver biopsy that was diagnostic of NASH. The aims of the study are: (1) to determine the prevalence of autoantibodies in patients with biopsy proven NASH, (2) to evaluate whether the presence of autoantibodies is associated with more severe hepatic inflammation and fibrosis in patients with NASH, and (3) to assess whether patients with elevated autoantibody titers and steatohepatitis have co-existing histologic features of autoimmune hepatitis (AIH).

MATERIALS AND METHODS

The study protocol was approved by the Institutional Review Board at RUSH-Presbyterian-St. Luke's Medical Center.

Subjects

The RUSH pathology database including all pathology reports issued between January 1999 and April 2003 was que-

ried using the keywords non-alcoholic steatohepatitis, NASH, and steatohepatitis. Two hundred and forty cases were identified. Review of clinical data including charts and electronic records yielded 80 patients who had documented testing for ANA or ASMA, had no history of alcohol abuse, and were not known to be taking medications associated with autoantibody production. Six additional patients with a history of a systemic autoimmune disease were excluded leaving 74 subjects for analysis.

Clinical Parameters

Clinical data including age, gender, and weight at the time of liver biopsy were obtained retrospectively. Antinuclear antibody and ASMA titers were measured at one point by the RUSH clinical laboratory on serial dilutions of sera by indirect immunofluorescence. Titers ≥1:40 were considered elevated. Additional laboratory results were collected including total cholesterol, triglycerides, total protein, albumin, globulin, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) levels.

Liver Histology

Liver biopsies from 86% (64/74) of patients included in this study were reviewed by a single pathologist (S.J.) who was blind to autoantibody titers. Biopsy slides from the remaining 10 patients had been returned to the outside institutions where they were performed and were not available for further review. The classification system described by Brunt et al,[7] was used to score components of NASH. Features of autoimmune hepatitis consisting of increased plasma cells and rosette formation were noted.[8]

Statistical Analysis

Normally distributed data are presented as mean ± SD. The Student *t* test was used to compare normally distributed variables between patients with and without elevated ANA titers. Non-normally distributed data are presented as median (range). The Mann-Whitney *U* test was used to compare non-normally distributed variables. Pearson's χ^2 test was used to compare categorical variables as a function of ANA status.

RESULTS

Demographics

The mean age of the study population was 45 ± 13 years. Fifty-nine percent (44/74) of subjects were women. The mean weight was 88 ± 19 kilograms.

Prevalence of Autoantibodies

Thirty four percent (25/74) of patients with biopsy proven NASH had positive ANA titers, including 8 with markedly elevated levels of ≥1:320. Many different ANA patterns were observed (Table 1). Anti-smooth antibody testing was performed in 48 patients. Six percent (3/48) of patients tested had ASMA titers of 1:40. One patient with an elevated ASMA level had an ANA titer of 1:80 in a speckled pattern. The other 2 ASMA positive patients were ANA negative.

TABLE 1. Antinuclear Antibody (ANA) Patterns in Patients With Elevated Titers

ANA Pattern	ANA Titer						Total
	1:40	1:80	1:160	1:320	1:640	1:1280	
Speckled	3	8	1	2	2		16
Nucleolar	1	2	1				4
Homogenous		1		1	1		3
Centromere						1	1
*Mixed					1		1
Total	4	11	2	3	4	1	25

*Speckled and homogenous.

Clinical Parameters and ANA Status

Women were more frequently ANA positive then men (20/44 vs. 5/30 $P = 0.01$). Age and weight were similar between ANA positive and negative cases. There were no significant differences in laboratory values as a function of ANA status (Table 2). A similar proportion of patients in both groups had a globulin fraction greater than 4.0 g/dL.

Histologic Features and ANA Status

Considering all patients together, histologic scoring for NASH presented as median (range) was as follows: macrovesicular steatosis grade 2 (1–3), hepatocellular ballooning and disarray 0.5 (0-3), lobular inflammation 1 (0–3), portal inflam-

TABLE 2. Comparison of Laboratory Values in ANA Positive and Negative Patients

	ANA (+) Median (Range)	ANA (−) Median (Range)	*p*-Value
Total cholesterol (mg/dL)	201 (106–276)	193 (72–296)	0.20
Triglycerides (mg/dL)	216 (59–674)	146 (21–684)	0.08
ALT (U/L)	100 (20–324)	80 (2–313)	0.36
AST (U/L)	63 (15–310)	51 (15–362)	0.44
Total protein (g/dL)	7.5 (4.6–8.5)	7.5 (4.2–8.9)	0.46
Albumin (g/dL)	3.9 (1.5–4.6)	4.0 (1.6–4.7)	0.28
Globulin (g/dL)	3.4 (2.7–4.2)	3.4 (2.5–5.0)	0.85

Normal ranges: total cholesterol (<200 mg/dL), triglycerides (30–135 mg/dL), ALT (3–50 U/L), AST (5–55 U/L), total protein (6–8.2 g/dL), albumin (3.5–5 g/dL).

mation 0 (0–2), perisinusoidal fibrosis 0 (0–2), portal fibrosis 0 (0–4), and bridging fibrosis 0 (0–4). There were no significant differences in the severity of the histologic features between ANA positive and ANA negative patients (Table 3). However, there was a trend toward a higher frequency of bridging fibrosis or cirrhosis in the ANA negative group (7/44 vs. 0/20, $P = 0.06$).

Fifteen percent (3/20) of ANA positive patients had increased plasma cells and no ANA positive patient had rosette formation on liver biopsy. The frequency of increased plasma cells was similar between the ANA positive and negative groups (3/20 vs. 4/44, $P = 0.48$), whereas there was a trend toward a higher rate of rosette formation in the ANA negative patients (7/44 versus 0/20, $P = 0.06$). The 3 ANA positive patients with increased plasma cells on liver biopsy had titers of 1:320, 1:80, and 1:80 in a speckled pattern. One of the patients with an ANA titer of 1:80 had a globulin level of 4.0 g/dL, and none were cirrhotic. All 3 patients with increased plasma cells on biopsy met the criteria for probable autoimmune hepatitis according to the scoring system proposed by the International Autoimmune Hepatitis Group.[8] Although we cannot exclude co-existing AIH in these 3 cases, the predominant histologic features were those of NASH (Fig. 1).

DISCUSSION

We found that elevated ANA titers were common in patients with biopsy proven NASH, occurring in 34% of cases. Antinuclear antibodies were not associated with more severe hepatic inflammation or fibrosis in NASH patients, and were rarely associated with histologic features of AIH on liver biopsy. In fact, there were trends toward higher frequencies of bridging fibrosis or cirrhosis and rosette formation in the ANA negative group. Furthermore, none of the 25 ANA positive patients had clear histologic evidence of AIH, despite the finding that eight had ANA titers ≥ 1:320. Anti-smooth muscle antibodies were infrequent in our series of patients with NASH.

TABLE 3. Comparison of Histologic Features of NASH in ANA Positive and Negative Patients

	ANA (+) Median (Range)	ANA (−) Median (Range)	*p*-Value
Macrovesicular steatosis	2.5 (1–3)	2 (1–3)	0.75
Hepatocellular ballooning	1 (0–3)	0 (0–3)	0.72
Lobular inflammation	1 (0–2)	1 (0–3)	0.82
Portal inflammation	1 (0–1)	0 (0–2)	0.51
Perisinusoidal fibrosis	0 (0–1)	0 (0–2)	0.94
Portal fibrosis	0 (0–2)	0.5 (0–4)	0.77
Bridging fibrosis	0 (0–2)	0 (0–4)	0.46

Features of NASH were scored from 0–3 with the exception of portal fibrosis and bridging fibrosis, which were scored 0–4.[7]

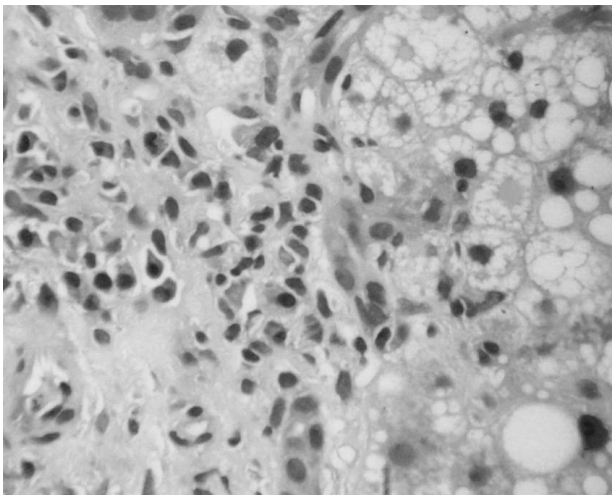

FIGURE 1. Microphotograph showing increased plasma cells in the portal area on the left, and severe lobular macrovesicular steatosis on the right (hemotoxylin and eosin stain, magnification ×100).

The results of this study show the importance of liver biopsy in patients with anti-nuclear antibodies and risk factors for NASH, particularly when features suggesting AIH are present as defined by the International Autoimmune Hepatitis Group.[8] Initiation of corticosteroid therapy in ANA positive patients with unsuspected NASH could be counterproductive. Careful histologic evaluation is needed in patients with NASH who have a plasma cell component to the inflammatory infiltrate to determine the dominant disease process and to guide therapy. NASH and AIH would be expected to co-exist in some cases, based on the overlapping demographic features associated with these diseases.[9,10]

The similar degree of inflammation and fibrosis in patients with NASH, with and without elevated ANA titers suggest that anti-nuclear antibodies most frequently are an epiphenomenon in this setting. Similarly, elevated ANA titers have been reported in other causes of chronic hepatitis including 14% to 23% of patients with hepatitis C.[11–13] Our findings are consisted with a preliminary report of elevated ANA titers in 35% of patients with NASH (5).

Weaknesses of the current study are related to the cross-sectional, retrospective design. We cannot exclude the possibility that patients with elevated liver enzymes were more likely to have a liver biopsy if they had an elevated ANA titer, thus inflating the estimated prevalence of ANA in patients with biopsy proven NASH. The observed prevalence rates for ANA might also have been influenced by selecting only patients with available ANA titers and a liver biopsy. More frequent testing of ANA in women with elevated liver enzymes could have contributed to the higher percentage of women in the ANA positive group. As a point of comparison, a survey of 2,500 female blood donors found that 5.6% had an ANA titer

≥1:40.[14] A longitudinal study is needed to further exclude an association between antinuclear antibodies and histologic progression in NASH.

In conclusion, 34% of patients with biopsy proven NASH had elevated ANA titers in this retrospective analysis. Our data suggest that anti-nuclear antibodies frequently represent a nonspecific antibody response in NASH that is not associated with histologic injury. The findings underscore the importance of performing a liver biopsy in patients with elevated ANA titers and risk factors for NASH before initiating corticosteroid therapy. A prospective study is needed to confirm these results.

REFERENCES

1. Dixon JB, Bhathal PS, O'Brien PE. Nonalcoholic fatty liver disease: predictors of nonalcoholic steatohepatitis and liver fibrosis in the severely obese. *Gastroenterology*. 2001;121:91–100.
2. Sanyal AJ, Campbell-Sargent C, Mirshahi F, et al. Nonalcoholic steatohepatitis: association of insulin resistance and mitochondrial abnormalities. *Gastroenterology*. 2001;120:1183–1192.
3. Marchesini G, Bugianesi E, Forlani G, et al. Nonalcoholic fatty liver, steatohepatitis, and the metabolic syndrome. *Hepatology*. 2003;37:917–923.
4. Neuschwander-Tetri BA, Caldwell SH. Nonalcoholic steatohepatitis: summary of an AASLD single topic conference. *Hepatology*. 2003;37: 1202–1219.
5. Tumiel M, Whitcomb BJ, Krawitt EL. Circulating antinuclear antibodies in patients with nonalcoholic steatohepatitis. [abstract] *Hepatology*. 1994; 20:409A.
6. Tajiri K, Takenawa H, Yamaoka K, et al. Nonalcoholic steatohepatitis masquerading as autoimmune hepatitis. *J Clin Gastroenterol*. 1997;25: 538–540.
7. Brunt EM, Janney CG, Di Bisceglie AM, et al. Nonalcoholic steatohepatitis: A proposal for grading and staging the histological lesions. *Am J Gastroenterol*. 1999;94:2467–2474.
8. Alvarez F, Berg PA, Bianchi FB, et al. International autoimmune hepatitis group report: review of criteria for diagnosis of autoimmune hepatitis. *J Hepatol*. 1999;31:929–938.
9. Reid AE. Nonalcoholic steatohepatitis. *Gastroenterology*. 2001;121: 710–723.
10. McFarlane IG. Autoimmune hepatitis: diagnostic criteria, subclassifications, and clinical features. *Clin Liver Dis*. 2002;6:605–621.
11. Clifford BD, Donahue D, Smith L, et al. High prevalence of serological markers of autoimmunity in patients with chronic hepatitis C. *Hepatology*. 1995;21:613–619.
12. Lenzi M, Bellentani S, Saccoccio G, et al. Prevalence of non-organ-specific autoantibodies and chronic liver disease in the general population: a nested case-control study of the Dionysos cohort. *Gut*. 1999;45: 435–441.
13. Peng Y-C, Hsieh S-C, Yang DY, et al. Expression and clinical significance of antinuclear antibody in hepatitis C virus infection. *J Clin Gastroenterol*. 2001;33:402–406.
14. Fritzler MJ, Pauls JD, Kinsella TD, et al. Antinuclear, anticytoplasmic, and anti-sjogren's syndrome antigen A (SS-A/Ro) antibodies in female blood donors. *Clin Immunol Immunopathol*. 1985;36:120–128.