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The Burden of NAFLD in Type 2 Diabetic Subjects from the General Population: a Nationwide Population-Based Follow-Up Study (NASHCO)

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Abbreviations

ALT : alanine amino-transferase

CKD : chronic kidney disease

CVD : cardiovascular disease

EHC : extra-hepatic cancer

FI : Forns Index

FLI : Fatty Liver Index

GGT : gamma glutamyl-transferase

HBP : high blood pressure

HR: Hazard ratio

NAFLD : Non-Alcoholic Fatty Liver Disease

NASH : Non-Alcoholic SteatoHepatitis

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Abstract

Background: The epidemiology and natural history of NAFLD in diabetes have been mainly investigated in the hospital setting. The goal of this study was to evaluate the characteristics of NAFLD and its impact on morbidity and mortality in type 2 diabetic subjects in a community setting.

Method: The study included 199,341 participants in the nationwide Constances cohort. After patients with excessive alcohol consumption, viral hepatitis or other causes of liver disease were excluded, 164,285 were analyzed and 8386 (5.3%) were considered to have type 2 diabetes. The non-invasive diagnosis of NAFLD and advanced fibrosis was made using a combination of the Fatty Liver Index and Forns Index. Median follow-up was 2.5 years.

Results: Diabetes increased the risk of NAFLD by 6 fold (adjusted OR 6.05, 95%CI 5.68-6.45) and the risk of advanced fibrosis by 3.76 fold (aOR 3.76, 95%CI 2.87-4.91) in NAFLD subjects. After controlling for confounders, the presence of NAFLD in diabetic subjects was associated with an increased risk of severe liver-related events (aHR 2.53, 95%CI 1.36-4.69), cardiovascular disease (aHR 2.71, 95%CI 1.72-4.26) and overall mortality (aHR 2.91, 95%CI 1.53-5.53). The risk of hepatic and extra hepatic complications in diabetic subjects with NAFLD significantly increased with the severity of fibrosis ($P < 0.05$).

Conclusion: This prospective, longitudinal study in a large community-based cohort provides real-world evidence of the risk for NAFLD and advanced fibrosis in diabetes, and its impact on liver disease progression, diabetes-related complications such as cardiovascular disease, and overall mortality. These data could be used to estimate real clinical and economic burden of NAFLD in diabetic subjects.

Key words: Obesity; type 2 diabetes; advanced fibrosis; Fatty Liver Index; Forns Index; cardiovascular disease; chronic kidney disease; extra-hepatic malignancy

Nonalcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease with an estimated worldwide prevalence ranging from 25% to 45%, concomitantly with the epidemic of obesity, type 2 diabetes and metabolic syndrome (1,2). Around 20% of patients with NAFLD have nonalcoholic steatohepatitis (NASH), the active form of NAFLD which may progress to cirrhosis, end-stage liver disease and hepatocellular carcinoma (3). In addition to liver-related complications, it has been suggested that NAFLD could promote cardiovascular disease, cancer and chronic kidney disease (4-6).

Type 2 diabetes (T2D) is a major risk factor of NAFLD and a driver of disease progression. A recent meta-analysis has reported high global prevalence rates of NAFLD, NASH and advanced fibrosis in diabetic patients (7). It has also been suggested that NAFLD is associated with an increased risk of diabetes-related complications such as cardiovascular disease or chronic kidney disease (8-10). However, the epidemiology and natural history of NAFLD in type 2 diabetes, as well as the link between NAFLD and the complications of diabetes have mainly been evaluated in patients consulting in clinics, which is not representative of the overall population of diabetic subjects (7-13). Indeed, diabetic patients followed in hospitals may have more advanced disease, while most diabetic subjects are followed by general practitioners. Moreover, the longitudinal data on the burden of NAFLD on diabetes-related complications are limited, and results are conflicting (9,13). Finally, mortality in NAFLD diabetic patients has only been reported in two relatively small retrospective studies (14,15).

The French Constances population-based cohort was designed as a large representative sample of the French adult population aged 18–69 years old, representative of gender and socioeconomic status including more than 200 000 subjects between 2012 and 2020 (16). This cohort is ideal for prospectively assessing longitudinal data in the general population and estimating the risk of death while eliminating the bias of a hospitalized cohort

with a more severe natural history of disease. The goal of the present study was to assess in unselected diabetic subjects 1) the prevalence of NAFLD and advanced fibrosis and examine risk factors associated with these conditions; and 2) to evaluate the impact of NAFLD on liver-related outcomes, diabetes-related complications such as cardiovascular disease, chronic kidney disease or extra-hepatic malignancies, and mortality.

Materials and Methods

Study Population

This longitudinal study was performed in data collected at baseline and follow-up from participants included in the Constances cohort between 2012 and 2020. This cohort was previously described (16,17). 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 in France that have a Health Screening Center (HSC) (16,18) affiliated with the national healthcare system, which represents 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 HBV or HCV 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 at baseline

Selected subjects were invited to complete questionnaires and to attend a Health Screening Center (HSC) for a comprehensive health examination. (16). Socio-demographic and lifestyle data were obtained by 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 using the AUDIT questionnaire (19). The number of glasses per day was converted into the amount of grams per day of alcohol (1 glass=10g of alcohol). To estimate the impact of smoking, we considered tobacco ever- versus never-use.

Health and morbidity data were recorded by a physician in the HSC. This included histories of high blood pressure, diabetes, cardiovascular disease (arteritis of lower limbs, myocardial infarction, stroke, angina pectoris), cancer (colon, breast, prostate, lung, thyroid, ovary, uterus) and chronic kidney disease. Subjects were considered to be obese with a Body Mass Index (BMI) ≥ 30 kg/m², or ≥ 27 kg/m² if Asian ethnicity, and as overweight with a BMI [25-29.9], or [23-27] if Asian ethnicity. Abdominal obesity was defined by a 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 in men and 31 IU/L in women (20), and GGT at 55 IU/L in men and 45 IU/L in women. Diabetes was defined either on the basis of self-reporting and/or blood glucose >6.9 mmol/L and/or antidiabetic therapy. High blood pressure (HBP) was defined either 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 lipid-lowering drugs. Hypertriglyceridemia was defined by a blood triglyceride > 1.7 mm/L or a lipid-lowering drug. The metabolic syndrome was defined according to the international diabetes federation (21). Chronic kidney disease (CKD) was defined according to a glomerular filtration rate < 60 ml/mn/1.73m² calculated according to 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. It is based on 4 common anthropometric and biochemical measures: BMI, waist circumference, GGT and triglycerides (22). This score has been shown to have an accuracy (area under the receiver operating characteristic) 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 (23,24). Subjects with FLI >60 were considered to have NAFLD according to the literature. The calculation of the Forns index for the evaluation of liver fibrosis was possible using data available in the Constances cohort and according to the formula (22):

$$\frac{(e^{0.953 \times \log(\text{triglycerides})} + 0.139 \times \text{BMI} + 0.718 \times \log(\text{GGT}) + 0.053 \times \text{waist circumference} - 15.745)}{(1 + e^{0.953 \times \log(\text{triglycerides})} + 0.139 \times \text{BMI} + 0.718 \times \log(\text{GGT}) + 0.053 \times \text{waist circumference} - 15.745)} \times 100.$$

The Forns Index was validated as a marker of liver fibrosis in NAFLD in a cohort of 1557 biopsy-proven NAFLD patients (17). The score was calculated according to the published formula (25):

$$(7.811 - 3.131 \times \ln(\text{platelets count}) + 0.781 \times \ln(\text{GGT})) + 3.467 \times \ln(\text{age}) - 0.014 \times (\text{cholesterol}),$$

and applied to subjects with NAFLD defined by FLI >60 . The Previously published thresholds were used to rule out or rule in advanced fibrosis (F3/F4), < 4.2 and > 6.9 respectively. Subjects were classified as follows: non-NAFLD (FLI <30), NAFLD (FLI > 60), NAFLD with mild fibrosis

(FLI>60 and Forns Index <4.2), NAFLD with intermediate fibrosis (FLI>60 and Forns Index between 4.2 -6.9) and NAFLD with advanced fibrosis (FLI>60 and Forns Index >6.9).

Follow-up study

Constances data were linked to hospitalization data and death which was recorded in the Systeme National des données de Santé (SNDS) from 2012 to December 31, 2017 (26). SNDS data were available during this period in 127 291 Constances' participants. Exclusion criteria were as follows: excessive alcohol consumption, HBV or HCV infection, other chronic liver diseases. The follow-up period for each study participant was the length of time between the Constances baseline inclusion and December 31, 2017, the date of death or the last date of follow-up. International Classification of Diseases (ICD-10) codes were used to define liver-related events according to the recent expert panel consensus statement (compensated cirrhosis, hepatocellular carcinoma, decompensated cirrhosis) (27), cardiovascular disease (arteritis of lower limbs, myocardial infarction, stroke, angina pectoris), extra-hepatic cancer (colon, breast, prostate, lung, thyroid, ovary, uterus), chronic kidney disease (chronic kidney disease, diabetic nephropathy, hypertensive nephropathy, kidney failure), and overall mortality. Subjects with histories of cirrhosis-related complications or hepatocellular carcinoma, cardiovascular disease, extra-hepatic cancer or subjects with GFR < 60 ml/mn/1.73m² at baseline were excluded in the calculation of each category of incident events.

Statistical analysis

Constances uses a complex probability sampling design to select participants. Sampling weights were estimated in relation to this complex design and non-responses were estimated

by modeling sociodemographic characteristics of patients from medico-administrative databases.

Baseline distributions were expressed as means with their 95% confidence intervals (CI95%) for continuous variables and percentages for categorical variables. Continuous variables were compared by the Student t-test. Categorical variables were compared by using the Chi-2 test or Fisher's exact test. None of the variables had more than 5% of missing data therefore no missing data were imputed. Prevalences were estimated as the ratio of subjects with available results to the total number of included subjects, with their 95% confidence intervals (CI95%). Logistic regression models were built to identify risk factors associated to NAFLD, advanced fibrosis and comorbidities from the baseline data in diabetic subjects. Multivariate models included the usual clinically relevant risk factors for each dependent variable (NAFLD, diabetes, advanced fibrosis). Because BMI and triglycerides are part of the FLI calculation, they were not included in the analysis of NAFLD risk factors, while age and cholesterol, which are part of the Forns Index calculation, were not included in the analysis of advanced fibrosis risk factors.

In a second set of analyses, we performed a longitudinal analysis to assess the impact of NAFLD at baseline on clinical outcomes and overall mortality. The overall follow-up population was split into 3 groups: diabetes with NAFLD, diabetes without NAFLD and no diabetes. Mortality was studied with the standard Cox proportional hazards models and other clinical outcomes with the Fine-Gray model, including all-cause mortality as a competing event. The cumulative incidence function was estimated for each clinical outcome, and the Gray's test was used to estimate the differences in the cumulative incidence functions between the groups. A standard Kaplan-Meier curve was estimated for the all-cause mortality rate. As the number of clinical events per variable considered for adjustment was sometimes too low to

build multivariate models with a limited risk of overfitting, confounding was handled with stabilized inverse-probability weighting (IPW). A multinomial propensity score for belonging to the three compared groups was derived by multinomial regression models, from potential confounding factors such as socio-demographic characteristics (age, sex, geographic origin, level of education), clinical and anthropometric variables (BPH, metabolic syndrome and BMI), lifestyle (alcohol consumption, smoking), biological data (triglyceride, cholesterol and ALT) and antidiabetics use in diabetic subjects. The positivity assumption was graphically checked by looking at the overlap of the propensity score distributions between the groups and their common support. In order to avoid bias induced by extreme weights, we truncated extreme weights (>0.99) by shrinking them to 0.99. We used the standardized mean difference to assess the balance of covariates before and after weighting to ensure that weighting leads to comparable between the groups, with respect to the measured covariates. Standardized mean differences were used to assess the balance of covariates. An absolute standardized mean difference less than 0.10 indicates a balance of covariates (data available in appendix supplementary figure 1). Fine and Grey models weighted by the IPTW to estimate competing risk hazard ratios of liver-related events, cardiovascular disease, extra-hepatic malignancy, chronic kidney disease and Cox proportional hazards models weighted by the IPTW to estimate the cause specific hazard ratios for overall mortality. A 6-month latency period since inclusion was imposed so that the incidence of a clinical event could be attributed to the presence of diabetes and/or NAFLD. Thus, to avoid an immortal time bias, the follow-up of subjects began six months after the collection of baseline NAFLD status. The associations of fibrosis with clinical outcomes according to the presence of NAFLD were studied in diabetic and non-diabetic subjects by splitting each population into four groups: NAFLD with advanced fibrosis, NAFLD with intermediate fibrosis, NAFLD with minimal fibrosis and no NAFLD).

Multivariate analysis was performed using the standard Cox model containing confounding factors such as age, gender, obesity, diabetes, HBP, ALT and smoking. A P value < 0.05 was considered to be statistically significant. Analyses were performed using R Statistical Software version 4.0.3.

Results

At the time of analysis, baseline data were available for 199 341 participants. After excluding subjects who withdrew their consent, had a history of excessive alcohol consumption, chronic viral hepatitis, other causes of liver disease, or missing data for assessment of diabetes, 164 285 were included in the final analysis and defined as the overall population (Figure 1). A total of 8386 (5.3%, 95%CI 5.2-5.4) of these subjects were considered to have type 2 diabetes, 7138 (80.7%) had been previously diagnosed and 6204 (77.8%) were receiving anti-diabetic drugs (oral treatment in 6039 and insulin requirement in 165). The baseline characteristics of the overall population and subjects with or without diabetes are shown in Table 1.

The prevalence of NAFLD in diabetic subjects

The FLI could be estimated in 159 203 subjects, including 7189 with type 2 diabetes (Figure 1). The characteristics of subjects with or without an available FLI were similar (data not shown). With a FLI>60, the prevalence of NAFLD was 61.1% (95%CI 60-62.2) in diabetic and 15.2% (95%CI 15-15.3) in non-diabetic subjects. On multivariate analysis diabetes remained associated with NAFLD independently from age, gender, BMI, HBP, hypertriglyceridemia, hypercholesterolemia, elevated ALT and lifestyle such as tobacco use and moderate alcohol consumption (OR 6.05, 95%CI 5.68-6.45) (Supplementary Table 1). In diabetic subjects, the prevalence of NAFLD was higher in men than in women (65.6%, 95%CI 64.1-67.1 vs 55.6%, 95%CI 53.9-57.3), and increased with age and gender from 28.7% in women aged 18-27 years old to 74.5% in men aged older than 68 (Supplementary Table 1 and Supplementary Figure 2). In diabetic subjects, the prevalence of NAFLD according to BMI was 77.6% (95%CI 77.2-78.03) in obese subjects, 58.9% (95%CI 58.13-59.63) in overweight subjects and 20.9% (95%CI 19.84-22.1) in lean diabetic subjects. The prevalence of NAFLD was 85.4% (95%CI 83.1-87.7) and

57.4% (95%CI 56.2-58.6) in those with or without elevated ALT (Supplementary Table 1), respectively.

Factors associated with NAFLD in diabetic subjects

The general characteristics of diabetic subjects with or without NAFLD are shown in Table 2. Diabetic subjects with NAFLD were older and more often men than those without NAFLD, and had significantly more metabolic disorders, a significantly higher level of serum glucose and a higher prevalence of ALT above the normal threshold. The proportion of subjects receiving anti-diabetic treatment was significantly lower in NAFLD than in the non-NAFLD group (37.6%, 95%CI 36.1-39.0 vs 94.1%, 95%CI 93-95.2). In multivariate analysis adjusted for anti-diabetic treatment the independent parameters associated with NAFLD were age (OR 1.02, 95%CI 1.01-1.04), male gender OR 2.1, 95%CI 1.72-2.57), high blood pressure (1.63, 95%CI 1.28-2.08), hypercholesterolemia (OR 4.5, 95%CI 3.63-5.86), serum glucose >1.5 g/L (OR 1.23, 95%CI 1.01-1.51) and ALT above the normal threshold (OR 15.35, 95%CI 11.11-21.64) (Supplementary Table 2). Because part of FLI calculation, obesity and hypertriglyceridemia were not included in the multivariate analysis. A history of cardiovascular disease, extra-hepatic malignancy and chronic kidney disease were significantly more prevalent comorbidities in diabetic subjects with NAFLD than in those without (Table 2).

Prevalence and risk factors of advanced fibrosis in diabetic subjects with NAFLD

The Forns Index could be estimated in 25607 subjects with NAFLD (99.8%), including 4034 subjects with diabetes. According to a Forns Index >6.9, the prevalence of advanced fibrosis in diabetic subjects with NAFLD was 4.8% versus 0.3% in the absence of NAFLD (Table 2). The characteristics of NAFLD diabetic subjects according to the presence of advanced fibrosis are shown on Supplementary Table 3. The independent parameters associated with advanced

fibrosis in NAFLD diabetic subjects on multivariate analysis were male gender (OR=16.68, 95%CI 7.81-39.15), HBP (OR=2.25, 95%CI 1.0-5.01), elevated ALT (OR=15.36, 95%CI 8.24-29.93), insulin requirement (OR=8.55, 95%CI 4.31-17.63), diabetes-related vascular complications (OR=13.46, 95%CI 6.66-28.38,) and moderate alcohol consumption (OR=2.03, 95%CI 1.02-3.99) (Figure 2).

The prevalence of advanced fibrosis in NAFLD subjects without diabetes was 1.2% (vs diabetic NAFLD, $P<0.0001$). On multivariate analysis diabetes remained associated with advanced fibrosis independently from age, gender, HBP, elevated ALT, tobacco use and moderate alcohol consumption (OR 3.76, 95%CI 2.87-4.91). The rate of advanced fibrosis in diabetic subjects with NAFLD increased to 10.0 % in obese vs 2.8% in non-obese subjects, 10.1% in hypertensive vs 3.6% in non-hypertensive subjects, 9.0% and 0% of subjects with and without metabolic syndrome, respectively and 9.7% and 2.7% in subjects with and without elevated ALT, respectively. In terms of the severity of diabetes, the following parameters were associated with a significantly higher prevalence of advanced fibrosis: anti-diabetic treatment (7.3% vs 1.5%, $P<0.0001$), insulin therapy (13.9% vs 1.9%, $P<0.0001$), plasma glucose level $>1.5\text{g/L}$ (13% vs 2.8%, $P<0.0001$) or a history of diabetes-related complications such as cardiovascular and/or chronic kidney diseases (6.1% vs 3.9%, $P<0.0001$) (Supplementary Figure 3).

Clinical outcomes and mortality in diabetic subjects according to the presence of NAFLD

Data for the analysis of clinical outcomes and mortality (follow-up population) were available in 102509 participants who were included between 2012 and 2017 and who had no excessive alcohol consumption or known chronic liver disease (Supplementary Figure 4). Their baseline characteristics were not significantly different from those of the overall study population (data not shown). These subjects were divided into 3 groups: 2825 patients with diabetes and

NAFLD, 1976 with diabetes without NAFLD, and 97708 non-diabetic subjects (Supplementary Figure 4). After a median follow-up of 2.5 years (0.51-5.91), 402 subjects developed liver-related events, including 92 HCC, 783 cardiovascular-disease, 350 chronic kidney disease, 767 extra hepatic malignancies, 11 were transplanted and 785 died. The cumulative incidence of liver-related events, cardiovascular disease, extra-hepatic malignancies, chronic kidney disease and death were significantly influenced by the presence of NAFLD and diabetes (Figure 3 and Supplementary Figure 5). The probability of death at 2 and 5 years in diabetic subjects with and without NAFLD and non-diabetic subjects was 0.4% (95%IC 0.16-0.64), 0.4% (0.11-0.69) and 0.2% (0.17- 0.23), respectively and 1.9% (1.23-2.57), 0.8% (0.18-1.42) and 0.6% (0.54-0.66), respectively ($P < 0.0001$). Unadjusted and adjusted analysis for the risk of clinical outcomes and death according to the presence of NAFLD in diabetic subjects are shown in Table 3. The presence of NAFLD after adjustment for age, gender, geographic origin, educational level, BMI, HBP, hypercholesterolemia, ALT, smoking, alcohol consumption and anti-diabetic treatment, was associated with an increased risk of liver-related events (aHR 2.53, 95%CI 1.36-4.69). The risk of cardiovascular disease was significantly increased by the presence of NAFLD after adjustment (aHR 2.71, 95%CI 1.72-4.26). The risk of extra-hepatic cancer or chronic kidney disease was not significantly influenced by the presence of NAFLD in diabetic subjects (Table 3). For overall mortality, the risk of death was significantly increased by the presence of NAFLD in diabetic subjects after adjustment (aHR 2.91, 95%CI 1.53-5.53).

Clinical outcomes and mortality in diabetic subjects with NAFLD according to the grade of fibrosis

Of the 4801 diabetic participants who had available data on mortality, 2825 had NAFLD and were divided according to the grade of fibrosis: minimal fibrosis in 2567 (Forns Index < 4.2), intermediate in 91 (Forns Index 4.2-6.9) and advanced in 159 (Forns-Index > 6.9). The cumulative incidence of liver-related events, cardiovascular disease, extra hepatic malignancy and death, but not chronic kidney disease, were significantly different in this group according to the grade of fibrosis (data not shown). Table 4 shows univariate and multivariate analysis for the risk of clinical outcomes and death according to the grade of fibrosis in diabetic subjects with NAFLD, with diabetic subjects without NAFLD as the reference group. In univariate analysis, there was a positive relationship between the grade of fibrosis and the risk of hepatic events and cardiovascular disease. After adjustment for age, gender, obesity, diabetes, HBP, ALT and smoking, multivariate analysis showed that the risk of hepatic events and cardiovascular disease was significantly increased in those with advanced fibrosis (HR=5.43, 95%CI 2.49-14.54 and HR=3.78, 95%CI 1.94-6.25, respectively) There was a dose effect of fibrosis on the risk of death in univariate analysis (Table 4). In multivariate analysis, the risk of death was significantly increased in diabetic NAFLD subjects with advanced fibrosis (HR=5.14, 95%CI 2.24-9.32). Supplementary Table 4 shows univariate and multivariate analysis for the risk of clinical outcomes and death according to the grade of fibrosis in non-diabetic subjects with NAFLD. Compared with NAFLD diabetic subjects, the risk was globally lower among NAFLD non-diabetic subjects. Only the risk of death was positively related with the grade of fibrosis.

Discussion

To our knowledge, this is the largest European survey to prospectively evaluate the burden of NAFLD on clinical outcomes and mortality in diabetic subjects in a community setting. Around 20% of our diabetic population was undiagnosed at inclusion. Our results confirm the high prevalence of NAFLD and the lower rate of advanced fibrosis in this unselected population compared to hospital cohorts. After controlling for confounders, diabetes increased the risk of NAFLD by 6 fold and the risk of advanced fibrosis by 3.76 fold in NAFLD subjects. The main predictors of advanced fibrosis in NAFLD diabetic subjects were elevated ALT and the severity of diabetes, independent from additional metabolic risk factors, alcohol or smoking. The presence of NAFLD in diabetic subjects was associated with an increased risk of liver-related events, diabetes-related complication such as cardiovascular disease, and overall mortality, after controlling for the usual risk factors. Among NAFLD diabetic subjects, the grade of fibrosis was a strong predictor of morbidity and overall mortality, with a 3-5 fold increase in risk of liver-related events, cardiovascular disease and death in those with advanced fibrosis.

Our results reflect the situation of diabetic subjects in the community setting. The CONSTANCES is a large population-based cohort representative of the French general population aged 18 and over (16). 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 (23-25). The performance of the Forns Index has already been shown to be relatively good as a marker of fibrosis in NAFLD in several studies (17,28,29). It was also shown to be accurate in the prediction of the development of severe liver disease in patients at risk of NAFLD (30).

Our study confirms the high prevalence of NAFLD in subjects with type 2 diabetes i.e. 61%, which was close to that reported in European diabetic patients and which increased with age (7). The highest prevalence of NAFLD reached 85% and was found in subjects with diabetes and elevated ALT, a high-risk population that requires screening. On the other hand, only 25% of NAFLD subjects with diabetes had elevated ALT, confirming the low diagnostic sensitivity of transaminases for the former. Interestingly, compared to diabetic subjects without NAFLD, those with NAFLD had higher levels of serum glucose and were less frequently treated with antidiabetic drugs, suggesting the importance of diabetes control to prevent NAFLD.

The impact of diabetes as a driver of fibrogenesis in NAFLD patients has been extensively investigated. One metaanalysis in selected diabetic patients with biopsy-proven NAFLD reported a 17% rate of advanced fibrosis (7). The screening of a large cohort of diabetic patients from Hong Kong with FibroScan reported a prevalence of extensive fibrosis, defined by liver stiffness >9.6 kPa, of around 18% (11). However, the study population included patients followed at a hospital clinic who probably had more severe diabetes than the general population of diabetic subjects. The prevalence of advanced fibrosis in NAFLD diabetic subjects in our study was 4.8%, which is close to that reported in 2 small community-based studies using FibroScan or MRE in diabetic subjects (31,32). The prevalence of advanced fibrosis increased to around 10% in NAFLD diabetic subjects with additional metabolic factors, and around 13% in those with advanced diabetes assessed by a high serum level of glucose or the need for insulin therapy. The link between the severity of diabetes and fibrosis may be explained by the worsening of hepatic insulin resistance and the release of multiple pro-inflammatory mediators and pro-diabetogenic hepatokines in patients with NASH and fibrosis, thus promoting diabetes (33). As previously shown in an aging population from Rotterdam, we confirmed that the combination of diabetes and NAFLD was associated with a significantly

higher rate of advanced fibrosis than NAFLD alone with our data on NAFL status in the overall population (34).

It is difficult to assess whether diabetes promotes NAFLD and its progression or whether NAFLD patients are at risk of developing type-2 diabetes using a cross sectional design (35). Several longitudinal studies in large cohorts have reported an increased risk of NAFLD, cirrhosis, HCC or cirrhosis-related death in diabetic patients (36-38). There are only a few longitudinal studies of the progression of liver disease in diabetic patients with NAFLD, as well as on the impact of NAFLD on diabetes-related complications, and these are mostly in hospital cohorts (9,13,14,15). In one of these studies, steatosis was assessed in 2343 diabetic patients who underwent CT scan for other indications and was not predictive of liver or cardiovascular complications (13). However, a similar study in 2103 diabetic patients attending clinics showed that NAFLD increased the risk of cardiovascular events by 1.87 fold after adjusting for confounders (9). Moreover, in a small study including 132 patients with biopsy-proven NAFLD, diabetes was associated with greater risk of the occurrence of cirrhosis or liver-related death (15). In the NHANES cohort, the combination of diabetes and chronic liver disease was associated with an increased risk of mortality compared to each condition on its own (39). The impact of NAFLD on mortality in diabetic patients was assessed in a small community-based cohort and showed an increased risk of overall death in diabetics with NAFLD after 10.1 years of follow-up, independent from other risk factors (14).

Our large community-based cohort confirms that NAFLD is associated with adverse liver-related events, including the occurrence of HCC, in diabetic subjects with a Hazard Ratio of 2.5 (95%CI 1.36-4.69) after adjustment for a large number of confounding factors. Liver-related events were assessed according to the recent expert statement which provided a reference standard to code compensated cirrhosis, HCC and decompensated cirrhosis (27). The risk of

incident cardiovascular disease was also found to be increased in NAFLD diabetic subjects with an adjusted HR of 2.71 (95%CI 1.72-4.26). Our results were adjusted for antidiabetic treatment but not statins, which may influence the risk of CVD. While the risk of incident CKD was increased in diabetic subjects, it was not significantly influenced by the presence of NAFLD. This result may be partly explained by the use of ICD-10 coding rather than eGFR changes, which is a more sensitive method to diagnose CKD events. Both NAFLD and type 2 diabetes are known to be associated with an increased incidence of many cancers (5,40). We found that there was a significantly higher frequency of incident extra-hepatic malignancies in diabetic than in non-diabetic subjects. However, despite this tendency, the presence of NAFLD did not significantly increase the risk of incident cancer. Longer follow-up is probably needed. Finally, in our study overall mortality was significantly influenced by the presence of NAFLD in diabetic subjects with an adjusted HR of 2.91 (95%CI 1.53-5.53), which is similar to that (2.2, 95% CI 1.1-4.2) in a previous study (14). However, the probability of death in diabetics with NAFLD was lower in our unselected population, i.e. 1.9% at 5 years, compared to the probability of death reported in 2 previous studies, i.e. 11.9% at 10 years (7,14,15). Poorer outcomes in the latter studies may be explained by the design as both were retrospective analyses of selected diabetic patients who mainly underwent liver biopsy for the diagnosis of NAFLD. Like in previous studies in biopsy-proven NAFLD patients, we found a dose dependent effect between the grade of fibrosis and clinical outcomes or overall mortality (41). Diabetic subjects with NAFLD and advanced fibrosis had not only the highest significant risk of liver-related event, but also the highest risk of cardiovascular disease and all cause death.

Our study has several limitations. The causality and temporality between metabolic risk factors or comorbidities and NAFLD could not be confirmed due to the cross-sectional design. For example, we cannot exclude that diabetes is a consequence rather than a cause of NAFLD.

Regarding the severity of diabetes, we used rough surrogates such as glucose levels or insulin need. Unfortunately, glycated hemoglobin which is the gold standard was not available in our study population. Another limitation is the use of non-invasive serum biomarkers to assess the presence of NAFLD and advanced fibrosis. Imaging methods, such as US or Fibroscan which have a better accuracy than biomarkers for the detection of NAFLD and fibrosis, are difficult to apply on a large scale such as in the general population. Because AST measurement was not performed in subjects of CONSTANCES cohort, we were not able to confirm our data with other fibrosis biomarkers such as FIB4 or NAFLD fibrosis score. Finally, we were not able to assess the impact of NASH in our study population, liver biopsy being the gold standard for this diagnosis.

In conclusion, this large, prospective community-based cohort provides real-world evidence that diabetes and NAFLD have an additive effect on liver injury, clinical outcomes and mortality. Advanced fibrosis should be mainly screened in diabetic NAFLD subjects with elevated ALT and/or severe diabetes. Patients with NAFLD, especially when it is associated with significant fibrosis, should be closely monitored for diabetes-related complications such as cardiovascular disease. Overall, the long-term impact of interventional strategies based on specific treatment of NAFLD in diabetic subjects requires further studies.

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Table 1: General characteristics at baseline of the overall population, type 2 diabetic and non-diabetic subjects with no excessive alcohol consumption nor HBV/HCV infection.

Overall population	Non-diabetic subjects <i>n=155,879</i>	Type 2 diabetic subjects <i>n=8386</i>	<i>P value*</i>
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n=164,265

Age, yrs, mean (95%CI)	48.5 (48.4-48.6)	48.0 (47.9-48.1)	56.2 (55.9-56.5)	<0.0001
Male gender, % (95%CI)	43.5 (43.3-43.7)	42.8 (42.7-42.9)	55.5 (54.4-56.5)	<0.0001
Obesity, % (95%CI)	11.9 (11.7-12.0)	9.8 (9.7-9.9)	49.5 (48.4-50.6)	<0.0001
BMI, kg/m², mean (95%CI)	24.9 (24.9-24.9)	24.7 (24.6-24.8)	29.8 (29.7-29.9)	<0.001
Waist circumference, cm, mean (95%CI)	85.1 (85.0-85.2)	84.3 (84.2-84.4)	99.7 (99.4-100.0)	<0.0001
Hypertriglyceridemia, % (95%CI)	12.3 (12.2-12.5)	10.5 (10.4-10.6)	45.8 (44.7-46.9)	<0.0001
Hypercholesterolemia, % (95%CI)	11.6 (11.4-11.8)	9.7 (9.5-9.9)	48.2 (46.7-49.6)	<0.0001
High Blood Pressure, % (95%CI)	12.1 (11.9-12.3)	11.8 (11.1-12.5)	20.5 (19.6-21.4)	<0.0001
Metabolic syndrome, % (95%CI)	15.5 (15.3-15.7)	12.9 (12.8-13.0)	66.1 (64.9-67.3)	<0.0001
ALT>N, % (95%CI)	10.0 (9.9-10.2)	9.8 (9.7-9.9)	13.5 (12.8-14.3)	0.001
GGT>N, % (95%CI)	12.6 (12.4-12.8)	12.0 (11.9-12.1)	23.6 (22.7-24.5)	<0.0001
<u>History of:</u>				
Cardiovascular disease, % (95%CI)	10.0 (9.8-10.1)	9.0 (8.9-9.1)	27.2 (26.3-28.2)	<0.0001
Extrahepatic cancer, % (95%CI)	3.6 (3.5-3.7)	3.3 (3.2-3.4)	5.0 (4.5-5.4)	0.001
Chronic Kidney disease, % (95%CI)	3.5 (3.3-3.7)	3.5 (3.4-3.6)	5.6 (5.2-60.0)	0.04
Antidiabetic drug, % (95%CI)	3.1 (3.0-3.2)	-	74.1 (74.0-74.2)	
Metformin, % (95%CI)	2.1 (2.0-2.2)	-	56.5 (56.2-56.7)	
Sulfamid, % (95%CI)	0.21 (0.18-0.24)	-	4.1 (3.0-5.2)	
DPP4 inhibitor, % (95%CI)	0.20 (0.13-0.23)	-	3.5 (2.4-4.6)	
GLP1 inhibitor, % (95%CI)	0.11 (0.08-0.14)	-	2.2 (1.2-3.1)	
Insulin, % (95%CI)	0.1 (0.1-0.2)	-	2.0 (1.6-2.3)	
Lipid-lowering drug, % (95%CI)	9.7 (9.6-9.8)	8.6 (8.5-8.7)	10.3 (10.0-10.6)	<0.001
Statin, % (95%CI)	7.1 (6.7-7.6)	6.6 (6.4-6.8)	7.6 (6.5-8.6)	ns
Fibrate, % (95%CI)	1.8 (1.0-2.7)	1.4 (0.9-1.9)	2.3 (1.3-3.3)	ns
HMG CoA inhibitors, % (95%CI)	0.6 (0.1-1.1)	0.6 (0.1-1.1)	0.4 (0.2-0.6)	ns
Antihypertensive drug, % (95%CI)	11.3 (11.2-11.4)	8.3 (8.2-8.4)	16.6 (16.4-16.8)	<0.001
Angiotensin II inhibitor, % (95%CI)	4.4 (3.9-4.8)	3.3 (2.9-3.8)	6.6 (6.3-7.0)	<0.001
Conversion enzyme inhibitor, % (95%CI)	2.8 (2.3-3.2)	1.5 (1.3-2.3)	3.1 (2.5-3.6)	0.0013
Betablquers, % (95%CI)	2.4 (1.9-2.8)	1.4 (0.9-1.9)	3.8 (3.0-4.7)	0.0093
Calcic inhibitor, % (95%CI)	1.8 (1.3-2.3)	1.1 (0.6-1.6)	2.1 (1.6-2.6)	0.061
Diuretic, % (95%CI)	1.1 (0.6-1.5)	1.1 (0.6-1.6)	1.0 (0.4-1.9)	ns

CI: confidence interval, GGT: gamma glutamyl transferase, ALT: alanine amino transferase

*Non-diabetic versus diabetic subjects

Table 2. General characteristics, advanced fibrosis and history of comorbidities according to the presence of NAFLD in diabetic subjects. The presence or absence of NAFLD was defined as FLI>60 or <30 respectively

	Diabetic w/o NAFLD n=1838	Diabetic with NAFLD n=4278	P value
Age, yrs, mean (95%CI)	52.7 (52.6-52.8)	58.1 (58.1-58.1)	<0.001
Male gender, % (95%CI)	43.2 (40.9-45.5)	60.6 (59.1-62.0)	<0.001
Obesity, % (95%CI)	30.6 (28.4-32.7)	66.7 (65.2-68.1)	<0.001
Hypertriglyceridemia, % (95%CI)	9.9(8.5-11.3)	73.8 (72.5-75.2)	<0.001
High Blood Pressure, % (95%CI)	18.9 (16.8–21.0)	89.4 (88.5-90.4)	<0.001
Hypercholesterolemia, % (95%CI)	13.6 (12.1-15.2)	71.2 (69.5-72.8)	<0.001
Glucose, mmol/L, mean (95%CI)	7.5 (7.5-7.5)	8.3 (8.2-8.4)	<0.001
Antidiabetic drugs, % (95%CI)	94.1 (93-95.2)	37.6 (36.1-39.0)	<0.001
Metabolic syndrome, % (95%CI)	10.9 (9.1-12.7)	96.4 (96.4-97.5)	<0.001
ALT>N, % (95%CI)	4.7 (3.8-5.7)	25.1 (24.5-25.6)	<0.001
Advanced fibrosis, % (95%CI)	ND	4.8 (4.2- 5.4)	-
History of:			
Cardiovascular disease, % (95%CI)	10.8 (9.4-12.2)	37.5 (36.0-38.9)	<0.001
Extrahepatic cancer, % (95%CI)	5.6 (4.5-6.7)	7. 8 (7.0-8.6)	0.025
Chronic Kidney disease, % (95%CI)	3.9 (3.6-4.2)	6.0 (5.1-6.9)	0.02

CI : confidence interval, ALT : alanine amino transferase

Table 3: Unadjusted and adjusted cause specific Hazard Ratio in diabetic subjects according to the presence of NAFLD

	Unadjusted analysis		IPTW adjusted analysis	
	Diabetic with NAFLD (vs. diabetic without NAFLD)		Diabetic with NAFLD (vs. diabetic without NAFLD)	
	HR (95%CI)	<i>P</i>	aHR (95%CI)	<i>P</i> *
Hepatic events	3.70 (2.01-6.81)	<0.001	2.53 (1.36-4.69)	0.003
Cardiovascular disease	2.82 (1.79-4.44)	<0.001	2.71 (1.72-4.26)	<0.001
Extra hepatic cancer	1.38 (0.80-2.36)	<i>ns</i>	1.31 (0.76-2.24)	<i>ns</i>
Chronic kidney disease	1.77 (0.91-3.43)	<i>ns</i>	1.71 (0.90-3.26)	<i>ns</i>
Death	2.73 (1.46-5.10)	0.002	2.91 (1.53-5.53)	0.001

*Covariates of PS model: age, gender, BMI, HBP, ALT, TGs, cholesterol, tobacco use, alcohol consumption, schooling, geographic origin and anti-diabetic treatment.

HR: hazard ratio, aHR: adjusted hazard ratio, CI: confidence interval

Table 4: Univariate and multivariate analysis of outcome risk according to fibrosis (Forns Index <4.2: no fibrosis; FI 4.2-6.9: intermediate fibrosis, FI>6.9: advanced fibrosis) in diabetic subjects with NAFLD. The reference group is diabetic subjects without NAFLD (FLI<30)

	Univariate analysis			Multivariate analysis*		
	NAFLD w/o fibrosis n= 2567	NAFLD with intermediate fibrosis n= 91	NAFLD with advanced fibrosis n= 159	NAFLD w/o fibrosis n= 2567	NAFLD with intermediate fibrosis n= 91	NAFLD with advanced fibrosis n= 159
	HR (95%CI)	HR (95%CI)	HR (95%CI)	HR (95%CI)	HR (95%CI)	HR (95%CI)
Hepatic events	1.63 (1.01-2.61) [§]	2.16 (1.31-3.57) [§]	6.14 (3.12-9.7) [§]	1.34 (0.82-2.17)	2.34 (0.03-5.91)	5.43 (2.49-14.54) [§]
Cardiovascular disease	1.40 (0.66-3.01)	3.71 (2.94-5.60) [§]	4.08 (2.01-6.00) [§]	1.20 (0.52-2.77)	2.69 (0.10-6.95)	3.78 (1.94-6.25) [§]
Extra-hepatic cancer	1.04 (0.46-2.74)	1.43 (0.69-2.93)	1.51 (0.13 -4.83)	1.06 (0.65-1.73)	1.27 (0.05 -4.65)	1.41 (0.8 -6.01)
Chronic kidney disease	1.31 (0.55-3.09)	1.93 (0.68-3.95)	0.98 (0.12-4.78)	2.01 (0.99-3.89)	1.19 (0.03-4.26)	1.01 (0.2- 5.11)
Death	1.46 (0.78-2.71)	2.39 (1.07-3.19) [§]	5.39 (1.33-8.31) [§]	2.01 (0.99-3.89)	1.08 (0.12-3.91)	5.14 (2.24-9.32) [§]

*Cox model, adjusted for age, gender, obesity, diabetes, HBP, ALT and smoking.

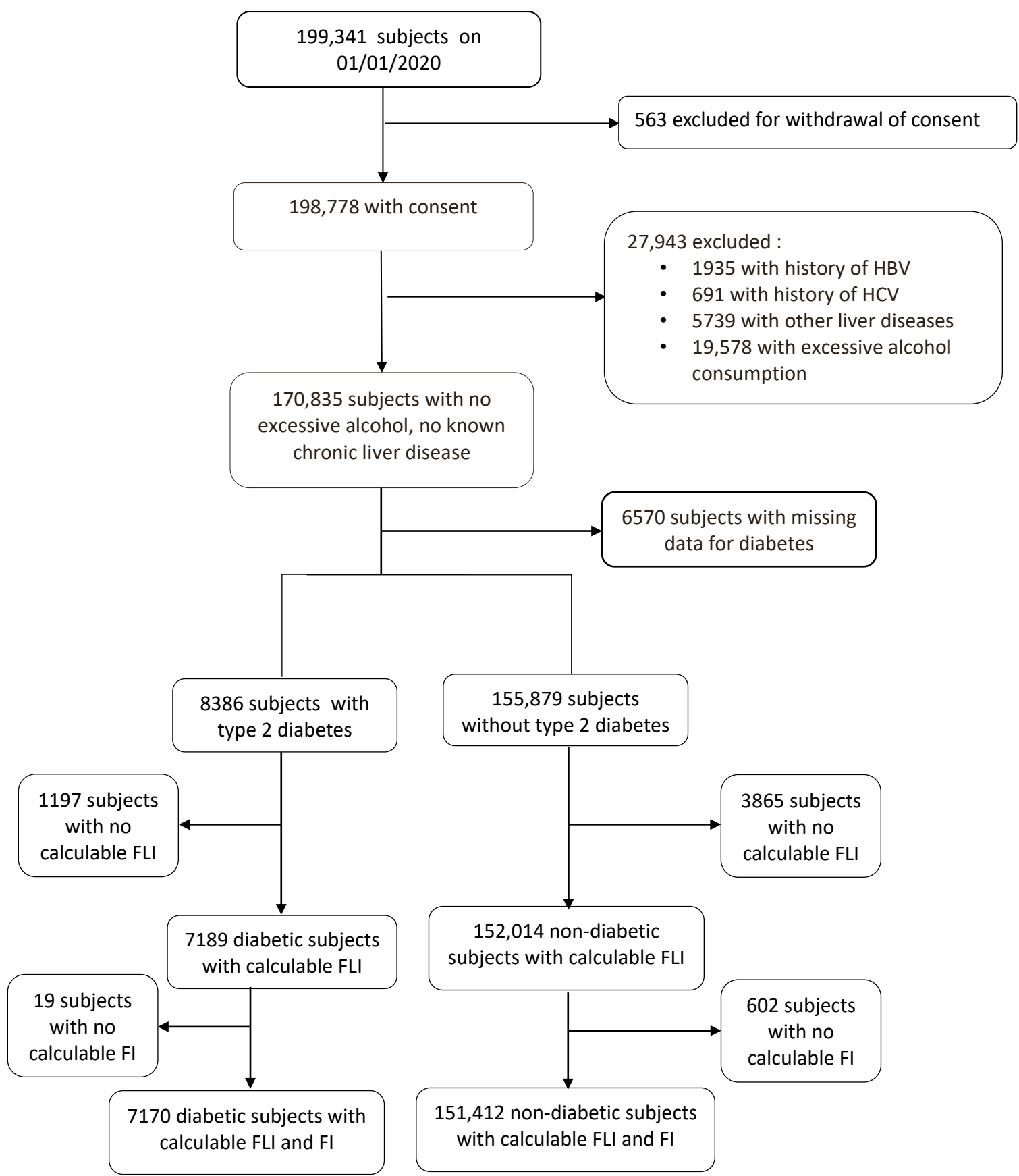
§ P<0.05

Figure legend

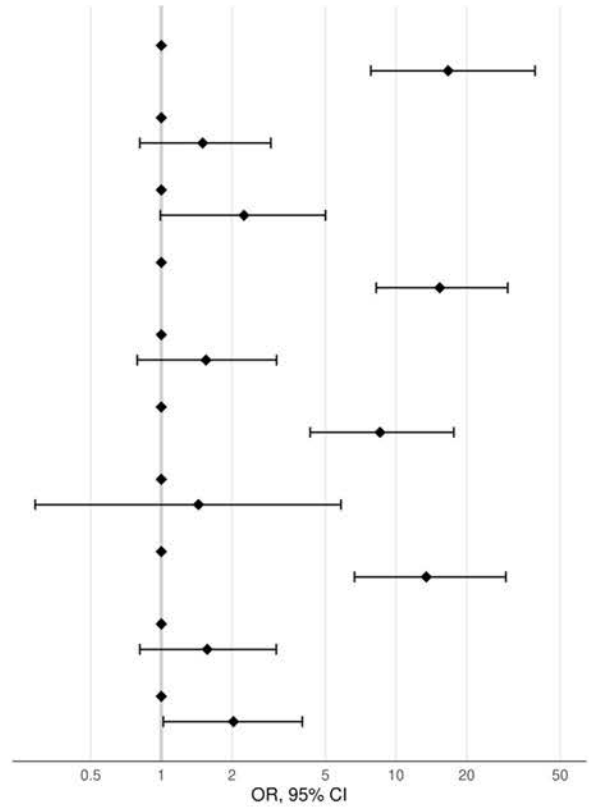
Figure 1: Flow chart

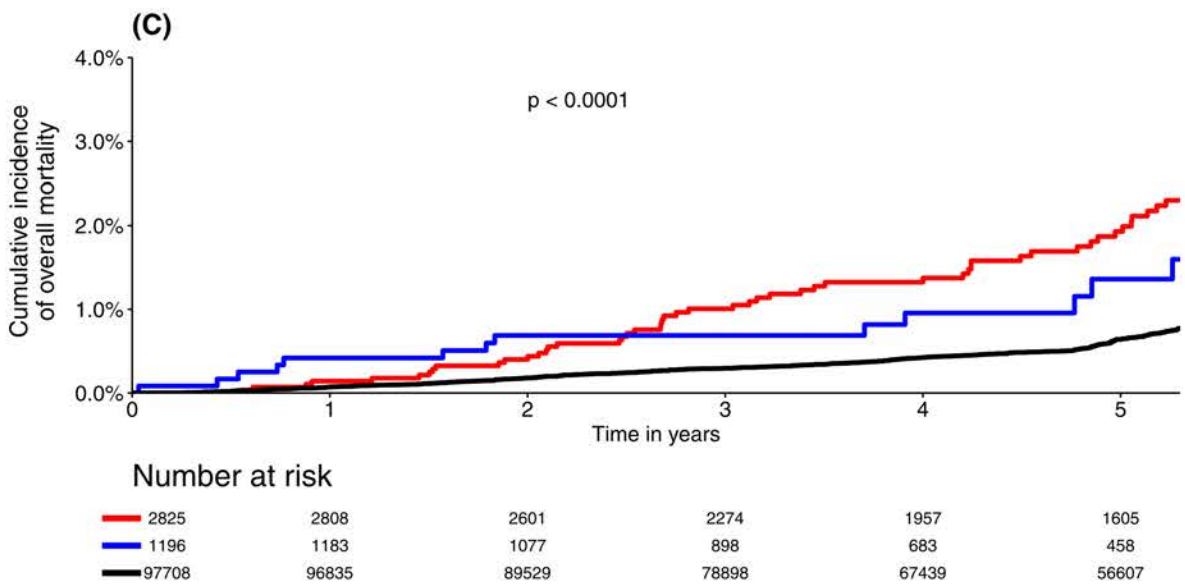
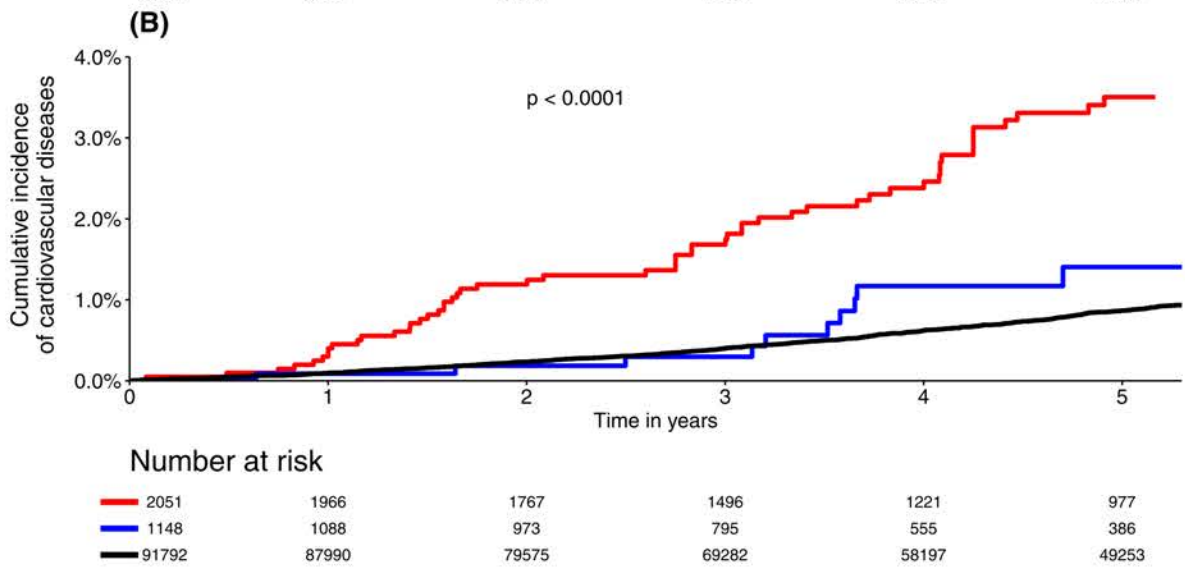
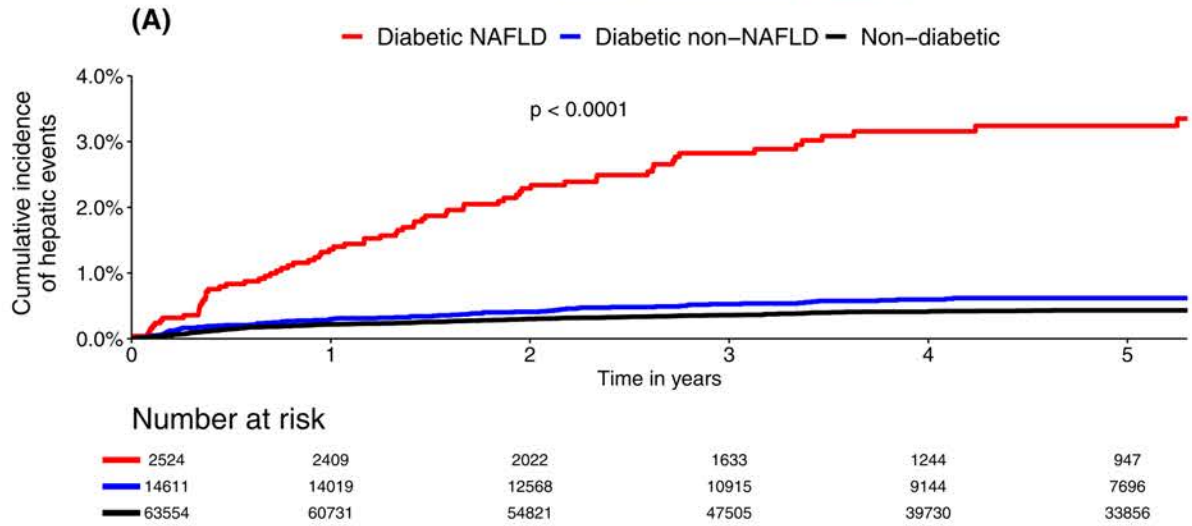
Figure 2: Multivariate analysis of risk factors associated with advanced fibrosis (Forns Index >6.9) among diabetic subjects with NAFLD. The reference group is diabetic subjects with NAFLD and no fibrosis (Forns Index <4.2).

Figure 3: Curves of cumulative incidence of liver-related events (a), cardiovascular events (b) and death (c) according to the presence of NAFLD and diabetes.



Gender	Female		
	Male	16.7 (7.8–39.1)	$p < 0.001$
Obesity	No		
	Yes	1.5 (0.8–2.9)	$p = 0.20$
HBP	No		
	Yes	2.2 (1.0–5.0)	$p < 0.05$
Elevated ALT	No		
	Yes	15.4 (8.2–29.9)	$p < 0.001$
Hyperglycemia	No		
	Yes	1.6 (0.8–3.1)	$p = 0.21$
Insulin requirement	No		
	Yes	8.6 (4.3–17.6)	$p < 0.001$
Oral antidiabetic	No		
	Yes	1.4 (0.3–5.8)	$p = 0.63$
Previous diabetes complications	No		
	Yes	13.5 (6.7–29.4)	$p < 0.001$
Tobacco	No		
	Yes	1.6 (0.8–3.1)	$p = 0.18$
Alcohol	No		
	Yes	2.0 (1.0–4.0)	$p = 0.041$





Supplementary

Supplementary Table 1

Supplementary Table 2

Supplementary Table 3

Supplementary Table 4

Supplementary Figure 1: Propensity scores covariate balance assessment. A) overlap assessment ; B) absolute standard mean difference

Supplementary Figure 2: Distribution of NAFLD according to age and gender in diabetic subjects

Supplementary Figure 3: Prevalence of advanced fibrosis according to the severity of diabetes in NAFLD diabetic subjects. Diabetes complications: history of cardiovascular disease and/or chronic kidney disease.

Supplementary Figure 4: Flow-chart of patients included in the follow-up study

Supplementary Figure 5: Cumulative incidence of a) extra hepatic malignancy and b) chronic kidney disease according to the presence of NAFLD and diabetes.

Supplementary Table 1 : Univariate and multivariate analysis of the prevalence of NAFLD according to risk factors among overall population, diabetic and non-diabetic subjects

		Overall population n=170,355				Diabetic subjects n=7189				Non-diabetic subjects n= 152,014			
Risk factors		NAFLD % (95%CI)	P	OR (95%CI)	P*	NAFLD % (95%CI)	P	OR (95%CI)	P*	NAFLD % (95%CI)	P	OR (95%CI)	P*
Age (yrs)	18-28	6.2 (5.8-6.6)	<0.0001	1.02 (1.01-1.02)	<0.0001	50.7 (42.5-58.9)	<0.0001	1.02 (1.01-1.03)	<0.0001	5.8(5.4-6.2)	0.0001	1.02(1.02-1.02)	<0.0001
	29-38	11.5 (11.1-11.9)				49.3 (45.9-52.7)				10.4(10.0-10.8)			
	39-48	17.0 (16.6-17.3)				55.9 (53.1-58.7)				14.1(13.8-14.5)			
	49-58	15.2 (14.9-15.5)				60.6 (58.0-63.2)				13.5(13.2-13.8)			
	59-68	26.4 (25.9-26.9)				61.4 (59.5-63.3)				23.4(23.0-23.8)			
	>68	35.4 (34.2-36.6)				72.5 (69.8-75.1)				23.5(22.4-24.6)			
Gender	Female	10.9 (10.7-11.1)	<0.0001	1	<0.0001	55.6 (53.9-57.3)	<0.0001	1	<0.0001	8.5(8.3-8.7)		1	<0.0001
	Male	26.7 (26.4-27.1)		2.83 (2.73-2.93)		65.6 (64.1-67.1)		1.76 (1.46-2.12)		24.1(23.8-24.4)		2.98(2.87-3.09)	
BMI	Lean	5.0 (4.9-5.1)	<0.0001	NA	-	20.9 (19.8-22.1)	<0.0001	NA	-	4.4(4.3-4.5)	<0.0001	NA	-
	Overweigh	22.7 (22.4-23.0)				58.0 (58.1-59.6)				21.3(20.9-21.7)			
	Obese	63.5 (62.8-64.2)				75.6 (77.2-78.0)				58.8(58.0-59.6)			
Diabetes	No	15.2 (15.0-15.3)	<0.0001	1	<0.0001	NA	-	NA	-	NA	-	NA	-
	Yes	61.1 (60.0-62.2)		6..05 (5.68-6.45)									

HBP	No	10.6 (10.4-10.8)	<0.0001	1	<0.0001	25.2 (23.3-27.1)	<0.0001	1	<0.0001	10.0(9.8-10.2)	<0.0001	1	<0.0001
	Yes	42.9 (42.3-43.5)		3.16 (3.03-3.54)		87.4 (86.3-88.5)		5.91 (4.87-7.16)		33.8(33.2-34.4)		3.01(2.89-3.13)	
Hypertriglyceridemia	No	13.8 (13.6-14.0)	<0.0001	NA	-	32.4 (30.9-33.9)	<0.0001	NA	-	12.7(12.5-12.9)	<0.0001	NA	-
	Yes	49.3 (48.6-50.0)		89.7		39.6(38.8-40.4)							
Hypercholesterolemia	No	14.1 (13.9-14.3)	<0.0001	1	<0.0001	32.9 (31.4-34.4)	<0.0001	1	<0.0001	13.1(12.9-13.2)	<0.0001	1	<0.0001
	Yes	47.5 (46.8-48.2)		6.23 (5.76-6.80)		89.0 (80.0-90.0)		3.81 (1.01-11.78)		36.9(36.1-37.7)		4.26(3.99-4.54)	
Elevated ALT	No	15.1 (14.9-15.2)	<0.0001	1	<0.0001	57.4 (56.2-58.6)	<0.0001	1	<0.0001	12.6(12.4-12.8)	<0.0001	1	<0.0001
	Yes	42.0 (41.2-42.8)		3.34 (3.15-3.54)		85.4 (83.1-87.7)		15.5 (10.83-22.68)		38.0(37.2-38.8)		3.21(3.03-3.41)	

* Logistic regression. Because BMI and triglycerides are part of FLI calculation, their OR was not provided (NA). Age was considered as continuous variable in the analysis.

OR: odds ratio, CI: confidence interval, ALT: alanine amino transferase, HBP: high blood pressure; NA: non-applicable

Supplementary Table 2. Univariate and multivariate analysis of risk factors of NAFLD among diabetic subjects

		Univariate analysis			Multi variate analysis		
		% NAFLD	95%CI	P value	OR	95%CI	P value *
Age (yrs)	18-28	36.1	31.0-41.2	<0.0001	1.02	1.01-1.04	0.0401
	29-38	55.8	54.7-56.9				
	39-48	62.6	61.1-64.1				
	49-58	76.8	75.5-78.1				
	59-68	76.9	75.6-78.1				
	>68	67.8	65.9-69.8				
Gender	Female	61.0	58.8-63.2	<0.0001	1.00	1.72-2.57	<0.0001
	Male	78.6	76.8-80.3		2.10		
HBP	No	67.8	66.1-69.5	<0.0001	1.00	1.28-2.08	<0.0001
	Yes	78.6	76.0-81.3		1.63		
Hypercholest.	No	40.1	37.8-42.4	<0.0001	1.00	3.63-5.86	<0.0001
	Yes	95.5	94.6-96.4		4.5		
Glucose>1.5g/L	No	9.4	8.9-10.0	<0.0001	1.00	1.01-1.51	0.0483
	Yes	75.6	73.8-77.3		1.23		
Elevated ALT	No	66.8	65.2-68.4	<0.0001	1.00	11.11-21.64	<0.0001
	Yes	91.6	89.3-93.9		15.35		
Hypertriglyc.	No	39.7	37.4-42.0	<0.0001	ND		
	Yes	95.4	94.5-96.3		ND		
Obesity	No	54.6	52.4-56.8	<0.0001	ND		
	Yes	84.7	83.1-86.2		ND		

*Logistic regression adjusted for anti-diabetic treatment. Because part of FLI calculation,, obesity and hypertriglyceridemia were not included in multivariate analysis.

OR: odds ratio, CI: confidence interval, ALT: alanine amino transferase

Supplementary Table 3. Characteristics of diabetic subjects with NAFLD according to the presence of advanced fibrosis. Presence or absence of fibrosis was defined according to Forns Index >6.9 or < 4.2.

	Diabetic NAFLD w/o fibrosis	Diabetic NAFLD with advanced fibrosis	
	<i>n=1283</i>	<i>n=168</i>	<i>P Value</i>
Age, yrs, mean (95%CI)	52 (51.5-52.5)	64 (63.4-64.6)	<i><0.0001</i>
Male gender, % (95%CI)	55.5 (51.4 – 59.5)	95.1 (91.6-98.6)	<i>0.0001</i>
Obesity, % (95%CI)	64.5 (60.6-68.4)	69.2 (61.7-76.7)	<i>ns</i>
Hypertriglyceridemia, % (95%CI)	49 (37.5 - 53.9)	64.6 (60.7-68.5)	<i>ns</i>
High Blood Pressure, % (95%CI)	24.2 (20.6 - 27.7)	45.7 (53-57.2)	<i>0.03</i>
Glucose> 1.5g/l, % (95%CI)	4.7 (1.5 - 7.8)	9.8 (8.9 - 10.7)	<i><0.001</i>
Insulin therapy, % (95%CI)	2.8 (1.4-3.0)	4 .1 (1.1 – 7)	<i>ns</i>
Diabetes-related complications*, % (95%CI)	25.9 (24.6 - 27.3)	42.0 (34.5 - 49.5)	<i><0.0001</i>
ALT>N, % (95%CI)	25.5 (21.9-29)	68.6 (61 - 76.2)	<i><0.0001</i>
Smoking, % (95%CI)	59.2 (54.8-63.3)	92.3 (87.5 - 97.2)	<i>0.0002</i>

CI : confidence interval, ALT : alanine amino transferase

*History of cardiovascular disease and or chronic kidney disease

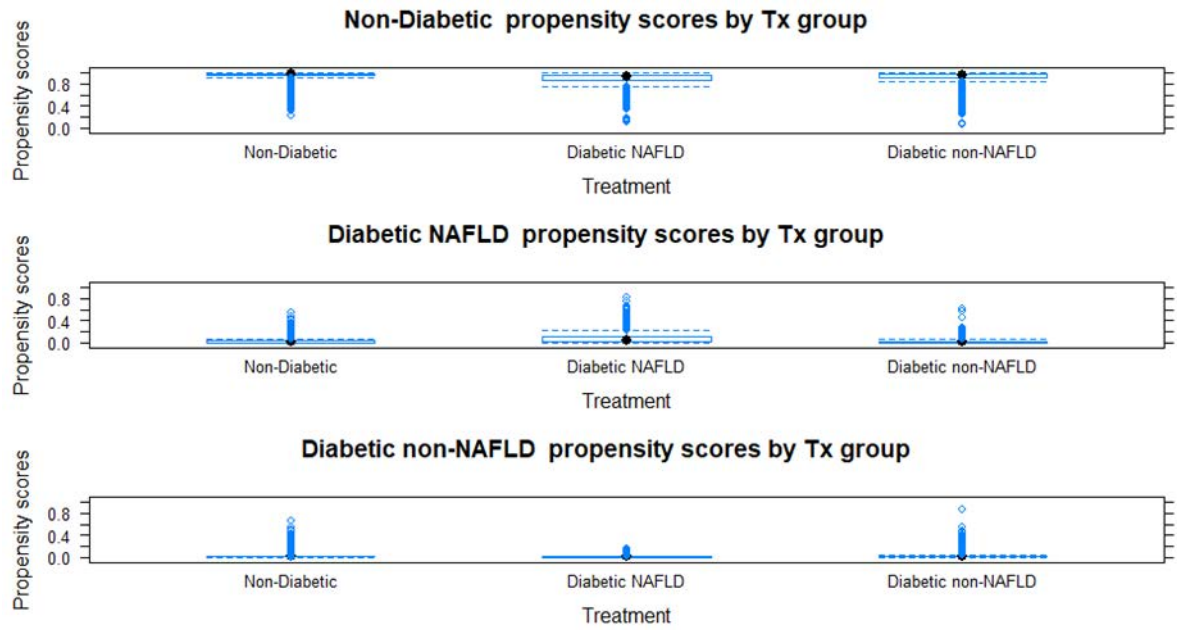
Supplementary Table 4: Univariate and multivariate analysis of outcome risk according to the grade of fibrosis (Forns Index <4.2: no fibrosis; FI 4.2-6.9: intermediate fibrosis, FI>6.9: advanced fibrosis) in non-diabetic subjects with NAFLD. The reference group is non-diabetic subjects without NAFLD (FI<30)

	Univariate analysis (reference group= non-Diabetic non-NAFLD, n=83346)			Multivariate analysis * (reference group= non-Diabetic non-NAFLD, n=83346)		
	NAFLD with advanced fibrosis n= 156	NAFLD with intermediate fibrosis n= 7028	NAFLD w/o fibrosis n= 7161	NAFLD with advanced fibrosis n= 156	NAFLD with intermediate fibrosis n= 7028	NAFLD w/o fibrosis n= 7161
	HR (95%CI)	HR (95%CI)	HR (95%CI)	HR (95%CI)	HR (95%CI)	HR (95%CI)
Hepatic events	1.62 (1.17, 2.24) [§]	1.07 (0.83, 1.37)	1.23 (0.86-1.78)	1.53 (1.07-2.18) [§]	1.02 (1.01-1.03) [§]	1.23 (0.84-1.80)
Cardiovascular disease	1.40 (0.20- 9.94)	0.66 (0.45-0.96) [§]	0.95 (0.70-1.27)	1.16 (0.17-8.16)	0.55 (0.38-0.81) [§]	0.80 (0.59-1.07)
Extra-hepatic cancer	1.45 (0.36-5.80)	0.85 (0.66- 1.11)	0.75 (0.57-0.99) [§]	1.43 (0.35-5.76)	0.85 (0.66-1.10)	0.76 (0.57-1.00) [§]
Chronic kidney disease	5.09 (1.27-20.47) [§]	1.11 (0.71-1.73)	1.24 (0.79-1.95)	3.64 (0.90-14.66)	0.77 (0.49-1.19)	(0.47-1.16)
Death	1.50 (1.17-1.92) [§]	1.49 (1.16-1.91) [§]	1.26 (0.18-8.93)	1.45 (1.12-1.87) [§]	1.43 (1.10-1.85) [§]	1.19 (0.17-8.51)

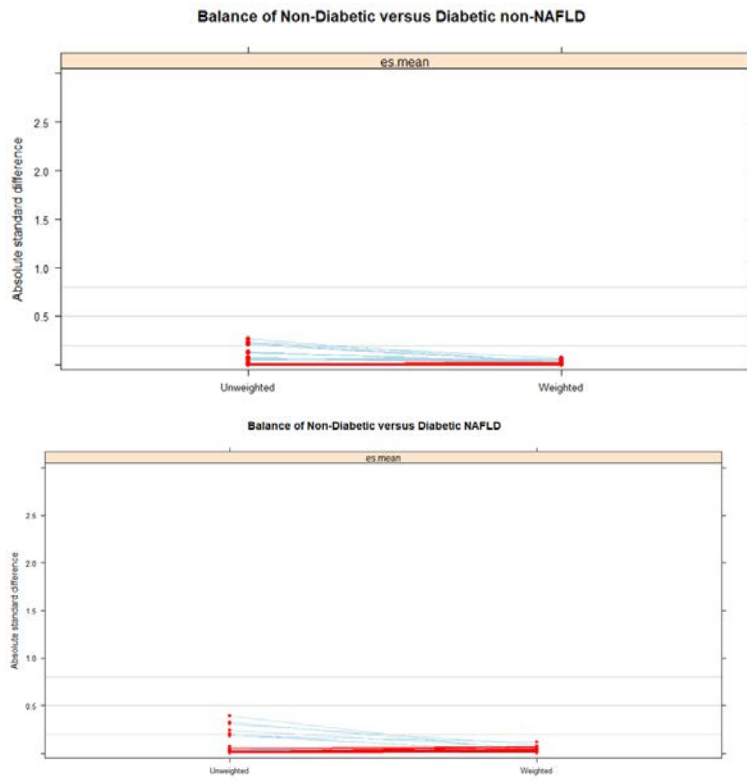
*Cox model, adjusted for age, gender, obesity, diabetes, HBP, ALT and smoking.

[§] P<0.05

A



B



Balance of Diabetic NAFLD versus Diabetic non-NAFLD

