Characteristics, Etiologies and Trends of Hepatocellular Carcinoma in Patients without Cirrhosis: A United States Multicenter Study

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ABSTRACT

Background: Limited data exists on the burden and features of non-cirrhotic hepatocellular carcinoma (HCC) in the United States.

Aims: To evaluate characteristics, etiologies, trends, and outcomes of non-cirrhotic HCC from 2000 to 2014 at 5 large US centers. **Methods:** Patient, tumor and liver disease etiology data were manually collected. The presence of underlying cirrhosis was assessed based on published criteria.

Results: Of 5,144 eligible patients with HCC, 11.7% had no underlying cirrhosis. Non-cirrhotic patients were older (64.1 vs 61.2 years), more frequently females (33.9% vs 20.8%), and less frequently Black (8.3% vs. 12.4%) ($p<0.001$ for all). Among non-cirrhotic patients, non-alcoholic fatty liver disease (NAFLD) was the most common liver disease (26.3%), followed by hepatitis C (HCV) (12.1%), and hepatitis B (10%). As of 2014, there was increased percentage of cirrhotic HCC and a decline in non-cirrhotic HCC mainly due to significant annual increases in cirrhotic HCC due to HCV [0.96% ($p<0.0001$)] and NAFLD [0.66% ($p=0.003$)]. Patients with non-cirrhotic HCC had larger tumors (8.9 vs 5.3 cm), were less frequently within Milan criteria (15% vs 39%), more frequently underwent resection (43.6% vs 8%) ($p<0.001$ for all), and had better overall survival than cirrhotic HCC patients (median 1.8 vs 1.3 years, $p=0.004$).

Conclusions: Nearly 12% of HCCs occurred in patients without underlying cirrhosis, and NAFLD was the most common liver disease in these patients. During the study period, the frequency of non-cirrhotic HCC decreased whereas that of cirrhotic HCC increased. Although non-cirrhotic patients presented with more advanced HCC, their survival was better.

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INTRODUCTION

Hepatocellular carcinoma (HCC) is the third leading cause of cancer-related deaths worldwide, causing more than 700,000 deaths per year[1]. HCC incidence rates have significantly increased in the United States (US), surpassed only by thyroid cancer[2-5]. Further, HCC mortality rate is the highest of all cancers in the US[4].

While viral hepatitis and alcohol have traditionally been the main liver diseases driving the occurrence of HCC[6,7], the new millennium has witnessed the emergence of non-alcoholic fatty liver disease (NAFLD) as a major contributor to the burden of HCC, not only in the US and Western countries but also at the global level[8-11]. Over the past 2 decades, NAFLD cirrhosis has been increasingly recognized as an important cause of HCC[12-18]. Recent studies based on national data have shown a significant and recent increase in NAFLD contribution to the HCC burden and mortality in the US[8,10,19,20]. In a study of United Network for Organ Sharing (UNOS) registry from 2002-2012, liver transplantation for HCC related to NAFLD has increased 4-folds compared to a two-fold increase for HCC related to hepatitis C virus (HCV), making NAFLD the most rapidly growing indication for liver transplantation for HCC in the US[19]. Between 2001-2013, HCV followed by NAFLD were the major contributors to rising rates of cirrhosis and HCC among US Veterans[10]. In a study of the Surveillance, Epidemiology and End Results (SEER) registries with Medicare-linkage files for HCC between 2004-2009, a 9% annual increase in NAFLD related HCC was observed, exceeded only by a 13% annual increase in HCV related HCC. However, whether NAFLD contribution to the expanding HCC burden is exclusively via the cirrhosis-carcinogenesis pathway was not clear from these studies.

The development of HCC in a non-cirrhotic setting has long been recognized as an uncommon event in patients with chronic liver disease, occurring primarily in the setting of hepatitis B virus (HBV) infection [21,22]. Accumulating data from different case reports and studies over the past 2 decades have shown the same phenomenon occurs in the setting of non-cirrhotic NAFLD[23-28]. The mechanisms of carcinogenesis in the setting of non-cirrhotic NAFLD are not entirely clear, but the chronic inflammatory state associated with obesity and commonly seen with NAFLD,

insulin resistance and lipotoxicity may alter hepatocyte proliferation and different modes of hepatocyte death, thus promoting a carcinogenic milieu[29-32].

There seems to be geographical differences in the frequency of non-cirrhotic HCC in the setting of NAFLD; only 15% of patients with NAFLD and HCC were not cirrhotic in an Australian study[33], compared to 27% in a single center US study[34], and up to 50% in European and Japanese studies[35-38]. However, beside population differences, some of these studies were based on data from single centers, had small study size, lacked specific criteria to define the non-cirrhotic status, or included only patients referred for surgical resection. The proportion of non-cirrhotic HCC in the US has not been adequately quantified.

While the trends of HCC related to specific underlying liver diseases in select populations in the US have been recently described based on the UNOS, Veterans Administration (VA), and SEER databases, these studies relied on International Classification of Diseases (ICD) codes for identifying underlying liver diseases or presence of obesity or diabetes to infer the presence of NAFLD[8,10,19]. No other large-scale data with direct ascertainment of the underlying etiology of liver disease and cirrhotic status is yet available in the US to allow for high-resolution evaluation of all comers with HCC. With the limitations in current literature[39], it is unclear whether the rising incidence of NAFLD in the US has resulted in higher number of non-cirrhotic HCC. If this were true, it would have a major public health impact as the proportion of HCC linked to NAFLD is anticipated to be on the rise with the global and growing epidemic of obesity and metabolic syndrome.

To address these questions, we studied 5,144 patients with HCC seen at 5 major liver centers across the United States (US) over a 14.5 year period between 2000-2014. The primary objectives of this study were to 1) characterize the frequency and characteristics of non-cirrhotic HCC, 2) assess the trends of percentage of non-cirrhotic HCC over the study period, and 3) determine the contribution of NAFLD to the burden of non-cirrhotic HCC. As secondary

objectives, we report on the treatment modalities these patients received and their survival compared to cirrhotic patients with HCC.

METHODS

HCC case identification and characterization

Using the institutional Cancer Registry at each participating site, all adult patients 18 years of age or older with HCC seen at the study centers (Indiana University School of Medicine, Indianapolis, IN; MD Anderson Cancer Center, Houston, Texas, MD, Vanderbilt University School of Medicine, Nashville, Tennessee; Atrium Health, Charlotte, NC, Columbia University Vagelos College of Physicians and Surgeons, New York, NY) and their affiliate hospitals from January 2000 to June 2014 were identified. We performed manual chart review of each identified case to verify the diagnosis and extract the data. The data were managed centrally by the data coordinating center at Indiana University. The study was reviewed and approved by each participating site's Institutional Review Board.

The diagnosis of HCC required histological and/or radiographic evidence consistent with the American Association for Study of Liver Disease (AASLD) guidelines[40,41]. Histology was most often confirmed at the time of resection, transplantation, or death and not as the primary diagnostic modality for HCC across centers. Patients were excluded from this study if they had 1) uncertain HCC diagnosis; 2) fibrolamellar HCC; 3) cholangiocarcinoma; 4) tumor recurrence as the reason for referral to our institution; and 5) insufficient data in the medical records.

Demographics, comorbidities, clinical data and laboratory values and tumor characteristics were collected at the time of HCC diagnosis. For the tumor size, the largest tumor diameter was recorded for each patient. The presence of macrovascular invasion or distant metastases, TNM staging and whether the HCC was within Milan criteria were captured[42,43]. Although the Milan criteria were originally developed in patients with cirrhosis, these criteria are now universally known and provide easy summary of tumor burden in terms of size and number of lesions,

therefore were used to define the HCC burden in non-cirrhotic patients in this study. All treatment modalities received throughout the disease course were manually extracted from medical records.

Determination of comorbidities and underlying liver disease

Physicians' documentation and/or presence of confirmatory laboratory tests were used to assess for the presence of the following medical co-morbidities: diabetes mellitus, hypertension, dyslipidemia, coronary artery disease, and peripheral vascular disease, as well as the following underlying liver disease etiologies: HCV, HBV, alcohol, autoimmune hepatitis, primary biliary cholangitis, primary sclerosing cholangitis, hemochromatosis, and alpha-1-antitrypsin deficiency. Alcohol abuse was defined as history of more than 3 drinks a day, clinical documentation of alcoholism/alcohol abuse, enrollment in a substance abuse treatment program, or history of alcoholic hepatitis[44]. NAFLD diagnosis required either a diagnosis assigned by the managing hepatologist and/or evidence of hepatic steatosis, either by imaging or by histology in the absence of an alternative liver disease [45]. The rare liver disease category included diseases like amyloidosis, sarcoidosis, cardiac cirrhosis, drug-induced chronic liver disease, and environmental exposure. Patients were classified as having unclear or unknown etiology of liver disease if, after extensive chart review, there was sufficient information to make a diagnosis of underlying etiology of disease, but no clear underlying etiology was found or if there was not sufficient data to make a diagnosis.

Definition of the cirrhotic and non-cirrhotic status

Patients were classified into 4 cirrhosis categories according to criteria published by Mittal et al[44]: 1) level 1 evidence (very high probability) of no cirrhosis, which requires histology and imaging evidence; 2) level 2 evidence (high probability) of no cirrhosis, which lacks histology but is based on imaging and laboratory criteria; 3) confirmed cirrhosis, which is based on histological, imaging, clinical or laboratory criteria; or 4) unclassified if there was insufficient data to classify into any of the above cirrhosis categories. The AST to Platelet Ratio Index (APRI)

Fibrosis-4 (FIB-4), and Model for End-Stage Liver Disease (MELD) were calculated for all patients using the formulas published and validated in the original papers[46-49]. Presence or absence of liver-related complications (ascites, hepatic encephalopathy, varices, spontaneous bacterial peritonitis, renal failure, portal vein thrombosis and hepatic hydrothorax) was collected starting the time of HCC diagnosis until the last documentation available in medical records.

Validation of Mittal's level 2 (high probability) criteria for absence of cirrhosis

The Mittal criteria were constructed to increase confidence in the cirrhotic or non-cirrhotic status assignment of the subgroup of patients who lacked liver histology for confirmation (Table 1) [44]. While the criteria for confirmed cirrhosis and level 1 evidence (very high probability) for absence of cirrhosis are clinically sound, the performance of their proposed criteria for level 2 evidence (high probability) of absence of cirrhosis has not been validated. The level 2 criteria for "no cirrhosis" consist of APRI <1 based on laboratory results available nearest to HCC diagnosis within 6 months before and 4 weeks after HCC diagnosis; in addition to no features suggestive of cirrhosis on abdominal imaging performed nearest to HCC diagnosis within 3 years before HCC diagnosis; and 2 of 3 test values in normal range based on laboratory results available nearest to HCC diagnosis within 6 months before and 4 weeks after HCC diagnosis [albumin >3.5 g/L, platelets 200,000/mL, or international normalized ratio (INR) <1.1]. By adding imaging and laboratory criteria for absence of cirrhosis to APRI, the aim was to enhance the specificity of these criteria and thus reducing the risk of misclassification when identifying non-cirrhotic status in the absence of liver histology.

To validate the level 2 Mittal criteria for the absence of cirrhosis, we randomly selected 200 patients from a large database of 1020 patients who underwent liver biopsy as part of their clinical care at Indiana University. Patients had different underlying liver disease etiologies; 100 had histologically proven cirrhosis and 100 had no histological evidence cirrhosis (16 patients with fibrosis stage 0, 29 patients with fibrosis stage 1, 34 patients with fibrosis stage 2, and 21 patients with fibrosis stage 3). Applicability of level 2 non cirrhosis criteria was assessed after

collection of laboratory data, imaging findings and evidence of complications. The area under the receiver operating characteristic curve (AUROC), sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were computed for the level 2 no-cirrhosis criteria, using liver biopsy as the reference test. The performance parameters of level 2 Mittal criteria for identifying non-cirrhotic status were: AUROC 0.80 (95% CI: 0.74-0.86), sensitivity 62.0%, specificity 98.0%, PPV 96.9%, and NPV 72.0%.

Survival status

Patient survival was ascertained from Cancer Registries and medical records. For patients who are still alive or died with an unknown date of death, the date of last contact available in the medical record was used to define the time of censoring for the survival analysis.

Statistical Methods

Categorical variables were summarized and compared using chi-square test. Continuous variables were summarized and compared using ANOVA and t-test. Simple linear regression was used to evaluate the annual trends in terms of percentage of respective non-cirrhotic HCC, NAFLD, HBV, HCV, or alcohol etiology over the year. A sensitivity analysis was performed for the sub-cohort when patients who had level 2 no cirrhosis were excluded. HCV was considered the primary etiology of liver disease regardless of other etiology, i.e., it could be either HCV and Alcohol, HCV and HBV with or without alcohol, or HCV alone; HBV was considered the primary etiology of liver disease if no HCV was presented, i.e., HBV and Alcohol, or HBV alone. Overall survival time was defined from the date of HCC diagnosis to the date of death, censored at the date of last contact. The overall survival probability was estimated by the Kaplan-Meier method and compared between groups using the log-rank test. A Cox proportional hazards regression model was used to evaluate the association between the risk factors and the overall survival. The following risk factors were considered for the Cox model: patient demographics [age, body mass index (BMI), sex, and race], medical history and comorbid conditions (date of HCC diagnosis, diabetes, hypertension, dyslipidemia, coronary artery disease, and peripheral

vascular disease), laboratory values [alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin, alkaline phosphatase, albumin, platelets, creatinine, INR, and alpha fetoprotein (AFP)], tumor characteristics (tumor size and anatomic stage), treatment modalities (resection, liver transplantation, catheter delivered therapy, sorafenib, stereotactic body radiation therapy (SBRT), radiofrequency ablation (RFA), microwave ablation, palliative/hospice care, other, none, and unknown), and underlying etiology (HCV, HBV, alcohol, NAFLD, and unknown/unclear). Variables that were significant at $p<0.10$ in the univariate analysis were included in a stepwise selection procedure to select which variables would be included in the final Cox multivariate regression model. Variables of clinical significance including age, BMI, sex, race, platelets, diabetes, and underlying etiology were always retained in the model. P-value <0.05 was considered statistically significant. All analyses were performed using SAS version 9.4 (SAS Institute; Cary, NC).

RESULTS

Prevalence of non-cirrhotic HCC in the study cohort

The study flow diagram is shown in **Supplementary Figure 1**. Of 6,250 identified patients, 5,144 met the inclusion criteria for HCC and had classifiable cirrhotic status; of whom 11.7% had no evidence of cirrhosis (7.7% by level 1 and 4% by level 2 criteria) and 88.2% had confirmed cirrhosis. There were no differences between level 1 and 2 “no-cirrhosis” patients in terms of their age, sex, race, BMI, frequency of obesity, diabetes, other comorbidities or frequency of alcohol abuse (**Supplementary Table 1**). The majority of HCC occurred in a cirrhotic background for all specific liver diseases, including HBV and NAFLD **(Table 2).**

Characteristics of patients with non-cirrhotic HCC

Non-cirrhotic patients (level 1 or 2 no cirrhosis, combined) were older (64.1 ± 14.0 vs 61.2 ± 9.9 years, $p <0.001$), more frequently females (34% vs 21%, $p <.001$) and Asian (8% vs 3%, $p <0.001$) but less frequently Black (8% vs 12%, $p <0.001$), compared to cirrhotic patients with

HCC (**Table 2**). They had lower frequency of obesity (22.1% vs 30.7%, p<0.001), diabetes (34.1% vs 38.7%, p=0.02) and alcohol abuse (17.1% vs 46.2%, p<0.001), but higher frequency of hypertension (68.4% vs 61.5%, p=0.001), dyslipidemia (38.0% vs 22.0%, p<0.001), coronary artery disease (22.0% vs 16.7%, p=0.001), and peripheral vascular disease (11.5% vs 7.4%, p<0.001). As expected, non-cirrhotic patients had higher albumin (3.9 ± 0.6 vs 3.3 ± 0.7 g/dL, p<0.001) and platelet count (278.9 ± 118.9 vs 148.4 ± 101.4 K/mm^3, p<0.001) but lower serum total bilirubin (1.0 ± 2.0 vs 2.2 ± 3.3 mg/dL, p<0.001), INR (1.1 ± 0.2 vs 1.3 ± 0.4, p<0.001), APRI (1.0 ± 1.8 vs 3.1 ± 6.6, p<0.001) and MELD scores (8.9 ± 3.4 vs 12.2 ± 5.6, p<0.001) and better performance status than cirrhotic patients with HCC (**Table 2**).

Trends of non-cirrhotic HCC over the study period

From January 2000 to June 2014, the percentage of non-cirrhotic HCC cases declined, whereas the percentage of cirrhotic HCC cases increased (p<0.001; **Figure 1**). The same trend was observed when only level 1 no cirrhosis patients were included in this analysis (p<0.001; **Supplementary Figure 2).** We also analyzed the trends of cirrhotic and non-cirrhotic HCC evaluated per underlying liver disease etiology **(Figure 2)**. From 2000 to 2014, there were no statistically significant changes in the HCV frequency (annual increase 0.37%, p=0.28) and NAFLD frequency (annual increase 0.76%, p=0.12) among non-cirrhotic HCC. In contrast, the increases were statistically significant among cirrhotic HCC (annual increase 0.96% for HCV frequency, p<0.0001, and 0.66% for NAFLD frequency, p=0.003). Overall, there was an annual 0.74% net reduction in non-cirrhotic HCC frequency (p<0.001). Cirrhotic and non-cirrhotic HCC burdens contributed by alcohol and HBV showed only significant decline for cirrhotic HCC related to HBV (**Figure 2**).

Etiology of liver disease in non-cirrhotic HCC

Among all HCC patients in the study cohort (**Table 2**), HCV was the most common liver disease associated with HCC (49%), followed by NAFLD (14.9%), alcohol (12.7%) and HBV (7.4%).

About 12.5% of the study cohort had the underlying liver disease status documented as unclear or unknown.

Among non-cirrhotic HCC patients, NAFLD was the most common liver disease occurring in 26.3% of patients, followed by HCV in 12.1%, HBV in 10%, then alcohol in 7.3% of the patients.

In cirrhotic HCC patients, HCV was the most common liver disease associated with HCC (53.8%), followed by alcohol (13.5%), NAFLD (13.4%), and HBV (7.1%).

Tumor Characteristics in patients with non-cirrhotic HCC

Non-cirrhotic patients had significantly larger tumors (8.9 ± 4.8 vs 5.3 ± 3.9 cm, p<0.001), more frequently had HCC diagnosed incidentally (18.9% vs 11.2%, p<0.001) or for symptoms work-up (60.2% vs 55.3%, p=0.03) and less frequently as part of surveillance (16.9% vs 29.1%, p<0.001) than cirrhotic patients (**Table 3**). They more frequently had histological confirmation of HCC (90.6% vs 62.3%, p<0.001). HCC in non-cirrhotic patients was more advanced (stage III or IV) (52.3% vs 45.5%, p=0.002) and more frequently presented as single (37.0% vs 34.4%, p<0.001) or large multinodular lesions (24.7% vs 21.5%, p<0.001) with vascular invasion or extrahepatic spread (36.3% vs 33.5%, p<0.001). Consequently, these patients less frequently presented within the Milan Criteria (14.9% vs 39.2%, p<0.001) than cirrhotic patients.

Treatment and survival of patients with non-cirrhotic HCC

Patients with non-cirrhotic HCC more frequently underwent surgical resection (43.6% vs 8.0%, p<0.001) but less frequently received liver transplantation (2.8% vs 19.2%, p<0.001), catheter delivered therapies (35.7% vs 42.7%, p=0.001) or palliative care-hospice services (16.5% vs 26.8%, p<0.001) compared to patients with cirrhotic HCC (**Table 4**). Patients with non-cirrhotic HCC had better median overall survival than patients with cirrhotic HCC [1.80 years (95% CI: 1.56-2.09) vs 1.32 years (95% CI: 1.25-1.39), p=0.004]. Non-cirrhotic patient's overall survival was significantly higher at 1 (63.5% vs 56.3%, p<0.001) and 3 years (35.2% vs 30.3%,p= 0.02) but not different at 5 years from diagnosis (25.7% vs 23.4%, p= 0.26) compared to patients with cirrhotic HCC (**Table 5 and Figure 3**).

Risk factors associated with increased mortality in patients with non-cirrhotic HCC were INR (HR 1.96, 95% CI 1.21–3.18, p=0.006), AFP (HR 1.00, 95% CI 1.00–1.00, p<0.001), anatomic stage of HCC [stage III vs stage I (HR 2.01, 95% CI 1.22–2.81, p<0.001, and stage IV vs stage I; HR 2.12, 95% CI 1.45–3.10, p<0.001), and hospice care (HR 1.85, 95% CI 1.35-2.54, p<.001), whereas albumin (HR 0.65, 95% CI 0.51-0.83, p<.001), surgical resection (HR 0.24, 95% CI 0.17–0.34, p<.001) and liver transplantation (HR 0.16, 95% CI 0.05–0.53, p=0.002) were associated with reduced risk of death (**Table 6**). The etiology of underlying liver disease did not affect mortality in patients with non-cirrhotic HCC.

DISCUSSION

In this large US multicenter study, HCC arose in non-cirrhotic liver background in nearly 12% of patients. NAFLD was the most common liver disease in these patients followed by HCV. NAFLD has contributed to both non-cirrhotic and cirrhotic HCC burden over the 14.5 year study period. However, the net effect in the cohort as of the year 2014 was increased percentage of cirrhotic HCC and a decline in non-cirrhotic HCC.

While this study and others from the US and UK have shown that NAFLD is a rapidly increasing cause of HCC[8,9,19], it was not previously investigated whether NAFLD contribution is mainly through the cirrhosis-carcinogenesis pathway and/or non-cirrhotic-carcinogenesis pathway. Our study shows that NAFLD is contributing to the enlarging HCC burden via both the non-cirrhotic and cirrhotic pathways. The majority of HCC contributed by NAFLD and HCV were via the cirrhosis-HCC pathway, thus explaining the overall rise in cirrhotic HCC. The observed increase in the percentage of non-cirrhotic HCC related to NAFLD was not significant or large enough to result in increasing the total burden of non-cirrhotic HCC. This is possibly because the contribution of other liver diseases to the non-cirrhotic HCC burden was not large enough and the fraction contributed by NAFLD was not substantial as of the year 2014.

NAFLD (and HBV as expected) were the only etiologies significantly more common in non-cirrhotic HCC. Indeed, patients with non-cirrhotic HCC were twice as likely to have NAFLD than patients with cirrhotic HCC (26.3% vs 13.4%, p <0.001), highlighting the importance of further investigating the mechanisms of HCC development in the absence of cirrhosis in patients with NAFLD. This finding is consistent with the findings of a recent systematic analysis showing that in the absence of cirrhosis, patients with NASH have a higher risk of HCC than patients with other liver diseases[50].

With the advent of effective cures, US national predictions project a decline in the contribution of HCV to the cirrhosis and HCC burdens and liver transplant utilization coinciding with a rise in NAFLD impact on these burdens and resources[19,20,51,52]. Thus, in this context, the observed significant rise in cirrhotic NAFLD HCC in this study is an important finding that confirms current AASLD guideline recommendations of surveillance for HCC in patients with NAFLD cirrhosis[40]. Our observation of a rise in the percentage of non-cirrhotic HCC cases related to NAFLD, albeit not statistically significant as of the end of study in 2014, will require further follow up study of the trend in the setting of the ongoing epidemic of obesity and NAFLD.

The non-cirrhotic HCC group included significantly more women than the cirrhotic HCC group, a finding seen in another study[53]. The reason for this disparity is unclear but may be related to a higher prevalence of NAFLD observed in women compared to men in this study (23.3% vs 12.4%, p<0.001).

The proportion of Asians patients was also higher in the non-cirrhotic group, probably due to higher proportion of HBV which was the underlying disease in 73.9% in non-cirrhotic Asian patients compared to 60% in cirrhotic Asian patients(p=0.8).

Similar to the findings of the Mittal et al study[44], slightly higher proportion of HCV patients had non-cirrhotic HCC (12.1%) vs those with HBV (10%). While HBV is more carcinogenic than HCV, the prevalence and time of acquisition of HBV (at birth or childhood in endemic areas in Asia and Africa vs adulthood in the Western countries) may be the reason for this observation.

The overall rate of non-cirrhotic HCC in this study (11.7%) is within the range of reported rates from prior US and European studies (6.9%-27.8%)[9,44,53,54]. The overall rate of non-cirrhotic HCC related to NAFLD (20.7%) was close to that reported in recent UK (22.8%)[9] and German (22.2%)[55] studies but lower than other studies reporting these rates by disease etiology[34-38]. These differences may be due to population differences and varying criteria for ascertaining the cirrhotic status between studies. We used the Mittal criteria to determine cirrhotic status after we validated the Mittal level 2 criteria for absence of cirrhosis in an independent cohort and found them to be highly specific (specificity of 98%). This should facilitate other investigators to define the absence of cirrhosis in their cohort studies with high confidence.

Non-cirrhotic patients with HCC presented at older age and with larger and more advanced HCC that was more commonly outside the Milan criteria, finding confirming those noted in other studies [53,56,57]. The significant difference in tumor size is probably due to lack of HCC surveillance in the majority of patients with non-cirrhotic HCC, who were not known to have underlying liver disease prior to the HCC presentation. Regular HCC surveillance within 2 years prior to HCC diagnosis was documented in 14.7% of non-cirrhotic patients, which is higher than the proportion of HBV patients (10%) in this group, reflecting variability in practice patterns and adherence to guidelines. The reason for imbalance between cirrhotic and non-cirrhotic patients in frequency of metabolic and vascular comorbidities (non-cirrhotics with lower frequency of obesity and diabetes but higher frequency of hypertension, dyslipidemia and coronary artery disease), is unclear. Similar to the findings in Weinmann et al study[55], patients with NAFLD and non-cirrhotic HCC in our cohort were older, more frequently females, had a higher BMI and higher frequency of metabolic and coronary artery disease (data not shown).

Non-cirrhotic patients more frequently underwent resection than liver transplantation and had better median overall survival than cirrhotic HCC. The median overall survival of non-cirrhotic patients was better than cirrhotic patients despite presenting with more advanced tumors,

probably due to normal underlying liver function, lack of portal hypertension associated with cirrhosis and better performance status at presentation. Nevertheless, the prognosis for all patients with HCC, with or without cirrhosis, remains poor in the US at the present.

While etiology of liver disease did not affect mortality in the non-cirrhotic patients, INR, albumin, AFP, anatomic stage of HCC, hospice care, surgical resection and liver transplantation influenced the risk of death in our study. Unlike findings of a prior single center study that assessed 143 patients with non-cirrhotic HCC specifically referred for surgical resection, age or sex did not influence the risk of death in our study[58].

This study has several limitations. As with any retrospective study, some data points were missing (e.g. on performance status or histology), but our manual review of each individual patient's chart significantly reduced missing data points and allowed collection of meaningful data to permit high resolution examination of these patients and their tumors characteristics. The higher rate of unclear or unknown underlying liver etiology in non-cirrhotic patients may hint at less frequent hepatology evaluation of the underlying liver disease etiology prior to resection and locoregional therapies in this group. Another limitation is that beyond the presence or absence of cirrhotic background in the resection sample, the exact stage of fibrosis was not consistently given in the pathology reports and we failed to systematically collect such data when available. However, we augmented the our data by the use of APRI and FIB4 score, which both show that the majority of patients fell below the cirrhotic range for these tests. Similar to other studies, there is referral bias in this study as these patients were seen at tertiary care centers. Although our findings may be applicable to HCC patients seen in other tertiary centers in the US, they may not be generalizable to HCC patients seen in the community. The reliance on non-histological criteria to determine the absence of cirrhosis in a small subset (4%) may have resulted in misclassification. However, we validated the performance of the level 2 Mittal criteria for absence of cirrhosis in an independent cohort and found these criteria to be

highly specific. Further, when we excluded level 2 no cirrhosis patients from the analysis, the trends of cirrhotic and non-cirrhotic HCC remained the same, thus increasing the confidence in our observation.

Despite these limitations, this study has several strengths. It included the largest number of patients with non-cirrhotic HCC in the literature to date. We validated and systematically applied specific criteria to determine the cirrhotic status. The manual review of each participant's chart allowed us to collect extensive data to phenotype the patients and tumors. We did not use ICD codes or presence of comorbidities to infer the presence of NAFLD, rather we directly verified the etiology of underlying liver disease including NAFLD based on manual chart reviews. Unlike other large cohorts with a primary focus on transplantation or resection, our dataset includes four liver transplant centers and one national cancer center each offering a range of treatment modalities. Finally, the study period from 2000-2014 allowed us to assess the trends of non-cirrhotic HCC and its associated liver diseases.

In conclusion, non-cirrhotic HCC account for nearly 12% of all HCC cases in this large multicenter US study. NAFLD was the most common liver disease in non-cirrhotic HCC, and a leading cause of cirrhotic HCC in this population. Although NAFLD is contributing to the rising HCC burden via both the non-cirrhotic and cirrhotic stages, its contribution to non-cirrhotic HCC burden was not significant or large enough to result in increasing the total burden of non-cirrhotic HCC as of the end of this study in 2014.

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Figure Legends

Figure 1:

Panel A: Non-cirrhotic HCC: Percentage of non-cirrhotic patients from 2000 to 2014

Panel B: Cirrhotic HCC: Percentage of cirrhotic patients from 2000 to 2014

Figure 2:

Time Trends of the Percentage of Non-Cirrhotic and Cirrhotic HCC per Underlying Liver Disease Etiology

Figure 3:

Survival of HCC patients with and without underlying cirrhosis

Table 1. Mittal's definitions for classification of cirrhosis categories

Cirrhosis Category	Definition
Level 1 evidence of no cirrhosis (very high probability)	No evidence of cirrhosis on resection specimen or liver biopsy performed within 1 year before or at time of HCC diagnosis. **AND** No features suggestive of cirrhosis on abdominal imaging available nearest to HCC diagnosis within 3 years before HCC diagnosis.
Level 2 evidence of no cirrhosis (high probability)	APRI <1 based on laboratory results available nearest to HCC diagnosis within 6 months before and 4 weeks after HCC diagnosis. **AND** No features suggestive of cirrhosis on abdominal imaging performed nearest to HCC diagnosis within 3 years before HCC diagnosis. **AND** Two of 3 test values in normal range based on laboratory results available nearest to HCC diagnosis within 6 months before and 4 weeks after HCC diagnosis (albumin >3.5 g/L, platelets >200,000/mL, or international normalized ratio <1.1).
Confirmed Cirrhosis	Documented cirrhosis on resection specimen or liver biopsy performed any time before or at time of HCC diagnosis. **OR** Features suggestive of cirrhosis on abdominal imaging performed nearest to HCC diagnosis within 3 years before HCC diagnosis. **OR** Documented presence of ascites, varices, or hepatic encephalopathy. **OR** Abnormal values on 2 of 3 laboratory tests available nearest to HCC diagnosis within 6 months before and 4 weeks after HCC diagnosis (albumin <3.0 g/L, platelets <200,000 mL, international normalized ratio >1.1).
Unclassified	Insufficient information to classify in any cirrhosis category.

From Mittal et al[44]. Right to use table obtained from Elsevier.

Table 2: Characteristics of the patients with hepatocellular carcinoma with and without underlying cirrhosis

Variable	Cirrhosis		p-value
	No N=605	Yes N=4,539	
Age (Years)	64.1 ± 14.0	61.2 ± 9.9	<0.001
Male Sex	400 (66.1%)	3,597 (79.2%)	<0.001
Race			
- White	420 (70.0%)	3,149 (70.4%)	<0.001
- Black	50 (8.3%)	556 (12.4%)	
- Hispanic	59 (9.8%)	452 (10.1%)	
- Asian	46 (7.7%)	140 (3.1%)	
- Other	25 (4.2%)	174 (3.9%)	
Center*			
Atrium Health	55 (9.6%)	509 (89%)	<0.001
Columbia	61 (11.3%)	470 (87.2%)	
Indiana University	135 (10.8%)	1054 (84.3%)	
MD Anderson	329 (15.6%)	1728 (82.1%)	
Vanderbilt	25 (2.9%)	778 (90.3%)	
BMI (kg/m2)	27.3 ± 6.1	28.6 ± 6.0	<0.001
Obesity	134 (22.1%)	1,392 (30.7%)	<0.001
Diabetes	205 (34.1%)	1,749 (38.7%)	0.02
Hypertension	412 (68.4%)	2,777 (61.5%)	0.001
Dyslipidemia	229 (38.0%)	992 (22.0%)	<0.001
Coronary Artery Disease	132 (22.0%)	752 (16.7%)	0.001
Peripheral Vascular Disease	69 (11.5%)	332 (7.4%)	<0.001
History of Alcohol Abuse	102 (17.1%)	2,075 (46.2%)	<0.001
HIV Positive	4 (0.7%)	55 (1.2%)	0.34
Total Bilirubin (mg/dL)	1.0 ± 2.0	2.2 ± 3.3	<0.001
Albumin (g/dL)	3.9 ± 0.6	3.3 ± 0.7	<0.001
Platelets (K/mm^3)	278.9 ± 118.9	148.4 ± 101.4	<0.001
Creatinine (mg/dL)	1.0 ± 0.7	1.1 ± 0.8	0.72
INR	1.1 ± 0.2	1.3 ± 0.4	<0.001
MELD Score	NA	12.2 ± 5.6	
APRI Score	1.0 ± 1.8	3.1 ± 6.6	<0.001
APRI Category			
- <1.0	450 (78.5%)	1,077 (25.8%)	<0.001
- 1.0-2.0	77 (13.4%)	1,143 (27.4%)	
- >2.0	46 (8.0%)	1,950 (46.8%)	

Variable	Cirrhosis		p-value
	No N=605	Yes N=4,539	
FIB-4 Category			
- <1.60	194 (34.4%)	212 (5.1%)	<.0001
- 1.60-3.60	255 (45.2%)	874 (21.1%)	
- >3.60	115 (20.4%)	3,066 (73.8%)	
Performance Status			
- 0 (KPS 90 or 100)	190 (49.2%)	799 (38.4%)	<0.001
- 1 (KPS 70 or 80)	130 (33.7%)	773 (37.1%)	
- 2 (KPS 50 or 60)	48 (12.4%)	313 (15.0%)	
- 3 (KPS 30 or 40)	16 (4.1%)	164 (7.9%)	
- 4 (KPS 10 or 20)	2 (0.5%)	33 (1.6%)	
Underlying liver disease			
- AIH/PBC/PSC	4 (0.7%)	72 (1.6%)	<0.001
- Alcohol alone	44 (7.3%)	611 (13.5%)	
- HBV + Alcohol	10 (1.7%)	59 (1.3%)	
- HBV alone	50 (8.3%)	265 (5.8%)	
- HC/A1ATD	3 (0.5%)	78 (1.7%)	
- HCV + Alcohol	11 (1.8%)	1,053 (23.2%)	
- HCV + HBV (+/- alcohol)	10 (1.7%)	178 (3.9%)	
- HCV alone	52 (8.6%)	1,211 (26.7%)	
- NAFLD	159 (26.3%)	608 (13.4%)	
- Rare etiologies	5 (0.8%)	16 (0.4%)	
- Unclear/Unknown	257 (42.5%)	388 (8.5%)	

Abbreviations: BMI: body mass index, HIV: human immunodeficiency virus, INR: international normalized ratio, MELD: Model for End-Stage Liver Disease, APRI: AST to Platelet Ratio Index, HCV: hepatitis C virus, AIH: autoimmune hepatitis. PBC: primary biliary cholangitis, PSC: primary sclerosing cholangitis, HC: hemochromatosis, A1ATD: alpha-1-antitrypsin deficiency, NA: not applicable, NAFLD: non-alcoholic fatty liver disease. * Remaining % in each center data indicate % with unclassified cirrhotic status.

Table 3: Tumor characteristics in patients with and without underlying cirrhosis

Variable	Cirrhosis		p-value
	No N=605	Yes N=4,539	
Tumor Size (cm)	8.9 ± 4.8	5.3 ± 3.9	<.001
AFP Category			
- <20	242 (45.1%)	1,711 (42.7%)	0.01
- 20-200	96 (17.9%)	942 (23.5%)	
- >200	199 (37.1%)	1,358 (33.9%)	
- Part of surveillance	84 (16.9%)	1,075 (29.1%)	<0.001
- Incidental	94 (18.9%)	416 (11.2%)	<0.001
- Symptoms work-up	299 (60.2%)	2,045 (55.3%)	0.03
- Other	4 (0.8%)	6 (0.2%)	0.005
- NA/Unknown	17 (3.4%)	189 (5.1%)	0.10
Regular Surveillance within 2 Years before HCC?			
- Yes	73 (14.7%)	1206 (32.5%)	<0.001
- Unknown	160 (32.3%)	1432 (38.6%)	
Method of Diagnosis			
- Histology	548 (90.6%)	2828 (62.3%)	<.001
- Imaging	568 (93.9%)	4257 (93.8%)	0.92
- Other	5 (0.8%)	20 (0.4%)	0.19
- Unknown	0 (0.0%)	4 (0.1%)	0.46
Anatomic Stage			
- Stage I	184 (33.6%)	1278 (31.9%)	<0.001
- Stage II	77 (14.1%)	905 (22.6%)	
- Stage IIIA	81 (14.8%)	414 (10.3%)	
- Stage IIIB	54 (9.9%)	619 (15.4%)	
- Stage IIIC	16 (2.9%)	44 (1.1%)	
- Stage IVA	31 (5.7%)	210 (5.2%)	
- Stage IVB	104 (19.0%)	537 (13.4%)	
Anatomic Stage Category			
- Stage I or II	261 (47.7%)	2183 (54.5%)	0.002
- Stage III or IV	286 (52.3%)	1824 (45.5%)	
Tumor Differentiation			
- Well	139 (29.8%)	757 (33.1%)	0.056
- Moderate	206 (44.2%)	1066 (46.6%)	
- Poor	117 (25.1%)	450 (19.7%)	
- Undifferentiated / anaplastic	4 (0.9%)	15 (0.7%)	
Tumor Stage			

Variable	Cirrhosis		p-value
	No N=605	**Yes N=4,539**	
- Single	222 (37.0%)	1545 (34.4%)	<0.001
- 3 tumors < 3 cm	12 (2.0%)	478 (10.6%)	
- Large multinodular	148 (24.7%)	966 (21.5%)	
- Vascular invasion or extrahepatic spread	218 (36.3%)	1503 (33.5%)	
Tumor within Milan Criteria	90 (14.9%)	1774 (39.2%)	<0.001

Abbreviations: AFP: Alfa fetoprotein.

Table 4: Treatment modalities offered to patients with HCC, stratified according to Cirrhosis Status

Variable	Cirrhosis		p-value
	No N=605	Yes N=4,539	
Treatment Modalities			
- Resection	264 (43.6%)	362 (8.0%)	<0.001
- Liver Transplantation	17 (2.8%)	873 (19.2%)	<0.001
- Catheter Delivered Therapy	216 (35.7%)	1,939 (42.7%)	0.001
- Sorafenib	131 (21.7%)	921 (20.3%)	0.43
- SBRT	54 (8.9%)	348 (7.7%)	0.27
- RFA and/or Microwave Ablation	53 (8.8%)	502 (11.1%)	0.08
- Palliative/Hospice Care	100 (16.5%)	1,218 (26.8%)	<0.001
- Other	83 (13.7%)	313 (6.9%)	<0.001
- None	19 (3.1%)	246 (5.4%)	0.01
- Unknown	42 (6.9%)	271 (6.0%)	0.34

Abbreviations: SBRT: stereotactic body radiation therapy; RFA: radiofrequency ablation

Table 5: Survival of Patients with HCC with and without underlying cirrhosis

Survival	Cirrhosis		p-value
	No N=605	Yes N=4,539	
Overall (years), median (95% CI)	1.80 (1.56-2.09)	1.32 (1.25- 1.39)	0.004
One Year	63.5%	56.3%	<.001
Three Year	35.2%	30.3%	0.02
Five Year	25.7%	23.4%	0.26

Abbreviations: CI: Confidence Interval.

Table 6: Variables associated with mortality among patients with non-cirrhotic HCC

Variable	HR	95% CI	p-value
Age at Diagnosis	1.00	0.99 – 1.01	0.67
BMI at Diagnosis	0.99	0.96 – 1.01	0.22
Platelets	1.00	1.00 – 1.00	0.12
INR	1.96	1.21 – 3.18	**0.006**
Albumin	0.65	0.51 – 0.83	**<0.001**
AFP	1.00	1.00 – 1.00	<0.001
Female Sex	1.23	0.92 – 1.66	0.16
Race			
Asian vs White	0.84	0.37 – 1.95	0.69
Black vs White	0.80	0.50 – 1.29	0.36
Hispanic vs White	1.11	0.76 – 1.63	0.58
Other vs White	1.11	0.58 – 2.14	0.75
Diabetes	1.20	0.89 – 1.60	0.22
Anatomic Stage			
Stage II vs Stage I	0.88	0.55 – 1.40	0.58
Stage III vs Stage I	2.01	1.44 – 2.81	**<0.001**
Stage IV vs Stage I	2.12	1.45 – 3.10	**<0.001**
Resection	0.24	0.17 – 0.34	**<0.001**
Liver Transplantation	0.16	0.05 – 0.53	**0.002**
Palliative/Hospice Care	1.85	1.35 – 2.54	**<0.001**
Etiologies			
Alcohol Alone vs Unclear/Unknown	1.02	0.64 – 1.63	0.92
HBV vs Unclear/Unknown	1.60	0.67 – 3.81	0.29
HCV vs Unclear/Unknown	1.24	0.80 – 1.91	0.33
NAFLD vs Unclear/Unknown	0.98	0.71 – 1.36	0.92

Abbreviations: BMI: body mass index; INR: international normalized ratio; AFP: Alfa fetoprotein; HR: Hazard Ratio; CI: Confidence Interval.

Supplemental Material

Supplemental Table 1

Characteristics of patients with HCC without underlying cirrhosis, separated based on Level 1 vs. Level 2 criteria for the absence of cirrhosis

Supplemental Figure 1:

Study Flow Diagram

Supplemental Figure 2:

Time Trends of the Percentage of Non-Cirrhotic (Level 1 Criteria)) and Cirrhotic HCC in the Overall Cohort

Panel A:

Non-cirrhotic HCC by level 1 criteria: Percentage of non-cirrhotic patients by level 1 criteria from 2000 to 2014

Panel B:

Cirrhotic HCC: Percentage of patients with HCC and underlying cirrhosis over time

Supplementary Table 1: Characteristics of patients with HCC without underlying cirrhosis, separated based on Level 1 vs. Level 2 criteria for the absence of cirrhosis

Variable	Overall N=605	Evidence of No Cirrhosis		p-value
		Level 1 N=397	Level 2 N=208	
Age (Years)	64.1 ± 14.0	63.3 ± 14.1	65.5 ± 13.7	0.06
Males	400 (66.1%)	264 (66.5%)	136 (65.4%)	0.78
Race				
- White	420 (70.0%)	279 (71.0%)	141 (68.1%)	0.52
- Black	50 (8.3%)	35 (8.9%)	15 (7.2%)	
- Hispanic	59 (9.8%)	33 (8.4%)	26 (12.6%)	
- Asian	46 (7.7%)	29 (7.4%)	17 (8.2%)	
- Other	25 (4.2%)	17 (4.3%)	8 (3.9%)	
BMI (kg/m^2)	27.3 ± 6.1	27.4 ± 6.3	27.1 ± 5.9	0.53
Obesity	134 (22.1%)	85 (21.4%)	49 (23.6%)	0.54
Diabetes	205 (34.1%)	135 (34.2%)	70 (33.8%)	0.92
Hypertension	412 (68.4%)	267 (67.6%)	145 (70.0%)	0.53
Dyslipidemia	229 (38.0%)	155 (39.2%)	74 (35.7%)	0.40
Coronary Artery Disease	132 (22.0%)	79 (20.1%)	53 (25.6%)	0.11
Peripheral Vascular Disease	69 (11.5%)	41 (10.4%)	28 (13.6%)	0.24
History of Alcohol Abuse	102 (17.1%)	61 (15.6%)	41 (19.9%)	0.17
ALT (units/L)	65.2 ± 82.1	73.2 ± 90.8	50.3 ± 59.6	0.001
AST (units/L)	88.1 ± 149.0	101.7 ± 180.3	63.2 ± 50.4	0.003
Total Bilirubin (mg/dL)	1.0 ± 2.0	1.1 ± 2.4	0.9 ± 1.1	0.19
Alkaline Phosphatase (units/L)	178.8 ± 214.0	186.3 ± 247.4	164.7 ± 130.5	0.24
Albumin (g/dL)	3.9 ± 0.6	3.9 ± 0.6	3.9 ± 0.6	0.65
Platelets (K/mm^3)	278.9 ± 118.9	267.1 ± 116.3	300.8 ± 120.8	0.001
Creatinine (mg/dL)	1.0 ± 0.7	1.0 ± 0.8	1.1 ± 0.7	0.65
INR	1.1 ± 0.2	1.1 ± 0.2	1.1 ± 0.3	0.6496
AFP (ng/mL)	27,536.6 ± 149,888.1	28,778.2 ± 174611.7	25,287.5 ± 89,493.5	0.79
MELD Score	8.9 ± 3.4	8.9 ± 3.5	8.8 ± 3.2	0.74
APRI Score	1.0 ± 1.8	1.2 ± 2.2	0.6 ± 0.4	<.0001
APRI Category				
- <1.0	450 (78.5%)	259 (70.2%)	191 (93.6%)	<0.001
- 1.0-2.0	77 (13.4%)	67 (18.2%)	10 (4.9%)	
- >2.0	46 (8.0%)	43 (11.7%)	3 (1.5%)	
Performance Status				
- 0 (KPS 90 or 100)	190 (49.2%)	141 (55.1%)	49 (37.7%)	0.006

Variable	Overall N=605	Evidence of No Cirrhosis		p-value
		Level 1 N=397	Level 2 N=208	
- 1 (KPS 70 or 80)	130 (33.7%)	75 (29.3%)	55 (42.3%)	
- 2 (KPS 50 or 60)	48 (12.4%)	31 (12.1%)	17 (13.1%)	
- 3 (KPS 30 or 40)	16 (4.1%)	9 (3.5%)	7 (5.4%)	
- 4 (KPS 10 or 20)	2 (0.5%)		2 (1.5%)	
Underlying liver disease				
- AIH/PBC/PSC	4 (0.7%)	4 (1.0%)	0 (0.0%)	
- Alcohol alone	44 (7.3%)	24 (6.0%)	20 (9.6%)	
- HBV + Alcohol	10 (1.7%)	6 (1.5%)	4 (1.9%)	
- HBV alone	50 (8.3%)	34 (8.6%)	16 (7.7%)	
- HC/A1ATD	3 (0.5%)	1 (0.3%)	2 (1.0%)	
- HCV + Alcohol	11 (1.8%)	7 (1.8%)	4 (1.9%)	0.01
- HCV + HBV (+/- alcohol)	10 (1.7%)	6 (1.5%)	4 (1.9%)	
- HCV alone	52 (8.6%)	37 (9.3%)	15 (7.2%)	
- NAFLD	159 (26.3%)	123 (31.0%)	36 (17.3%)	
- Rare etiologies	5 (0.8%)	4 (1.0%)	1 (0.5%)	
- Unclear/Unknown	257 (42.5%)	151 (38.0%)	106 (51.0%)	
Tumor Size	8.9 ± 4.8	8.9 ± 4.9	9.1 ± 4.8	0.61
Anatomic Stage Category				
- Stage I or II	261 (47.7%)	192 (54.4%)	69 (35.6%)	<0.001
- Stage III or IV	286 (52.3%)	161 (45.6%)	125 (64.4%)	
Tumor Differentiation				
- Well	139 (29.8%)	93 (28.6%)	46 (32.6%)	
- Moderate	206 (44.2%)	158 (48.6%)	48 (34.0%)	0.01
- Poor	117 (25.1%)	71 (21.8%)	46 (32.6%)	
- Undifferentiated / anaplastic	4 (0.9%)	3 (0.9%)	1 (0.7%)	
Tumor Stage				
- Single	222 (37.0%)	164 (41.3%)	58 (28.6%)	
- 3 tumors < 3 cm	12 (2.0%)	10 (2.5%)	2 (1.0%)	
- Large multinodular	148 (24.7%)	102 (25.7%)	46 (22.7%)	<0.001
- Vascular invasion or extrahepatic spread	218 (36.3%)	121 (30.5%)	97 (47.8%)	
Tumor within Milan Criteria	90 (14.9%)	74 (18.6%)	16 (7.7%)	<0.001
Overall Survival (Years)	2.5 ± 2.8	3.0 ± 3.1	1.6 ± 1.9	<0.001

Abbreviations: BMI: body mass index, HIV: human immunodeficiency virus, INR: international normalized ratio, MELD: Model for End-Stage Liver Disease, APRI: AST to Platelet Ratio Index, HCV: hepatitis C virus, AIH: autoimmune hepatitis. PBC: primary biliary cholangitis, PSC: primary sclerosing cholangitis, HC: hemochromatosis, A1ATD: alpha-1-antitrypsin deficiency, NAFLD: non-alcoholic fatty liver disease.