The NAFLD Burden on Mortality and Morbidities in General Population: a Community-Based Longitudinal Study (NASH-CO study)

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Abbreviations

NAFLD: non-alcoholic fatty liver disease

CVD: cardiovascular disease

EHC: extrahepatic cancer

CKD: chronic kidney disease

NASH: non-alcoholic steatohepatitis

HCC: hepatocellular carcinoma

HBV: hepatitis B virus

HCV: hepatitis C virus

FLI: fatty liver index

SNDS: Système National des Données de Santé

CNIL: Commission Nationale Informatique et Libertés

AUDIT: alcohol use disorders identification test

BMI: body mass index

HSC: Health Screening Center

GGT: gamma glutamyl-transferase

ALT: alanine aminotransferase

HD-cholesterol: high-density lipoprotein cholesterol

TG: triglyceride

WHO: world health organization

HBP: high blood pressure

CKD-EPI: Chronic Kidney Disease-Epidemiology Collaboration

MAFLD: metabolic associated fatty liver disease

T2DM: type 2 diabetes mellitus

AST: aspartate aminotransferase

FIB-4: fibrosis-4

FI: Forns Index

ICD-10: International Classification of Diseases 10th revision

GFR: glomerular filtration rate

SD: standard deviation

IPTW: inverse-probability of treatment weighting

HR: hazard ratios

CI: confidence interval

DAA: direct-acting antiviral

Data availability

Data from this study are protected by health data regulations from the French National Commission on Informatics and Liberty (Commission Nationale de l'Informatique et des Libertés). Data can be made available upon reasonable request to the steering committee of the Constances cohort study, after legal verification of the use of the data.

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Ethics approval

The research was conducted in accordance with both the Declarations of Helsinki and Istanbul. The study was approved by the "Commission Nationale Informatique et Libertés" (CNIL), and ethical approval was obtained from the Institutional Review Board of the French National Institute of Health.

Patient consent statement:

All participants provided written informed consent for the use of their personal data for research.

Author contributions to the manuscript

Oumarou Nabi, Nathanaël Lapidus, Karine Lacombe, and Lawrence Serfaty: study concept and design; acquisition of data; analysis and interpretation of data; drafting of the manuscript; critical revision of the manuscript for important intellectual content; statistical analysis.

Jerome Boursier, Philippe Mathurin, Victor de Ledinghen: analysis and interpretation of data; drafting of the manuscript; critical revision of the manuscript for important intellectual content.

Jean-Michel Petit, Sofiane Kab, Adeline Renuy, Marie Zins: critical revision of the manuscript for important intellectual content.

Abstract

Background: The impact of Non-alcoholic fatty liver disease (NAFLD) on morbidity and mortality has yet to be documented at the general population level. This study aimed to assess whether NAFLD was associated with morbidities and mortality, and to estimate its impact on health status and mortality.

Methods: The study population consisted of 137,206 participants from Constances cohort. Non-invasive diagnosis of NAFLD and advanced fibrosis was performed using the fatty liver index and Forns index, respectively. Constances data were linked to health care and hospitalization data to identify liver-related events, cardiovascular diseases (CVD), extrahepatic cancers (EHC), chronic kidney disease (CKD) and all-cause mortality.

Results: The prevalence of NAFLD was 18.3% in subjects without other chronic liver diseases, among whom 2.7% had fibrosis. NAFLD after IPTW-weighted remained associated with an increased risk of death (HR 1.26, 95%CI 1.01–1.57), hepatic-related complications (HR 2.48, 95%CI 1.99–3.29), CVD (HR 1.42, 95%CI 1.30–1.55), EHC (HR 1.11, 95%CI 1.01–1.28) and CKD (HR 1.81, 95%CI 1.53–2.07) compared to those without chronic liver diseases risk factors (Non-NAFLD). In the trend analysis over the study period of inclusion and compared to Non-NAFLD, NAFLD have showed a fastest growing cause of hepatic events (HR 1.38, 95% CI 1.07–1.76 per year), CVD (HR 1.08, 95% CI 1.03–1.12), CKD (HR 1.16, 95% CI 1.07–1.25), and death (HR 1.39, 95% CI 1.39–1.50).

Conclusion: This large community-based cohort showed that NAFLD was associated with excess morbidity and mortality and demonstrated a fastest-growing trend.

Lay summary

This study provide evidence that, NAFLD has become a major public health problem with a significant negative impact on morbidity and mortality. Moreover, with the increasing prevalence of diabetes, obesity and metabolic syndrome, the prevalence of NAFLD and the morbidity and mortality related to NAFLD will continue to progress.

Key words: NAFLD; advanced fibrosis; Fatty Liver Index; Forns Index; cardiovascular diseases; chronic kidney disease; extrahepatic malignancies, mortality.

Introduction

Non-alcoholic fatty liver disease is a common cause of chronic liver disease(1). This syndrome ranges from simple steatosis to non-alcoholic steatohepatitis (NASH) with varying amounts of fibrosis and cirrhosis(2). Cohort studies have reported that the severity of liver fibrosis was the best predictor of liver-related mortality, but also of overall mortality in patients with biopsy-proven NAFLD(3). As a major risk factor for cirrhosis and hepatocellular carcinoma (HCC), NAFLD is possibly becoming the leading cause of liver transplantation and liver-related mortality(4,5). In addition to liver-related complications, NAFLD may promote CVD, extrahepatic malignancy, or CKD(6–8). However, few studies have investigated the burden of NAFLD at a general population level especially in Europe. The French Constances population-based cohort was designed as a large sample, representative for age, gender, and socioeconomic status of the French adult population aged 18–69 years, composed of more than 220,000 subjects included between 2012 and 2021(9). Using this cohort, the present study aimed to assess the burden of NAFLD on liver-related morbidity, extrahepatic morbidities (CVD, EHC, and CKD), and overall mortality in the general population.

Materials and Methods

Study sample

We conducted a longitudinal study with data collected at baseline and follow-up from participants included in the Constances cohort between 2012 and 2019. This cohort has been previously described(9,10). Subjects recruited in the Constances cohort were included in the present analysis if they were at least 18 years old and had no hepatitis B virus (VHB) and or hepatitis C virus (HVC) infection, history or ongoing chronic hepatic disease such as autoimmune hepatitis, Wilson’s disease, hemochromatosis, alpha-1 antitrypsin deficiency, or known hepatotoxic drug intake (systemic corticosteroid therapy, methotrexate, tamoxifen, amiodarone), or hazardous alcohol use (defined by daily consumption above 30 grams per day

in men and 20 grams per day in women). Next, the overall sample was split into two groups: (i) non-NAFLD subjects, corresponding to those with the blood test Fatty Liver Index (FLI) <60; (ii) NAFLD subjects, corresponding to those with FLI ≥60. To assess the burden of fibrosis on morbidity and mortality, the NAFLD population was divided into three groups according to the result of the blood test Forns Index: those with mild fibrosis, those with intermediate fibrosis, and those with advanced fibrosis (Supplementary Methods). Constances data were linked to the National Healthcare Database (Système National des Données de Santé [SNDS]) that provided data on hospitalization and occurrence of death. The research was conducted in accordance with both the Declarations of Helsinki and Istanbul. The study was approved by the Commission Nationale Informatique et Libertés (CNIL), and ethics clearance was obtained from the Institutional Review Board of the French National Institute of Health (Authorization number: 910486). All participants provided written informed consent for the use of personal data for research.

Data collection at inclusion

Socio-demographics, comorbidities, and lifestyle data were obtained through standardized self-administreted questionnaires. Socio-demographics data included age, gender, occupation and employment status, education level, and geographic origin. Alcohol consumption was assessed using the alcohol use disorders identification test (AUDIT) questionnaire(11). The number of glasses per day was converted into grams per day of alcohol (1 glass=10 g of alcohol).

The health and morbidity data included history of cancer (colon, breast, prostate, lung, thyroid, ovary, and uterus), cardiovascular events (arteritis of lower limbs, myocardial infarction, stroke, and angina pectoris), blood hypertension, and diabetes. Body mass index (BMI) was calculated as the ratio of weight to height squared and obesity was defined if BMI ≥30 kg/m² (≥27.5 kg/m² if Asian ethnicity). Blood samples were made on a venous blood

sample after a 12-hour fasting period, and analyses were performed in the Health Screening Center (HSC) laboratories according to standards common to all. Available biological data of interest were blood glucose, serum creatinine, gamma glutamyl transferase (GGT), alanine aminotransferases (ALT), total cholesterol, high-density lipoprotein cholesterol (HD-cholesterol), triglycerides (TGs), and platelets. ALT was considered to be elevated at a threshold of 40 IU/L, and GGT at a threshold of 55 IU/L in men and 45 IU/L in women(12). Diabetes was defined by a blood glucose >6.9 mmol/L after a 12-h fast according to the world health organization (WHO) definition(13) and/or antidiabetic therapy. High blood pressure (HBP) was defined based on self-reporting, antihypertensive therapy, measured systolic blood pressure ≥140 mmHg and/or diastolic ≥90 mmHg. Hypercholesterolemia was defined by a blood cholesterol >5.5 mm/L and/or use of a lipid-lowering drugs. Hypertriglyceridemia was defined by blood triglyceride >1.7 mm/L or the use of lipid-lowering drugs. The metabolic syndrome was defined according to the international diabetes federation(14). Chronic kidney disease at baseline was defined as a glomerular filtration rate <60 mL/min/1.73m^2 calculated according to the CKD-EPI equation (Chronic Kidney Disease-Epidemiology Collaboration) (Supplementary Methods).

Definition of NAFLD, metabolic associated fatty liver disease (MAFLD), and liver fibrosis

The fatty liver index (FLI) was chosen as a surrogate marker of NAFLD(15). It has been successfully cross-validated in external populations(16). Subjects with FLI ≥60 were considered to have NAFLD based on the literature. The score was calculated according to the published formula:

$$FLI = 100\frac{e^{0.953 \times log(trigycerides) + 0.139 \times BMI + 0.718 \times log(GGT) + 0.053 \times waist\ circumference}}{1 + e^{0.953 \times log(trigycerides) + 0.139 \times BMI + 0.718 \times log(GGT) + 0.053 \times waist\ circumference}}$$

The MAFLD was defined base on the published criteria(17) : the evidence of hepatic steatosis, or any of the following three conditions: overweight/obesity, presence of type 2 diabetes mellitus (T2DM), or evidence of metabolic dysregulation.

As the measurement of aspartate aminotransferase (AST) was not available in Constances for the calculation of the Fibrosis-4 (FIB-4) as well as other scores, the Forns Index (FI)(18) was chosen as a surrogate marker of liver fibrosis. It has been previously validated as a marker of liver fibrosis in a large cohort of biopsy-proven NAFLD (10). Moreover, FI has been shown as accurate predictor of morbidities and mortality in NAFLD patients(19). It was calculated according to the published formula(18):

$$FI = 7.811 - 3.131 \times log(platelet\ count) + 0.781 \times log(GGT) + 3.467 \times log(age) - 0.014 \times total\ cholesterol.$$

Thus, the previously published thresholds were used to rule in or rule out advanced fibrosis (F3/F4), <4.2 and >6.9, respectively. Subjects were classified as follows: non-NAFLD (FLI <60), NAFLD (FLI ≥60), NAFLD with mild fibrosis (FLI ≥60 and Forns index <4.2), NAFLD with intermediate fibrosis (FLI ≥60 and Forns index 4.2–6.9), and NAFLD with advanced fibrosis (FLI ≥60 and Forns index >6.9).

Follow-up study

Constances data were linked with the National Healthcare Database (SNDS), which provided data on medication, long-term chronic disease benefits, hospitalization and occurrence of death from January 1, 2012, to December 31, 2019(20). SNDS data were available during this period for 169,303 Constances participants. The follow-up period for each study participant was the time elapsed between the Constances baseline inclusion and December 31, 2019, the date of death, or the last date of follow-up, whichever occurred first. The International Classification of Diseases (ICD-10) code was used to define liver-related events according to the recent expert panel consensus statement (compensated cirrhosis, hepatocellular carcinoma, decompensated cirrhosis)(21), CVD (arteritis of lower limbs, myocardial infarction, stroke, angina pectoris), EHC (colon, breast, prostate, lung, thyroid, ovary, uterus), CKD (chronic kidney disease, diabetic nephropathy, hypertensive nephropathy, kidney failure)

(Supplementary Table 1), and overall mortality. Subjects with histories of cirrhosis-related complications or hepatocellular carcinoma, CVD, or EHC, or subjects with glomerular filtration rate (GFR) <60 mL/min/1.73 m^2 at baseline were excluded in the calculation of each category of incident events.

Patient and public involvement

Constances is a large prospective epidemiological cohort for generalist use characterized by a wide coverage of health problems and determinants and intended to serve as an epidemiological research infrastructure open and accessible to the scientific community to carry out ancillary projects on a variety of research questions. The projects resulting from this cohort do not involve the participation of patients or members of the public in the design, conduct, communication or dissemination plans of these projects. Therefore, this work did not involve Patient and Public Involvement statement.

Statistical analysis

Continuous and categorical variables were expressed as means ± standard deviation (SD) and as counts and percentages, respectively (Supplementary Methods). We performed a longitudinal analysis to estimate the association of NAFLD with morbidities and overall mortality in the general population. This sample was split into two groups: participants without NAFLD (FLI <60) and those with NAFLD (FLI ≥60). Due to the fact that FLI scores have a grey zone, we performed sensitive analyses regarding to the FLI cut-off. Then study population was split into three groups according to the FLI cut-off: FLI <30, FLI 30-60 and FLI ≥60 (NAFLD). To estimate the burden of liver fibrosis on the incidence of morbidities and mortality in NAFLD patients we split study sample into four groups: participants without NAFLD (FLI <60), those with NAFLD and mild fibrosis (FLI ≥60 and FI <4.2), those with NAFLD and intermediate fibrosis (FLI ≥60 and FI 4.2–6.9), and those with NAFLD and advanced fibrosis (FLI ≥60 and FI >6.9).

The cumulative incidence function was estimated for each clinical outcome, and Gray's test was used to estimate the differences in the cumulative incidence functions between the groups. Standard Kaplan-Meier estimators were calculated for the all-cause mortality rate.

Propensity-score-based stabilized inverse-probability of treatment weighting (IPTW) was applied to handle confounding. Logistic and multinomial propensity scores for belonging to either one of the compared groups were derived from potential confounding factors such as socio-demographic characteristics (age, sex, geographic origin, education level), clinical and anthropometric variables (high blood pressure, diabetes, metabolic syndrome and BMI, waist circumference), lifestyle (smoking, soft drink, coffee intake, and regular exercise practice), and biological data (TGs, cholesterol, and ALT). The positivity assumption was graphically checked. We used the standardized mean difference to assess the balance of covariates before and after weighting. An absolute standardized mean difference less than 0.10 was deemed satisfactory. IPTW-weighted Fine and Gray models were used to estimate hazard ratios (HRs) and their 95% confidence interval (CI) of liver-related events, CVD, EHC, and CKD with regards to the competing risk of death; and IPTW-weighted Cox proportional hazard models were used to estimate hazard ratios for overall mortality. We carried out sensitivity analyses using Cox models adjusted for confounders deemed clinically relevant to check the robustness of the results of the analysis with propensity scores (Supplementary Methods). Subjects presenting an HCC in the 6 months following inclusion were excluded from the hepatic events analyses, so that they could not be attributed to a too recent change in the status of chronic liver conditions. Finally, in order to assess the trend of the burden of NAFLD on the clinical outcomes and overall mortality over time, we tested the interaction between the study period and the risk groups in the previous models.

Considering mounting controversy about NAFLD and MAFLD, we performed sub-analysis to compare outcomes on NAFLD versus MAFLD (more details in supplementary methods). A p

value <0.05 was considered to be statistically significant. Analyses were performed using R Statistical Software version 4.1.0.

Results

At the time of analysis, baseline data were available for 199,341 participants. After excluding subjects who withdrew their consent, had other causes of liver diseases, or missing data for NAFLD assessment, and participants whose data were not available in the SNDS and those who were not meet inclusion criteria, 137,206 were included in the final analysis and defined the overall sample (Figure 1). Their baseline characteristics were not different from those of the excluded participants (data not shown, available on request). A total of 25,753 had NAFLD, which corresponded to 18.3% (95%CI 18.2–18.4) of the general population without HCV, HBV, hazardous alcohol use, and other chronic liver diseases. The baseline characteristics of the study sample are reported in Table 1.

Clinical outcomes and overall mortality according to the presence of NAFLD

After a median follow-up of 3.62 years (range 0.07–7.95), 649 subjects developed a hepatic event (including 119 with HCC) (Supplementary Table 2), 1901 had an incident cardiovascular event, 410 evolved towards CKD, 1854 were diagnosed with an extrahepatic malignancy, and 685 died. The cumulative incidence of liver-related events, CVD, CKD, and overall mortality were significantly associated with the presence of NAFLD compared to those without NAFLD (Figure 2 and Supplementary Figure 1 and 2). The liver-related events risk factors according to the presence of NAFLD are shown in Supplementary Table 3 and 4. Raw and IPTW-weighted analyses for the risk of clinical outcomes and overall mortality according to the presence of NAFLD are shown in Table 2 (and Supplementary Table 5). The presence of NAFLD compared to subjects without NAFLD after IPTW-weighted remained associated with an increased risk of liver-related events (HR 2.48, 95%CI 1.99–3.29), CVD

(HR 1.42, 95%CI 1.30–1.55), extrahepatic cancers (HR 1.11, 95%CI 1.01–1.28), CKD (HR 1.81, 95%CI 1.53–2.07), and overall mortality (HR 1.26, 95%CI 1.01–1.57). The results remained stable in sensitivity analysis after adjustment for confounding factors deemed clinically relevant (Supplementary Table 6 and 7).

In the trend analysis over the study inclusion period, NAFLD was associated with a growing cause of hepatic events (interaction HR 1.38, 95%CI 1.07–1.76 per year) and all-cause mortality (HR 1.39, 95%CI 1.29–1.50 per year). NAFLD also showed an increasing association over the study period regarding CVD (interaction HR 1.08, 95% CI 1.03–1.12 per year), CKD (interaction HR 1.16, 95% CI 1.07–1.25 per year), but not extrahepatic malignancies (Table 3).

The risk of liver-related events, extrahepatic malignancies, CKD, and overall mortality remained significantly associated with the presence of NAFLD compared to those with MAFLD. (Supplementary Figure 3, 4, and 5 and Supplementary Table 8, 9, 10, and 11).

NAFLD-related fibrosis burden on clinical outcomes and overall mortality

To estimate the burden of liver fibrosis on the incidence of morbidities and mortality in NAFLD patients, we split the NAFLD study population into three groups: 13326 NAFLD with mild fibrosis (FLI ≥60 and FI <4.2), 10638 NAFLD with intermediate fibrosis (FLI ≥60 and FI 4.2–6.9) and 535 NAFLD with advanced fibrosis (FLI ≥60 and FI >6.9). Liver-related and extrahepatic clinical outcomes as well as overall mortality according to NAFLD-related fibrosis degree and compared to Non-NAFLD are shown in Figure 3 (and Supplementary Figure 6 and 7). Raw and IPTW-weighted analyses of the risk of clinical outcomes and overall mortality according to the fibrosis degree are shown in Table 4 (and Supplementary Table 12 and 13). Compared to non-NAFLD, there was a dose-dependent effect of the severity of fibrosis on the liver-related and extrahepatic clinical outcomes as well as overall mortality excepted to extrahepatic cancers, i.e., mild, intermediate and advanced, in NAFLD

subjects for the risk of hepatic events (HR 6.67, 95% CI 3.46-12.85, p<0.001, HR 8.26, 95% CI 5.92-11.53, p<0.001, HR 56.72, 95% CI 12.31-261.39, p<0.001 respectively), CVD (HR 1.15, 95% CI 1.02-1.47, p=0.007, HR 1.22, 95% CI 10.9-1.65, p=0.022, HR 5.22, 95% CI 2.49-10.92, p<0.001 respectively), CKD (HR 2.46, 95% CI 1.18-5.11, p=0.016, HR 3.07, 95% CI 1.80, 6.86, p=0.031, HR 3.77, 95% CI 2.31-6.13, p<0.001 respectively) and all-cause mortality (HR 1.92, 95% CI 1.37-2.26, p=0.048, HR 3.49, 95% CI 2.55-4.77, p<0.001, HR 3.87, 95% CI 2.11-7.12, p<0.001 respectively). These results remained unchanged in the sensitivity analysis after adjustment for the confounding factors deemed clinically relevant (Supplementary Table 14).

Discussion

To our knowledge, this study is the first European survey to prospectively evaluate the burden of NAFLD in terms of clinical outcomes and mortality in a community setting. The scores for non-invasive markers such as the Fatty Liver Index and Forns Index have been validated in several studies as surrogates markers of NAFLD and fibrosis in the general population and have been shown to accurately detect fatty liver and advanced fibrosis (5,33–36). Compared to non-NAFLD subjects, those with NAFLD were older, mostly males, and had more metabolic disorders and liver injuries, such as ALT above the normal threshold and liver fibrosis. Our study showed that: 1) NAFLD, regardless of the severity of the disease, was associated with high risk of liver-related events, extrahepatic clinical outcomes, and overall mortality; 2) there was an exposure-response relationship between the grade of liver fibrosis and the occurrence of cardiovascular events and extra-hepatic malignancies; 3) NAFLD showed a growing increasing cause trend of overall mortality, liver-related morbidity, and leading and the most common cause of extrahepatic morbidities, such as CVD and CKD.

Findings from community-based studies are conflicting and continue to fuel controversy. Several prospective Asian community-based studies have shown conflicting results. Indeed,

contrary to Lee et al. who demonstrated from a Korean population-based cohort that NAFLD was associated with a higher risk of digestive and esophageal malignancies(25), Zhou et al. reported from a Chinese cohort that patients with NAFLD had a benign prognosis(26). Lazo et al. found no association between NAFLD or non-alcoholic steatohepatitis and all-cause mortality, CVD, cancer, or liver disease in the US general population(27). In a recent meta-analysis, Mantovani et al. showed that NAFLD is associated with increased long-term risk of fatal or non-fatal CVD events but a moderately increased long-term risk of developing extrahepatic cancers(28,29). Our results showed a strong association of clinical outcomes such as liver-related complications or extrahepatic clinical outcomes (CVD, CKD) and all-cause mortality. As in previous studies in biopsy-proven NAFLD patients(3,25,30–32), we found a dose-response relationship between the grade of fibrosis and the incidence of clinical outcomes or all-cause mortality. Our results support that NAFLD is associated with an increased risk of hepatic and extrahepatic clinical outcomes and worse long-term prognosis.

Our results are particularly relevant for patient management and the improvement of monitoring. HCV and heavy alcohol consumption were once considered the first and second cause of chronic liver disease, respectively, ahead of HBV, which ranked third(33,34). Our results from a large community-based cohort suggest that NAFLD is now one of the leading causes with growing trends of all-cause mortality, liver-related morbidity, and extrahepatic morbidities, such as CVD and CKD in western countries. Previous studies have demonstrated the increasing trend in NAFLD-related hepatic complications and liver transplants worldwide(4,5). In the US, although HCV remained the leading etiology of chronic liver disease among new liver transplant waitlist registrations, NASH demonstrated the greatest increase from 2004 to 2013 and had already become the second leading etiology of liver transplantation in 2013(4). This tendency had already been observed since 2011 from the US National Health and Nutrition Examination Surveys collected from 1988 to 2008. Those

surveys showed that the main causes of chronic liver diseases remained stable during the study period, except NAFLD, which increased steadily(35). Since 2011, the landscape of HCV treatment has evolved rapidly, with the approval of direct-acting antiviral (DAA) agents. In 2017, Goldberg et al.(33) have theorized that the burden of active HCV infection on cirrhosis and HCC (compensated or decompensated) has decreased since the introduction of highly efficacious DAA therapy. The rising prevalence of NAFLD coupled with the stabilization and expected decline in HCV-related clinical outcomes due to DAA therapy will soon result in NAFLD overtaking HCV as the leading etiology of chronic liver disease. Another potential explanation for these observations might be etiology-specific differences in disease progression. In our study, the prevalence of metabolic disorders (such as diabetes, hyperlipidemia or high blood pressure) was significantly higher in NAFLD subjects. In addition to the higher metabolic disorders, in our study population, as in several other studies(36,37), NAFLD subjects seem to have more and a poorer lifestyle (smoking and regular sports practice). Although these associations with the prevalence of NAFLD are not new, it has been demonstrated that metabolic disorders such as diabetes, high blood pressure, dyslipidemia, smoking, and lack of regular exercise practice contribute to disease progression and NAFLD-related mortality(36–39). On top of liver-related complications and mortality, several previous studies(36,40) have demonstrated that extrahepatic clinical outcomes, such as CVD, extrahepatic malignancies, and CKD, and their related mortality were strongly influenced by metabolic disorders and amplified in the presence of NAFLD. Thus, due to the rising prevalence of obesity, diabetes, and metabolic syndrome in the last two decades, we could observe a high incidence of NAFLD and its resulting complications(41,42). These results underscore the need for a comprehensive approach to NAFLD patient management and suggest the need to reinforce and focus efforts on preventing and treating metabolic

disorders, and to advise patients to reduce and maintain appropriate body weight and to optimize lifestyle regardless of the level of fibrosis.

Our study has several limitations. The non-invasive biomarkers we used to assess the prevalence of NAFLD or advanced fibrosis may lead to a (probably non-differential) misclassification bias and decrease the power of our analyses to identify associations with clinical outcomes or mortality. However, the performance of imaging methods such as ultrasound or elastometry, which have been used for the screening of NAFLD and fibrosis, also have such limitations(43,44). Magnetic resonance imaging or liver biopsy, which are the gold standards for NAFLD diagnosis and measuring liver injuries, cannot be used on a large scale, such as in the general population. Another limitation is the use of the Forns Index rather than FIB-4 for the assessment of fibrosis since FIB-4 is the recommend test.

In conclusion, this large general population-based cohort showed that NAFLD was associated with excess morbidity and mortality and demonstrated an increasing trend of NAFLD burden on morbidities and all-causes mortality. In addition, given the increasing rates of obesity and diabetes, NAFLD may soon become the first cause of the burden of hepatic and extrahepatic morbidities and mortality in western countries.

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Table 1: General characteristics of study sample and according to chronic liver diseases

	Overall sample N=137,206	Non-NAFLD (FLI <60) N=111,453	NAFLD (FLI ≥60) N= 25,753
Age (years), mean [SD]	46.8 [13.3]	45.6 [13.2]	52.4 [11.8]
Male sex, n (%)	62,223 (45.4)	46,895 (41.4)	15,328 (66.9)
Obesity (BMI ≥30 kg/m^2), n (%)	16,628 (12.1)	3341 (3.0)	13,287 (58.0)
HBP, n (%)	14,485 (10.6)	8370 (7.6)	6115 (27.3)
Waist circumference (cm), mean [SD]	84.7[12.9]	80.8 [9.5]	103.9 [9.5]
Diabetes, n (%)	6419 (5.3)	2473 (2.2)	3946 (15.6)
Hypercholesterolemia, n (%)	10,094 (11.5)	5991 (5.4)	4103 (18.5)
ALT >N, n (%)	15,410 (11.3)	7404 (6.5)	8006 (35.0)
GGT >N, n (%)	13,790 (10.1)	6232 (5.5)	7558 (33.0)
FI >6.9, n (%)	-	-	517 (2.7)
Geographic origin, n (%)			
European	126,590 (93.9)	105,612 (94.2)	20,978 (92.7)
Overseas France	1206 (0.9)	978 (0.9)	228 (1.0)
Asian	961 (0.7)	844 (0.8)	117 (0.5)
Sub-Saharan African	1382 (1.0)	1119 (1.0)	263 (1.2)
North African	3318 (2.5)	2490 (2.2)	828 (3.7)
Other	1306 (1.0)	1087 (1.0)	219 (1.0)
Education level (years), n (%)			
≤8	4142 (3.0)	2788 (2.5)	1354 (5.9)
9–15	87,961 (64.7)	70,863 (62.6)	17,098 (74.6)
≥16	43,949 (32.3)	39,491 (34.9)	4458 (19.5)
Tobacco >10 pack/year, n (%)	48,389 (36.3)	38,870 (34.4)	9519 (41.5)
Soft drink ≥1/d, n (%)	8306 (4.0)	4012 (3.6)	4294 (18.7)
Exercise ≥2h/w, n (%)	34,585 (25.9)	27,529 (24,7)	7056 (27.4)
Coffee ≥1 cup/d, n (%)	39,618 (28.9)	31,764 (28.5)	7854 (30.5)

Table 2: Raw (unweighted) and IPTW-weighted associations between clinical outcomes and presence of NAFLD (Reference group: subjects without NAFLD, FLI <60)

	Unweighted		IPTW-weighted *	
	NAFLD (FLI ≥60) N=25,753		NAFLD (FLI ≥60) N=25,753	
	HR (95% CI)	p	HR (95% CI)*	p
Hepatic events	2.83 (2.42–3.32)	<0.001	2.48 (1.99–3.29)	<0.001
Cardiovascular diseases	2.95 (2.79–3.12)	<0.001	1.42 (1.30–1.55)	<0.001
Extrahepatic malignancies	1.46 (1.35–1.57)	<0.001	1.11 (1.01–1.28)	0.020
Chronic kidney disease	3.24 (2.78–3.77	<0.001	1.81 (1.53–2.07)	<0.001
Death	2.31 (1.95–2.74)	<0.001	1.26 (1.01–1.57)	0.004

*Adjusted for age, sex, geographic origin, education level, high blood pressure, diabetes, metabolic syndrome and BMI, waist circumference, smoking, soft drink, coffee intake, and regular exercise, TGs, cholesterol, and ALT

Table 3: Time-dependent risk of clinical outcomes and mortality according to the presence of NAFLD (reference group = non-NAFLD, FLI <60)

	NAFLD (FLI ≥60)	
	HR (95%CI)	p
Hepatic events	1.38 (1.07–1.76)	0.015
Cardiovascular diseases	1.08 (1.03–1.12)	0.041
Extrahepatic malignancies	1.03 (0.89–1.09)	0.798
Chronic kidney disease	1.16 (1.07–1.25)	0.006
Death	1.39 (1.29–1.50)	<0.001

Table 4: IPTW-weighted* associations between grade of fibrosis and clinical events or overall mortality
(reference group: Non-NAFLD (FLI <60)

	NAFLD with mild fibrosis (FLI ≥60 and FI <4.2) n=13297		**NAFLD with intermediate fibrosis (FLI ≥60 and FI 4.2–6.9) n=10638**		**NAFLD with advanced fibrosis (FLI ≥60 and FI >6.9) n=535**	
	HR (95% CI)	p	HR (95% CI)	p	HR (95% CI)	p
Hepatic events	6.67 (3.46-12.85)	<0.001	8.26 (5.92-11.53)	<0.001	56.72 (12.31-261.39)	<0.001
Cardiovascular diseases	1.15 (1.02-1.47)	0.007	1.22 (10.9-1.65)	0.022	5.22 (2.49-10.92)	<0.001
Extrahepatic malignancies	0.93 (0.68-1.28)	0.715	1.07 (0.73-1.58)	0.654	1.21 (1.07-2.59)	0.008
Chronic kidney disease	2.46 (1.18-5.11)	0.016	3.07 (1.80, 6.86)	0.031	3.77 (2.31-6.13)	<0.001
Death	1.92 (1.37-2.26)	0.048	3.49 (2.55-4.77)	<0.001	3.87 (2.11-7.12)	<0.001

*Adjusted for age, sex, geographic origin, education level, high blood pressure, diabetes, metabolic syndrome, BMI, waist circumference, smoking, soft drink, coffee intake, and regular exercise, TGs, cholesterol, and ALT

Figure Legends

Figure **1**: Flow chart

Figure **2**: Cumulative incidence of (A) overall mortality, (B) hepatic events according to the presence of NAFLD

Figure **3:** Cumulative incidence of (A) overall mortality, (B) hepatic events according to the grade of fibrosis related to NAFLD subjects