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Patient Determinants for Histologic Diagnosis of NAFLD in the Real World: A TARGET-NASH Study

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Much of the current data on nonalcoholic fatty liver disease (NAFLD) are derived from biopsy-based studies that may introduce ascertainment and selection bias. Selection of patients for liver biopsy has implications for clinical practice and the reported epidemiology of NAFLD. The aim of this study was to determine patient factors predictive of histologic versus empiric clinical diagnosis of NAFLD in real-world practice. Adults from TARGET-NASH were included in this study. Descriptive statistics are provided for the cohort and compare the characteristics of histologic NAFLD versus patients with clinically diagnosed NAFLD, followed by logistic regression and machine-learning models to describe predictors of liver biopsy. The records of 3,474 subjects were analyzed; median age was 59 years, 59% were female, 75% were White, and median body mass index was 32 kg/m^2. Using histologic and/or clinical criteria, a diagnosis of nonalcoholic steatohepatitis was made in 37%, and cirrhosis in 33%. Comorbid conditions included cardiovascular disease (19%), mental health diagnoses (49%), and osteoarthritis (10%). Predictors of a biopsy diagnosis included White race, female sex, diabetes, and elevated alanine aminotransferase (ALT). ALT increased the odds of liver biopsy by 14% per 10-point rise. Machine-learning analyses showed non-White patients with ALT <69 had only a 0.06 probability of undergoing liver biopsy. ALT was the dominant variable that determined liver biopsy. *Conclusions*: In this real-world cohort of patients with NAFLD, two-thirds of patients did not have a liver biopsy. These patients were more likely to be non-White, older, with a normal ALT, showing potential gaps in or knowledge about this population. (*Hepatology Communications* 2021;5:938-946).

Nonalcoholic fatty liver disease (NAFLD) is a progressive disease including nonalcoholic fatty liver (NAFL), nonalcoholic steatohepatitis (NASH), and cirrhosis due to NAFLD.[1] One in three people in the United States have some degree of excess fat in the liver,[2,3] and up to 5% of the population may have NASH.[4] NAFLD, in most cases, is preceded by elements of the metabolic syndrome, particularly type 2 diabetes and obesity (body mass index [BMI] ≥ 30 kg/m^2), which have a national prevalence of 10.2%[5] and 39.6%,[6] respectively. Given the increase in the incidence of both diabetes and obesity in the United States, as well as increased mortality associated with NAFLD progression,[7] there is heightened awareness of the impact of NAFLD in the medical community.[5,89]

The medical community has learned a tremendous amount about NAFLD over the last two decades.

Abbreviations: ALT, alanine aminotransferase; BMI, body mass index; CART, classification and regression analysis; FDA, Food and Drug Administration; FIB-4, Fibrosis-4 index; NAFL, nonalcoholic fatty liver; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; NFS, NAFLD fibrosis score; VCTE, vibration-controlled transient elastography.

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However, much of what is known about the natural history of NAFLD has been inferred from cohorts seen in academic centers, with liver biopsies that are subject to inherent selection and detection bias, or from administrative claims databases that lack granularity of clinical data. Data from solely biopsy-confirmed cohorts may be subject to a biased representation of liver enzymes levels that are poor predictors of disease severity, and socio-economic bias around access to care, insurance, age, and comorbid conditions. Therefore, there is a critical unmet need to identify which patients with suspected NAFLD undergo liver biopsy and what types of patients are diagnosed on clinical grounds alone.

Additionally, clinical trials investigating therapeutic agents for NAFLD are ongoing, with multiple products either in phase 2 or 3 trials. These trials enrolled highly selected patients with relatively few comorbid conditions, and results of these trials may not be generalizable to a real-world population of patients with NAFLD.[10] Accordingly, there is an unmet need for studies describing NAFLD in the real-world setting (i.e., regular practice, outside of clinical trials), from community and academic centers alike, where pragmatic clinical and noninvasive methods for the diagnosis of NAFLD, NASH, and disease severity are often used.

Thus, to complement the existing knowledge base, TARGET-NASH was designed to follow the course of patients with NAFLD over time in clinical practice at hepatology, gastroenterology and endocrinology practices, and to provide real-world data on the clinical effectiveness of NAFLD therapeutics

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once available. The aim of this study is to distinguish characteristics of patients whose diagnosis was established using clinical criteria compared with liver histology, and to describe the clinical characteristics of patients diagnosed with NAFLD in a real-world setting.

Materials and Methods

COHORT

TARGET-NASH is a longitudinal observational cohort of pediatric and adult patients with NAFLD managed at academic (i.e., teaching hospitals/universities) and community (i.e., private practice) sites in the United States, representing hepatology, gastroenterology, and endocrinology practices. At the date of enrollment, consented patients provided access to their medical records for 3 years before the date of enrollment, and were then followed prospectively for at least 5 years. Clinical information from the electronic medical records, including patient narratives, laboratory results, pathology reports, and imaging data, were extracted and uploaded into a secured database. Specifically, demographic data, patient comorbidities, concomitant medications, interventions for NAFLD, and liver-disease progression were recorded in the database as well as adverse outcomes. Approvals from central or local institutional review boards were obtained before subject enrollment. Full details of the study methodology have been previously described.[11] This analysis included patients ≥18 years old enrolled in TARGET-NASH between August 1, 2016, and March 4, 2019. Patients enrolled in interventional clinical trials were excluded.

LIVER DISEASE CASE DEFINITION

All patients in the TARGET-NASH cohort had a diagnosis of NAFLD at enrollment by their treating physician. Method of diagnosis was left to the judgment of the treating physician, to reflect how patients are defined as NAFLD in usual clinical practice. Participants in TARGET-NASH were classified following enrollment as having NAFL, NASH, or NAFLD cirrhosis, either by biopsy if one was available or according to clinical criteria defined by the TARGET-NASH protocol.[11] Specifically, NAFL was defined as the presence of hepatic steatosis not attributable to other causes (e.g., alcohol consumption) and without evidence of biochemical or histological inflammation. NASH was defined as the presence of steatohepatitis on biopsy or both steatosis and elevated alanine aminotransferase (ALT) levels in a patient with obesity, type 2 diabetes, dyslipidemia, or metabolic syndrome. A diagnosis of cirrhosis was based on clinical determination (i.e., before clinical decompensation events, laboratory assessments, imaging) and/or liver biopsy. These criteria were selected with an emphasis on pragmatic, inexpensive, readily available patient information. Fibrosis-4 index (FIB-4) scores were calculated retrospectively with available data. Diagnostic criteria are listed in Supporting Table S1 and were previously described.[11,12]

STATISTICAL ANALYSIS

Differences in proportions and means of patient characteristics by severity of liver disease and biopsy status (NAFL, NASH, NASH cirrhosis) were compared using a chi-squared test and analysis of variance, respectively. Bivariate comparisons and relevant clinical variables informed a traditional logistic regression model with standard stepwise elimination to find the odds of predicting which patient characteristics influenced the chance of liver biopsy. This analysis was limited to biopsies performed within 1 year of enrollment, to ensure that all relevant clinical variables were contemporaneous and within the same prevalent cross-sectional window. The comparator group had never had a liver biopsy documented in the medical record.

Further predictive analysis was performed to find patient phenotypes (i.e., multiple patient characteristics that grouped together rather than single independent patient attributes) that influenced liver biopsy and histological diagnosis of NAFLD. This was performed using tree ensemble classification methods (e.g., classification and regression analysis [CART], boosted trees). Tree ensemble methods are a data-driven nonparametric approach estimating the association between independent variables and the predicted probability of a given outcome. Compared with regression analyses, which estimate an average effect of an outcome, this method allows for the

estimation of the outcome of interest by subgroups of patient characteristics.[13] Observations with a similar probability of the outcome are partitioned into nodes or branches of the classification tree using a learning algorithm.[14,15]

Boosted trees were used to estimate the importance of patient characteristics in predicting whether a patient received a biopsy. Boosted trees repeatedly apply the classification algorithm over multiple classification trees using the error in the previous tree to inform the following classification tree.[16] The boosted tree algorithm included 100 trees with three splits per tree. For all analyses, race, presence of comorbid disease, and clinical indicators of liver disease were modeled as categorical variables. Laboratory values (ALT, alkaline phosphatase, total bilirubin, albumin), BMI, and age were examined as continuous variables. All analyses were performed in SAS (version 9.4) and JMP Pro (SAS Institute, Inc., Cary, NC).

Results

OVERALL REAL-WORLD COHORT

A total of 3,474 patients ≥18 years of age were enrolled in TARGET-NASH from August 2016 to March 2019. The median age was 59 years, 59% were female, and 75% were White. The study population was stratified by severity of liver disease into patients with NAFL (n = 1,052, 30%), NASH (n = 1,293, 37%), and NAFLD cirrhosis (n = 1,129, 33%). Overall, 66% of the study population were obese (BMI ≥ 30 kg/m^2), with a median BMI of 32 kg/m^2. Mean BMI increased with increasing severity of liver disease from 31 kg/m^2 among patients with NAFL to 35 kg/m^2 among patients with cirrhosis (P < 0.0001). More than 50% of the cohort had a history of diabetes, hypertension, or hyperlipidemia at enrollment. The prevalence of features of the metabolic syndrome increased with increasing severity of liver disease. Patients with cirrhosis were 43% more likely to have a history of diabetes at enrollment, and 24% more likely to have a history of hypertension compared to patients with NAFL (P < 0.0001) (Table 1).

The proportion of patients with a history of cardiovascular disease (19%), depression or use of antidepressant medications (39%), and osteoarthritis (10%) also increased significantly with progressive disease severity (P < 0.0001). Patients with NAFLD cirrhosis were 1.67 times more likely to be depressed than patients with NAFL (Table 1).

Less than 10% of patients had documented use of pharmaceutical interventions (vitamin E, ursodeoxycholic acid, pioglitazone, and liraglutide) that have been used for the treatment of NAFLD. Antidiabetic medications were used by 145 (4%) patients who did not have a diagnosis of diabetes. Other disease states that might use an antidiabetic medication like polycystic ovarian syndrome (PCOS), which made up <2% of the cohort, were uncommon. Use of potentially anti-NASH interventions varied across severity of liver disease.

PATIENTS WITH A LIVER BIOPSY

Overall, 33% (n = 1,141) of patients had a liver biopsy in the 3-year period before enrollment. Among patients who had undergone a biopsy, 40% (n = 455) of patients had the procedure within 1 year of their enrollment date. Of patients with a biopsy within 1 year, 171 (38%) had cirrhosis, mean (SD) FIB-4 score of 3.2 (2.1), and 260 (57%) had NASH mean (SD) FIB-4 score of 1.4 (1.0). In bivariate comparisons of patients with a recent biopsy versus those who were never biopsied, patients with a histologic diagnosis of NAFLD were younger, female, White, with features of the metabolic syndrome. Mental health diagnoses (e.g., anxiety, depression) were also more common among patients with a biopsy, as were autoimmune and rheumatologic diagnoses (Table 2).

PATIENTS WITHOUT A LIVER BIOPSY

In total, 766 patients were diagnosed with NASH based on clinical criteria. Overall, 100% met the abnormal ALT and steatosis on imaging criteria as part of the diagnostic criteria. The following secondary indicators (patients may have more than one) were present: BMI ≥ 30 kg/m^2 569 (74%), type 2 diabetes 322 (42%), and dyslipidemia 413 (54%). These patients had a mean (SD) FIB-4 score of 1.3 (1.2). Cirrhosis was diagnosed on clinical grounds in 597 patients according to criteria in Supporting Table S1. Vibration-controlled transient elastography (VCTE) criteria and/or secondary clinical criteria were VCTE stiffness ≥16 kPa (83 [13.9%]) and VCTE stiffness between 12.5 and 16 kPa and at least one secondary

TABLE 1. DEMOGRAPHIC AND CLINICAL CHARACTERISTICS OF ADULTS WITH NAFLD IN THE TARGET-NASH COHORT

	Severity of Liver Disease*				
Patient Attribute	NAFL (n = 1,052 [30.2%])	NASH (n = 1,293 [37.2%])	NAFLD Cirrhosis (n = 1,129 [32.5%])	All Participants (n = 3,474)	*P* Value
Age at study entry (years)					
Median (n)	58.0 (1,051)	56.0 (1,293)	62.0 (1,129)	59.0 (3,473)	<0.0001
Mean (SD)	56.8 (13.6)	53.4 (13.8)	61.1 (9.8)	56.9 (13.0)	
Sex, n (%)					
Female	557 (52.9)	826 (63.9)	664 (58.8)	2,047 (58.9)	<0.0001
Race, n (%)					
White	617 (58.7)	965 (74.6)	1,006 (89.1)	2,588 (74.5)	<0.0001
Black or African American	74 (7.0)	88 (6.8)	21 (1.9)	183 (5.3)	
Asian	266 (25.3)	130 (10.1)	30 (2.7)	426 (12.3)	
Ethnicity, n (%)					
Hispanic or Latino	121 (11.5)	166 (12.8)	139 (12.3)	426 (12.3)	0.35
Not Hispanic or Latino	881 (83.8)	1,078 (83.4)	956 (84.6)	2,915 (84.0)	
BMI (kg/m^2) at enrollment†					
Median (n)	29.0 (1017)	33.0 (1270)	34.0 (1107)	32.0 (3394)	<0.0001
Mean (SD)	30.9 (7.3)	33.9 (7.4)	34.7 (7.5)	33.3 (7.6)	
Diabetes, n (%)					
Yes	333 (31.7)	605 (46.8)	811 (71.8)	1,749 (50.3)	<0.0001
Hypertension, n (%)					
Yes	618 (58.7)	840 (65.0)	983 (87.1)	2,441 (70.3)	<0.0001
Hyperlipidemia, n (%)					
Yes	542 (51.5)	873 (67.5)	719 (63.7)	2,134 (61.4)	<0.0001
Cardiovascular disease, n (%)					
Yes	162 (15.4)	201 (15.5	280 (24.8)	643 (18.5)	<0.0001
Mental health diagnosis, n (%)					
Yes	404 (38.4)	644 (49.8)	655 (58.0)	1,703 (49.0)	<0.0001
Osteoarthritis, n (%)					
Yes	65 (6.2)	135 (10.4)	156 (13.8)	356 (10.2)	<0.0001
Autoimmune/rheumatologic, n (%)					
Yes	58 (5.5)	101 (7.8)	79 (7.0)	238 (6.9)	0.09
≥1 abdominal imaging study‡	777 (73.9)	1,176 (91.0)	1,074 (95.1)	3,027 (87.1)	<0.0001
Enrolling center type					<0.0001
Academic	471 (44.8)	799 (61.8)	889 (78.7)	2,159 (62.1)	
Community	581 (55.2)	494 (38.2)	240 (21.3)	1,315 (37.9)	
Enrolling center specialty					0.0472
Gastroenterology/hepatology	1,024 (97.3)	1,274 (98.5)	1,113 (98.6)	3,411 (98.2)	
Endocrinology	28 (2.7)	19 (1.5)	16 (1.4)	63 (1.8)	

*Severity of liver disease at the time of enrollment.
†BMI calculated based on the most recent height and weight measurement up to or at the time of enrollment. Some cells may not add to 100, as very small proportions were excluded for readability.
‡Inclusive of magnetic resonance imaging, computed tomography, or ultrasound.

indicator (14 [2.3%]). A total of 540 (91%) patients met at least two secondary indicators: (1) evidence of ascites on imaging or paracentesis performed (231 [40%]); (2) evidence of portal hypertension on any imaging study (361 [63%]); (3) any varices or portal gastropathy noted on EGD (264 [66%]); (4) platelet count below 140,000 (441 [76%]); (5) cirrhosis noted as present or possible on any imaging study (509 [88%]); and (6) splenomegaly noted as present on any imaging study (240 [51%]) (Supporting Table S2).

TABLE 2. PATIENT ATTRIBUTES OF ADULTS WITH NAFLD WITH A LIVER BIOPSY WITHIN 1 YEAR OF ENROLLMENT*

Patient Attribute‡	Liver Biopsy (n = 455)	No Liver Biopsy (n = 2,333)	*P* Value
Age at study entry (years)			
Median (n)	56 (455)	59 (2332)	<0.0001
Mean (SD)	53.5 (13.4)	57.3 (13.3)	
Sex, n (%)			
Female	284 (62.4)	1,327 (56.9)	0.03
Race, n (%)			
White	381 (83.7)	1,642 (70.4)	<0.0001
Black or African American	30 (6.6)	119 (5.1)	
Asian	15 (3.3)	365 (15.6)	
Ethnicity, n (%)			
Hispanic or Latino	68 (15.0)	279 (12.0)	0.07
Not Hispanic or Latino	371 (81.7)	1,956 (83.9)	
BMI (kg/m^2) at enrollment†			
Median (n)	33.0 (448)	33.0 (2,179)	0.0032
Mean (SD)	34.6 (8.4)	33.5 (7.7)	
Diabetes, n (%)			
Yes	250 (54.9)	1,105 (47.4)	0.0007
Dyslipidemia, n (%)			
Yes	286 (62.9)	1,390 (59.6)	0.01
Mental health diagnosis, n (%)			
Yes	247 (54.3)	1,065 (45.6)	<0.0001
Osteoarthritis, n (%)			
Yes	60 (13.2)	216 (9.3)	0.01
Sleep apnea, n (%)			
Yes	116 (25.5)	461 (19.8)	0.0030
Autoimmune/rheumatologic, n (%)			
Yes	40 (8.8)	125 (5.4)	0.0045
FIB-4 score			
Median (n)	1.59 (404)	1.42 (2033)	0.0033
ALT (IU/L)			
Median (n)	51.0 (429)	33.0 (2,246)	<0.0001
Mean (SD)	73.0 (72.1)	43.6 (35.0)	
ALP (IU/L)			
Median (n)	81.0 (423)	81.0 (2,221)	0.3086
Mean (SD)	95.5 (47.8)	92.3 (49.5)	
Total bilirubin (mg/dL)			
Median (n)	0.6 (426)	0.6 (2,221)	0.05
Mean (SD)	0.7 (0.8)	0.8 (0.9)	
Albumin (g/dL)			
Median (n)	4.3 (415)	4.2 (2,196)	0.0008
Mean (SD)	4.2 (0.5)	4.1 (0.6)	
Platelets (10^3/uL)			
Median (n)	211 (409)	211 (2,119)	0.12
Mean (SD)	216 (79.7)	207 (91.0)	

Note: Analysis excludes 686 patients who had a biopsy more than 1 year before enrollment. Academic centers included those institutions with a teaching component; centers not meeting this criterion were considered community.
*Index date: For participants with no biopsy, outcome variables are assessed at the time of enrollment. This group had no biopsy at any time in any available records. For participants with a biopsy, outcome variables are assessed at the time of biopsy.
†BMI calculated based on the most recent height and weight measurement up to or at the time of the index date.
‡History of comorbid disease or medications were indication of disease before or at the time of the index date. Lab values and results from the imaging or scan were within 6 months before or after the index date. Multiple other clinical variables were analyzed but excluded from the table for readability.
Abbreviation: ALP, alkaline phosphatase.

Patients with cirrhosis diagnosed on clinical grounds had a mean (SD) FIB-4 score of 6.0 (5.6).

PREDICTORS OF LIVER BIOPSY

A multivariable model was created to show independent predictors of liver biopsy. Patients were 1.5% less likely to have a biopsy with each year of advancing age, or 15% less likely per decade. Men were 26% less likely to have a biopsy, and non-White patients were 51% less likely to have a biopsy. Among the features of the metabolic syndrome (hyperlipidemia, diabetes, hypertension, and obesity), diabetes was the only independent patient attribute influencing the odds of having had a biopsy, increasing the odds by 43%. ALT elevation, as measured closest to the time of biopsy, increased the odds of biopsy by approximately 1% per point or 14% per 10-point increase (Table 3).

In CART analysis, the patient phenotypes that had the lowest probability of undergoing liver biopsy to confirm diagnosis of NALFD or assess disease severity were non-White patients with ALT <69 IU/mL (6%). White patients with ALT between 29 and 69 IU/mL had only 21% probability of biopsy. This fell to 10% when the ALT was <29 IU/mL. In boosted tree analysis, ALT was the most important single variable in determining who had a liver biopsy.

TABLE 3. INDEPENDENT PREDICTORS OF HAVING HAD A BIOPSY DIAGNOSIS OF NAFLD

Attribute	Odds Ratio	95% Confidence Interval
Age	**0.985**	**0.974, 0.995**
Sex (male vs. female)	**0.743**	**0.560, 0.985**
Race (non-White vs. White)	**0.483**	**0.329, 0.709**
Type 2 diabetes	**1.432**	**1.073, 1.911**
Hyperlipidemia	1.256	0.928, 1.702
Anxiety/depression	**1.576**	**1.191, 2.086**
Autoimmune/rheumatologic conditions	1.502	0.925, 2.437
ALT	**1.014**	**1.011, 1.017**
Albumin	1.246	0.971, 1.600

Note: A forward, step-wise logistic regression model was fit (significance level for entry = 0.25; significance level to remain in the model = 0.1) with the following predictors of liver biopsy: age, sex, race (White vs. non-White), BMI, type 2 diabetes, hyperlipidemia, depression/anxiety, osteoarthritis, sleep apnea, autoimmune/rheumatologic conditions, thrombocytopenia, ALT, total bilirubin, albumin, and evidence of ascites from imaging or paracentesis. Final model n = 288 biopsy within 1 year of enrollment; n = 1,709 no biopsy ever. Bold faced values are statistically significant.

Discussion

The TARGET-NASH cohort represents a large real-world longitudinal observational study of patients with NAFLD followed in clinical practice in the United States. Patients were included based on real-world diagnosis of NAFLD by their treating provider. Disease severity was determined by biopsy findings where available or with pragmatic clinical case definitions. Herein are the cross-sectional data collected at time of enrollment from over 3,400 patients. In the entire cohort, 70%, had advanced disease with either NASH or NAFLD cirrhosis. Among those with a biopsy within the year before enrollment, 79% had advanced disease. As shown in cohorts in academic practices, features of the metabolic syndrome (obesity, diabetes, dyslipidemia, and hypertension) were prevalent in this population and increased in frequency as liver disease progressed. Cardiovascular disease, osteoarthritis, and depression were common comorbid conditions, illuminating the challenges of treating patients with NAFLD with diet and exercise alone.

Currently, there are no Food and Drug Administration (FDA)–approved medications for the treatment of NASH. Although intent cannot always be derived from chart review, this study does allow insight into the frequency with which some medications with potentially anti-NASH properties are used in clinical practice. Evidence or opinions exist for the use of vitamin E,[17] pioglitazone,[17] liraglutide,[18] ursodeoxycholic acid,[19] and metformin[20] in the treatment of NASH.[1] These medications are likely prescribed for the *a priori* treatment of NASH in the case of vitamin E and ursodeoxycholic acid, and potentially among the 4% of patients taking antidiabetic medications without a clear diagnosis of diabetes. Some of these medications, like metformin, could have alternate intent, such as the treatment of prediabetes or PCOS. Overall, however, these off-label medications for NASH are not widely adopted in this cohort (a finding similar to an analysis at academic centers[21]), nor is there a clear correlation of use by disease severity.

Patient characteristics that led to biopsy in real-world practice included elevated ALT values and type 2 diabetes. Other patient attributes that led to biopsy were female sex and comorbid mental health disorders like anxiety and depression. Although not reaching statistical significance as an independent predictor, autoimmune/rheumatologic disease was significant in bivariate comparisons. Psychiatric medications and/or immune-mediated disease may influence the decision process to obtain a liver biopsy due to a concern for autoimmune hepatitis or potential drug-induced liver injury. These findings are generally in accord with the American Association for the Study of Liver Diseases practice guidelines for liver biopsy in patients with an elevated ALT or suspected NAFLD.[1] Overall, however, it is notable that only a minority of patients diagnosed with NAFLD under real-world conditions had a liver biopsy. Once FDA-approved medications are available, patients may be treated on the basis of noninvasive clinical criteria rather than having a histologic diagnosis of NAFLD/NASH. Therefore, understanding differences in these populations becomes important, as responses to therapy may vary in real-world populations compared with the well-defined cohorts of patients recruited into clinical trials. Equally important will be the ability to identify real-world patients with similar characteristics as those in clinical trials, without resorting to liver biopsy.

Data gleaned from industry-sponsored clinical trials or biopsy-driven studies like the National Institutes of Health NASH Clinical Research Network (CRN) have greatly advanced the knowledge and understanding of the natural history of NAFLD. A cohort

such as TARGET-NASH is complementary to these efforts because of its analysis of patients who either do not participate in clinical trials or in populations that are not routinely biopsied. In this study, two different analyses showed predictors for patients having a histologic diagnosis of NAFLD. Viewed comparatively, this study shows independent patient attributes that were associated with a *decreased* odds of having had liver biopsy, such as older patients, non-White patients, men, and those with lower ALT values. The parallel CART analysis defines patient phenotypes and confirms that non-White patients with an ALT <69 IU/mL are the least likely to be biopsied, with a 6% probability, and that any patient—regardless of race–with ALT level <1.5 × upper limit of normal is infrequently diagnosed by biopsy, with a probability that ranges between 10% and 21%. It is well established that ALT is a suboptimal surrogate for disease severity. As many as 25% of patients with NAFLD and 19% of patients with NASH may have a normal ALT,[22] yet ALT was the dominant variable that determined who was biopsied in real-world clinical practice. How a normal ALT is defined and how a normal ALT range may vary across different laboratories may play a role in its utility as a diagnostic tool as well.[23-25] Ideally, information learned from the TARGET-NASH cohort will confirm and add to what has been learned by the NASH CRN and others, and that in clinical trials, efficacy does correlate to real-world clinical effectiveness.

The strengths of this study are its real-world setting and the large sample size. These data also contain granularity of clinical data that are lacking from larger health care claims databases. A real-world setting with many patients diagnosed using clinical criteria will reflect how most patients with NAFLD are diagnosed in the future and how treatment decisions may be made once FDA-approved pharmacotherapy is available. This study also identifies the work that needs to be undertaken in clinical practice to proactively identify patients with NAFLD and significant fibrosis, as these are the patients for whom pharmacotherapy will likely be targeted. The large number of study participants also allows for detailed analyses of niche patient populations not included in clinical trials due to disease severity, comorbid conditions, or contraindicated medications.

Limitations that may apply to other large studies of disease states in administrative billing databases do not necessarily apply to this study. Misclassification of diagnoses and patient data may be present, but is minimized by using direct review of medical records in 100% of study participants, and expert adjudication of diagnoses and histological data rather than relying on International Classification of Diseases 9/10 or administrative billing codes. Misclassification may occur in determining which patients had a biopsy. Those included in the no-biopsy group had no liver biopsy in the 3 years before enrollment, nor any comment on prior liver biopsy in the medical record; therefore, a distant, undocumented biopsy in the past is an unlikely possibility. Incomplete data capture or patient loss of follow-up may also contribute to misclassification; however, this is expected to be a nondifferential bias. This study was also set forth with the *a priori* goal of studying NAFLD in a real-world setting with attention to detail in regard to NAFLD-specific variables, rather than data collected from a large cohort of patients in which NALFD was not the main focus.

Other limitations should be considered. Health care provider intent cannot always be determined from the medical record. There are always undocumented patient and provider factors that may influence the decision to perform a liver biopsy or pursue a specific course of therapy. Among these are use of noninvasive assessments of disease severity such as clinical prediction scores. It is interesting to note that FIB-4 scores were lower among patients with biopsy-diagnosed cirrhosis (3.28) compared with clinical cirrhosis (4.53); perhaps this was because the diagnosis of cirrhosis was less obvious. Although we can report scores *post hoc* from patients who had a biopsy and from those who did not, we cannot determine whether these scores were ever calculated by providers or used to inform diagnosis and management; thus, these scores were not used in predicative modeling. Patients in this study were mostly Caucasian (75%), and while representing community and academic practices, were limited to primarily gastrointestinal and hepatology practices rather than primary care. As the study relied on provider diagnosis of NAFLD, this may reduce misclassification, but real-world primary care diagnosis and treatment of NAFLD is deserving of future study. Finally, in an effort to devise pragmatic diagnostic criteria for NASH, the study uses clinical definitions for disease from a panel of experts that has not yet been validated.

In summary, patients with NAFLD included in the TARGET-NASH study commonly had advanced disease, yet only a minority had had a liver biopsy.

Patients had the expected metabolic syndrome comorbid conditions that become more prevalent as liver disease severity advances. Important findings from this real-world cohort include additional comorbid conditions that might limit current therapies for NAFLD. The high frequency of cardiovascular disease, osteoarthritis, and rheumatologic disease may make exercise interventions difficult. The high prevalence of mental health diagnoses, such as depression and anxiety, likely limits adherence to dietary interventions. The *ad hoc* method for liver biopsy in the real world underscores the need for better systematic methods of diagnosis and assessments of disease severity, especially as new interventions for NASH become available. These data provide context for the selection bias that may be present in many registries and randomized controlled trials of therapies for NAFLD, where biopsy is required for inclusion. Liver biopsy is not widely prevalent in clinical practice for NAFLD management, especially among older, non-White, men with near-normal or normal ALT levels.

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