Comorbidities Are Associated with Fibrosis in NAFLD Subjects: A Nationwide Study (NASH-CO Study)

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Comorbidities are associated with fibrosis in NAFLD subjects: a nationwide study (NASH-CO study)

Short Title: NAFLD and comorbidities

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Summary

Background: The relationship between the severity of NAFLD and extra-hepatic events such as cardiovascular disease (CVD), extra-hepatic cancer (EHC) or chronic kidney diseases (CKD) has not been clearly investigated in the general population.

Aims: The aim of this study was to assess whether the severity of fibrosis in NAFLD subjects was associated with extra-hepatic diseases based on non-invasive markers in a large population-based cohort.

Methods: The study population included a cohort of 118,664 participants from the nationwide CONSTANCES cohort. After excluding individuals with excessive alcohol consumption and other causes of liver disease, 102,344 were included. The non-invasive diagnosis of NAFLD and fibrosis was performed using a combination of the Fatty Liver Index (FLI) and the Forns Index (FI). The history of CVD or EHC was recorded by a physician and CKD was defined by a glomerular filtration rate < 60 ml/mn.

Results: The prevalence of NAFLD (FLI>60) was 18.2%, 10% with mild fibrosis (FI<4.2), 7.7% with intermediate fibrosis (FI 4.2-6.9) and 0.4% with advanced fibrosis (FI>6.9). The prevalence of CVD, EHC or CKD increased significantly with the severity of fibrosis (P<0.0001). When adjusted for demographic, metabolic risk factors and smoking, NAFLD with intermediate or advanced fibrosis remained associated with CVD (OR 1.36, p<0.0001 and OR 3.07, p<0.0001, respectively), EHC (OR 1.24, p=0.001 and OR 1.64, p=0.004, respectively) and CKD (OR 1.18, p=0.03 and OR 2.09, p<0.0001, respectively).

Conclusions: In a large adult population-based cohort, there is a dose-dependent relationship between the severity of fibrosis and CVD, EHC or CKD in NAFLD subjects.

Key words: cardiovascular disease, cancer, colorectal cancer, chronic kidney disease, Fatty Liver Index, Forns index

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) which is associated with the epidemic of obesity, type 2 diabetes and metabolic syndrome, is the most common cause of chronic liver disease (1). NAFLD is a clinico-pathological syndrome that ranges from simple steatosis to non-alcoholic steatohepatitis (NASH) with varying amounts of fibrosis, and cirrhosis (2). Long-term cohort studies have reported that the severity of liver fibrosis was the best predictor of liver-related as well as overall mortality in patients with biopsy-proven NAFLD (3). In addition to liver-related complications, it has been suggested that NAFLD could promote cardiovascular disease, cancer and chronic kidney disease (4-6). However, few studies have investigated the relationship between comorbidities and NAFLD, or the role of fibrosis in the general population.

The French CONSTANCES population-based cohort was designed to be a large sample of the French adult population aged 18-69, and representative of gender and socioeconomic status including more than 200 000 subjects between 2012 and 2018 (7). This is an ideal cohort to assess the epidemiology of NAFLD and advanced fibrosis in the French general adult population. The aim of the present study was to assess the relationship between NAFLD, fibrosis and comorbidities such as cardiovascular disease, extra-hepatic cancer or chronic kidney disease using non-invasive markers.

METHODS

Study Population

This cross-sectional study was performed in data collected at baseline from participants included in the Constances cohort between 2012 and 2018. This cohort was previously described (8). Briefly, Constances is a "general purpose" epidemiological cohort designed to

be representative of the French general population including more than 200 000 adults aged 18 years and over at baseline, and living in 21 departments which have a Health Screening Center (HSC) in France (7,9), affiliated with the national healthcare system, representing approximately 50 million people. Subjects recruited in Constances were included in the present analysis if they were at least 18 years old, with no history of excess alcohol consumption defined by a daily consumption > 30 grams per day in men and 20 grams per day in women, no history of chronic hepatitis virus infection, or history of other liver diseases excepted NAFLD. 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 (INSERM). All participants provided written informed consent for the use of personal data for research.

Data collection

Socio-demographic and lifestyle data were obtained from a standardized self-administered questionnaire at home. Socio-demographic data included age, gender, occupation and employment status, education and geographic origin. Alcohol consumption was assessed on the AUDIT questionnaire (10). The number of glasses per day was converted into the number of grams of alcohol per day (1 glass=10g of alcohol). To estimate the impact of smoking, we considered tobacco ever- versus never-use. Sport practice was also recorded and considered as less or more than 2 hours a week.

Health and morbidity data were recorded by a physician in HSC. This included a history of cancer (colon, breast, prostate, lung, thyroid, ovary, uterus), high blood pressure, cardiovascular events (arteritis of lower limbs, myocardial infarction, stroke, angina pectoris), and diabetes. The Body Mass Index (BMI) was calculated as the ratio of weight to height squared. Subjects were considered to be obese when the BMI ≥30 kg / m^2, or ≥25 kg /

m^2 if Asian ethnicity, and overweight when the BMI [25-29.9], or [23-24.9] if Asian ethnicity. Abdominal obesity was defined according to the waist circumference ≥94 cm for men (≥90 cm if Asian ethnicity) and ≥80 cm for women. Blood samples were taken from a venous blood sample after a 12-hour fast and analyses were performed in the HSC laboratories according to common standards. Biological data included blood glucose, serum creatinine, gamma glutamyl transferase (GGT), alanine aminotransferases (ALT), total cholesterol, HDL-cholesterol, triglyceride (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. Diabetes was defined by a blood glucose >6.9mmol/L after a 12-hour fast according to the WHO definition and/or antidiabetic therapy. High blood pressure (HBP) was defined on the basis of 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 (11). Chronic kidney disease (CKD) was defined according to a glomerular filtration rate <60 ml/mn/1.73m2 which was calculated with the CKD-EPI equation (Chronic Kidney Disease-Epidemiology Collaboration).

Definition of NAFLD and liver fibrosis

The fatty liver index (FLI) was chosen as a surrogate marker of NAFLD. This is based on 4 common anthropometric and biochemical measures: body mass index, waist circumference, gamma-glutamyl-transferase and triglycerides (12). This score has an accuracy of 0.84 (95%CI 0.81-0.87) for detecting steatosis in the general population and has been successfully cross-validated in external populations (13,14). According to the literature, subjects with

FLI>60 were considered to have NAFLD. The Forns index could be determined to evaluate liver fibrosis with data available from the Constances cohort (age, gamma-GT, platelets, cholesterol) The Forns Index was previously validated as a marker of fibrosis in a cohort of patients with biopsy-proven NAFLD (8). The score was calculated according to the published formula (15) and applied to subjects with NAFLD defined as FLI>60. Thresholds to rule-out or rule-in advanced fibrosis (F3/F4), were <4.2 and >6.9, respectively, as previously published. Thus, subjects were classified as follows: non NAFLD, FLI<30, indeterminate NAFLD, FLI 30-60, 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.

Statistical analysis

A complex probability sampling plan was used to select participants from Constances. The complex design of the survey and non-response to the survey were then weighted by modeling the socio-demographic characteristics of patients from medico-administrative databases. To take into account a non-response and to estimate representatives of the French general population, crude prevalences were weighted by modeling socio-demographic characteristics and health status of patients from administrative databases (16).

Continuous variables were expressed as means ± standard deviation (SD) and qualitative variables as percentages and their 95% confidence intervals (CI95%). Categorical variables were compared using the Chi-square test or the Fisher exact test. Continuous variables were compared using the Student t test. No variables had more than 5% of missing data thus missing data were not calculated. The prevalence was estimated as the ratio of subjects with available results to the total number of subjects included, with their 95% confidence

intervals (CI95%). Multivariate logistic regression models containing the covariates of interest (age, gender, BMI class, diabetes, blood hypertension, hypertriglyceridemia, hypercholesterolemia, ALT, sports practice and smoking) were constructed for each dependent variable. Full models containing risk factors were adjusted to estimate odds ratios (OR) of various risk-factors and their 95% confidence intervals (CI). Non-NAFLD subjects (FLI <30) were the reference group for all analyzes.

To confirm the relationship between NAFLD and comorbidities, we performed sensitivity analyses using a propensity score. The propensity score was calculated by multivariate logistic regression to estimate the probabilities of patients having NAFLD (FLI > 60) or not (FLI < 30). Variables included in the model were age, sex, BMI and smoking. Assessment of the probability of having NAFLD according to these covariates before weighting by the propensity score is shown in Figure 1a Suppinfo. The standardized means differences (SMD) of pre-weighting and post-weighting were used to assess and compare the balance of characteristics between the risk groups. An absolute post-weighting SMD > 0.10 was considered to be a significant imbalance of characteristics (Figure 1b Suppinfo). We then performed a one-to-four (1:4) matching by the Greedy matching method. A total of 13418 NAFLD subjects were matched to 53672 non-NAFLD subjects. The relationship between NAFLD and comorbidities was then estimated using logistic regression with patients matched on the propensity score.

Due to a mean prevalence of NAFLD reported in cohorts from the general population of 20 to 40%, to be able to detect an increase in the risk of NAFLD associated with various risk factors as low as 10% (OR of 1.10) in a population with a prevalence of NALFD as low as 20%, the required sample size was 28,374 subjects for a level of significance of 0.05 with a power > 0.90.

A P value < 0.05 was considered to be statistically significant. The R software package used for analysis (3.4.3 version).

RESULTS

At the time of analysis, data were available for 119,150 participants. After excluding subjects who withdrew consent and had a history of excessive alcohol consumption, chronic viral hepatitis or other causes of liver diseases, 102,344 were included in the final analysis as representative of the overall population (Fig 1).

The general characteristics of the overall population are shown in Table 1. A history of cardiovascular disease (CVD) or extra-hepatic cancer (EHC) was present in 6893 (7.1%, CI95% 7-7.3) and 5134 (4.5%, 95%CI 4.3-4.6) subjects respectively, and chronic kidney disease (CKD) was present in 3078 (3.1%, 95%CI 3-3.2) subjects.

NAFLD and fibrosis in overall population

FLI could be estimated in 97,472 subjects (Fig 1). The characteristics of subjects with or without an FLI were similar (data not shown). For FLI > 60, the adjusted prevalence of NAFLD was 18.2% (95%CI 17.9-18.4) in the overall population. The adjusted prevalence of NAFLD with mild, intermediate or advanced fibrosis according to the Forns Index was 10% (95% CI 9.8-10.2), 7.7% (95% CI 7.5-7.8) and 0.4% (95%CI 0.3-0.5), respectively. The general characteristics of subjects in relation to the presence of NAFLD and fibrosis are shown in Table 2. Subjects with NAFLD were older than subjects without NAFLD and more frequently male, with more prevalent metabolic disorders and smoking. The severity of fibrosis in NAFLD subjects was related to age, male gender, waist circumference, diabetes, high blood pressure, metabolic syndrome and smoking (Table 2). The characteristics of subjects with indeterminate NAFLD were in-between NAFLD and non-NAFLD subjects.

Prevalence of comorbidities according to NAFLD and the severity of fibrosis

The prevalence of comorbidities in subjects with NAFLD increased significantly according to the grade of fibrosis as shown in Figure 2. The prevalence of CVD in subjects without NAFLD,

in NAFLD subjects with mild, intermediate or advanced fibrosis were respectively 5 (95%CI 4.8-5.2) vs 7.4 (95%CI 6.8-7.9) vs 19.2 (95%CI 18.3-20.1) vs 33% (95%CI 28.5-37.5) (P<0.0001). The prevalence of EHC in subjects without NAFLD, in NAFLD subjects with mild, intermediate or advanced fibrosis were respectively 3.9 (95%CI 3.8-4.1) vs 3.5 (95%CI 95% CI 3.1-3.8) vs 7.3 (95%CI 6.7-7.9) vs 17.1% (95%CI 13.5-20.8) (P<0.0001). The prevalence of CKD in subjects without NAFLD, in NAFLD subjects with mild, intermediate or advanced fibrosis were respectively 2.9 (95% CI 2.8-3.1) vs 3.4 (95%CI 95% CI 3.0-3.8) vs 4.5 (95%CI 4.0-5.0) vs 7.6% (95%CI 5.1-10.1) (P<0.0001).

Multivariate analysis of the association between the severity of NAFLD and comorbidities

We first analyzed the relationship between comorbidities and NAFLD status, with patients without NAFLD as a reference group (Table 3A). After adjustment for age, gender, overweight/obesity, diabetes, high blood pressure, hypercholesterolemia and smoking, both indeterminate NAFLD and NAFLD remained associated with CVD (OR 1.15, 95% CI 1.05-1.25, p=0.002 and OR 1.25, 95% CI 1.12-1.41, p=0.0001, respectively) and EHC (OR 1.12, 95% CI 1.01-1.24, p=0.02 and OR 1.37, 95% CI 1.20-1.58, p<0.0001, respectively). After adjustment for the same risk factors, CKD was no longer associated with NAFLD, although there was a trend (OR 1.16, 95%CI 0.98-1.37, p=0.07). We then analyzed the relationship between the severity of NAFLD and comorbidities (Table 3B). When adjusted for age, gender, overweight/obesity, diabetes, high blood pressure, hypercholesterolemia and smoking, there was a dose-dependent effect of the severity of fibrosis, i.e. mild, intermediate and advanced, in NAFLD subjects for the risk of CVD (OR 0.93, 95%CI 0.81-1.06, p>0.05, OR 1.36, 95%CI 1.21-1.53, p<0.0001 and OR 3.07, 95%CI 2.36-3.98, p<0.0001, respectively), EHC (OR 1.13, 95%CI 0.97-1.31, p>0.05, OR 1.24, 95%CI 1.08-1.41, p=0.001 and OR 1.64, 95%CI 1.15-2.29, p=0.004, respectively) and CKD (OR 1.03, 95%CI 0.87-1.22, p>0.05, OR 1.18, 95%CI

1.01-1.39, p=0.03 and OR 2.09, 95%CI 1.42-2.98, p<0.0001, respectively). Only the risk of CVD was significantly increased in subjects with indeterminate NAFLD (OR 1.16, 95%CI 1.06-1.27, p=0.001).

Multivariate analysis of the risk and type of extra hepatic cancer according to NAFLD and fibrosis

Multivariate analysis of the relationship between NAFLD/fibrosis and the type of extra-hepatic cancer in men and women is shown on Table 4. When adjusted for age, gender, overweight/obesity, diabetes, high blood pressure, hypercholesterolemia and smoking, NAFLD remained associated with extra-hepatic cancer with a dose-dependent effect of fibrosis in both men and women (Table 4). After adjustment for the same risk factors, extra-hepatic malignancies associated with NAFLD in men were prostate (OR=1.38, 95%CI 1.07-1.79, p=0.01) and colon cancer (OR=1.16, 95%CI 1.12-1.19, p<0.0001). There was a significant severity-dependent effect of fibrosis for prostate cancer, ie mild, intermediate and advanced (OR 1.6, CI95% 1.13-2.28, p=0.008, OR 1.91, 95%CI 1.18-3.10, p=0.008, and OR 2.13, 95%CI 1.19-3.8, p=0.01, respectively) while the risk of colon cancer was only significantly increased in NAFLD subjects with advanced fibrosis (OR 2.19, 95%CI 1.07-6.54, p=0.01). When adjusted for the same risk factors in women, extra-hepatic malignancies associated with NAFLD were breast (OR 1.09, 95%CI 1.08-1.09, p<0.0001) and lung cancer (OR 1.12, 95%CI 1.06-1.17, p<0.0001). The risk of breast cancer was only significantly increased in NAFLD subjects with advanced fibrosis (OR 1.49, 95% CI 1.08-2.04, p=0.01), while there was no significant association between lung cancer and the severity of fibrosis. Colon cancer was only associated with NAFLD in subjects with advanced fibrosis (OR 1.15, 95% CI 1.12-1.18, p<0.0001).

Propensity analysis of the risk of comorbidities in NAFLD subjects

NAFLD subjects were matched with non-NAFLD subjects according to age, gender, BMI and smoking using a propensity score. NAFLD remained significantly associated with CVD (OR 1.02, 95% CI 1.01-1.02, $p<0.0001$), EHC (OR 1.01, 95% CI 1.01-1.02, $p<0.0001$) and CKD (OR 1.01, 95% CI 1.01-1.03, $p<0.0001$) in these subjects.

DISCUSSION

This study in a large sample of subjects, representative of the adult French population with no excessive alcohol consumption or chronic viral hepatitis, suggests that the relationship between NAFLD and comorbidities such as cardiovascular disease (CVD), extra-hepatic cancer (EHC) and chronic kidney disease (CKD), mainly depends on the severity of liver fibrosis. NAFLD with intermediate or advanced fibrosis was associated with a high prevalence of CVD, EHC and CKD and remained associated with these comorbidities when adjusted for age, sex, metabolic disorders and smoking. Malignancies associated with NAFLD and fibrosis were prostate and colon cancer in men, and breast and lung cancer in women.

The CONSTANCES population-based cohort is a large, representative sample of the French general population aged 18 and over (7). The lower prevalence of obesity or diabetes than expected in our study population (17) is mainly due to the exclusion of subjects with excessive alcohol consumption or viral hepatitis. Scores for non-invasive markers such as the Fatty Liver Index (FLI) and Forns Index were determined with prospective data. The FLI has been validated in several studies as a marker of NAFLD in the general population and has been shown to be highly accurate in detecting fatty liver (12-14). Moreover, a high FLI score has been associated with an increased risk of coronary heart disease (18). The Forns Index has already been shown to have a relatively good performance as a marker of fibrosis in NAFLD in several studies (8,19-21). It was also recently shown to be accurate in the prediction of the development of severe liver disease in patients at risk of NAFLD (22). In this study the Forns Index was only used in subjects with NAFLD based on an FLI > 60, and we identified NAFLD subjects with minimal or advanced fibrosis. NAFLD subjects with an intermediate Forns Index score, in the “grey zone”, were considered to have intermediate fibrosis. The significant increase in the prevalence of usual risk factors in NAFLD subjects

with mild, intermediate and advanced fibrosis in our population supports the accuracy of this classification in this study.

We found a significant and dose-dependent relationship between subjects with NAFLD and intermediate or advanced fibrosis and a history of CVD, EHC or CKD after adjustment for other known risk factors, but no association in subjects with NAFLD and minimal fibrosis. Although BMI is a factor in the calculation of the FLI, NAFLD remained associated with an increased risk of CVD, EHC and CKD when NAFLD and non-NAFLD subjects were matched for age, gender, BMI and smoking. NAFLD has been linked to an increased risk of CVD in a number of cross-sectional and longitudinal surveys (4). Few studies have investigated the role of the severity of NAFLD on the risk of CVD in the general population. Two studies in patients with biopsy-proven NAFLD have reported a positive, graded relationship between carotid-artery intimal medial thickness and the histological severity of NAFLD, including the stage of fibrosis (23,24). A metaanalysis has shown that the risk of developing fatal and non-fatal CVD events was higher in patients with severe NAFLD (25). Finally, CVD was the main cause of death in longitudinal studies performed in patients with biopsy proven NAFLD and baseline fibrosis was the strongest predictive factor (3). Some of the mechanisms that may increase the risk of CVD in NAFLD are systemic inflammation, endothelial dysfunction, hepatic insulin resistance, oxidative stress, and altered lipid metabolism (4). The second most frequent cause of death after CVD in patients with NAFLD is malignancy (3). Besides HCC and colorectal cancer, little attention has been paid to the association between the severity of NAFLD and other type of cancers. In our study, after adjustment for the usual risk factors, NAFLD and fibrosis remained associated with prostate and colon cancer in men, and breast and lung cancer in women. In a large cohort of subjects from South Korea, the development of colon cancer in men and breast cancer in women was associated with

NAFLD, when adjusted for the usual risk factors (26). Interestingly, high values of non-invasive fibrosis markers were strongly associated with the development of all cancers. In another study from China in patients with biopsy-proven NAFLD, colon adenoma or advanced neoplasms were associated with NASH but not with simple steatosis (27). The mechanisms of the association between NAFLD and extra-hepatic malignancies are not fully understood. It has been suggested that hyperinsulinemia and the proinflammatory state that is associated with hypoadiponectinemia could promote carcinogenesis in NAFLD patients, both conditions which are worsened in advanced liver disease (5). We also evaluated chronic kidney disease (CKD) in our population, defined by a glomerular filtration rate < 60 ml/mn/1.73m2. CKD was associated with NAFLD and advanced fibrosis, after adjustment for other risk factors such as diabetes and HBP. In a meta-analysis using the same definition of CKD, the presence and severity of NAFLD were associated with an increased risk and severity of CKD (6).

Our study has several limitations. The causality and temporality between the comorbidities and NAFLD could not be confirmed due to the cross-sectional design. For example, we cannot exclude that NAFLD could occur after a cardiovascular event. The influence of NAFLD on cardiovascular and cancer events should be evaluated in our cohort in a longitudinal study before drawing any conclusions. Our study was also limited because we could not evaluate the influence of NASH on the relationship between NAFLD and comorbidities. Unfortunately, there are no validated non-invasive markers for the diagnosis of NASH, which can still only be confirmed by liver biopsy. However, a number of longitudinal studies in patients with biopsy-proven NAFLD have demonstrated that fibrosis rather than NASH injury was the best predictor of overall mortality.

In conclusion, this large adult population-based cohort study confirms that NAFLD may be associated with a high prevalence of CVD, EHC and CKD, and that this association mainly depends on the grade of liver fibrosis when adjusted for the usual risk factors. It is still not known whether NAFLD plays a direct pathogenic role in the development of CVD, EHC or CKD, or whether it is a surrogate marker of these events perhaps by the worsening of the metabolic syndrome. Nevertheless, screening for CVD, extra-hepatic cancers such as colon and prostate cancer in men and breast and lung cancer in women, or CKD should be performed in NAFLD subjects, especially those with advanced fibrosis. Specific treatments of NAFLD should evaluate whether interventional strategies influence the occurrence or the severity of extra-hepatic complications.

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Table 1: General characteristics of overall population with no excessive alcohol consumption or chronic viral hepatitis

Variables	Overall population (N=102,344)
Age, *yrs, mean (SD)*	47.2 (13.6)
Male gender, *% (95% CI)*	45.4 (45.1-45.7)
Waist circumference, *cm, mean (SD)*	84.6 (12.9)
Overweight, *% (95% CI)*	30.0 (30.3-30.6)
Obesity, *% (95% CI)*	12.3 (12.1-12.5)
Diabetes mellitus, *% (95% CI)*	3.7 (3.7-3.9)
High blood pressure*,% (95% CI)*	11.6 (11.4-11.8)
Hypertriglyceridemia*,% (95% CI)*	12.5 (12.3-12.7)
Hypercholesterolemia,*% (95% CI)*	8.1 (8.0-8.3)
Metabolic syndrome*,% (95% CI)*	13.4 (13.2-13.6)
Smoking *% (95% CI)*	45.6 (45.2 - 45.9)
History of cardiovascular disease,*% (95% CI)*	7.1 (7.0 - 7.3)
History of extrahepatic cancer*,% (95% CI)*	4.5 (4.3 - 4.6)
Chronic kidney disease*,% (95% CI)*	3.1 (3.0 - 3.2)

SD : standard deviation, CI : confidence interval,

Table 2: Characteristics of subjects according to NAFLD and fibrosis. No NAFLD: FLI<30; Indeterminate NAFLD: FLI 30-60; NAFLD with mild fibrosis: FLI>60 and FI<4.2; NAFLD with intermediate fibrosis: FLI>60 and FI 4.2-6.9; NAFLD with advanced fibrosis: FLI>60 and FI>6.9.

Variables	**No NAFLD (n=62,444)**	**Indeterminate NAFLD (n=18,764)**	**NAFLD with mild fibrosis (n=8278)**	**NAFLD with intermediate fibrosis (n=7498)**	**NAFLD with advanced fibrosis (n=427)**
Age, *yrs, mean (SD)*	41.5 (12.9)	48.3 (12.7)	44.9 (11.5)	58.4 (7.8)	65 (4.9)
Male gender, *% (95% CI)*	36.7 (36.4-37.1)	63.5 (62.8-64.1)	58.4 (57.3 - 59.5)	76.9 (75.9 - 77.8)	92.9 (90.5-95.4)
Waist circumf., *cm, mean (SD)*	77.3 (7.8)	91.6 (6.1)	103.7 (9.8)	105.1 (10.0)	107.3 (9.5)
Overweight, *% (95% CI)*	17.9 (17.6-18.2)	64.5 (63.8-65.2)	34.3 (33.3 - 35.3)	40.3 (39.2 - 41.4)	36.4 (31.8-40.9)
Obesity, *% (95% CI)*	0.6 (0.5-0.7)	13.4 (12.9-13.8)	64.1 (63.1 - 65.2)	57.3 (56.2 - 58.5)	59.9 (55.2-64.5)
Diabetes mellitus, *% (95% CI)*	1.4 (1.3-1.5)	5.4 (5.1-5.8)	9.6 (8.9 - 10.3)	27.6 (26.5 - 28.7)	47.2 (42.5-52)
High blood pressure,*% (95% CI)*	3.8 (3.6-3.9)	11.5 (11.0-11.9)	12.0 (11.3 - 12.7)	31.1 (30 - 32.1)	56.4 (51.7-61.2)
Hypertriglyceridemia,*% (95% CI)*	2.5 (2.4-2.7)	20.5 (19.9–21.0)	49.3 (48.2 - 50.3)	43.4 (42.3 - 44.5)	42.6 (37.9-47.3)
Hypercholesterolemia,*% (95% CI)*	3.7 (3.5-3.9)	10.7 (10.2-11.1)	70.3 (69.4 - 71.3)	66.6 (65.6 - 67.7)	53.3 (48.5-58)
Metabolic syndrome,*% (95% CI)*	1.3 (1.2-1.4)	18.8 (18.1-19.4)	60.1 (58.8 - 61.4)	72.7 (71.5 - 73.9)	80.8 (77-84.5)
Smoking *% (95% CI)*	42.5 (42.1-42.9)	47.8 (47.0-48.5)	50.5 (49.3 - 51.7)	57.7 (56.5 - 58.9)	76.7 (72.2 - 81.1)

SD : standard deviation, CI : confidence interval,

Table 3A: Association between NAFLD and cardiovascular disease, extra-hepatic cancer or chronic kidney disease. No NAFLD: FLI<30; Indeterminate NAFLD: FLI 30-60; NAFLD: FLI>60 .

	Cardiovascular disease		**Extra-hepatic cancer**		**Chronic kidney disease**	
Class	**OR (95% CI)**	***P* value***	**OR (95% CI)**	***P* value***	**OR (95% CI)**	***P* value***
No NAFLD	Reference	-	Reference	-	Reference	-
Indeterminate status of NAFLD	1.15 (1.05-1.25)	*0.002*	1.12 (1.01-1.24)	*0.02*	1.03 (0.91-1.16)	*ns*
NAFLD	1.25 (1.12-1.41)	*0.0001*	1.37 (1.20-1.58)	*<0.0001*	1.16 (0.98-1.37)	*0.07*

*Adjusted on age, gender, overweight/obesity, diabetes, high blood pressure, hypertriglyceridemia, hypercholesterolemia, smoking and sport practice. OR : odds ratio, CI : confidence interval,

Table 3B: Association between the severity of NAFLD and cardiovascular disease, extra-hepatic cancer or chronic kidney disease

	Cardiovascular disease		**Extra-hepatic cancer**		**Chronic kidney disease**	
Class	**OR (95% CI)**	***P* value***	**OR (95% CI)**	***P* value***	**OR (95% CI)**	***P* value***
No NAFLD	Reference	-	Reference	-	Reference	-
Indeterminate status of NAFLD	1.16 (1.06-1.27)	*0.001*	1.10 (0.99-1.21)	*ns*	1.06 (0.94-1.20)	*ns*
NAFLD with mild fibrosis	0.93 (0.81-1.06)	*ns*	1.13 (0.97-1.31)	*ns*	1.03 (0.87-1.22)	*ns*
NAFLD with intermediate fibrosis	1.36 (1.21-1.53)	*<0.0001*	1.24 (1.08-1.41)	*0.001*	1.18 (1.01-1.39)	*0.03*
NAFLD with advanced fibrosis	3.07 (2.36-3.98)	*<0.0001*	1.64 (1.15-2.29)	*0.004*	2.09 (1.42-2.98)	*<0.0001*

*Adjusted on age, gender, overweight/obesity, diabetes, high blood pressure, hypertriglyceridemia, hypercholesterolemia, smoking and sport practice. OR : odds ratio, CI : confidence interval,

Table 4: Risk and type of extra-hepatic cancer in overall NAFLD subjects and according to the severity of fibrosis

	Overall NAFLD		**NAFLD with mild fibrosis**		**NAFLD with intermediate fibrosis**		**NAFLD with advanced fibrosis**	
	OR (CI 95%)	***P* value***	**OR (CI 95%)**	***P* value***	**OR (CI 95%)**	***P* value***	**OR (CI 95%)**	***P* value***
Extra hepatic cancer in men	**1.24 (1.06-1.44)**	***0.007***	**1.21(0.93-1.57)**	***ns***	**1.28 (1.04-1.58)**	***0.02***	**1.77 (1.19-2.59)**	***0.004***
Prostate	1.38 (1.07-1.79)	*0.01*	1.60 (1.13-2.28)	*0.008*	1.91 (1.18-3.10)	*0.008*	2.13 (1.19-3.80)	*0.01*
Lung	2.61 (0.83-8.28)	*ns*	1.49 (015-14.54)	*ns*	3.52 (0.73-17.10)	*ns*	3.30 (0.98-3.77)	*ns*
Colon	1.16 (1.12-1.19)	*<0.0001*	1.03 (0.36-2.97)	*ns*	1.46 (0.71-3.04)	*ns*	2.19 (1.07-6.54)	*0.01*
Thyroid	1.16 (0.48-2.80)	*ns*	0.81 (0.40-1.86)	*ns*	1.24 (0.40-3.87	*ns*	1.98 (0.23-17.07)	*ns*
Extra hepatic cancer in women	**1.27 (1.06-1.51)**	***0.008***	**1.13 (0.98-1.29)**	***ns***	**1.30 (1.03-1.63)**	***0.02***	**1.47 (1.16-1.84)**	***0.001***
Ovarian	0.90 (0.70-1.34)	*ns*	0.45 (0.15-1.37)	*ns*	1.78 (0.50-6.37)	*ns*	2.87 (0.78-10.60)	*ns*
Uterus	0.99 (0.61-1.62)	*ns*	0.91 (0.64-1.36)	*ns*	0.61 (0.31-1.20)	*ns*	0.99 (0.51-1.91)	*ns*
Breast	1.09 (1.08-1.09)	*<0.0001*	1.08 (0.88-1.32)	*ns*	1.03 (0.73-1.44)	*ns*	1.49 (1.08-2.04)	*0.01*
Colon	1.44 (0.84-2.49)	*ns*	1.25 (0.47-3.35)	*ns*	1.07 (0.73-4.30)	*ns*	1.15 (1.12-1.18)	*<0.0001*
Lung	1.12 (1.06-1.17)	*<0.0001*	0.87 (0.49-1.03)	*ns*	1.05 (0.88-1.77)	*ns*	1.15 (0.86-2.99)	*ns*
Thyroid	1.55 (0.91-2.66)	*ns*	1.01 (0.43-2.36)	*ns*	1.23 (0.76-2.02)	*ns*	1.97 (0.96-4.06)	*ns*

*Adjusted on age, gender, overweight/obesity, diabetes, high blood pressure, hypercholesterolemia, and smoking. Reference group is non NAFLD (FLI<30)

OR : odds ratio, CI : confidence interval.

Figure legend

Figure 1: Flow chart

Figure 2: Prevalence of cardiovascular disease, extrahepatic cancer and chronic kidney among NAFLD subjects according to grade of fibrosis. No NAFLD: FLI<30; NAFLD with mild fibrosis: FLI>60 and FI<4.2; NAFLD with intermediate fibrosis: FLI>60 and FI 4.2-6.9; NAFLD with advanced fibrosis: FLI>60 and FI>6.9. All prevalence are significantly different, excepted extra-hepatic cancer prevalence between non NAFLD and NAFLD subjects with mild fibrosis. P value according to Chi2