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Significance of autoantibody seropositivity in children with obesity and non-alcoholic fatty liver disease

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Summary

Background: Autoantibodies are frequently positive in adults with nonalcoholic fatty liver disease (NAFLD) without concurrent autoimmune hepatitis (AIH). The clinical significance of this is unknown in children.

Objective: To determine the prevalence of autoantibody positivity in pediatric NAFLD and to evaluate its association with disease severity.

Methods: Multicenter, retrospective study of patients ≤18 years of age with biopsy-confirmed NAFLD. Descriptive statistics were used and groups were compared using Wilcoxon-Mann Whitney or χ^2 testing, and multivariable logistic regression was used for binary or ordinal outcomes.

Results: One hundred and thirty six patients with a median age of 14 years were included. The median body mass index *Z*-score was 2.5 (interquartile range 2.2, 2.6). Positive antinuclear antibody (ANA), anti-smooth muscle antibody (ASMA), anti-liver-kidney microsomal antibody, or any combination of autoantibodies were observed in 22%, 14%, 0%, and 33% of patients, respectively. The proportion of patients with a steatosis score ≥2 was significantly higher in those with positive ANA ($P = .045$). In the multivariable regression analysis, positive ANA was associated with increased odds of steatosis score ≥2 (odds ratio, 5.91; 95% confidential interval,

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AUTHOR CONTRIBUTIONS

P.L.V. conceptualized and designed the study, coordinated and supervised data collection, drafted the initial manuscript, and reviewed and revised the manuscript. M.M. conceptualized and designed the study, coordinated and supervised data collection, carried out the initial analyses, drafted the initial manuscript, and reviewed and revised the manuscript. T.Y. drafted the initial manuscript, carried out the initial analyses, assisted with interpretation of the data and critically reviewed the manuscript for important intellectual content. S.A.X., A.C.A., K.B., and S.O. participated in the interpretation of the data, critically reviewed and revised the manuscript for important intellectual content. All authors were involved in writing the paper and had final approval of the submitted and published versions.

CONFLICT OF INTEREST

None of the authors have any potential conflicts of interest to declare.

1.50-23.26), after controlling for potential confounders. No other significant histology differences were seen between the groups.

Conclusions: Positive ANA and ASMA are common in children with NAFLD; however, anti-LKM positivity is not. ANA positivity is associated with more severe steatosis.

Keywords

antinuclear antibody; anti-smooth muscle antibody; elevated liver transaminases; liver biopsy; non-alcoholic steatohepatitis

1 | INTRODUCTION

Children with overweight or obese body mass index (BMI), when referred to gastroenterology clinics with elevated serum aminotransferase levels, are most often diagnosed with non-alcoholic fatty liver disease (NAFLD).[1] The second most common diagnosis in those referred for suspected NAFLD is autoimmune hepatitis (AIH).[1] While these two conditions may co-exist, it is crucial to identify and appropriately treat AIH as soon as possible, so as to prevent liver disease progression.[2,3] The most recent guidelines of the North American Society of Pediatric Gastroenterology Hepatology and Nutrition recommend that patients referred for suspected NAFLD should be tested for AIH with serum levels of specific autoantibodies, which for AIH are the antinuclear (ANA), anti-smooth muscle (ASMA) and anti-liver kidney microsomal (anti-LKM) autoantibodies.[4] These autoantibodies (particularly the ANA and ASMA) can also be positive in 6%-15% of healthy subjects,[5–7] as well as in approximately one third of patients with NAFLD who do not have concurrent AIH.[1,8] Therefore, in those with autoantibody positivity, a liver biopsy should be considered for exclusion of AIH.[1,4]

Autoantibody positivity in the context of NAFLD without AIH has been a topic of scientific interest for years. Through cross-sectional studies, investigators have attempted to discern whether patients with NAFLD and positive autoantibodies have more severe liver disease compared to their counterparts with NAFLD and negative autoantibodies. These studies, which have been performed across the world, have yielded inconsistent results. Initial reports in adults suggested that autoantibody positivity was associated with more advanced fibrosis and more significant necroinflammatory activity.[8,9] Subsequent studies found no association between autoantibody positivity and inflammation or fibrosis, but reported a link between presence of autoantibodies and milder steatosis severity.[3,10,11] Pediatric data on the topic are very limited. Patton et al investigated the association between autoantibody positivity and histologic patterns in a large cohort of 176 children with NAFLD.[12] This study failed to identify any previously seen associations in adults, but did find that ASMA positivity was an independent predictor of increasing NAFLD activity scores (NAS), with an overall OR 2.8 [95% 1.4-5.4]. Further, the prevalence of positive ASMA in the entire pediatric cohort was much higher (32%) than what had been previously reported in the adult literature.

Given the inconsistent results of prior studies and paucity of pediatric data, the objective of this study was to determine the prevalence of autoantibody positivity in a multicenter cohort

of children with overweight or obese BMI and biopsy-confirmed NAFLD, and to investigate associations with histologic disease severity.

2 | METHODS

2.1 | Subjects and study design

This was a multicenter, retrospective study of patients ≤18 years of age with overweight or obese BMI and biopsy-confirmed NAFLD, followed from 2009-2018 at Cincinnati Children's Hospital Medical Center (CCHMC) and Yale-New Haven Children's Hospital (YNHCH). NAFLD was defined histologically as the presence of steatosis involving at least 5% of the hepatocytes in the absence of evidence of other etiologies of chronic liver disease. Patients with secondary causes of hepatic steatosis (eg, genetic or medication-induced), histological steatosis score <1, evidence of other/concurrent liver diseases and history of weight loss surgery were excluded. Institutional Review Board approval and a waiver of informed consent were obtained at both institutions (CCHMC & YNHCH) prior to the initiation of data collection.

Clinical records were reviewed to collect clinical characteristics (age, sex, ethnicity, anthropometrics) and laboratory data obtained within 3 months of the liver biopsy, including levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyl transferase (GGT), direct bilirubin, fasting serum insulin, hemoglobin A1c (HbA1c), albumin, lipid profile, immunoglobulin G (IgG), ANA, ASMA, anti-LKM, were also collected. Severity of obesity was classified as overweight (BMI: 85th to <95th percentile for age and sex based on CDC growth charts), obese class I (BMI: 95th to <120% of the 95th percentile), obese class II (BMI: 120% to <140% of the 95th percentile), or obese class III (BMI ≥ 140% of the 95th percentile).[13]

All three antibodies were measured by indirect immunofluorescence, using human Hep-2 cells for ANA, using the commercial test NOVA-LITE DAPI Hep-2 ANA, and mouse liver/kidney tissue for ASMA and anti-LKM. ANA positivity was defined by the commercial technique as ≥1:80, while an undetectable ANA was reported as <1:80. Any ASMA and anti-LKM positivity was reported according to the commercial techniques, which differed at each institution: ASMA ≥1+ or elevated F-actin ASMA >20, and anti-LKM ≥1+ or any detectable antibody titers. Diagnosis of type 2 diabetes mellitus (T2DM) at the time of liver biopsy was defined as hemoglobin A1C (HbA1c) >6.4%, oral glucose tolerance test with plasma glucose >200 mg/dL at 2 hours, or confirmation of T2DM diagnosis by an endocrinologist.

Typical clinical indications for liver biopsy at our institutions include concerns for: (1) severe NAFLD, as evidenced by: (a) persistently (3-6 months) elevated ALT and/or lack of improvement in serum ALT levels after appropriate implementation of lifestyle changes with subsequent BMI z-score improvements; (b) cardiometabolic risk factors associated with more severe NAFLD, such as type 2 diabetes or OSA; (c) evidence of splenomegaly and/or increased liver stiffness on magnetic resonance elastography); or (2) concern for a secondary underlying liver disease (eg, AIH due to positive autoantibodies with elevated serum immunoglobulin G levels).

The classification developed by the non-alcoholic steatohepatitis (NASH) Clinical Research Network was used to score severity of steatosis (scored 0-3), lobular inflammation (0-3), hepatocyte ballooning (0-2) and fibrosis (0-4), and the NAS was calculated (sum of scores for steatosis, lobular inflammation and ballooning; range 0-8).[14] A NAS of ≥5 was used to distinguish those with mild vs severe liver disease during the analyses. Significant fibrosis in this pediatric cohort was defined as fibrosis at or above stage 2. The biopsies were also reviewed by experienced hepatopathologists for features of autoimmune hepatitis.

2.2 | Statistical analyses

Descriptive statistics (medians and interquartile ranges [IQRs] for continuous variables and frequencies and percentages for categorical variables) were used to present the demographics and clinical characteristics of the cohort. Chi-square testing was used to compare normal categorical variables, Mann-Whitney *U* test for continuous data. Multivariable logistic regression was used for binary or ordinal outcomes. Statistical analyses were performed using Stata MP v.14.2 (StataCorp, College Station, Texas). Significance was set as *P* value ≤ .05.

3 | RESULTS

A total of 136 children with biopsy-confirmed NAFLD were included in this study (Table 1). The median age at the time of biopsy was 14 years (range 5-18), 60% of the patients were male, 68% were non-Hispanic and their median body mass index (BMI) z-score was 2.5 (IQR 2.2, 2.6). T2DM was diagnosed in 7 (5%) patients. Medication data are included in Table 1; patients included in the cohort were not taking medications associated with drug induced liver injury resembling AIH.[15] All patients in the cohort underwent testing for ANA, while 1 patient was not tested for ASMA and 2 patients were not tested for anti-LKM. Positive ANA, ASMA, anti-LKM, or any combination autoantibodies (ANA, ASMA or anti-LKM) was observed in 22% (30/135), 14% (19/135), 0% (0/134), and 33% (45/136) of patients, respectively. Only four patients (3%) were both ANA and ASMA positive. None of the patients met histological criteria for AIH, and none with positive autoantibodies had a concurrent diagnosis of another autoimmune disease, such as juvenile idiopathic arthritis, systemic lupus erythematosus, psoriasis, or celiac disease. No patients had positive hepatitis B or C serology studies, which had been obtained prior to the liver biopsy. Patients continued to be followed at our centers and none were subsequently diagnosed with autoimmune hepatitis.

The group of patients with positive autoantibodies had a higher proportion of females (53% vs 33%, $P = .022$) and an absence of patients with T2DM (0% vs 8%, $P = .056$). Otherwise, there were no differences in the remaining demographic and clinical characteristics between patients with and without positive autoantibodies (Table 2). In terms of laboratory values, patients with positive autoantibodies had significantly lower serum ALT, AST, total cholesterol, and low-density lipoprotein-cholesterol (LDL-C) levels at time of biopsy compared to those with negative autoantibodies (Table 2). The distinct histological features and the overall liver disease severity were not different between patients with and without positive autoantibodies (Table 2, Figure 1), with the exception of patients with ANA

positivity. The proportion of patients with moderate-to-severe steatosis (steatosis score ≥2) was significantly higher in those with positive ANA compared to those with negative ANA (90% vs 72%, respectively; $P = .045$). The severity of steatosis, ballooning, lobular/portal inflammation and NAS were not statistically different between the groups (Table 3). After controlling for age, sex, ethnicity, BMI z-score and the presence of T2DM, positive ANA was associated with increased odds of moderate-to-severe steatosis (adjusted odds ratio, 5.91; 95% confidential interval, 1.50-23.26).

Autoantibody positivity was not associated with more severe stages of fibrosis. Specifically, 31% of patients with positive autoantibodies had advanced fibrosis (stage >2) compared to 34% of patients who had negative autoantibodies ($P = .730$; Table 2). Similar findings were observed in patients with or without ANA positivity (Table 3).

Notably, there were no statistically significant associations between ASMA positivity or those with both ANA and ASMA positivity, and ALT level, degree of lobular inflammation, degree of steatosis or degree of fibrosis (data not shown).

4 | DISCUSSION

In this large pediatric cohort with biopsy-confirmed NAFLD, we found that one third (33%) of patients had positive ANA, ASMA or a combination of autoantibodies that are also implicated in autoimmune hepatitis; importantly no patients were positive for anti-LKM antibodies. These results suggest that positive autoantibodies are more common in children and adolescents with confirmed NAFLD than the general healthy pediatric population, with reports of 11.2% prevalence.[16] Autoantibody positivity was associated with similar prevalence of cardiometabolic risk factors, such as obesity severity and dyslipidemia, with the exception of T2DM which was absent in those with autoantibodies. Histologically, autoantibody positivity was not associated with liver disease severity, with the exception of the subgroup of patients with positive ANA who had increased steatosis severity independent of known confounders.

The prevalence of autoantibody positivity we observed was in agreement with previous reports from the adult NAFLD literature, which range from 21%-36% among adults with NAFLD who are in their 5th - 6th decade of life.[3,8–11] However, in these studies, the relationship between autoantibody positivity and histologic disease severity has been inconsistent. Vuppalanchi et al studied the largest cohort of patients with histologically confirmed NAFLD (n = 864) and showed that those with positive autoantibodies were less likely to have a steatosis score of ≥2.[10] As expected, the NAS was lower in those with positive autoantibodies as well. Similarly, two other smaller studies have revealed a similar association between autoantibody positivity and a decreased likelihood of steatosis severity.[3,11] In contrast, one study from the U.S.[9] and one from Japan,[8] both of which have investigated similar sized cohorts (n = 225 and n = 212, respectively), failed to reveal an association between steatosis severity and autoantibody positivity. They did however show an association between autoantibody positivity and fibrosis severity,[9] as well as a link between ANA positivity and necroinflammation.[8] The latter was no longer significant in the multivariate analyses, which ultimately revealed that ANA positivity was solely associated

with obesity severity. Finally, autoantibody positivity was not associated with advanced fibrosis, neither in our cohort nor in adults.[10,17]

Adult data may not be directly comparable to our pediatric cohort, as there are considerable differences in the clinical characteristics between the patients studied. The prevalence of T2DM in the adult cohorts ranged from 28%-41%,[8–10] whereas in our study only 5% of the patients had T2DM. This is significant because it has been previously hypothesized that autoantibody positivity is an epiphenomenon of the insulin resistance seen in patients with NAFLD.[11] If this were true in pediatrics, we would expect the prevalence of autoantibody positivity to be much lower than that of adult NAFLD, which is not the case. This finding suggests that either the proposed hypothesis by Loria et al is false, or that it does not apply to pediatric NAFLD. Another significant difference between previously published adult studies and our current cohort is the inclusion of patients with cirrhosis. The proportion of patients with cirrhosis in the adult studies ranged from 11%-22%.[8,10] In our cohort, there were no patients with cirrhosis as is typical in pediatric NAFLD cohorts. Cirrhosis is associated with an impaired gut barrier, which in turn allows the passage of pathogens that can trigger autoimmune processes, such as the development of autoantibody seropositivity.[18] In addition, the pathologic patterns in the context of pediatric NAFLD may differ in terms of more portal inflammation and less ballooning.[19] All reported studies on this topic have been cross-sectional in nature, thus precluding further investigations on the timing of that seropositivity (eg, if cirrhotic patients always had a positive ANA or if this occurred following their progression to cirrhosis). Lastly, the referral bias that exists in studies of patients with confirmed NAFLD may be different between adult and pediatric NAFLD cohorts, as different criteria and thresholds may exist to biopsy an adult vs a young child with presumed NAFLD.[20]

The only pediatric report that, to our knowledge, has addressed the association between autoantibody positivity and histologic disease severity in children with NAFLD is that of Patton et al.[12] In that study, the investigators reviewed data collected prospectively within the framework of the NASH Clinical Research Network to determine whether routinely measured serologic markers can serve as predictors of histologic outcomes. Of the 176 children included, 18% were ANA positive and 32% were ASMA positive. In that cohort, the proportion of patients with ASMA positivity increased in parallel with increasing NAS (20% among those with NAS 1-3 were ASMA positive vs 45% among those with NAS 6-7) and this remained an independent predictor after multivariable analysis. The remaining histologic features, particularly steatosis, inflammation and fibrosis, were not associated with antibody positivity. We were not able to replicate these findings in our study. In our cohort, we had a lower proportion with high NAS score,[12] as only 12% of our patients had a NAS 6-7 vs 24% in the study by Patton et al. The only other significant difference between the report by Patton et al. and our study was the lower prevalence of ASMA positivity, which may have contributed to a type II error and the differences in the results seen. Notably, to our knowledge, no study to date has identified anti-LKM positivity in patients with NAFLD, as was observed in this cohort. Thus, the identification of this specific autoantibody should prompt investigation for type II AIH with a liver biopsy, following exclusion of hepatitis C infection due to its association with anti-LKM1 positivity.[21]

In our study, patients with positive autoantibodies were found to have lower levels of serum aminotransferases, total cholesterol and LDL-C at the time of liver biopsy than their counterparts with negative autoantibodies. This finding is likely reflective of the clinical practices regarding liver biopsy decision-making. Following exclusion of conditions such as viral hepatitis B or C infections, autoantibody positivity alone can be sufficient evidence for clinicians to proceed with a liver biopsy, considering that autoimmune hepatitis is the second most common diagnosis made in children with overweight or obese BMI and suspected NAFLD, and should not be missed.[1] Conversely, in the absence of autoantibody positivity, at our institutions, patients undergo liver biopsies if there are concerns that they may have more severe NAFLD (steatohepatitis, advanced fibrosis), as suggested by the presence of multiple cardiometabolic risk factors (eg, dyslipidemia, T2DM etc.)[4,22] or significantly and persistently elevated levels of serum aminotransferases.[1,12]

Interestingly, in spite of the lower aminotransferase levels seen in children with positive autoantibodies, those with positive ANA were more likely to have severe steatosis than those with negative ANA. This, as aforementioned, is in contrast with adult literature.[3,8–11] It has been previously suggested that autoantibody positivity in patients with NALFD is an epiphenomenon and a reflection of the patient's human leukocyte antigen (HLA) haplotype and immune predisposition.[23] The majority of pediatric AIH is associated with HLA-DRB1*0301.[24] In a study of 85 adults with biopsy-proven NAFLD, investigators performed genetic testing to explore associations between HLA haplotypes and disease severity.[25] HLA-DRB1*03 was associated with an increased risk of NASH, while HLA-B*27 was associated with severe steatosis. Further study of HLA haplotypes in children with NAFLD may identify specific disease subtypes based on immune predisposition, and provide a link between autoantibody positivity and the increase in steatosis severity observed in our cohort.

Strengths of this study include the large sample size, multicenter design and inclusion of biopsy-confirmed patients with NAFLD. Furthermore, we did note and adjust for the presence of other autoimmune conditions in patients with positive autoantibodies.

Limitations of this study include its retrospective design. Specifically, we did not have access to saved sera for standardized evaluation of autoantibody characterization. Despite this limitation, the samples were analyzed at CLIA-approved laboratories. Of fundamental value is the multicenter nature of the study design, as with the reporting of data from multiple centers, the results are more generalizable to other pediatric centers. Also, not all patients had a serum IgG measured as some centers only order an IgG when AIH is of significant clinical concern (eg, in the context of highly positive autoantibody titers, unclear histology, etc.). Lastly, this study investigated a cohort of children with clinical features concerning enough to warrant a liver biopsy to confirm the diagnosis of NAFLD. This referral bias may have affected the results, which may not be representative of the entire spectrum of disease severity within pediatric NAFLD.

4.1 | Summary and conclusions

Autoantibody positivity is common (33%) in pediatric NAFLD. We found that children with positive autoantibodies have lower liver transaminase levels, but more severe steatosis, and

no other associations with histologic severity of disease. Further investigations are needed to elucidate the significance of autoantibody positivity, with a potential for HLA subtypes to play a role. Although AIH was not diagnosed in this cohort of children with biopsy-proven NAFLD, autoantibody positivity should prompt an investigation for AIH, including an evaluation of liver histology, following exclusion of conditions such as viral hepatitis infections. Ultimately, all patients with NAFLD should be managed according to current guidelines regardless of autoantibody positivity.

Abbreviations:

AIH	autoimmune hepatitis
ALT	alanine aminotransferase
ANA	antinuclear antibody
anti-LKM	anti-liver-kidney microsomal antibody
ASMA	anti-smooth muscle antibody
AST	aspartate aminotransferase
BMI	body mass index
GGT	gamma glutamyl transpeptidase
HbA1c	hemoglobin A1c
IgG	immunoglobulin G
IQR	interquartile range
LDL-C	low-density lipoprotein cholesterol-Cholesterol
NAFLD	non-alcoholic fatty liver disease
NAS	NAFLD Activity Score
NASH	non-alcoholic steatohepatitis
T2DM	type 2 diabetes mellitus

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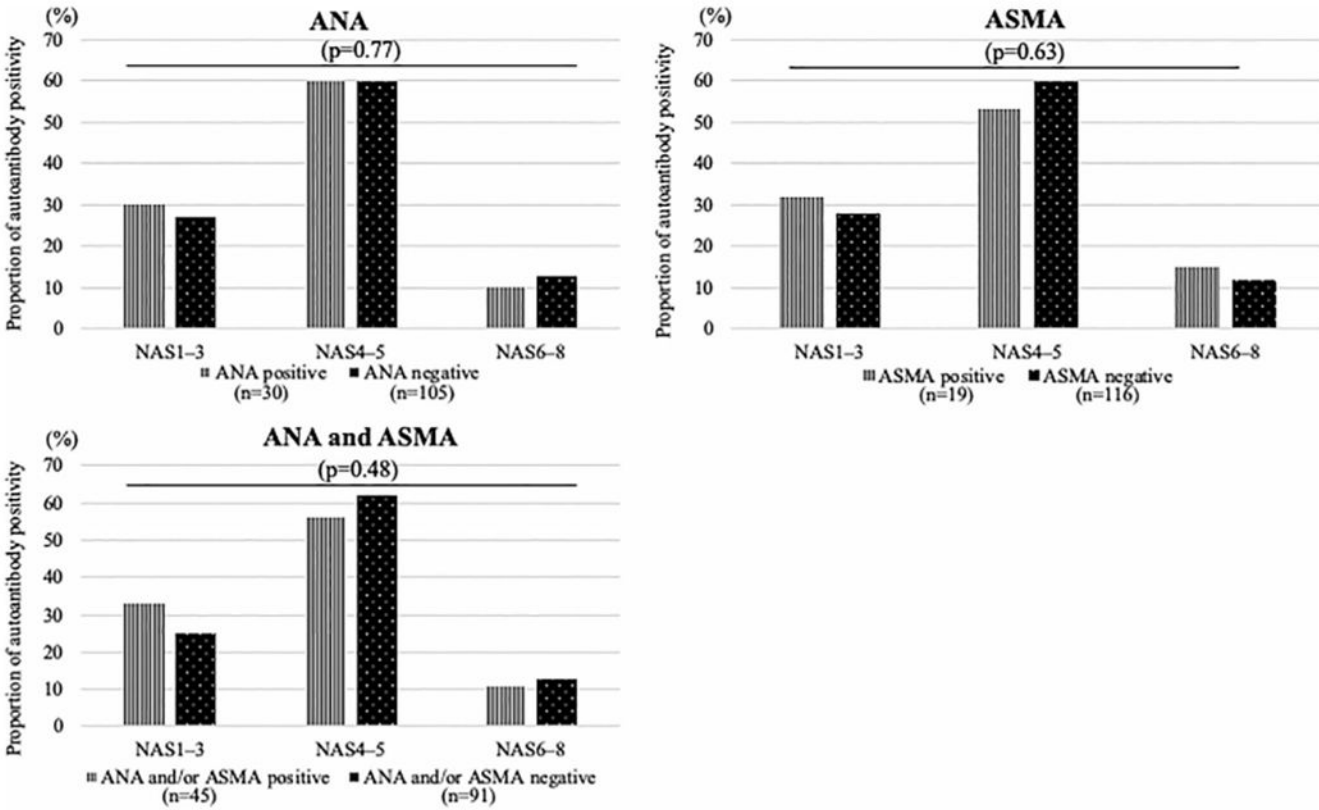

FIGURE 1.

Comparison of the proportion of patients with positive autoantibodies by nonalcoholic fatty liver disease (NAFLD) Activity Score severity

TABLE 1

Demographic and baseline clinical, laboratory and histologic characteristics of the study cohort

Variable	Study cohort, n = 136
Age (y)	14 (11, 16)
Female sex; n (%)	54 (40%)
Ethnicity/Race	
Hispanic n (%)	43 (32%)
Non-hispanic	93 (68%)
Asian	4 (3%)
Black	8 (6%)
White	79 (58%)
Unknown	2 (1%)
BMI (z-score)	2.46 (2.2, 2.6)
Obesity; n (%)	
Overweight	19 (14%)
Obese Class I	36 (26%)
Severe obesity Class II	57 (42%)
Severe obesity Class III	24 (18%)
Medication list	
Anticonvulsants	7 (5%)
Antihypertensives	11 (8%)
Ciprofloxacin for acne	1 (1%)
Insulin	3 (2%)
Metformin	31 (23%)
Psychiatric drugs	35 (26%)
Proton pump inhibitor	16 (12%)
Vitamin D supplementation	34 (25%)
Laboratory data	
ANA positivity; n (%)	30/135 (22%)
ASMA positivity; n (%)	19/135 (14%)
Anti-LKM antibody positivity; n (%)	0/134 (0%)
ANA or ASMA positivity; n (%)	45/136 (33%)
ANA and ASMA positivity; n (%)	4/135 (3%)
ALT (U/L)	111 (72, 174)
AST (U/L)	59 (40, 89)
GGT (U/L)	52 (35.5, 80)
Direct bilirubin (mg/dL)	0.05 (0.05, 0.1)
Serum insulin (nU/L) (n = 99)	27.7 (19, 36.2)
HbA1c (%) (n = 110)	5.3 (5.0, 5.6)

Variable	Study cohort, n = 136
Albumin (g/dL)	4.2 (4.0, 4.5)
Prothrombin-INR	0.99 (0.96, 1.02)
Cholesterol (mg/dL) (n = 112)	167 (141, 185)
LDL-C (mg/dL) (n = 110)	98 (76, 111)
HDL-C (mg/dL) (n = 112)	37 (31, 42)
Triglyceride (mg/dL)	145.5 (109.5, 209)
Elevated immunoglobulin G; n (%) (n = 53)	3 (6%)
Histology data	
Steatosis score	2 (2, 3)
Patients with steatosis ≥2; n (%)	104 (76%)
Lobular inflammation score	1 (1, 2)
Ballooning score	1 (0, 1)
NAFLD activity score (NAS)	4 (3, 5)
Patients with NAS ≥5; n (%)	60 (44%)
Portal inflammation score	1 (0, 1)
Fibrosis stage	1 (0, 2)
Fibrosis stage 0	40 (29%)
Fibrosis stage 1	51 (38%)
Fibrosis stage 2	30 (22%)
Fibrosis stage 3	15 (11%)
Patients with fibrosis ≥2; n (%)	45 (33%)

Note: Data are presented as medians and interquartile ranges or N (%). Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; GGT, gamma glutamyl transpeptidase; HbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein-Cholesterol; LDL-C, low-density lipoprotein-Cholesterol; NAFLD, non-alcoholic fatty liver disease; NAS, NAFLD Activity Score.

TABLE 2

Comparison of the clinical, laboratory and histology findings between patients grouped by autoantibody status

Variable	ANA or ASMA autoantibody positive (N = 45)	ANA or ASMA autoantibody negative (N = 91)	*P* value
Age	14 (11, 16)	14 (11, 15)	.780
Female sex; n (%)	24 (53%)	30 (33%)	.022
Hispanic ethnicity; n (%)	14 (31%)	29 (32%)	.929
BMI z-score	2.4 (2.1, 2.6)	2.5 (2.2, 2.7)	.082
Severe obesity; n (%)	25 (56%)	56 (62%)	.504
AIH; n (%)	0 (0%)	0 (0%)	-
T2DM; n (%)	0 (0%)	7 (7.7%)	.056
Laboratory investigations			
ALT (U/L)	84 (60, 127)	118 (85, 191)	.009
AST (U/L)	48 (30, 68)	69 (43, 104)	<.001
GGT (U/L)	43 (32, 71)	54 (39, 85)	.081
Direct bilirubin (mg/dL)	0.05 (0.05, 0.1)	0.05 (0.05, 0.1)	.452
HbA1c (%) (n = 110)	5.3 (4.95, 5.45)	5.3 (5.0, 5.65)	.390
Serum Insulin (nU/L) (n = 99)	24.1 (15.9, 37.9)	27.8 (20.1, 33.2)	.422
Albumin (g/dL)	4.2 (4, 4.5)	4.3 (4, 4.6)	.262
Prothrombin-INR	0.99 (0.95, 1.02)	0.99 (0.96, 1.03)	.737
Cholesterol (mg/dL) (n = 112)	158 (138, 173)	171 (143, 197)	.041
LDL-C (mg/dL) (n = 110)	90 (73, 103)	103 (81, 118)	.004
HDL-C (mg/dL) (n = 112)	39 (31, 43)	37 (31, 42)	.231
Triglyceride (mg/dL)	141 (105, 199)	147 (112, 214)	.447
Elevated immunoglobulin G; n (%)	1/25 (4%)	2/28 (7%)	.621
Histology data			
Steatosis	2 (2, 3)	2 (1, 3)	.480
Patients with steatosis ≥2; n (%)	37 (82%)	67 (74%)	.266

Variable	ANA or ASMA autoantibody positive (N = 45)	ANA or ASMA autoantibody negative (N = 91)	*P* value
Lobular inflammation	1 (1, 2)	1 (1, 2)	.363
Ballooning	1 (0, 1)	1 (0, 1)	.147
NAFLD activity score (NAS)	4 (3, 5)	4 (3, 5)	.143
Patients with NAS ≥5, n (%)	16 (36%)	44 (48%)	.157
Portal inflammation	1 (0, 1)	1 (0, 1)	.403
Fibrosis stage	1 (0, 2)	1 (0, 2)	.374
Fibrosis stage 0	24 (26%)	16 (36%)	.704
Fibrosis stage 1	36 (40%)	15 (33%)	
Fibrosis stage 2	20 (22%)	10 (22%)	
Fibrosis stage 3	11 (12%)	4 (9%)	
Patients with fibrosis ≥2, n (%)	14 (31%)	31 (34%)	.730

Note: Data are presented as N (%) for categorical variables, and medians and interquartile ranges.

Abbreviations: AIH, autoimmune hepatitis; ALT, alanine aminotransferase; ANA, anti-nuclear antibody; ASMA, anti-smooth muscle antibody; AST, aspartate aminotransferase; BMI, body mass index; GGT, gamma glutamyl transpeptidase; HbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein-Cholesterol; INR, International Normalized Ratio; LDL-C, low-density lipoprotein-Cholesterol; NAFLD, non-alcoholic fatty liver disease; NAS, NAFLD Activity Score; T2DM, type 2 diabetes mellitus.

TABLE 3

Comparison of the clinical, laboratory and histology findings between patients grouped by ANA status

Variable	ANA positive (N = 30)	ANA negative (N = 105)	*P* value
Age	14 (12, 16)	14 (11, 15)	.609
Female sex; n (%)	17 (57%)	37 (35%)	.035
Hispanic ethnicity; n (%)	12 (40%)	31 (30%)	.277
BMI z-score	2.4 (2.2, 2.5)	2.5 (2.2, 2.7)	.195
Severe obesity; n (%)	17 (57%)	63 (60%)	.743
T2DM; n (%)	0 (0%)	7 (6.7%)	.146
Laboratory investigations			
ALT (U/L)	87 (59, 127)	114 (78, 186)	.100
AST (U/L)	52 (30, 71)	64 (41, 98)	.056
GGT (U/L)	52 (34, 73)	51 (36, 81)	.470
Direct bilirubin (mg/dL)	0.05 (0.05, 0.1)	0.05 (0.05, 0.1)	.740
HbA1c (%) (n = 110)	5.35 (5.1, 5.7)	5.3 (5.0, 5.6)	.293
Serum insulin (nU/L) (n = 99)	23.8 (17.4, 39)	27.8 (19.2, 33.6)	.806
Albumin (g/dL)	4.4 (4.1, 4.5)	4.2 (4, 4.6)	.396
Prothrombin-INR	1.00 (0.96, 1.02)	0.99 (0.96, 1.02)	.811
Cholesterol (mg/dL) (n = 112)	160 (137, 180)	168 (142, 186)	.388
LDL-C (mg/dL) (n = 110)	97 (74, 106)	98 (76, 115)	.247
HDL-C (mg/dL) (n = 112)	38 (29, 41)	37 (31, 42.5)	.967
Triglyceride (mg/dL)	143 (105, 188)	147 (110, 213)	.625
Elevated immunoglobulin G; n (%)	1/20 (5%)	2/33 (6%)	.871
Histology data			
Steatosis	2 (2, 3)	2 (1, 3)	.879
Patients with steatosis ≥2; n (%)	27 (90%)	76 (72%)	.045
Lobular inflammation	1 (1, 2)	1 (1, 2)	.607
Ballooning	1 (0, 1)	1 (0, 1)	.141
NAFLD activity score (NAS)	4 (3, 5)	4 (3, 5)	.388
Patients with NAS ≥5, n (%)	11 (37%)	49 (47%)	.331
Portal inflammation	1 (0, 1)	1 (0, 1)	.812
Fibrosis stage	1 (0, 2)	1 (0, 2)	.589
Fibrosis stage 0	30 (29%)	10 (33%)	.962
Fibrosis stage 1	39 (37%)	11 (37%)	
Fibrosis stage 2	24 (23%)	6 (20%)	
Fibrosis stage 3	12 (11%)	3 (10%)	
Patients with fibrosis ≥2, n (%)	9 (30%)	36 (34%)	.661

Note: Data are presented as N (%) for categorical variables, and medians and interquartile ranges. Abbreviations: ALT, alanine aminotransferase; ANA, anti-nuclear antibody; AST, aspartate aminotransferase; BMI, body mass index; GGT, gamma glutamyl transpeptidase; HbA1c, hemoglobin

A1c; HDL-C, high-density lipoprotein-Cholesterol; LDL-C, low-density lipoprotein-Cholesterol, NAFLD, non-alcoholic fatty liver disease; NAS, NAFLD Activity Score; T2DM, type 2 diabetes mellitus.