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Effect of Metabolic Traits on the Risk of Cirrhosis and Hepatocellular Cancer in Non-alcoholic Fatty Liver Disease

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Abstract

Non-alcoholic fatty liver disease (NAFLD) is now the most common liver condition. Predicting its progression could help clinicians manage and potentially prevent complications. We evaluated the independent and joint effects of metabolic traits on the risk of cirrhosis and hepatocellular carcinoma (HCC) among patients with NAFLD. We assembled a retrospective cohort of patients with NAFLD diagnosed at 130 facilities in the Veterans Administration between 1/1/2004 and 12/31/2008 with follow-up through 12/31/2015. We performed competing risk, adjusted cause-specific Cox models to evaluate the effects of metabolic traits (diabetes, hypertension, dyslipidemia, obesity) as additive or combined indicators on time to develop cirrhosis or HCC or a composite endpoint of both. Of the 271,906 patients, 22,794 developed cirrhosis, and 253 developed HCC during a mean of 9 years follow up. At baseline, the mean BMI was 31.6 (SD, 5.6), 28.7% had diabetes, 70.3% hypertension, and 62.3% had dyslipidemia with substantial overlap among the these traits. The risk of progression was the lowest in patients with only one or no metabolic trait. There was a stepwise increase in risk with each additional metabolic trait. Compared to patients with no metabolic trait, patients with both hypertension and dyslipidemia had 1.8-fold higher risk of progression to cirrhosis/HCC (hazard ratio (HR) =1.8, 95% CI=1.59-2.06); the risk was 2.6-fold higher in patients with diabetes, obesity, dyslipidemia and hypertension (HR=2.6, 95% CI=2.3,2.9). These associations were stronger for HCC. Diabetes had the strongest association with HCC in this cohort. **Conclusions**: Each additional metabolic trait increased the risk of cirrhosis and HCC in patients with NAFLD. Diabetes conferred the highest risk of progression to HCC. Diabetic patients with co-existing hypertension and obesity may be important targets for secondary prevention.

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Background

Non-alcoholic fatty liver disease (NAFLD) is now the most common liver disease in the U.S and much of the developed world[1-3], with a general population prevalence of 20 to 30%.[1] As NAFLD increases the risk of cirrhosis and hepatocellular cancer (HCC),[4] it will likely become the most common cause of these conditions in the coming years.[1] Screening NAFLD patients for cirrhosis or HCC is not an option because of the sheer size of NAFLD population and the absence of accurate screening biomarkers. For such screening to be cost-effective, we need better risk stratification to guide targeted screening. Predicting progression by better understanding risk factors would also allow clinicians to more effectively plan secondary prevention efforts in NAFLD, including those related to HCC surveillance.

NAFLD shares risk factors with the other manifestations of metabolic syndrome: diabetes, hyperlipidemia, obesity and hypertension. Several studies support the relationship between metabolic traits like diabetes and obesity and the likelihood of developing nonalcoholic steatohepatitis (NASH) and advanced fibrosis[5-7]—both surrogates of progressive disease in NAFLD. Hypertension and dyslipidemia (specifically, hypertriglyceridemia and low high-density lipoprotein [HDL]) may also be associated with NAFLD severity, although previous studies reported mixed results.[9,10] These metabolic traits are also readily identifiable and potentially modifiable, rendering them as ideal targets not just for risk stratification but risk modification as well.

While the available studies point to a probable association between metabolic traits and NAFLD progression, there remains doubt about the strength and extent of that association. Most available cohort studies followed only a limited number of NAFLD patients with incomplete risk factor data and few incident cirrhosis or HCC cases.[11] The frequent co-occurrence of these metabolic traits and their interplay further complicates the examination of each traits' specific contribution to cirrhosis and hepato-carcinogenesis, knowledge that would be useful to both predicting prognosis and preventing complications.

To fill this gap in the literature, we conducted a large retrospective cohort study of over 270,000 patients with NAFLD. Patients were followed over an average of 9 years to evaluate the independent and joint effects of metabolic traits on the subsequent risk of cirrhosis and HCC.

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METHODS

Data Source

We used data from the national VHA Corporate Data Warehouse (CDW) and Central Cancer Registry (CCR). CDW includes all laboratory test results, inpatient and outpatient utilization, and diagnosis codes. CDW also contains information from annual Alcohol Use Disorders Identification Test (AUDIT-C) screen and Vital Status files.[12,13] AUDIT-C has been used to screen over 90% of VA outpatients nationwide since 2004.[13] CCR is a centralized repository for over 750,000 VHA patients with cancer and includes information on date of diagnosis, primary site, and histology.

Study Cohort

We evaluated all patients 18 years to 80 years who had at least one visit to any VHA hospital in the nation between January 1, 2003 and December 31, 2011. As reported previoulsy,[4] patients were classified as having NAFLD if they had two or more elevated ALT values (≥40 IU/ml for men and >31 IU/ml for women) in the ambulatory setting and more than 6 months apart, with no positive serologic laboratory testing for HBV (i.e., HBV surface antigen) or HCV (*i.e*., HCV RNA). We excluded patients with any alcohol related ICD-9 codes or positive AUDIT-C scores (≥ 4 in men and ≥3 in women) any time prior to or during study follow up. We also excluded patients with evidence of rare chronic liver disorders (hereditary hemochromatosis, primary biliary cirrhosis, primary sclersoing cholangitis, alpha-1 antitrypsin disease, or autoimmune hepatitis) defined based on diagnostic codes. This combined definition was highly predictive of NAFLD diagnosis based on explicit chart review.[4] We used the date of first elevated ALT as the index date of follow up for NAFLD diagnosis. We included NAFLD patients with an index date from January 1, 2004 to December 31, 2008 in this analysis because AUDIT-C was implemented in the VA in 2004. We used 2008 as the cut-off to define study cohorts to allow sufficient follow up (minimum 5 years) for all patients. We followed patients to December 31, 2015 to examine study outcomes.

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Because our goal was to examine the risk of incident cases of cirrhosis and HCC, and to avoid reverse causation (where cirrhosis may predispose to diabetes for example) we excluded patients with prevalent diagnoses of cirrhosis or HCC, defined as having a diagnosis date any time before or within 2 years of the NAFLD diagnosis date. We also excluded patients who died within 2 years of index. Patients had to have evidence of clinical follow up (>1 visit) beyond the first 2 years of index to be included in the analysis.

Variable Specification

***Outcomes**:* We used a hierarchical approach to define the occurrence of HCC, as described previously.[4] Briefly, we defined HCC by extracting patients in our cohort with 2 instances of diagnosis codes for HCC (155.0 in the absence of 155.1) in the inpatient, outpatient or fee basis files of the CDW data. We then examined the VA CCR for patients with possible HCC diagnosis. For patients who had an ICD-9 code but was not identified as having HCC in the CCR data, we conducted a manual review of the VA electronic medical record (EMR) for each discordant patient to determine their true HCC status. This hierarchical approach ensured high validity of all the captured HCC cases.

We classified patients as having cirrhosis if they had ≥2 outpatient or ≥1 inpatient ICD-9 code for cirrhosis (571.5) or its complications (i.e., ascites, encephalopathy, varices with or without bleeding) or if they had persistently high FIB-4 values. We calculated serial FIB-4, starting any time prior to the index date of follow up until the end of follow up. We calculated FIB-4 using laboratory results from AST, ALT and platelet tests performed in ambulatory settings and recorded within 6 months of each other, as previously described: FIB-4 = Age (years) × AST (U/L) / [PLT(109/L)×$ALT^{1/2}$ (U/L)]. We used cut-off >2.67 to define high FIB-4 because it was shown to be highly predictive of the presence of advanced fibrosis/cirrhosis in patients with NAFLD.[15] We classified patients as having persistently high FIB-4 if they had > 2 values >2.67 within 2 years of each other. When comparing our FIB-4 in NAFLD patients with cirrhosis codes, most of those with cirrhosis (68.1%) had persistently high FIB-4. We used the date of first instance of the cirrhosis code or high FIB-4 to define diagnosis date of cirrhosis.

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We obtained all-cause mortality data from VA Vital Status file. Vital Status combines data from Medicare, VA, Social Security, and VA Compensation and Pension Benefits to determine date of death (sensitivity 98.3% and specificity 99.8% relative to National Death Index).

Our primary endpoint was progression to the composite endpoint of either cirrhosis or HCC, whichever was diagnosed first, because both represent important landmarks in NAFLD progression. We also modeled progression to HCC and cirrhosis as secondary endpoints.

***Metabolic Traits**:* The primary exposure variables of interest included obesity, diabetes, hypertension and dyslipidemia. We defined diabetes and hypertension by ≥2 outpatient or ≥1 inpatient ICD-9 code or > 1 filled prescription of diabetes medications (oral hypoglycemic medications or insulin) or anti-hypertensives respectively; we used the first evidence of the condition as the date of diagnosis. The key features of metabolic syndrome-associated dyslipidemia included high serum triglycerides and low HDL levels.[16] We defined dyslipidemia by examining serial laboratory values for abnormal serum triglycerides (≥200) and/or HDL (<40) starting from the value within one year prior to or after and closest to the index and then updated during the follow-up time. We used height (one time) and annual weight values within any time before to one year after and nearest to the index date to define baseline body mass index (BMI) and then updated it yearly. We used the median weight in the event of multiple values in the same year.

***Covariates*:** Other covariates included age, gender, race/ethnicity (non-Hispanic white, non-Hispanic black, Hispanic, and other) and healthcare utilization measured as the number of clinic visits in the first 2 years of NAFLD index.

Statistical Analyses

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We performed a set of cause-specific Cox proportional hazard models for the composite (cirrhosis or HCC) endpoint and each secondary endpoint. We defined each person follow up time as the time between NAFLD index and the diagnosis of cirrhosis, HCC, death, or December 31, 2015, whichever came first. Patients who did not develop events (cirrhosis, HCC, or death) by the end of follow-up were censored. Death was treated as a competing risk event.

The primary exposure variables included the 4 metabolic traits: diabetes (yes vs. no), hypertension (yes vs. no), dyslipidemia defined as either low HDL (>40 *vs*. <40 mg/dl) or high hypertrigylcerides (>200 *vs*. <200 mg/dl), and obesity (BMI >30 vs. <30). We modeled the first 3 traits (diabetes, hypertension and dyslipidemia) as time-varying covariates. We considered using BMI as time-varying covariate but opted against it because BMI did not change in a meaningful manner (>10% change) in most patients. Given the chronicity of metabolic traits, once diagnosed, we assumed that patients continued to have the given trait for the duration of the follow up.

Because it is common for an individual to have multiple metabolic traits, we used two alternative strategies to investigate the joint effect of the metabolic traits. First, we modeled them as additive indicators (as number of traits), and in the second strategy, we created indicator variables representing different combinations that are frequently observed in the cohort. We only included the most frequent metabolic syndrome profiles that showed evidence of developing the endpoints in the models. We adjusted all models with relevant demographic variables (age, gender, race/ethnicity), modeled as time-independent effects.

Secondary and sensitivity analyses

We excluded patients with an expanded definition of prevalent cirrhosis or HCC from this analysis. Because we used the FIB-4 cut off >2.67 to define cirrhosis, and because some patients with cirrhosis can have FIB-4 that fall between 1.3 and 2.67 (indeterminate range), it is plausible that we included some patients with cirrhosis in our cohort. We examined the robustness of our inclusion/exclusion criteria by limiting the cohort to patients with baseline

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FIB-4 <1.30 (NPV for absence of advanced fibrosis/cirrhosis at the low cutoff <1.3 = 90%).[15] We also excluded patients who developed cirrhosis or HCC within 3 years of index.

HCC can occur in the absence of cirrhosis in NAFLD. It is unclear if metabolic traits are associated with HCC in patients without cirrhosis. We ran the cause specific Cox model to examine metabolic traits associated with HCC among patients without baseline or incident cirrhosis by censoring cirrhosis patients at cirrhosis diagnosis date. We also assumed that cirrhosis preceded HCC in the subset of patients whose cirrhosis was recorded after HCC diagnosis date, and censored these patients in the analysis.

In an additional analyses, we modeled metabolic traits as 5 variables (diabetes, hypertension, obesity, hypertriglyceridemia, low HDL) to assess the potentially independent effects of different patterns of lipid abnormalities.

Last, although we focused on the association of metabolic traits with liver disease progression in our NAFLD cohort, we also examined in a secondary analysis the effect of these traits on all-cause mortality.

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RESULTS

Patient characteristics

We identified 271,906 patients with NAFLD. The mean age at the time of first ALT elevation was 55.5 year (standard deviation, SD 12.8 years), 94.3% were men, 69.1% were white, 11.5% were African American (AA), and 5.4% were Hispanic. At baseline, the mean BMI was 31.6 (SD, 5.6), 28.7% had diabetes and 70.3% had hypertension. Over 92% of patients had >1 laboratory test to determine HDL and triglycerides values at baseline. Of these, 52.5% patients had HDL value < 40 mg/dl and 34.2% had triglycerides >200 mg/dl. (**Table 1**)

There was a significant overlap between the metabolic traits at baseline (**Figure 1 and Supplementary Table 1**). In total, 18.1% had only one trait, 26.1% had 2, 26.3% had 3 and 17.5% had all 4 traits; only 5.8% did not have any metabolic trait. At baseline, the most common joint profiles were hypertensive patient with dyslipidemia (12.6%), obese patients with hypertension and dyslipidemia (17.6%), and patients with all 4 metabolic traits (17.5%). The prevalence of metabolic traits increased during follow-up. Specifically, 45.7%, 86.3%, and 83.9% of patients had diabetes, hypertension and dyslipidemia at any time during follow up. By the end of follow up, 23.8% of patients had all 4 metabolic traits. Supplementary Figure 1 displays the cumulative incidence of hypertension, diabetes and dyslipidemia in our cohort.

Patients in general had low likelihood of liver fibrosis as indicated by the mean FIB-4 of 1.1 (SD, 0.57) at baseline. On average, patients had 2.7 (SD 0.5) visits to the VA in the first 2 years following index date.

During mean follow-up of 9.3 (SD 2.7) years, 22,794 progressed to advanced fibrosis/cirrhosis (referred to as cirrhosis from here onwards), 253 patients were diagnosed with HCC, and 31,829 patients died.

Individual associations of metabolic traits and cirrhosis and HCC

When examining each metabolic trait separately, each one was significantly associated with an increased risk of the composite outcome (incident cirrhosis or HCC). For example, the

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risk of progressing to cirrhosis or HCC (the composite endpoint) was 3.5-fold higher in patients with hypertension than those without hypertension (adjsuted hazard ratio [HR]=3.52, 95% confidence interval [CI]=3.34-3.71). Patients with diabetes had 1.9-fold higher risk of developing the composite endpoint than those without diabetes. Dyslipidemia had modest effect with hazard ratio of 1.32 whereas obesity was not associated with the composite outcome in the unadjusted analyses. We found similar associations between individual metabolic traits and risk of HCC or cirrhosis, although the effect of diabetes was stronger for HCC than for other outcomes. (**Table 2**).

In the multivariable models, the associations between each of the metabolic traits and progression to the composite outcome were attenuated but persisted in statistical significance and direction for all traits. Patients with hypertension, diabetes, dyslipidemia or obesity had 59%, 31%, 24% and 10% increased risk of developing the composite endpoint compared with their counterparts. (**Table 3**).

Diabetes was the only factor independently associated with the risk of HCC in the multivariable model. Patients with diabetes had ~2.8-fold higher risk of progressing to the HCC than those without diabetes (adjusted HR=2.77, 95% CI=2.03-3.77). Obesity and dyslipidemia were associated with a modest 31% increase in HCC risk, however, these associations did not reach statistical significance. Similarly, hypertension was not associated with the risk of HCC.

The associations between metabolic traits and cirrhosis alone were similar to those observed for the composite outcome (**Table 3**).

Joint associations of metabolic traits with cirrhosis and HCC

Compared with patients with one or no trait, the risk of progression to the composite endpoint increased to 1.33 (95% CI, 1.26-1.40), 1.61 (95% CI=1.53-1.69) and 2.03 (95% CI=1.93-2.13) for having 2, 3 and 4 traits, respectively (**Figure 2 and Supplementary Table 2**). A similar, yet stronger, trend emerged for the HCC outcome; the risk of progression HCC increased to 2.5 (95% CI=1.40-7.72) and 3.9 (95% CI=2.2 – 7.2) for having 3 and 4 traits,

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respectively. The effect of number of conditions on cirrhosis was similar to its effect on the composite outcome.

The metabolic traits often co-exist in the same patients; the specific combination was also associated with the risk of progression to the endpoints. **Table** 4 displays the comparative risk of developing the composite endpoint in patients with different trait combinations. Compared to patients with no metabolic trait, patients with both hypertension and dyslipidemia had 1.8-fold higher risk of progression to cirrhosis/HCC (adjusted HR =1.81, 95% CI=1.59-2.06); the risk was 2.6-fold higher in patients with diabetes, obesity, dyslipidemia and hypertension (adjusted HR=2.57, 95% CI=2.26-2.9). The effect of specific combinations of metabolic traits was stronger for HCC. Compared to patients with obesity alone, the risk of progression to HCC was the highest in patients with obesity, diabetes, and hypertension (adjusted HR=8.63), those with diabetes, hypertension and dyslipidemia (adjusted HR=5.55), and those with all 4 metabolic traits (adjusted HR=6.42); although the latter 2 associations did not reach statistical significance, perhaps due to power limitations.

Compared with whites, Hispanics had the highest risk of progression whereas AA had the lowest risk; these effects were independent of the metabolic trait effects. The risk of progression increased with age and was lower in women than men (**Table 3**).

Secondary and sensitivity analyses

The key findings for diabetes, hypertension and obesity did not change in magnitude or direction when we restricted analyses to patients with high likelihood of absence of advanced fibrosis/cirrhosis (i.e., FIB-4 <1.30 at baseline) or extended timeframe to define prevalence cirrhosis or HCC. In total 64 HCC cases developed among NAFLD patients in the absence of cirrhosis. The median time to HCC in patients without cirrhosis was 6.6 years (25^{th}, 75^{th} percentile, 4.1 to 9.0 years) compared with 5.8 years (25^{th}, 75^{th} percentile 4.0 to 8.1 years) in the primary analysis. The effects of obesity, diabetes and dyslipidemia were stronger (adjusted HRs 1.19, 2.15, and 1.73, respectively) among these patients, than the associations in the overall analysis (adjusted HRs 1.09, 1.31, and 1.23, respectively).

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Hypertension was not associated with the risk of progression to HCC in the absence of cirrhosis. We found similar results in the sensitivity analysis limited to patients with baseline FIB-4 <1.30, providing convergent validity to our findings.

The key findings did not change when we modeled metabolic traits as 5 variables. Low HDL was associated with a high risk of progression to the composite endpoint (adjusted HR =1.30, 95% CI=1.17,1.45) whereas hypertriglyceridemia had a modest effect (adjusted HR =1.07, 95% CI=1.04-1.11) (**Supplementary Table 3)**. We found similar trends for progression to HCC but the associations did not reach statistical significance.

We also examined the association between metabolic traits and risk of overall mortality in our cohort. Patients with hypertension had a 2-fold higher risk of death during study follow up. Similarly, diabetes and dyslipidemia were associated with 58% and 15% higher risk of death, respectively. In contrast, obesity had an inverse association with the risk of death (adjusted hazard ratio, 0.81, 95% CI=0.79-0.83). (**Supplementary Table 4**).

DISCUSSION

In this large cohort study of patients with NAFLD, we found that all four metabolic traits -- diabetes, obesity, hypertension and hyperlipidemia were individually and jointly associated with an increased risk of developing incident cirrhosis and HCC. Higher burden of coexisting metabolic traits was linked with higher risk in this cohort with relatively mild liver disease at inception.

We were also able to disentangle the effects of individual traits. Although all individual traits had similar modest association with the risk of progression to cirrhosis (and the composite endpoint of cirrhosis or HCC), our results support a stronger effect of diabetes on the risk of progression to HCC than the other metabolic traits. The relative risks associated with diabetes ranged from 5.5 to 8.6 in this study and had a significant additive effect on the overall HCC risk. For example, in individuals with obesity and hypertension, concomitant diabetes was associated with a substantial increase in the risk of progression to

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HCC – with hazard ratio of 1.07 in the absence of diabetes to 8.63 in the presence of diabetes (**Table 4**).

We also found modest effects of obesity, hypertension and dyslipidemia on risk of NAFLD progression. For example, the risk of progression was 10% to 30% higher in patients with *versus* those without obesity. Previous metaanalyses of studies, most of which included general population cohorts, showed that individuals with obesity (as measured by BMI) had a 50% to 85% increased risk of incident HCC compared with non-obese individuals.[17-18] Our study extends these findings to individuals with established NAFLD. We also found statistically significant associations between the risk of progressive disease and hypertension or dyslipidemia. However, our results suggest that the effect of dyslipidemia may differ based on the individual components. For example, low HDL was consistently associated with a high risk of progression to the composite endpoint (HR=1.22, 95% CI=1.18,1.26) and HCC (HR=1.33, 95% CI=0.85, 2.08) (Supplementary Table 3). Low HDL cholesterol is a common feature of type 2 diabetes[19] and obesity, and has been linked with cancer risk in previous studies of patients with diabetes.[20] In contrast, elevated triglycerides had a small effect in our study. Overall, the metabolic risk factors we identified might serve as important targets for secondary prevention to modify the progression of NAFLD to cirrhosis and HCC.

Our results provide the first data on the strength and extent of the associations between metabolic traits and HCC in non-cirrhotic liver - a key knowledge gap. We conducted separate analyses to address this gap (**Table 5**). In total, 64 of the 253 (25%) HCCs in our study cohort developed in patients who did not have any evidence of cirrhosis or advanced fibrosis at baseline or during follow-up – a proportion similar to that reported in previous studies.[21-23] The effects of obesity or dyslipidemia on the HCC risk (adjusted HRs 1.19 and 1.73, respectively) were slightly stronger than their corresponding effects in the overall analysis (adjusted HRs 1.10 and 1.31, respectively). Diabetes was the strongest risk factor associated with HCC risk in the absence of cirrhosis. Specifically, the risk of progressing to HCC was more than 2-fold higher in patients with diabetes than those without. We found similar results in the sensitivity analysis limited to patients with baseline

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FIB-4 <1.3 (**Table 5**). Although we did not directly examine the effect of diabetes on the risk of progression from cirrhosis to HCC, a recent study showed a moderate association with a HR of 1.3.[24] Our results, (with HRs >2.0) show that magnitude of diabetes effect may be stronger at the earlier stages in disease spectrum. Controlling diabetes at earlier stages of disease (*i.e.*, before progression to advanced fibrosis/cirrhosis) might have a larger impact on overall incidence of complications including HCC.

Our data may also inform HCC screening efforts in NAFLD. Based on our results, a hierarchical, stepwise, risk-stratification approach may be one way to investigate targeted screening. Prioritizing diabetic patients with co-existing hypertension and obesity for cirrhosis surveillance (with non-invasive tests for fibrosis) may be effective and cost-effective, and needs to be evaluated. The risk of progression in this subgroup was 2.5-2.8% at 10 years, approaching the risk in community-based cohorts with HCV (estimated ~3-4% at 10 years following infection).[25] HCC surveillance, with imaging and serological biomarkers, may then be limited to patients with cirrhosis. Although we identified diabetes as an important risk factor for progression to HCC in the absence of cirrhosis, over 40% of patients in our cohort had diabetes – therefore future studies should examine the determinants and mechanisms of HCC in this group to arrive at additional risk-stratification schemes and biomarkers to aid in the screening and prevention.

Identifying these metabolic traits in clinical practice or population-based management should not prove too challenging. To ease such efforts, we used previously validated diagnostic codes for diabetes and hypertension to identify these conditions in the database, and we used BMI as a marker of obesity because it is objective, routinely performed, and readily available in electronic medical records. Beyond guiding screening efforts, the metabolic risk factors we identified might also serve as important targets for secondary prevention to modify the progression of NAFLD to cirrhosis and HCC.

Our study has several limitations. Our NAFLD definition captures patients with clinically apparent and relevant NAFLD. We recognize that it does not include individuals who may have some evidence of hepatic steatosis yet who *never* develop abnormal ALT. Recent longitudinal studies have shown the presence of liver fat (or non-alcoholic fatty liver,

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NAFL) *per se* may be largely inconsequential; these individuals have similar overall and liver-related outcomes as those without NAFLD[26,27] Given these data, our current research focused on NAFLD individuals with evidence of ALT elevation – a large group with a known increased risk of developing HCC[28]. Furthermore, our previous data showed that most of these patients with clinically apparent NAFLD nonetheless remain unrecognized in routine practice.[29] Second, our observational design precludes casual inferences with certainty. However, the strength of association, consistency with previous studies, temporality, dose-response relation with additional traits, and biological plausibility lend support for causality. Third, though reverse causation might explain the observed associations – such as diabetes reflecting an early manifestation of cirrhosis – we believe this unlikely. Most of the patients had risk factors at baseline. We excluded patients with progression in the first 2 years of their index diagnosis to minimize this potential bias in the primary analysis. Further, our results did not change in the sensitivity analysis that excluded patients who had progressed in the first 3 years of their index. Fourth, we might have misclassified the cirrhosis outcome as the database did not contain universal biopsy or imaging data. To prevent this, we used previously validated diagnosis codes and combined information from serial FIB-4 values to define cirrhosis, and tested the robustness of our findings in sensitivity analysis restricted to patients with FIB-4 <1.30 – a cut-off associated with a high NPV for advanced fibrosis/cirrhosis.[15] To further examine the construct validity of cirrhosis definition, we examined the effect of incident cirrhosis on all cause mortality in a post-hoc analysis (Supplementary Table 5). Incident cirrhosis was associated with an 80% increase in the risk of death – showing that cirrhosis is a key driver of all cause mortality in NAFLD. We may have also misclassified the risk factors. Laboratory lipid values could fluctuate over time (with or even without treatment). However, the results did not change in a sensitivity analysis that used a restrictive definition (>2 abnormal values > 30 days apart) of dyslipidemia, rendering misclassification bias unlikely. Indeed, our approach to define dyslipidemia based on the objective laboratory test values represents a significant strength to the study. BMI is a common measure of obesity, but there are more sensitive and sophisticated markers of obesity – such as D dual-energy X-ray absorptiometry, abdominal

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computed tomography, and bioelectrical impedence analysis – unavailable in our database. However, these measures are not likely to be widely implemented in routine practice, limiting their utility in population level screening and risk-modification interventions. Fifth, some risk factors and intermediate outcomes, like smoking status and NASH were unavailable. Future studies will need to investigate if these modify our observed strong associations. Though the metabolic traits present targets for secondary prevention, our data do not examine treatments; only future trials of risk reduction will reveal if such strategies are effective. Last, our study population consisted mostly of men. It is plausible that the mechanisms of progressive liver disease may be different among different genders. Our results are derived from patients who sought care in the VA healthcare system, and although the generalizability of the biologic process of progression probably extends from men veterans to men nonveterans, further research would be needed to confirm that.

In summary, metabolic traits increased the risk of cirrhosis and HCC in this large cohort of patients with NAFLD. Diabetes had the strongest association with HCC in the presence or absence of cirrhosis. We were able to explain more of the progression risk when we considered metabolic traits jointly rather than individually. Our findings highlight the need for comprehensive evaluation of diabetes, hypertension, dyslipidemia and obesity for prevention of future morbidity and mortality in NAFLD. Although, several treatments for NAFLD are currently in the pipeline, these therapies may fail to stem the rising tide of cirrhosis and HCC in NAFLD if we do not simultaneously target co-existing metabolic traits.

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Figure Legends

Figure 1: Change in the proportion of patients with different combinations of metabolic traits over time**.** See Supplementary Table 1 for details.

Figure 2: Adjusted associations between the number of metabolic traits and the time to the composite endpoint (cirrhosis or HCC) or HCC only by cause-specific Cox regression. The results for cirrhosis only endpoint were similar to those for the composite endpoint. See Supplementary Table 2 for details.

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Table1. Baseline characteristics of patients with nonalcoholic fatty liver disease (NAFLD)

Characteristic, N (%)	NAFLD (n=271,906)
Age, mean (SD)	54.52 (12.86)
Gender	
Men	256,359 (94.28)
Women	15,547 (5.72)
Race/ethnicity	
White	187,964 (69.13)
African American	31,495 (11.58)
Hispanic	14,675 (5.40)
Other races	7,357 (2.71)
Unreported	30,415 (11.19)
BMI, mean (SD)	31.64 (5.62)
Hypertension	191,266 (70.3)
Diabetes	78,065 (28.71)
Dyslipidemia*	
HDL <40	131,636 (52.49)
Triglycerides ≥200	86,181 (34.16)
Any of the above	158,225 (62.43)
FIB4, mean (SD)	1.09 (0.57)

* 21,109 (7.76%) and 19,651 (7.22%) patients had missing data on HDL and triglycerides at baseline

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Table 2. Unadjusted associations between demographic and clinical characteristics and the time to the composite endpoint (cirrhosis or HCC), HCC, or cirrhosis only endpoints.

Variables	Cirrhosis or HCC	HCC Only	Cirrhosis Only
Age	1.07(1.07,1.07)	1.07(1.06,1.09)	1.07(1.07,1.07)
Female (ref: male)	0.51(0.48,0.55)	0.26(0.10,0.70)	0.51(0.48,0.55)
Race (ref: white)			
African American	0.70(0.67,0.74)	0.56(0.34,0.90)	0.70(0.67,0.73)
Hispanic	0.83(0.78,0.88)	0.80(0.45,1.43)	0.83(0.78,0.88)
Other groups	0.75(0.68,0.81)	0.80(0.36,1.81)	0.74(0.68,0.81)
Unreported	0.81(0.78,0.85)	0.97(0.65,1.45)	0.81(0.78,0.85)
Body mass index >30	1.02(0.99,1.05)	1.30(1.01,1.69)	1.02(0.99,1.05)
Hypertension	3.52(3.34,3.71)	3.80(2.18,6.65)	3.53(3.35,3.72)
Diabetes	1.89(1.84,1.94)	3.57(2.72,4.69)	1.89(1.84,1.94)
Dyslipidemia	1.32(1.27,1.37)	1.74(1.13,2.69)	1.32(1.27,1.37)

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Table 3. Adjusted associations between demographic and clinical characteristics and the time to the composite endpoint (cirrhosis or HCC), HCC or cirrhosis only endpoints

Variables	Cirrhosis or HCC	HCC Only	Cirrhosis Only
Age	1.07 (1.07,1.07)	1.07 (1.06,1.09)	1.07 (1.07,1.07)
Female (ref: male)	0.91 (0.85,0.99)	0.57 (0.21,1.56)	0.91 (0.85,0.99)
Race (ref: white)			
African American	1.00 (0.96,1.05)	0.58 (0.32,1.05)	1.00 (0.96,1.05)
Hispanic	1.11 (1.04,1.18)	0.93 (0.49,1.76)	1.11 (1.05,1.19)
Other groups	0.97 (0.89,1.06)	0.94 (0.38,2.28)	0.97 (0.88,1.06)
Unreported	0.83 (0.79,0.87)	1.01 (0.67,1.54)	0.83 (0.79,0.87)
Body mass index >30	1.10 (1.07,1.13)	1.31 (0.98,1.74)	1.09 (1.06,1.13)
Hypertension	1.59 (1.50,1.68)	1.25 (0.65,2.42)	1.59 (1.51,1.69)
Diabetes	1.31 (1.27,1.35)	2.77 (2.03,3.77)	1.31 (1.27,1.34)
Dyslipidemia	1.24 (1.19,1.28)	1.31 (0.84,2.04)	1.23 (1.19,1.28)

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Table 4. Adjusted associations between types of metabolic traits and time to the composite, HCC or cirrhosis only endpoints.

Variables	Composite endpoint	HCC Only	Cirrhosis Only
Age	1.07(1.07,1.07)	1.07(1.06,1.09)	1.07(1.07,1.07)
Female (ref: male)	0.91(0.85,0.99)	0.57(0.21,1.55)	0.91(0.85,0.99)
Race (ref: white)			
African American	1.01(0.96,1.06)	0.58(0.32,1.05)	1.01(0.96,1.05)
Hispanic	1.11(1.05,1.19)	0.93(0.49,1.76)	1.12(1.05,1.19)
Other groups	0.97(0.89,1.06)	0.94(0.38,2.28)	0.97(0.89,1.06)
Missing	0.83(0.79,0.87)	1.01(0.67,1.54)	0.83(0.79,0.87)
Metabolic trait groups (Ref=No trait)			
Obesity	0.99(0.78,1.25)	--	0.99(0.79,1.26)
Dyslipidemia	1.08(0.93,1.26)	2.10(0.23,18.78)	1.08(0.92,1.26)
Hypertension	1.46(1.27,1.68)	1.48(0.18,12.33)	1.47(1.28,1.69)
Obesity and dyslipidemia	1.11(0.94,1.31)	2.30(0.24,22.19)	1.11(0.94,1.31)
Obesity and hypertension	1.49(1.28,1.73)	1.07(0.10,11.76)	1.50(1.29,1.73)
Diabetes and hypertension	1.79(1.53,2.09)	1.47(0.13,16.27)	1.80(1.54,2.10)
Hypertension and dyslipidemia	1.81(1.59,2.06)	1.69(0.23,12.65)	1.81(1.59,2.06)
Obesity, hypertension and	1.90(1.67,2.16)	2.58(0.35,19.06)	1.90(1.67,2.17)

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dyslipidemia			
Diabetes, hypertension, dyslipidemia	2.24(1.96,2.56)	5.55(0.76,40.43)	2.24(1.96,2.56)
Obesity, diabetes, hypertension	2.02(1.74,2.35)	8.63(1.11,66.99)	2.02(1.73,2.35)
Diabetes, obesity, hypertension and dyslipidemia	2.57(2.26,2.92)	6.42(0.89,46.07)	2.56(2.26,2.92)

** For metabolic trait groups, we used patients with obesity as the reference group because there were no HCC cases in patients without any metabolic trait.

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Table 5. Sensitivity analyses

	Limiting to patients with FIB-4<1.30 at baseline (n = 131,276)		**Extending the lag time to define incident cirrhosis and HCC** (n=262,366)		**HCC in the absence of cirrhosis** (n=271,906)
Variables	**Cirrhosis or HCC (n=**2891**)**	**HCC (n=**30**)**	**Cirrhosis or HCC (n=**18,685**)**	**HCC (n=230)**	**HCC (n=64)**
Age	1.05 (1.05,1.06)	1.08 (1.04,1.11)	1.06 (1.06,1.07)	1.08 (1.06,1.09)	1.10 (1.06,1.13)
Female (ref: male)	1.27 (1.13,1.42)	1.50 (0.45,4.93)	0.89 (0.81,0.96)	0.64 (0.24,1.73)	1.31 (0.31,5.49)
Race (ref: white)					
African American	0.71 (0.64,0.79)	1.11 (0.43,2.89)	0.99 (0.94,1.04)	0.59 (0.32,1.10)	1.30 (0.55,3.08)
Hispanic	1.00 (0.88,1.13)	0.41 (0.06,3.03)	1.09 (1.02,1.17)	1.04 (0.55,1.98)	--
Other groups	0.68 (0.56,0.82)	0.78 (0.11,5.69)	0.94 (0.85,1.04)	0.84 (0.31,2.26)	1.61 (0.39,6.68)
Unreported	0.80 (0.72,0.88)	0.86 (0.31,2.42)	0.82 (0.78,0.87)	1.05 (0.67,1.62)	0.82 (0.35,1.92)
Body mass index >30	1.08 (1.02,1.14)	1.16 (0.62,2.18)	1.10 (1.06,1.13)	1.45 (1.07,1.97)	1.19 (0.69,2.07)
Hypertension	1.64 (1.47,1.83)	1.11 (0.32,3.83)	1.58 (1.48,1.69)	1.79 (0.78,4.12)	0.78 (0.27,2.27)
Diabetes	1.47 (1.39,1.56)	2.56 (1.32,4.99)	1.31 (1.27,1.35)	2.73 (1.97,3.78)	2.15 (1.20,3.85)
Dyslipidemia	1.41 (1.29,1.55)	1.55 (0.54,4.45)	1.27 (1.21,1.32)	1.25 (0.78,2.00)	1.73 (0.72,4.12)

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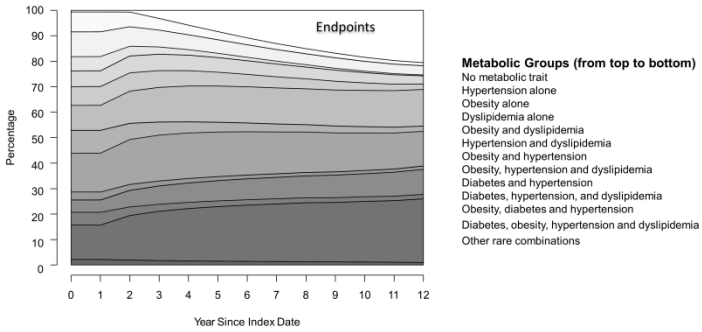

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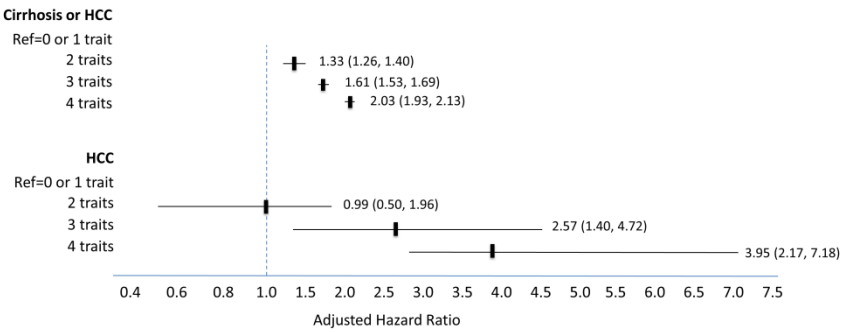

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