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Clinical, laboratory and histological associations in adults with nonalcoholic fatty liver disease

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Abstract

The NASH Clinical Research Network (NASH CRN) was formed to conduct multi-center studies on the etiology, contributing factors, natural history, and treatment of nonalcoholic steatohepatitis (NASH). The aim of this study was to determine the associations of readily available demographic, clinical and laboratory variables with the diagnosis of NASH and its key histological features, and determine the ability of these variables to predict the severity of nonalcoholic fatty live disease (NAFLD). A total of 1,266 adults were enrolled in NASH CRN studies between October 2004 and February 2008 of whom 1,101 had available liver histology. The median age was 50 years; 82% were white and 12% Hispanic. The median BMI was 33 kg/m^2; 49% had hypertension and 31% type 2 diabetes. On liver biopsy, 57% were judged to have definite NASH and 31% bridging fibrosis or cirrhosis. Using data from the 698 patients with liver biopsies within 6 months of clinical data, patients with definite NASH were more likely to be female and have diabetes, higher levels of AST, ALT, alkaline phosphatase, GGT and HOMA-IR. Progressive models for predicting histological diagnoses performed modestly for predicting steatohepatitis or ballooning (area under receiver operating characteristic curves ranged from 0.71 to 0.79), and better for advanced fibrosis (AUC 0.73–0.85).

Conclusion—Readily available clinical and laboratory variables can predict advanced fibrosis in adults with NAFLD but additional information is needed to reliably predict the presence and severity of NASH. Prospective studies of this well-characterized population and associated tissue bank samples offer a unique opportunity to better understand the cause and natural history of NAFLD and develop more precise means for noninvasive diagnosis.

Keywords

nonalcoholic steatohepatitis; nonalcoholic fatty liver disease; liver fibrosis; cirrhosis; alanine aminotransferase; biological markers

Nonalcoholic fatty liver disease (NAFLD) affects 10–30% of the general U.S. population and can progress to significant fibrosis and cirrhosis.[1] When nonalcoholic steatohepatitis (NASH) is present, the 5 and 10-year survivals are estimated at 67% and 59% respectively.2 The presence of NASH and early fibrosis is currently established only by liver biopsy; noninvasively determining who has NASH and who is at risk for progressing to cirrhosis remains challenging.3 Serum aminotransferases are routinely measured to detect liver disease, but their specificity and sensitivity for NASH, fibrosis or cirrhosis is low4 and the results may vary considerably over time 5, [6] and among laboratories.[7]

The NASH Clinical Research Network (NASH CRN) was initiated by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) in 2002 to conduct multi-center, collaborative studies on the etiology, contributing factors, natural history, complications, and treatment of NASH. To meet these goals, patients with the full spectrum of NAFLD or cryptogenic cirrhosis were enrolled in an observational Database study (Database), and patients with NASH into an adult treatment trial (PIVENS)8, 9 and a pediatric treatment trial (TONIC).10

The objectives of this paper are (1) to provide a description of all *adult* patients enrolled in NASH CRN studies, (2) to determine the associations of basic clinical variables with the diagnosis of definite NASH, stage of fibrosis, grade of inflammation and presence of hepatocellular ballooning injury, and (3) to determine the overall accuracy of models using only demographic and basic clinical variables to predict the presence of NASH, and the activity grade and fibrosis stage of NASH. A similar analysis of the clinical and histological features of NAFLD in children enrolled in the NASH CRN studies has been published.[11]

Methods

Study design

Patients with suspected or histologically proven NAFLD were enrolled into the Database observational study at nine U.S. medical centers: Case Western Reserve (Cleveland, Ohio); Duke University (Durham, North Carolina); Indiana University (Indianapolis, Indiana); Johns Hopkins University (Baltimore, Maryland); Saint Louis University (St. Louis, Missouri); University of California, San Diego; University of California, San Francisco; University of Washington (Seattle, Washington); and Virginia Commonwealth University (Richmond, Virginia). The data were stored, monitored and analyzed at the Data Coordinating Center at the Johns Hopkins Bloomberg School of Public Health.

The NASH CRN enrolled patients at least 2 years of age who met any one of the following criteria into the Database: (1) a histologic diagnosis of NAFLD; (2) a histologic diagnosis of cryptogenic cirrhosis; (3) suspected NAFLD based on imaging studies; (4) clinical evidence of cryptogenic cirrhosis. Patients were excluded if they had clinical or histological evidence of alcoholic liver disease or alcohol consumption during the two years before entry of more than 20 g daily for men and 10 g daily for women. Other exclusion criteria included evidence of other forms of chronic liver disease; history of total parenteral nutrition, biliopancreatic diversion, or bariatric surgery; short bowel syndrome; suspected or confirmed hepatocellular carcinoma; known HIV positive; conditions that were likely to interfere with study follow-up; or inability to provide informed consent. The enrollment

goals were a total of 1500 patients including 1125 adults and 375 children. Patients were enrolled from October, 2004 until February, 2008 and followed until September, 2009. Comprehensive data, including demographics, medical history, symptoms, medication use, diet and exercise habits, and routine laboratory studies were collected on all patients at entry and at annual visits for up to 4 years after enrollment. Interim liver biopsies were obtained during patient study involvement only when indicated for patient care. Study questionnaires administered at enrollment and at selected follow-up visits included AUDIT, Block Food Questionnaire, Skinner Lifetime Drinking History, Physical Activity, Modifiable Activity, and the MOS 36-Item Short-Form Health Survey. Specimens including whole blood as a source of DNA, serum and plasma, were collected at selected time points during follow-up for contemporaneous analysis or storage in a central repository.

Data collected and included in this analysis were also from patients entering the NASH CRN adult treatment trial, "Pioglitazone versus Vitamin E versus Placebo for the Treatment of Nondiabetic Patients with Nonalcoholic Steatohepatitis" (PIVENS).[8, 9] This study was designed to evaluate whether 96 weeks of treatment with either pioglitazone or vitamin E improved histological features of NASH and the entry criteria were more stringent than for enrollment in the Database observational study. Eligible patients were 18 years or older and had histological evidence of NASH without cirrhosis obtained no more than 6 months before randomization. The PIVENS trial was limited to patients without diabetes or a history of therapy to treat diabetes. Patients were excluded if they consumed > 20 grams of alcohol per day for females or >30 g/day for males on average, either currently or for a period of more than 3 consecutive months in the 5 years prior to screening. Additional exclusion criteria included any other form of chronic liver disease, the use of any medications thought to cause or affect NAFLD, the use of nonstable doses of lipid lowering medications, and alanine aminotransferase (ALT) levels > 300 U/L or a serum creatinine levels ≥ 2.0 mg/dL. Women of childbearing age who were pregnant, unwilling to use effective birth control or nursing were excluded. At baseline, all PIVENS patients underwent extensive data collection similar to that for the Database observational study, as well as a new liver biopsy if one had not been obtained in the previous 6 months.

Sample analysis

Routine laboratory studies were performed on fresh samples in CLIA certified laboratories at each clinical site according to standard clinical protocols. When liver biopsies were obtained as part of routine patient care, a small amount of extra liver tissue, if available, was frozen immediately in liquid nitrogen and stored at −80°C in a central repository. All biopsy specimens were formalin fixed, paraffin embedded and ten extra unstained slides were prepared locally that were sent to the CRN repository. Hematoxylin and Eosin, Masson's trichrome and Perls' iron stains were prepared by a central laboratory and reviewed centrally by the NASH CRN Pathology Committee, a group of 9 hepatopathologists who were masked to all clinical and identifying data. Biopsies were scored by consensus during Pathology Committee meetings using the previously published NASH CRN NAFLD Activity Score (NAS) and fibrosis score.[12]

Data analysis

The characteristics of the adult patients (ages 18 and over) enrolled in the Database or the PIVENS trial were analyzed descriptively. Subjects were divided into 3 mutually exclusive groups: 1) those with liver biopsies obtained within 6 months of clinical and laboratory data (contemporaneous liver biopsies), 2) those with the most recent liver biopsies obtained more than 6 months before clinical and laboratory data were obtained, and 3) those without an available liver biopsy. Cross-sectional analyses were then conducted of the first group of patients, that is, those who were enrolled in the Database or the PIVENS trial and had a liver

biopsy within 6 months of their baseline clinical data. The two main outcomes studied were 1) the presence of definite NASH versus borderline or no NASH and 2) stage 3 (bridging) or stage 4 (cirrhosis) fibrosis scores versus lower stages. Secondary histological outcomes included the presence of one or more of the following features: 1) ≥ 34% steatosis, 2) ≥ grade 2 lobular inflammation, 3) portal inflammation, 4) any ballooning, 5) NAFLD Activity Score ≥ 5, 6) any fibrosis, and 7) cirrhosis.

For these analyses, we examined the following basic predictor variables: aspartate aminotransferase (AST), and alanine aminotransferase (ALT) levels; demographic factors including age, gender, race and ethnicity; anthropometrics including body mass index (BMI) and waist circumference; and the presence of co-morbid conditions including hypertension and type 2 diabetes. We also examined additional clinical laboratory tests including: the AST/ALT ratio, gammaglutamyl transpeptidase (GGT), albumin, total protein, prothrombin time, platelet count, total cholesterol, HDL and LDL cholesterol, triglycerides, hemoglobin A1c (HbA1c), fasting glucose and insulin as well as the homeostasis model assessment (HOMA) index, and titers of anti-nuclear (ANA), anti-smooth muscle (ASMA) and anti-mitochondrial (AMA) antibodies.

To determine the factors associated with each outcome, bivariate and multivariate logistic regression analyses were used and progressive models were built using AST and ALT alone (Model 1), Model 1 plus demographic information (Model 2), Model 2 plus co-morbidities (Model 3), and finally Model 3 plus other standard laboratory studies (Model 4).

To determine the overall accuracy of these progressive prediction models for the predefined outcomes, areas under the receiver operating characteristic curves (AUROC) for each of the models were calculated. All analyses were conducted using SAS 9.2 (SAS Institute Inc, Cary, NC) and Stata 11 (StataCorp, College Station, TX).

Responsibility for Design, Study Safety and Data Quality

The NASH CRN studies were designed by subcommittees of the NASH CRN Steering Committee, the latter composed of principal investigators from each clinical site, the two co-chairs of the Pathology Committee, the principal investigator from the Data Coordinating Center, and the NIDDK scientific officer [all investigators in the NASH CRN and their positions and locations are listed in the appendix]. After approval by the Steering Committee, studies were approved by the respective institutional review boards at all involved sites. All enrolled patients gave written informed consent before data collection with special consent for genetic testing. The clinical protocols, consent forms and manual of operations were also reviewed and approved by a Data Safety Monitoring Board established by the NIDDK specifically for the NASH CRN. All studies were in compliance with Good Clinical Practice guidelines for human research quality standards. Investigators, coordinators and ancillary staff involved in data collection and entry were trained and certified for quality assurance. In addition, monthly data audits were performed by comparing entered data with source documents by the Data Coordinating Center throughout the NASH CRN studies.

Results

Study population

A total of 1,266 adults were enrolled into the NASH CRN Database (n=1,019) or PIVENS trial (n=247) between October 2004 and February 2008. Of these, 698 had a liver biopsy obtained within 6 months of clinical data collection (contemporaneous biopsy group), 403 had a biopsy more than 6 months before study data was collected, and 165 did not have biopsy data available. Of those classified as having contemporaneous liver biopsies, 53% had biopsies within 1 week of having blood tests, 60% within 4 weeks, 81% within 3

months and the remaining 19% between 3 and 6 months. For non-PIVENS patients with more than one biopsy, only the last biopsy was used for analysis. For PIVENS patients, the entry biopsy and contemporaneous laboratory and clinical data obtained within 6 months of the biopsy were used.

The characteristics, laboratory test results and biopsy features of the NASH CRN adult patients are given in Table 1. Overall, the median age was 50 years, 82% of patients were white and 12% Hispanic. The median BMI was 33 kg/m^2 and median waist circumference 108 cm; 49% had hypertension and 31% had type 2 diabetes. Combining these features, 61% met the National Cholesterol Education Program (NCEP) criteria[13] for the metabolic syndrome. Acanthosis nigricans, a cutaneous manifestation of insulin resistance, was noted in 12% of the entire cohort. Cirrhosis, either by clinical evidence or biopsy, was present in 14% of the entire cohort. The median AST was 41 IU/L (SD 23) and median ALT 56 IU/L (SD 36). An elevated alkaline phosphatase level with normal aminotransferase levels defined by local laboratory reference ranges was found in 4% and a positive AMA in 4%. There was no association between an isolated alkaline phosphatase elevation and a positive AMA. Of those with a biopsy at any time, 54% had ≥34% steatosis, 48% had ≥grade 2 lobular inflammation, 66% had ballooning, 57% met the criteria for “definite” NASH and 25% had bridging hepatic fibrosis or cirrhosis.

The major differences between those with contemporaneous liver biopsies and those without was the lower prevalence of diabetes and hypertension, lower glucose, lower HDL cholesterol, higher triglycerides and less advanced fibrosis in the contemporaneous biopsy group. The contemporaneous liver biopsy group included all of the PIVENS patients, who did not, by definition, have diabetes or cirrhosis. Interestingly, the prevalence of the metabolic syndrome as defined by the NCEP ATP-III criteria was similar in all groups despite the group differences in individual components that define the metabolic syndrome. Aminotransferase levels were also higher in the contemporaneous biopsy group, possibly reflecting more patients with lower enzyme levels because of “burnt out” NASH in the setting of advanced fibrosis in the other groups. Further analyses of the study cohort focused on the subgroup with contemporaneous liver biopsies.

Factors associated with definite NASH, ballooning and advanced fibrosis

Factors associated with definite NASH in patients with NAFLD and contemporaneous liver biopsies are shown in Table 2. Patients with NASH were more likely to be women, have diabetes and meet the NCEP criteria for the metabolic syndrome; they also had significantly higher levels of AST, ALT, GGT, triglycerides, HbA1c, HOMA-IR and lower levels of HDL cholesterol compared to those without definite NASH. Patients with NASH also had significantly more steatosis, lobular inflammation, ballooning and fibrosis as well as higher NAFLD Activity Scores. Portal inflammation was more likely to be greater than mild in those with definite NASH. There were no differences between the two groups in age, BMI, waist circumference, acanthosis nigricans or self-identified Hispanic ethnicity. Interestingly, autoantibodies were found more often in those without definite NASH compared to those with NASH. Overall, the same factors associated with definite NASH were also significantly associated with ballooning. This may reflect the dominant role that the presence of ballooning has in establishing a diagnosis of definite NASH.

The value of using ALT levels to screen for NASH in patients with NAFLD was examined using three different cutoffs for the upper reference range. Using a conservative cutoff of 19 U/L for women and 30 U/L for men,[14] the sensitivity and specificity for identifying NASH were 99% (95% CI=97%, 100%) and 8% (95% CI=5%, 12%) respectively. Using local laboratory-defined upper limits of normal, the sensitivity and specificity for identifying NASH were 75% (95% CI = 70%, 79%) and 45% (95% CI=39%, 51%) respectively.

Finally, setting the upper limit arbitrarily at 40 U/L, a common practice, the sensitivity and specificity for identifying NASH were 86% (95% CI=82%, 89%) and 32% (95% CI=27%, 38%) respectively.

The factors associated with different stages of fibrosis are shown in Table 3. This cohort included good representation of the fibrosis spectrum with 26% (N=183) having no evidence of fibrosis, 17% (N=118) having bridging fibrosis and 8% (N=54) having cirrhosis. The associations between the clinical characteristics and fibrosis stages were complex. In general, the associations found for NASH held true for fibrosis. In addition, patients with advanced fibrosis were significantly older and more likely to have diabetes and hypertension. The degree of obesity was not found to be a risk factor for advanced fibrosis but an increased waist circumference was a risk factor. Despite the association with diabetes, hypertension and increased waist circumference, meeting NCEP criteria for the metabolic syndrome was not a risk factor for advanced fibrosis.

As would be expected, patients with advanced fibrosis had higher prothrombin times, and lower albumin levels, hematocrits, white blood cell counts, and platelet counts. In some cases the relationship was not monotonic. For example, AST and ALT levels were highest with stage 2 and 3 fibrosis and were lower in patients with cirrhosis. The low AST/ALT ratio typical of NASH also reversed and was >1 in the group with cirrhosis. Cirrhosis was also associated with lower levels of LDL cholesterol and triglycerides, decreasing severity of histological features including steatosis, lobular inflammation, ballooning and a lower likelihood of having definite NASH. Finally, subjects of Hispanic ethnicity were equally distributed between definite NASH and not NASH, but overall had lower fibrosis scores and less advanced fibrosis.

Predictive Models for NASH and Fibrosis

The performance of the 4 progressive models for predicting the different histological outcomes is shown in Table 4. Serum levels of AST, ALT and the AST/ALT ratio together performed modestly for predicting steatosis (AUROC 0.60, 95%, confidence interval [CI] 0.55 to 0.64) but were somewhat better for other histologic features. The aminotransferase levels and their ratio alone were predictive of cirrhosis with an AUC of 0.81 (95% CI 0.75 to 0.88). Addition of the other basic clinical variables and laboratory tests improved the performance of the models somewhat for each of the pathological characteristics, with the full model having an AUROC of 0.79 for NASH and 0.96 for cirrhosis. Application of other scoring systems for fibrosis15⁻18 to this dataset did not demonstrate better diagnostic accuracy (results not shown) than the models developed here.

Discussion

Identifying patients at risk for developing cirrhosis and hepatocellular carcinoma from progressive NASH is challenging. Routinely available laboratory testing has proven to be inadequate and a variety of scoring systems based on clinical and laboratory parameters have been proposed but have not proven sufficiently reliable when evaluating individual patients.[19] However, performing biopsies in all patients with suspected NAFLD is problematic because of the high prevalence of disease, risks, costs and sampling variability.[20–22]

This study was undertaken using the largest prospectively enrolled cohort of adults with NAFLD with carefully characterized and uniform entry criteria to determine if rigorously evaluating a large cohort of adults with NAFLD would provide new insights into the value of routinely obtained clinical and laboratory data for diagnosing the presence and severity of NASH. The subjects were enrolled with variable times between their liver biopsies and

acquisition of clinical and laboratory data. To correlate histology with these data, the analyses focused on the 698 patients who had biopsies within six months of data collection, a period that would optimize enrollment while minimizing the chance of significant changes during this time. Comparing the group with contemporaneous biopsies to those without biopsies or biopsies more than six months before data acquisition demonstrated that the contemporaneous group was slightly biased to having a lower prevalence of diabetes, hypertension and cirrhosis (Table 1). The contemporaneous liver biopsy group was also similar overall to the group without liver biopsies, suggesting that the analysis was not biased by focusing only on patients willing or able to have liver biopsies.

Inherent to this study of NAFLD is the case ascertainment bias of studying only patients referred to tertiary care centers who then agree to participate in studies. Thus the findings may be most relevant to patients within the healthcare system who have been referred for subspecialist care and may not be applicable to the population as a whole or those seen only by primary care providers and not referred for further evaluation of possible liver disease.

Overall, the cohort of patients studied by the NASH CRN was similar to other large cohorts of NAFLD patients. It was enriched with patients having NASH (57%) compared to population studies suggesting a 10–30% prevalence of NASH when NAFLD is present.[1] The roughly 2:1 ratio of women to men may reflect a higher disease burden in women or, alternatively, gender differences among those pursuing and receiving healthcare. Population studies have not shown major gender differences in the prevalence of NAFLD detected by imaging. The cohort was 95% self-identified as white or Hispanic with relative underrepresentation of African Americans. This underrepresentation of African Americans likely reflects the recognized lower prevalence of NASH in African Americans as the demographic representation of African Americans in the geographic regions of the study sites was commensurate with the United States as a whole. About one third of patients did not meet NCEP criteria for the metabolic syndrome.13 NAFLD may be a sensitive early indicator of insulin resistance; whether the presence of NAFLD predicts the future development of the metabolic syndrome will require continued observation of these patients.

Additional useful observations for clinicians from this large cohort include the prevalence of acanthosis nigricans and autoantibodies. Acanthosis nigricans, previously thought to be rare in NASH, is a cutaneous manifestation of insulin resistance and was found in 12% of patients with NAFLD. Recognizing this regional hyperpigmentation, typically occurring in adults around the neck and over knuckles, elbows and knees provides clinicians with a physical clue to the presence of insulin resistance and affords the opportunity to educate patients on the underlying cause of this often unexplained skin change. The detection of autoantibodies during evaluation of patients with suspected liver disease can raise questions about unrecognized primary biliary cirrhosis or autoimmune hepatitis. This study identified a positive AMA without histologic evidence of PBC in 5% of patients, similar to that in a smaller study.[23] One third of patients had either a positive ANA or ASMA and 5% had both positive without histological evidence of autoimmune hepatitis. These observations confirm findings in smaller studies.[24–26]

Several clinical and biochemical parameters were associated with an increased likelihood of having NASH, but these differences were not quantitatively large (Table 2). It is worth noting that 16% of biopsies did not meet NASH criteria yet had a NAS ≥ 5, emphasizing the point, previously made, that the NAS is not a substitute for a diagnosis of NASH.[12] Larger biopsies are more likely to include findings that support a diagnosis of NASH,[21, 22] and consistent with this observation was the finding that the absence of definite NASH was more likely when the total biopsy core length was less than ten millimeters.

Identifying early fibrosis may identify patients at risk for progressing to cirrhosis over time. As shown in Table 3, there were a large number of differences in clinical and laboratory parameters associated with the progressive stages of fibrosis but these differences were generally not quantitatively large. Notable exceptions included the higher prevalence of diabetes and more advanced age with advanced fibrosis, the AST/ALT ratio which increases as fibrosis progresses and the relative thrombocytopenia known to occur with cirrhosis. These variables have consistently emerged in several studies as predictive of the presence of advanced fibrosis.[3, 16, 17, 19] Patients with decompensated cirrhosis were excluded from enrollment and thus other changes such as hypoalbuminemia and coagulopathy were not observed in those with cirrhosis.

Serum ALT levels are used to screen patients for unsuspected liver disease, but the value of ALT measurements for detecting patients with NASH has been questioned.[4, 27–29] Because there is uncertainty regarding how an elevated ALT should be defined, this large cohort with the full spectrum of NAFLD was analyzed using a conservative upper limit of normal,[14] a pragmatic upper limit of 40 U/L, and the upper limit as defined by the local laboratory where the test was performed. Laboratory reference ranges for ALT are quite variable, independent of analyzer characteristics, and may be unreliable for identifying ALT elevations.[7] Using any of these upper limits of normal did not provide sufficient sensitivity and specificity to make ALT measurement a reliable screening test to identify NASH in patients with NAFLD.

The prospective collection of high quality clinical and histological data from this large cohort of patients with NAFLD facilitated the development and testing of predictive models built on bivariate and multivariate analyses. Although these progressive models performed increasingly well in predicting established cirrhosis, they were only modestly successful in predicting definite NASH or advanced fibrosis (stages 3 and 4 combined). Algorithms of varying complexity have also been developed over the past two decades that use non-invasive measures to estimate steatosis,[30, 31] the presence of NASH,[32–36] and the stage of fibrosis.[16, 17, 35, 37–40] While the value of estimating steatosis has also been questioned,[32, 41] noninvasively identifying the presence of NASH or fibrosis would likely improve clinical management. Analysis of this cohort demonstrates that scoring systems based on readily available clinical and biochemical data cannot reliably identify NASH or fibrosis in patients suspected of having NAFLD. Clinical or laboratory measures that provide more information are needed and this information should reflect the underlying pathogenic processes.[3] As new evidence emerges to explain the mechanisms of lipotoxic liver injury and its associated fibrosis, this new knowledge may lead to more accurate non-invasive testing that can identify patients at risk for developing cirrhosis and hepatocellular cancer as a consequence of NASH.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Abbreviations

NASH	nonalcoholic steatohepatitis
NAFLD	nonalcoholic fatty liver disease
NASH CRN	NASH Clinical Research Network
CLIA	Clinical Laboratory Improvement Amendments
HOMO-IR	homeostasis model of assessment of insulin resistance

AUROC	area under the receiver operator characteristic curve
NAS	NAFLD activity score
NCEP	National Cholesterol Education Program
CI	confidence interval

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Table 1

Characteristics of adult patients with NAFLD enrolled in the NASH CRN studies

Variable	Proximity of liver biopsy to enrollment			No liver biopsy (n=165)	Total (n=1266)
	# 6 mos (n=698)	> 6 mos (n=403)	P*		
Demographics					
Male (%)	39	34	0.12	33	36
Age - yrs (median ∀ SD)	49	52	<0.0001	52	50∀12
White (%)	81	85	0.05	79	82
Hispanic (%)	14	7	0.0003	14	12
Clinical					
Hypertension (%)	44	56	0.0001	52	49
Type 2 diabetes (%)	22	42	<0.0001	40	31
Metabolic syndromeH (%)	62	59	0.34	57	61
Acanthosis nigricans positive (%)	13	9	0.02	13	12
severity scoreI (mean∀SD)	0.31	0.18	0.01	0.28	0.26∀0.81
Cirrhosis based on imaging or biopsy (%)	9	21	<0.0001	18	14
Anthropometric (median ∀ SD)					
Body mass index - kg/m^2	34	33	0.08	33	33∀6
Waist circumference - cm	108	106	0.01	106	108∀815
Waist to hip ratio	0.93	0.93	0.12	0.92	0.93∀0.08
Hepatology panel (median ∀ SD)					
AST - U/L	45	37	<0.0001	36	41∀22
Abnormal AST** (%)	48	35	<0.0001	27	41
ALT - U/L	65	44	<0.0001	45	56∀36
Abnormal ALT** (%)	65	41	<0.0001	42	55
AST/ALT	0.72	0.84	<0.0001	0.79	0.76∀0.30
Alkaline phosphatase - U/L	80	85	0.01	82	82∀30
Isolated abnormal alkaline phosphatase′ (%)	3	6	0.01	2	4
GGT - U/L	49	46	0.95	45	47∀40

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Variable	Proximity of liver biopsy to enrollment			No liver biopsy (n=165)	Total (n=1266)
	# 6 mos (n=698)	> 6 mos (n=403)	P*		
Globulin - g/dL	3.0	3.0	0.02	3.0	3.0∀0.5
Albumin - g/dL	4.2	4.3	0.58	4.2	4.2∀0.4
Bilirubin, total - mg/dL	0.7	0.7	0.05	0.7	0.7∀0.3
Bilirubin, direct - mg/dL	0.1	0.1	0.41	0.1	0.1∀0.07
International normalized ratio (mean∀SD)	1.02	1.04	0.07	1.04	1.03∀0.18
Hematology and other laboratory studies (median ∀ SD)					
Hematocrit - %	42	41	<0.0001	41	42∀4
White blood cells - 1K/mm^3	6.7	6.3	<0.0001	6.5	6.5∀1.8
Platelet count - 1K/mm^3	244	225	<0.0001	236	237∀70
Total cholesterol - mg/dL	195	188	0.002	184	192∀41
HDL cholesterol - mg/dL	42	45	0.0004	43	43∀12
LDL cholesterol - mg/dL	119	111	0.0002	110	117∀36
Triglycerides - mg/dL	152	141	0.03	134	144∀78
HbA1c - %	5.7	5.8	0.009	5.8	5.7∀0.7
Fasting serum glucose - mg/dL	96	99	0.002	96	97∀21
Fasting serum insulin - μU/mL	19	17	0.35	19	18∀12
HOMA-IR - mg/dLHΦU/mL/405	4.4	4.4	0.59	4.7	4.4∀3.4
ANA (% positive)	24	21	0.22	30	24
ASMA (% positive)	10	15	0.007	27	14
ANA + ASMA (% both positive)	3	5	0.06	12	5
AMA (% positive)	6	1	<0.0001	2	4
Ferritin - ng/mL	155	127	0.02	108	136∀153
Histology					
Steatosis (% ∃ 34%)	59	47	0.0001	--	54
Lobular inflammation (% ∃ grade 2)	48	44	0.15	--	47
Portal inflammation (% > mild)	20	30	0.0003	--	24
Ballooning (% any)	67	64	0.33	--	66
NAFLD Activity Score (% ∃5)	49	44	0.08	--	47
Presence of NASH (% definite)	58	55	0.29	--	57

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Variable	Proximity of liver biopsy to enrollment			No liver biopsy (n=165)	Total (n=1266)
	# 6 mos (n=698)	> 6 mos (n=403)	P*		
Fibrosis - score[2] (mean ∀ ∃ SD)	1.5	1.9	<0.0001	--	1.7∀1.3
Mallory bodies (% present)	28	33	0.05	--	30
Biopsy length (% < 10 mm)	13	14	0.67	--	14

[*] Comparison of patients with liver biopsies # 6 mos vs. > 6 mos from enrollment using chi-square test for binary predictors and logistic regression of group indicator on continuous predictors

[H] NCEP definition JAMA (2001) 285: 2486–2497

[I] 0=absent, 1=present on close inspection, 2=mild, 3=moderate, 4=severe

[**] Defined as > 1 ULN according to local reference ranges (ULN, upper limit of normal)

[′] Defined as alkaline phosphatase ∃ 1 ULN and AST < 1 ULN and ALT < 1 ULN according to local references ranges

[2] Fibrosis scored 0 for none; 1 for mild to moderate in zone 3 perisinusoidal *or* portal/periportal only; 2 for zone 3 perisinusoidal *and* portal/periportal; 3 for bridging; and 4 for cirrhosis

Table 2

Characteristics of adult patients with NAFLD with contemporaneous[*] biopsies and clinical factors by presence of definite NASH

Variable	Presence of Definite NASH[H]		
	No (n=291)	Yes (n=404)	P[I]
Demographics			
Male (%)	45	34	0.006
Age - yrs (median)	48	49	0.57
White (%)	82	80	0.49
Hispanic (%)	13	15	0.48
Clinical			
Hypertension (%)	40	47	0.07
Type 2 diabetes (%)	17	26	0.007
Metabolic syndrome[′] (%)	56	66	0.01
Acanthosis nigricans positive (%)	13	14	0.76
severity score[2] (mean)	0.26	0.34	0.22
Anthropometric (median)			
Body mass index - kg/m^2	33	34	0.87
Waist circumference - cm	108	109	0.51
Waist to hip ratio	0.93	0.94	0.53
Hepatology panel (median)			
AST - U/L	37	55	<0.0001
ALT - U/L	56	74	<0.0001
AST/ALT	0.68	0.74	0.03
Alkaline phosphatase - U/L	78	83	0.05
Isolated abnormal alkaline phosphatase[&] (%)	5	2	0.01
GGT - U/L	40	56	<0.0001
Globulin - g/dL	2.9	3.0	0.0004
Albumin - g/dL	4.2	4.2	0.18
Bilirubin, total - mg/dL	0.7	0.6	0.0007
Bilirubin, direct- mg/dL	0.1	0.1	0.41
International normalized ratio (mean)	1.01	1.03	0.22
Hematology and other laboratory studies (median)			
Hematocrit - %	42	43	0.09
White blood cells - 1K/mm^3	6.7	6.8	0.61
Platelet count - 1K/mm^3	249	239	0.25
Total cholesterol - mg/dL	194	196	0.33
HDL cholesterol - mg/dL	43	41	0.01
LDL cholesterol - mg/dL	120	119	0.98
Triglycerides - mg/dL	137	159	0.01

Variable	Presence of Definite NASH[H]		
	No (n=291)	Yes (n=404)	P[I]
HbA1c - %	5.6	5.7	0.0002
Fasting serum glucose - mg/dL	94	97	0.003
Fasting serum insulin - μU/mL	16	20	0.001
HOMA-IR - mg/dLHΦU/mL/405	3.8	5.0	<0.0001
ANA (% positive)	26	23	0.44
ASMA (% positive)	14	7	0.004
ANA + ASMA (% both positive)	5	1	0.0009
AMA (% positive)	4	8	0.06
Ferritin - ng/mL	129	174	0.003
Histology			
Steatosis (% ∃ 34%)	50	66	<0.0001
Lobular inflammation (% ∃ grade 2)	30	62	<0.0001
Portal inflammation (% > mild)	13	25	0.0002
Ballooning (% any)	22	100	<0.0001
NAFLD Activity Score (% ∃ 5)	16	73	<0.0001
Fibrosis - score** (mean)	0.9	2.0	<0.0001
Mallory Denk bodies (% present)	2	46	<0.0001
Biopsy length (% < 10 mm)	19	9	0.0001

*Within 6 months

*H*3 patients with missing data for presence of NASH

*I*Comparison of presence vs. absence of definite NASH using chi-square test for binary predictors and logistic regression of group indicator on continuous predictors

,NCEP definition

*2*0=absent, 1=present on close inspection, 2=mild, 3=moderate, 4=severe

&Defined as alkaline phosphatase ∃ 1 ULN and AST < 1 ULN and ALT < 1 ULN according to local references ranges

**Fibrosis scored 0 for none; 1 for mild to moderate in zone 3 perisinusoidal *or* portal/periportal only; 2 for zone 3 perisinusoidal *and* portal/periportal; 3 for bridging; and 4 for cirrhosis

Table 3

Characteristics of adult patients with NAFLD with contemporaneous* biopsies and clinical factors by fibrosis stage

Variable	Fibrosis stage[H] None (n=183)	Mild/Moderate (n=338)	Bridging (n=118)	Cirrhotic (n=54)	p[I]
Demographics					
Male (%)	41	41	28	39	0.07
Age - yrs (median)	45	48	54	57	<0.0001
White (%)	81	80	77	89	0.34
Hispanic (%)	19	14	10	4	0.02
Clinical					
Hypertension (%)	34	44	51	59	0.003
Type 2 diabetes (%)	11	21	29	50	<0.0001
Metabolic syndrome[1] (%)	60	64	64	52	0.31
Acanthosis nigricans positive (%)	13	15	11	11	0.65
severity score[2] (mean)	0.29	0.36	0.25	0.15	0.34
Anthropometric (median)					
Body mass index - kg/m^2	33	33	35	35	0.28
Waist circumference - cm	106	109	111	115	0.02
Waist to hip ratio	0.93	0.94	0.93	0.94	0.28
Hepatology panel (median)					
AST - U/L	35	50	59	52	<0.0001
ALT - U/L	56	70	78	46	<0.0001
AST/ALT	0.65	0.70	0.83	1.16	<0.0001
Alkaline phosphatase - U/L	77	79	89	100	<0.0001
Isolated abnormal alkaline phosphatase[&] (%)	3	4	2	6	0.60
GGT - U/L	38	46	67	78	0.0002
Globulin - g/dL	2.8	3.0	3.1	3.4	<0.0001
Albumin - g/dL	4.2	4.3	4.2	4.0	<0.0001
Bilirubin, total - mg/dL	0.7	0.7	0.6	0.8	0.006
Bilirubin, direct - mg/dL	0.1	0.1	0.1	0.2	0.04

Variable	Fibrosis stage[H] None (n=183)	Mild/Moderate (n=338)	Bridging (n=118)	Cirrhotic (n=54)	P[I]
International normalized ratio (mean)	0.99	1.01	1.03	1.16	<0.0001
Hematology and other laboratory studies (median)					
Hematocrit - %	42	43	42	41	0.004
White blood cells - 1K/mm^3	6.7	6.9	6.4	6.0	0.001
Platelet count - 1K/mm^3	254	254	228	148	<0.0001
Total cholesterol - mg/dL	197	196	198	170	<0.0001
HDL cholesterol - mg/dL	42	41	42	42	0.60
LDL cholesterol - mg/dL	122	122	119	97	0.0003
Triglycerides - mg/dL	149	160	147	124	0.09
HbA1c - %	5.6	5.7	5.8	5.9	0.0006
Fasting serum glucose - mg/dL	93	96	98	98	0.02
Fasting serum insulin - µU/mL	16	19	22	24	<0.0001
HOMA-IR - mg/dLHΦU/mL/405	3.5	4.6	5.9	5.9	<0.0001
ANA (% positive)	21	26	20	33	0.18
ASMA (% positive)	12	7	12	15	0.09
ANA + ASMA (% both positive)	5	1	4	4	0.03
AMA (% positive)	4	8	8	2	0.27
Ferritin - ng/dL	119	172	185	159	0.0009
Histology					
Steatosis (% ∃ 34%)	50	68	58	31	<0.0001
Lobular inflammation (% ∃ grade 2)	26	60	60	22	<0.0001
Portal inflammation (% > mild)	6	15	41	54	<0.0001
Ballooning (% any)	34	75	91	80	<0.0001
NAFLD Activity Score (% ∃5)	21	59	69	33	<0.0001
Presence of NASH (% definite)	15	70	88	61	<0.0001
Mallory Denk bodies (% present)	1	27	60	54	<0.0001
Biopsy length (% < 10 mm)	20	12	5	13	0.003

*Within 6 months

[H] 5 patients had missing data for fibrosis stage

[I] Comparison of categories of fibrosis using chi-square test for binary predictors and multinomial logistic regression of group indicators on continuous predictors

[′] NCEP definition

[2] 0=absent, 1=present on close inspection, 2=mild, 3=moderate, 4=severe

[&] Defined as alkaline phosphatase ∃1 ULN and AST < 1 ULN and ALT < 1 ULN according to local references ranges

Table 4

Areas under the ROC curves (AUROC) for discrimination of NASH and other histological features of adult NAFLD using common clinical features

	AUROC (95% CI)				
Histological finding	**Model 1: AST+ALT+ AST/ALT ratio***	**Model 2: AST+ALT+ AST/ALT ratio*+ Demographics[H]**	**Model 3: AST+ALT+ AST/ALT ratio*+ Demographics[H]+ Comorbidities[I]**	**Model 4: AST+ALT+ AST/ALT ratio*+ Demographics[H]+ Comorbidities[I]+ Other lab tests'**	**P: Model 1 vs. Model 4**
Number of predictors in model	3	7	13	36	
Presence of NASH					
Definite vs. borderline/none	0.71	0.72	0.73	0.79[2]	<0.0001
	(0.67, 0.75)	(0.68, 0.76)	(0.69, 0.77)	(0.76, 0.83)	
Fibrosis					
Any vs. none	0.72	0.74	0.78	0.84	<0.0001
	(0.67, 0.76)	(0.70, 0.78)	(0.74, 0.82)	(0.80, 0.87)	
Bridging/cirrhosis vs. < none/mild/moderate	0.73	0.75	0.77	0.85[&]	<0.0001
	(0.68, 0.78)	(0.70, 0.79)	(0.73, 0.81)	(0.82, 0.89)	
Cirrhosis vs. < cirrhosis	0.81	0.83	0.86	0.96	<0.0001
	(0.74, 0.88)	(0.77, 0.88)	(0.80, 0.92)	(0.93, 0.98)	
NAFLD Activity Score					
5+ vs. <5	0.73	0.74	0.74	0.80	<0.0001
	(0.69, 0.77)	(0.70, 0.78)	(0.70, 0.78)	(0.76, 0.83)	
Steatosis					
34%+ vs. <34%	0.59	0.61	0.61	0.68	0.0004
	(0.55, 0.64)	(0.57, 0.66)	(0.57, 0.66)	(0.64, 0.72)	
Lobular inflammation					
Grade 2+ vs. grade <2	0.72	0.73	0.74	0.76	0.006
	(0.68, 0.76)	(0.69, 0.77)	(0.70, 0.78)	(0.72, 0.80)	
Portal inflammation					
>Mild vs. #mild	0.64	0.66	0.67	0.75	0.0001
	(0.59, 0.70)	(0.60, 0.71)	(0.61, 0.72)	(0.70, 0.80)	

	AUROC (95% CI)				
Histological finding	**Model 1: AST+ALT+ AST/ALT ratio***	**Model 2: AST+ALT+ AST/ALT ratio*+ Demographics*H***	**Model 3: AST+ALT+ AST/ALT ratio*+ Demographics*H*+ Comorbidities*I***	**Model 4: AST+ALT+ AST/ALT ratio*+ Demographics*H*+ Comorbidities*I*+ Other lab tests'**	**P: Model 1 vs. Model 4**
Ballooning degeneration					
Few/many vs. none	0.70	0.71	0.72	0.79	<0.0001
	(0.66, 0.75)	(0.67, 0.75)	(0.68, 0.76)	(0.75, 0.83)	

Note: All models were based on 621 adult patients with NAFLD who had complete data on contemporaneous clinical factors and histological findings

*AST, ALT, AST/ALT ratio

*H*Age, race, gender, ethnicity

*I*Hypertension, Type 2 diabetes, body mass index, waist circumference, waist/hip ratio, acanthosis nigricans

'Alkaline phosphatase, GGT, globulin, albumin, total and direct bilirubin, international normalized ratio, hematocrit, white blood cells, platelet count, total cholesterol, triglycercides, HDL cholesterol, LDL cholesterol, HbA1c, HOMA-IR, fasting serum glucose, fasting serum insulin, autoimmune markers (ANA, AMA, ASMA), metabolic syndrome, ferritin

Prediction equations (β (SE)):

2−0.3(2.9) + 0.049(0.010)*AST(U/L) − 0.012(0.006)*ALT(U/L) − 0.89(0.51)*AST/ALT ratio + 0.005(0.010)*age(yrs) − 0.82(0.32) if male + 0.40(0.33) if Hispanic − 0.17(0.28) if white − 0.36(0.32) if diabetic + 0.14(0.21) if hypertensive − 0.010(0.039)*BMI(kg/m^2) + 0.10(0.32) if positive acanthosis nigricans + 0.003(0.020)*waist circumference(cm) − 0.2(2.2)*waist/hip ratio − 0.12(0.24) if metabolic syndrome + 0.21(0.42) if positive AMA − 0.07(0.23) if positive ANA − 0.81(0.36) if positive ASMA + 0.17(0.12)*HOMA-IR(mg/dLHΦU/mL/405) − 0.027(0.030)*fasting serum insulin (μU/mL) − 0.0053(0.0079)*fasting serum glucose (mg/dL) + 0.007(0.033)*hematocrit (%) + 0.051(0.055)*white blood cells(1K/mm^3) − 0.0030(0.0017)*platelet count(1K/mm^3) − 0.001(0.014)*total cholesterol (mg/dL) + 0.0037(0.0028)*triglycerides (mg/dL) − 0.037(0.018)*HDL cholesterol (mg/dL) − 0.003(0.014)*LDL cholesterol (mg/dL) + 0.00125(0.00045)*ferritin (ng/dL) − 0.0037(0.0035)*alkaline phosphatase (U/L) − 0.0004(0.0016)*GGT (U/L) + 0.25(0.21)*globulin (g/dL) + 0.12(0.29)*albumin (g/dL) − 0.74(0.32)*total bilirubin (mg/dL) + 0.41(0.95)*direct bilirubin (mg/dL) + 0.42(0.63)*international normalized ratio + 0.17(0.16)*HbA1c(%)

&−7.5(3.3) + 0.0063(0.0069)*AST(U/L) − 0.0012(0.0051)*ALT(U/L) + 0.55(0.43)*AST/ALT ratio + 0.031(0.012)*age(yrs) − 0.54(0.37) if male − 0.83(0.45) if Hispanic − 0.36(0.33) if white + 0.29(0.35) if diabetic + 0.11(0.25) if hypertensive + 0.049(0.046)*BMI(kg/m^2) − 0.43(0.41) if positive acanthosis nigricans - 0.004(0.023)*waist circumference(cm) + 3.1(2.5)*waist/hip ratio − 0.30(0.30) if metabolic syndrome + 0.03(0.50) if positive AMA − 0.32(0.28) if positive ANA + 0.33(0.39) if positive ASMA − 0.066(0.078)*HOMA-IR(mg/dLHΦU/mL/405) + 0.033(0.022)*fasting serum insulin (μU/mL) − 0.0014(0.0076)*fasting serum glucose (mg/dL) − 0.057(0.038)*hematocrit (%) + 0.15(0.07)*white blood cells(1K/mm^3) − 0.013(0.002)*platelet count(1K/mm^3) + 0.015(0.015)*total cholesterol (mg/dL) − 0.0055(0.0031)*triglycerides (mg/dL) − 0.033(0.020)*HDL cholesterol (mg/dL) − 0.021(0.015)*LDL cholesterol (mg/dL) + 0.00058(0.00040)*ferritin (ng/dL) + 0.0046(0.0039)*alkaline phosphatase (U/L) + 0.0033(0.0015)*GGT (U/L) + 0.76(0.25)*globulin (g/dL) + 0.51(0.34)*albumin (g/dL) − 0.18(0.36)*total bilirubin (mg/dL) − 1.1(1.3)*direct bilirubin (mg/dL) + 1.3(0.6)*international normalized ratio + 0.08(0.17)*HbA1c(%)