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Smoking and Severity of Hepatic Fibrosis in Nonalcoholic Fatty Liver Disease†

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Abstract

Background—Although many predictors of disease severity of nonalcoholic fatty liver disease (NAFLD) have been proposed, studies of the potential effects of specific environmental exposures on human NAFLD are lacking. Smoking increases insulin resistance. Given the pathophysiological role of insulin resistance in NAFLD, characterization of the influence of smoking in NAFLD is warranted.

Aim—To study the potential association between cigarette smoking and advanced fibrosis in NAFLD.

Methods—All adults enrolled in the NASH CRN studies between October 2004 and February 2008 who had liver biopsies were included (n=1091). Advanced fibrosis was defined as stages 3-4. Analyses were performed.

Results—Significant bivariate associations were demonstrated between advanced fibrosis and age, gender, ethnicity, diabetes, and smoking history. History of smoking >=10 pack-years was more common ($p<0.0001$) among patients with advanced fibrosis. Multivariate analysis demonstrated an association between smoking history of >=10 pack-years and advanced fibrosis (OR=1.63). Among non-diabetics, history of >=10 pack-years was associated with an OR of 2.48 for advanced fibrosis. High frequencies of advanced fibrosis were observed among diabetics (with or without >=10 pack-years history) and non-diabetics with >=10 pack-years history as compared to non-diabetics without significant smoking history.

Conclusions—Smoking history was associated with advanced liver fibrosis in this large multicenter cohort of NAFLD patients. The results indicate that smoking may enhance the progression of NAFLD partly through its effect on insulin resistance. Our results are consistent with recent animal studies suggesting that cigarette smoke may aggravate fatty liver. To our knowledge, this is the first study to show that cigarette smoking is associated with increased

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fibrosis severity in human NALFD, suggesting it may accelerate disease progression. These results may support a formal recommendation of smoking cessation in patients with NAFLD.

Keywords

Smoking; alcohol; nonalcoholic fatty liver disease; liver fibrosis; predictors

Introduction

Several factors have been associated with advanced histological disease in patients with chronic liver disease. Studies of factors associated with a higher likelihood of advanced liver fibrosis at the time of evaluation in nonalcoholic fatty liver disease (NAFLD) have predominantly focused on the assessment of demographic characteristics, anthropomorphic measurements, co-morbidities, and biochemical data. In this regard, there is evidence to support that age, obesity and type 2 diabetes (DM) are associated with liver fibrosis severity in NAFLD [1-4]. However, very limited data are available regarding the potential influence of specific exposures such as cigarette smoking on the course and severity of NAFLD.

The crucial role of insulin resistance in NAFLD is well recognized [5]. Several studies have demonstrated the association between insulin resistance and the severity of hepatic steatosis [6,7], necroinflammation [7], and fibrosis [7-9] in NAFLD. Consequently, environmental factors (such as smoking) which influence insulin activity may impact disease severity in NAFLD. The direct effects of smoking on insulin action have been demonstrated in studies with oral and intravenous glucose tolerance tests [10], and the euglycemic clamp technique [11]. Furthermore, epidemiological studies have shown that smoking appears to be a risk factor for glucose intolerance and DM [12,13] and that a dose response relationship may exist between smoking and incidence of diabetes [14]. In addition, an association between smoking and advanced liver fibrosis has been observed in certain chronic liver diseases including chronic hepatitis C [15-18] and primary biliary cirrhosis [19].

Therefore, the primary focus of this study was to assess the potential role of smoking on histological severity of disease in patients with NAFLD while specifically controlling for the potential roles of other relevant clinical, exposure, and socio-demographic variables including DM, BMI, gender, race, ethnicity, alcohol use history, household income, and age.

Patients and Methods

Research Design and Study Population

This study involved analysis of data collected as part of the multicenter Nonalcoholic Steatohepatitis Clinical Research Network (NASH CRN) [20]. The NASH CRN is sponsored by the National Institute of Diabetes & Digestive & Kidney Diseases (NIDDK). General information about the network and clinical, laboratory and histological observations in this well characterized patient cohort have been reported [20,21]. Extensive information is gathered in every subject including demographic characteristics, anthropomorphic measurements, smoking history, alcohol consumption history, medical history, medication use, laboratory tests and liver biopsy results. Among its goals, the NASH CRN seeks the generation of hypotheses that would lead to the use of database resources in order to further elucidate the pathogenesis of NAFLD and to identify determinants of disease progression and severity [20]. There were 1091 adult subjects enrolled in the NASH CRN studies between October 2004 and February 2008 who had liver biopsies. These 1091 subjects constitute the current study population. As of the date of this analysis, that number represents over 90% of the NASH CRN subjects with available liver biopsy.

NAFLD Diagnosis

Subjects have to meet specific criteria regarding the diagnoses of NAFLD in order to be enrolled in the observational Database study or adult treatment trial. Cirrhosis cases presumed to be due to NAFLD/NASH may be enrolled if there is documented absence of other potential etiologies of liver disease and clinical assessment of an almost certain NAFLD/NASH etiology. Patients with alcohol consumption of >140 g/ week (>70 g if female) in the 2 years prior to screening, or with suspected alcohol-related liver injury are excluded. In addition, other etiologies of chronic liver disease are carefully excluded. For the purposes of enrollment into the observational Database study, the diagnosis of NAFLD may be based on the histological diagnosis of NAFLD or cryptogenic cirrhosis as described above, or on imaging studies consistent with these. However, for this study, only subjects with available liver biopsy were included.

For the purposes of this study, NAFLD was defined based on the following criteria: 1. Histologic diagnosis of NAFLD or histologic diagnosis of cryptogenic cirrhosis; 2. Alcohol use history consistent with NAFLD as defined above; 3. Exclusion of liver disease of other etiologies including viral or autoimmune hepatitis, drug induced liver disease, and cholestatic or metabolic liver disease. These other potential etiologies were carefully investigated based on Database study specific criteria at screening.

Liver Biopsies

Liver biopsy slides stained with hematoxylin and eosin and Masson's trichrome were reviewed and scored centrally by NASH CRN pathologists. The stage of fibrosis was scored based on a Histologic Scoring System for NAFLD developed by Kleiner et al [21]. Advanced fibrosis was defined as stages 3 or 4.

Smoking History

A history of regular smoking was defined as having smoked ≥400 cigarettes (i.e., 20 packs) during the patient's lifetime or at least one cigarette a day for one year. Current smokers were defined as those who met the definition of regular smoking and that at the time of the interview reported smoking every day or some days. The amount of cigarettes smoked up to the time of study enrollment was quantified in pack-years (number of packs smoked per day multiplied by the number of years smoked). For the purposes of this study, a significant history of smoking was defined as a history of consumption of ≥ 10 pack-years.

Smoking history information was obtained from direct interview with each patient following this questionnaire:

1. Have you ever smoked tobacco cigarettes? Never (1), In the past but not anymore (2), Currently smokes cigarettes (3)
2. Did you smoke cigarettes regularly (“No” means less than 20 packs of cigarettes in a lifetime or less than 1 cigarette a day for one year)?
3. How old were you when you first started regular cigarette smoking?
4. How old were you when you (last) stopped smoking cigarettes *(code as “n” if you didn't stop smoking)?*
5. On the average of the entire time you smoked cigarettes, how many cigarettes did you smoke per day?

Other Variables

In addition, information regarding the following variables was obtained for analysis: Age, gender, ethnicity, body mass index (BMI), DM, intake of medications that may cause liver fibrosis, household income, and recruiting clinical center. Regarding alcohol use history, based on the NAFLD Database eligibility criteria, patients with a history of more than moderate alcohol consumption (>140 g/ week for males or >70 g/ week if female) in the 2 years prior to screening or with suspected alcohol-related liver injury are excluded from enrollment. Therefore, unless lifetime abstinence was reported, alcohol use history would be mild to moderate based on eligibility criteria. This definition is consistent with the consensus provided by the Dietary Guidelines for Americans, which defines moderate alcohol consumption as the consumption of up to two drinks per day for men and up to one drink per day for women [22]. Among study subjects, information regarding alcohol consumption was derived from the Lifetime Drinking History questionnaire. Patients were categorized based on their reported history of lifetime abstinence or not.

Statistical Analysis

The analysis used cross-sectional data obtained at the baseline visit of the prospectively enrolled study subjects. Bivariate analysis was performed to determine associations between smoking and other variables of interest and histological disease severity in patients with NAFLD. Student's t-test, or non-parametric tests where applicable, were used to compare continuous variables. Fisher's exact test was used to compare categorical variables. Significant smoking history and other variables that were found to be associated with advanced fibrosis by bivariate analysis as well as those considered potential confounders or of possible influence were included in forward and backward stepwise regression analyses. A significance level of $p=0.05$ was applied for retention and removal of the variables, respectively. The variables in the final logistic regression model were assessed for all 2-way interactions. Analysis of a possible dose-response relationship between pack-years and fibrosis stage was done. All statistical analyses were carried out by the Data Coordinating Center using the statistical analysis software SAS 8.0 (SAS Institute Inc., Cary, NC). Results are presented in tables with categorical variables presented as number and percentage and continuous variables presented as mean and standard deviation (SD).

Results

Patient Characteristics

Of 1091 patients with NAFLD, 687 (63%) were female. Mean age was 49±11.9 years. Most of the patients were white (77%) or Hispanic (11.3%); only a small portion of the subjects in this cohort (2.6%) were black. Significant smoking history of >=10 pack-years was reported by 257 (23.8%) subjects. A diagnosis of DM was present in 318 (29.2%) subjects in this cohort. Advanced liver fibrosis (stages 3-4) was present in 338 (31.3%) subjects. Steatohepatitis was present in 621 (56.9%) subjects and NAFLD Activity Score [21] was read centrally as >=5 in 511 (46.8%) subjects. These and other characteristics of the 1091 patients are summarized in Table 1.

Bivariate Analysis Results

As shown in Table 2, a significant smoking history was associated with advanced liver fibrosis by bivariate analysis. A significant bivariate association ($p=0.01$) was demonstrated between overall history of smoking (yes or no) and advanced liver fibrosis. Specifically, a history of smoking >=10 pack-years was significantly more frequent ($p<0.0001$) among patients with fibrosis stages 3-4 (32.3%) compared to those with fibrosis stages 0-2 (20.0%).

No association was demonstrated between current smoking status (current smoker vs. not) and liver fibrosis.

Table 2 shows further details regarding the differences between variables of interest according to early versus advanced stage of fibrosis. In addition to smoking history of >=10 pack-years, older age, female gender, non-hispanic ethnicity, DM, and lifetime alcohol history were significantly associated with advanced stage of disease on liver biopsy by bivariate analysis.

Table 3 shows a comparison between the characteristics of patients with a reported lifetime smoking history of >=10 pack years history versus others. Older age, Caucasian ethnicity, higher BMI, DM, history of alcohol use, and household income <15,000/year were more frequent among patients with history of >=10 pack years versus others by bivariate analysis. Advanced liver fibrosis was more frequent ($p<0.0001$) among patients with a history of >=10 pack years (42.6%) versus those without a smoking history of >=10 pack years (28.1%). No association was demonstrated between the presence of steatohepatitis or NAFLD activity score >=5 and smoking status or history. Further analysis comparing the 103 current smokers with other subjects in our patient cohort did not find an association between current smoking status and NAFLD activity score or its components of steatosis, inflammation, and ballooning (data not shown).

Multivariate Analysis Results

Variables associated with advanced fibrosis by bivariate analysis and others considered potential confounders were included in the multivariate analysis. These included age, gender, ethnicity, BMI, type 2 DM, history of >=10 pack-years, history of mild to moderate alcohol consumption (versus lifetime abstinence), and household income. As shown in Table 4, multivariate analysis demonstrated an association between smoking history of >=10 pack-years and advanced fibrosis, OR=1.63 (95% CI 1.19, 2.24). In addition, older age, DM, and alcohol use history were significant factors associated with advanced liver fibrosis in this cohort.

As shown in table 5, a significant interaction was demonstrated between diabetes and history of >=10 pack years ($p=0.0005$). Subsequent logistic regression stratifying for the relationship between pack years and fibrosis by presence or absence of diabetes demonstrated that smoking history of >=10 pack-years was associated with an OR of 2.48 (95% CI 1.68, 3.67) for advanced fibrosis, but only in non-diabetics. In order to scrutinize this observation further, further analysis of the rates of advanced fibrosis in the different groups regarding diabetes and smoking history was done. This demonstrated similar frequencies of advanced fibrosis among diabetics with >=10 pack-years history (45.0%) or without >=10 pack-years history (50.5%) and non-diabetics with >=10 pack-years history (41.2%), compared to a significantly lower frequency of advanced fibrosis among non-diabetics without significant smoking history (19.6%).

To investigate for possible gender-associated effects of smoking on liver fibrosis, the association between smoking history and advanced fibrosis was examined while stratifying by gender. Gender-stratified multivariate analysis did not demonstrate a gender related difference in the magnitude of the association between advanced liver fibrosis and history of smoking >=10 pack-years (data not shown).

Dose Response Relationship

Patients were categorized into one of four groups based on the number of pack-years smoked to investigate the existence of a dose response relationship. Table 6 shows the dose response relationship between pack-years and fibrosis stage ($p<0.0001$). To examine these

findings further, stratification by presence or absence of diabetes was done. Similar to the observations in multivariate analysis, among non-diabetics a dose response relationship between pack-years and fibrosis stage was clear ($p<0.0001$). However, the dose response relationship was not evident in diabetics, among whom advanced fibrosis stage was similarly frequent in all pack-years groups (Table 6).

Discussion

The results of this study demonstrate a significant association between smoking history and severity of liver fibrosis in a large cohort of 1091 subjects with biopsy-proven NAFLD. Previous studies have suggested a significant association between smoking history and severity of liver fibrosis in patients with other chronic liver diseases including hepatitis C and primary biliary cirrhosis [15-19]. The exact mechanism behind the increase in liver fibrosis induced by smoking in these liver conditions is unclear and may vary by disease, but potential factors have been proposed including enhancement of the pro-inflammatory cytokine environment in PBC [23,24], as well as possible induction of vascular endothelial growth factor cytokines and their receptors that may influence angiogenesis in hepatitis C leading to fibrosis progression (17).

The roles of insulin resistance, alterations in lipid metabolism, oxidative stress and pro-inflammatory cytokines in NAFLD are well recognized [25,26,27]. These and other factors, such as chronic hypoxia, may play a role in the smoking-related increased fibrosis in NAFLD. Although delineating the specific mechanisms underlying the association between smoking and liver fibrosis in NAFLD is beyond the scope of this manuscript, we discuss various possibilities.

Insulin resistance has been associated with the severity of hepatic steatosis [6,7], necroinflammation [7], and fibrosis [7-9] in NAFLD. Cigarette smoking has been shown to exert direct effects on insulin action in experimental studies [10,11]. Furthermore, epidemiological studies have shown that smoking appears to be a risk factor for glucose intolerance and DM development [12,13,14]. A recent cross-sectional study reported that subjects with NAFLD who smoked had a higher frequency of the metabolic syndrome compared to subjects with NAFLD who did not smoke [28]. Our results showed a significant interaction between history of smoking >=10 pack years and DM in a large population of subjects with NAFLD. There were similar high rates of advanced fibrosis among both diabetics (with or without smoking history) and non-diabetics with a smoking history, as compared to a low rate of advanced fibrosis among non-diabetics without significant smoking history. These results indicate that smoking may exacerbate NAFLD leading to more severe hepatic injury partly through an effect on insulin resistance. In this case, insulin resistance may be an intermediary risk factor. Accordingly, we observed that the effect of smoking as a predictive risk factor of advanced fibrosis in NAFLD is statistically evident among non-diabetics, but may have been obscured among diabetics because of confounding related to an intermediary variable effect.

This is further supported by the observation of an overall dose response relationship between pack years and fibrosis stage. After stratifying by presence of diabetes, this dose response relationship remained clearly evident among non-diabetic subjects. However, it was not seen when the analysis was limited to diabetic subjects only, among whom the presence of advanced fibrosis was similarly high across pack year groups.

Experimental data reporting that smoking worsens liver injury in NAFLD have recently emerged. A recent study demonstrated that exposure to cigarette smoke enhanced the liver steatosis elicited by a high fat diet in mice [29] via enhanced fatty acid synthesis through

inhibition of AMP-activated protein kinase phosphorilation in liver tissue. A previous study also in mice had reported increased hepatic steatosis in animals fed a high fat diet and exposed to cigarette smoke compared to those on a high fat diet only [30]. The observations from these animal studies suggest that alterations in lipid metabolism may also play a part the association between cigarette smoking and NAFLD.

Oxidative stress is a known mechanism of injury in NAFLD [31] Cigarette smoking is known to cause oxidative stress and have pro-inflammatory effects in other organs [32,33]. Bailey and collaborators recently reported that mice exposed to cigarette smoke and an ethanol-containing diet showed increased hepatic steatosis, inflammation, alpha smooth muscle actin, and collagen compared to mice on an ethanol containing diet alone [34]. In that study, cigarette smoke exposure was associated with increased CYP2E1, which is linked to the mechanism of oxidative injury in NASH [35]. A novel report by Azzalini and collaborators provides further evidence supportive of the concept that smoking exacerbates histological injury in NAFLD. In that study, obese Zucker rats exposed to cigarette smoke developed increased hepatocellular ballooning, lobular inflammation, up-regulated expression of genes involved in fibrogenesis, and increased oxidative stress compared to non-smoker and control rats [36].

Chronic hypoxia may also play a role in the smoking related increased liver fibrosis in NAFLD. Cigarette smoking is associated with hypoxia and this may enhance the known susceptibility of acinar zone 3 to relative ischemia. One study in mice showed that cigarette smoke exposure was associated with a greater increase in hypoxia inducible factors [34], which are responsible for cellular adaptation to oxygen deprivation and activate the transcription of target genes such as vascular endothelial growth factor. In an animal model of liver fibrosis, it has been shown that hypoxia inducible factor 1 alpha regulates the production of critical pro-fibrotic mediators during the development of liver fibrosis [37]. Another study showed that chronic intermittent hypoxia in mice on a high fat diet led to hepatic inflammation, extensive collagen deposition, and increases in lipid peroxidation and pro-inflammatory cytokines in liver tissue, which were not evident in control mice [38].

Tumor necrosis factor alpha, interferon-gamma and other pro-inflammatory cytokines play a key role in the pathogenesis and progression of NAFLD. It has been reported in human studies that smoking influences the cytokine milieu and increases levels of pro-inflammatory cytokines in the peripheral blood of smokers compared to nonsmokers [39]. In a mouse model, chronic intermittent hypoxia increased levels of proinflammatory cytokines in liver tissue including interleukin-1beta, interleukin-6, and tumor necrosis factor alpha [40]. Gender-related differences in immune response are also recognized. Interestingly, pro-inflammatory cytokines appear to be higher among female smokers compared to male smokers and to male and female nonsmokers [40]. Because of this, we performed a stratified analysis by gender, but we did not demonstrate any gender based differences in the association between smoking and liver fibrosis.

Based on the available experimental data, it is apparent that several factors may play a role in the smoking-related increased fibrosis in NAFLD. Future studies are needed to better characterize the mechanistic pathways involved in the smoking related liver fibrosis of human NAFLD.

Interestingly, although an association between liver fibrosis and smoking history was shown, no association was demonstrated between NASH and smoking history or current smoking. This was relatively surprising since it is well known that the presence of NASH is associated with liver fibrosis in NAFLD. A possible explanation is that the timing of the exposure may be important and a link between smoking and NASH may not be seen among patients with a

significant past history of smoking if they have not smoked for a long time. In our cohort, only 9.5% of the patients were current smokers whereas 28.5% had a history of smoking. A possible association with NASH may be found in a sample containing a higher proportion of current smokers.

The associations between older age and DM with liver fibrosis progression in NAFLD have been previously recognized and are supported by evidence generated by longitudinal paired biopsy studies and by cross-sectional studies [1-4,9]. History of mild to moderate alcohol consumption (versus lifetime abstinence) was associated with a lower risk of advanced liver fibrosis in this study. This interesting observation warrants further study. Mechanisms behind it may include alcohol related enhancement of insulin sensitivity, as it is known that mild to moderate alcohol use reduces insulin resistance and decreases the risk of diabetes [41] and the metabolic syndrome [42]. Another possible mechanism is the anti-inflammatory action of mild to moderate alcohol consumption [43]. Although the benefits of light to moderate alcohol consumption related to a lower risk of coronary artery disease related events are well recognized [44,45], the effects of mild to moderate alcohol consumption on the liver are less clear.

Our study also made pertinent observations regarding race or ethnicity and NAFLD. The small number of African Americans in our cohort of patients with NAFLD is consistent with recently reported variations in the prevalence of NAFLD in different racial or ethnic groups [46,47]. These findings do not appear to be due to under-recognition and under-referral and may indicate differences in genetic susceptibility [48]. Although population based studies [46,49] observed an increased prevalence of NAFLD among Hispanic patients, an increased number of Hispanic subjects with NAFLD or increased NAFLD severity related to Hispanic ethnicity was not observed in the current study of adult patients with NAFLD.

Specific strengths of this cross-sectional study include the prospective data collection. In addition, the multi-center design has allowed the enrollment of a very large number of subjects, which gives further strength to our findings, and also ensures the broader generalizability of our observations. Exclusion of other possible etiologies of liver disease was required and comprehensive in the study population. Furthermore, the diagnosis of NAFLD in this cohort was well documented by histology and this is a fundamental advantage compared to other studies that have used suggestive criteria for NAFLD diagnosis such as imaging studies or transaminases levels. In addition, all liver biopsies in this study were centrally reviewed by NASH CRN pathologists with specific expertise in NAFLD and with Histologic Scoring System for NAFLD developed by Kleiner et al [21].

We acknowledge that our study also has limitations. The study population might have been affected by referral and access biases or design limitations. However based on the large size and geographical span of the sample, it is reasonable to infer that the conclusions made regarding predictors of advanced fibrosis in NAFLD are applicable to patients with NAFLD in general. By design limitations we refer to selection bias that may lead to over-representation of the “healthier” or self-motivated end of affected subjects within the with NAFLD disease spectrum; and to the fact that the clinical definition of NAFLD excluding excessive alcohol use prohibits us to investigate the full spectrum of alcohol consumption compared to a population based study with advanced fibrosis as the outcome. The information regarding smoking history and alcohol consumption was obtained by patient interview and self report and this has inherent limitations. However, smoking history was obtained by direct patient interview with utilization of a carefully designed comprehensive questionnaire. The instrument used to obtain the alcohol consumption history was the Lifetime Drinking History questionnaire, a validated questionnaire with tested reliability and that was specifically designed to enhance recall. Therefore, the use of this specific

instrument to obtain lifetime alcohol consumption history may actually be considered a strength compared to other studies. Although exact quantitative alcohol intake was not categorized other than abstinent versus non abstinent, the rigorous entry criteria results in indirect quantification of alcohol intake; that being essentially equivalent to up to 1 drink daily for women and 1 to 2 drinks daily for men among non lifetime abstinents.

In summary, a statistically significant association between smoking and advanced liver fibrosis in NAFLD was demonstrated in this large multicenter cohort of NAFLD patients. Our results indicate that smoking may influence the progression of NAFLD partly through its effect on insulin resistance although several mechanistic pathways are likely involved. Age, DM and lifetime alcohol abstinence were also independently associated with advanced fibrosis in this cohort of patients with NAFLD. The risk of advanced fibrosis increased with presence of DM and with older age. In contrast, history of mild to moderate alcohol consumption (versus lifetime abstinence) was associated with a lower risk of advanced fibrosis. The association between lifetime abstinence and advanced liver fibrosis in patients with NAFLD warrants further study.

To our knowledge, this is the first study to show that smoking may accelerate the progression of human NALFD. Recent experimental animal data suggesting that cigarette smoking may aggravate NAFLD reinforce our findings. Taken together with the experimental data, our reported observations may support a recommendation of smoking cessation in patients with NAFLD.

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Abbreviations

NAFLD	Nonalcoholic Fatty Liver Disease
NASH CRN	Nonalcoholic steatohepatitis Clinical Research Network
DM	type 2 diabetes mellitus
BMI	body mass index
SD	standard deviation
OR	odds ratio

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Table 1

Characteristics of 1091 patients with NAFLD*

	n (%) or Mean ± SD#
Female Gender	687 (63.0%)
Age (years)	49.0 ± 11.9
Race / Ethnicity	
White	838 (77.0%)
Hispanic	123 (11.3%)
Black	28 (2.6%)
Other	100 (9.2%)
BMI	34.2 ± 6.4
Diabetes Mellitus Type 2	318 (29.2%)
History of Smoking	309 (28.5%)
Current smoker	103 (9.5%)
History of >=10 pack-years	257 (23.8%)
Pack-years	0 (0.0, 8.4)
Lifetime alcohol abstinence	543 (49.8%)
Medication exposures	
Amiodarone use	5 (0.5%)
Methotrexate use	4 (0.4%)
Household income <15,000/year	92 (8.6%)
Histology	
Steatohepatitis present	621 (56.9%)
NAFLD Activity Score >=5	511 (46.8%)
Advanced Fibrosis (Stages 3-4)	338 (31.3%)
Biopsy length, mm	18.8 ± 9.9

*Patients under age 18, patients without a biopsy, and patients with a NAS score of 0 were excluded.

#Median (IQR) is shown for pack-years variable because of its non-normal distribution.

Table 2

Characteristics of patients with NAFLD according to Early (Stages 0-2) versus Advanced (Stages 3-4) liver fibrosis*

	Stages 0-2 (n=742)	Stages 3-4 (n=338)	p-value
Female Gender	445 (60.0%)	238 (70.4%)	0.001
Age (years)	46.7 ± 11.9	54.0 ± 10.4	<0.0001
Race / Ethnicity			0.007
White	556 (75.1 %)	273 (80.8%)	
Hispanic	99 (13.4%)	22 (6.5%)	
Black	20 (2.7%)	8 (2.4%)	
Other	65 (8.8%)	35 (10.4%)	
Hispanic, yes vs. no	99 (13.3%)	22 (6.5%)	0.0008
BMI, kg/m^2	34.0 ± 6.3	34.9 ± 6.5	0.03
BMI >=30 kg/ m^2	515 (69.9%)	253 (74.9%)	0.10
Diabetes Mellitus Type 2	164 (22.1%)	154 (45.6%)	<0.0001
History of Regular Smoking, yes vs. no	194 (26.3%)	114 (33.8%)	0.01
Current smoker, yes vs. no	67 (9.1%)	34 (10.1%)	0.65
History of >=10 vs. <10 pack-years	147 (20.0%)	109 (32.3%)	<0.0001
Pack-years, median (IQR)	0 (0.0, 6.0)	0 (0.0, 16.5)	0.0002
Alcohol use, not abstinent vs. lifetime abstinent	389 (52.4%)	147 (43.5%)	0.007
Household income <15,000 vs. >=15,000/year	62 (8.5%)	30 (9.0%)	0.81
Biopsy length, mm	18.2± 9.5	19.9 ± 10.6	0.01

*Expressed as percent for categorical variables and mean (±SD) for continuous variables. Median (IQR) is shown for pack-years variable because of its non-normal distribution.

Table 3

Comparison between characteristics of patients with NAFLD who reported a lifetime history of smoking 10+ cigarette pack years versus those who did not

	<10 pack years (n=825)	10+ pack years (n=257)	P value
Female Gender	519 (62.9%)	163 (63.4%)	0.94
Age (years)	47.7 ± 12.0	52.9 ± 10.4	<0.0001
Race / Ethnicity			<0.0001
White	613 (74.5%)	220 (85.6%)	
Hispanic	111 (13.5%)	10 (3.9%)	
Black	20 (2.4%)	8 (3.1%)	
Other	79 (9.6%)	19 (7.4%)	
BMI	34.0 ± 6.4	35.1 ± 6.0	0.02
Diabetes Mellitus Type 2	224 (27.2%)	91 (35.4%)	0.01
Lifetime alcohol abstinence	449 (54.4%)	92 (35.8%)	<0.0001
Medication exposures			
Amiodarone use	3 (0.4%)	2 (0.8%)	0.34
Methotrexate use	3 (0.4%)	1 (0.4%)	1.00
Household income <15,000/year	61 (7.5%)	31 (12.4%)	0.02
Histology			
Steatohepatitis present	464 (56.2%)	155 (60.3%)	0.28
NAFLD Activity Score >=5	385 (46.7%)	124 (48.3%)	0.67
Advanced Fibrosis (Stages 3-4)	229 (28.1%)	109 (42.6%)	<0.0001

Table 4

Logistic Regression Model for Advanced Liver Fibrosis in a cohort of patients with NAFLD*

	OR (95% CI)	p-value
History of ≥10 vs. <10 pack-years of smoking	1.63 (1.19-2.24)	0.003
Age, years	1.06 (1.04-1.07)	<0.0001
DM, yes vs. no	2.44 (1.83-3.26)	<0.0001
Alcohol use, not lifetime abstinent versus lifetime abstinent	0.62 (0.46-0.82)	0.0008

*Whole Model Test <0.0001

*n=1072

Table 5

Logistic Regression Model for Advanced Liver Fibrosis in a cohort of patients with NAFLD*

	OR (95% CI)	p-value
History of ≥10 vs. <10 pack-years of smoking among non-diabetics†	2.48 (1.68-3.67)	<0.0001
History of ≥10 vs. <10 pack-years of smoking among diabetics†	0.80 (0.48-1.33)	0.38
Age, years	1.05 (1.04-1.07)	<0.0001
Alcohol use, not lifetime abstinent versus lifetime abstinent	0.62 (0.47-0.83)	0.001

*Whole Model Test <0.0001

*n=1072

† P value for pack years by diabetes interaction=0.0005

Table 6

Dose response relationship between pack-years by fibrosis stage

	Early fibrosis stage (Stages 0-2)	Advanced fibrosis stage (Stages 3-4)	p value*
	n (%)	n (%)	
Pack-years, all patients			<0.0001
<=5	544 (71.5)	217 (28.5)	
5.1-10	50 (78.1)	14 (21.9)	
10.1-20	51 (62.2)	31 (37.8)	
>20	89 (53.9)	76 (46.1)	
Pack-years, non-diabetics			<0.0001
<=5	443 (80.1)	110 (19.9)	
5.1-10	39 (84.8)	7 (15.2)	
10.1-20	35 (61.4)	22 (38.6)	
>20	56 (55.5)	45 (44.5)	
Pack-years, diabetics			0.41
<=5	101 (48.6)	107 (51.4)	
5.1-10	11 (61.1)	7 (38.9)	
10.1-20	16 (64.0)	9 (36.0)	
>20	33 (51.6)	31 (48.4)	

*Based on Cochran-Armitage trend test