Liver-related and extrahepatic events in patients with non-alcoholic fatty liver disease: a retrospective competing risks analysis

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Summary Background & Aim: Non-alcoholic fatty liver disease (NAFLD), and especially fibrotic non-alcoholic steatohepatitis, is associated with high risks of liver-related events (LRE) and extrahepatic events (EHE). We evaluated the competitive risk occurrence of LRE and EHE in a large cohort of biopsy-proven NAFLD stratified according to baseline severity of fibrosis.

Methods: Two thousand one hundred thirty-five patients with biopsy-proven NAFLD were enrolled. Observed cumulative incidence functions (CIFs) were used to evaluate the risk of LRE and EHE; cause-specific Cox model and predicted CIFs were fitted to identify predictors of LRE and EHE. A replication cohort of NAFLD patients with liver fibrosis severity estimated by liver stiffness measurement by transient elastography was also enrolled.

Results: Observed CIFs indicated that the 60-month probabilities of LRE and EHE were 0.2% and 3% in F0-F1, 2% and 3.8% in F2 and 9.7% and 6.4% in F3-F4 patients, respectively. The cause-specific Cox model indicated that in F0-F1 and F2 patients, age > 50 years (HR 2.7) was the only predictor of LRE, while age > 50 years (HR 2.96), previous cardiovascular events (CVE, HR 2.07), and previous extra-hepatic cancer (HR 2.36) were independent risk factors for EHE. In F3-F4 patients, age > 55 years (HR 1.73), obesity (HR 1.52), PLT < 150 000/mmc (HR 3.66) and log(GGT) (HR 1.77) were associated with LRE, while age > 55 years (HR 1.74) and previous CVE (HR 2.51) were independent predictors of EHE. Predicted CIFs for HE and EHE in F0-F1, F2 and F3-F4 patients stratified the risk of events. The results were externally replicated.

Conclusion: The likelihood of EHE in NAFLD patients is relevant and increases according to the severity of liver fibrosis, while the risk of LRE is negligible in F0-F1, low but clinically relevant in F2 and high in F3-F4 patients.

1 | INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is the most emergent cause of liver disease, affecting one quarter of the world population, with prevalence predicted to increase to 33.5% within the next few years, in line with the global epidemics of obesity and type 2 diabetes (T2D).1,2 Natural history studies of NAFLD patients show that the relative risk of liver-related complications has increased when compared to control populations.3-9 The two most frequent complications are cardiovascular events (CVE) and extra-hepatic cancers (EHC). NAFLD is becoming the most prevalent cause of liver-related events (LRE) that include liver decompensation (LD) and hepatocellular carcinoma (HCC), leading to end-stage liver disease and liver transplantation.10-13 In parallel to the upsurge of LRE, a recent American analysis also reported increased mortality due to EHC and CVE in patients with NAFLD.14 In this complicated scenario, multiple studies have shown that—as expected—the risk of developing LRE is higher in NAFLD patients with advanced liver fibrosis or cirrhosis.15 Furthermore, in the same clinical setting, other studies have reported an increased risk of both CVE and EHC in patients with more advanced fibrosis.16 Consistent with these results, two recent meta-analyses have shown that the risk of liver-related and overall mortality progressively increases according to the baseline severity of liver fibrosis.17,18 Despite the clinically relevant and heterogeneous burden of hepatic and extrahepatic complications of NAFLD and the effect of fibrosis on their occurrence, no definitive evidence exists regarding the natural history of NAFLD when LRE and extra-hepatic events (EHE) are considered as competing risks. This is a crucial point because, in complex epidemiological and clinical contexts, competing risks modelling is a powerful statistical method to adjust for the impact of clinical decision making in the presence of multiple major clinical endpoints.19 The rationale for using the competing risk approach for the study of the natural history of NAFLD patients lies in the fact that a competing risk is an event that either precludes or modifies the risk of occurrence of an event of interest (eg, LRE vs EHE, as in the present study).19 Furthermore, from a methodological point of view, time-to-event analysis using either the naive KaplanMeier estimator or the PH Cox model to estimate the probability of occurrence of the event of interest in the presence of competing risks could yield biased estimates. Cumulative incidence function (CIF) and competing risks models such as the cause-specific Cox (CSC) and/or the Fine-Gray (FG) models should be applied because they account for the effects of competing risks.20 In the present study, we evaluated the probability of developing LRE and EHE, considered as competing risks, in a large multicentre cohort of patients with a histological diagnosis of NAFLD stratified by fibrosis stage. We replicated these results in a large cohort of NAFLD patients in whom liver fibrosis was assessed non-invasively.

2 | METHODS

2.1 | Patient selection

We retrospectively analysed data from two multicentre cohorts of NAFLD patients with histological (training set) or non-invasive (replication set) assessment of liver fibrosis, prospectively recruited at the Gastrointestinal and Liver Unit of the Palermo University Hospital; at Centre d'Investigation de la Fibrose Hépatique of the Bordeaux University Hospital; at Division of Gastroenterology and Hepatology of McGill University Health Centre of Montreal; at Hepatology Unit of Ospedale San Giuseppe University of Milan; at Hospital Universitario Virgen del Rocío de Sevilla; at Department of Medicine and Therapeutics of the Chinese University of Hong Kong; at HepatoGastroenterology Department of Angers University Hospital; at the Swiss Liver Center; at Division of Gastroenterology, Department of Medical Sciences of University of Torino; and at Department of Pathophysiology and Transplantation, Ca' Granda IRCCS Foundation of Policlinico Hospital of University of Milan; and at Storr Liver Centre, Westmead Institute for Medical Research, Westmead Hospital and University of Sydney. Patients were included if they had received follow-up for at least 6 months. Other causes of liver disease were ruled out, including alcohol intake (>20 g/d) as evaluated by a questionnaire; viral (hepatitis B surface antigen, anti-hepatitis C virus and antihuman immunodeficiency virus negativity) and autoimmune hepatitis; hereditary hemochromatosis; and alpha-1

antitrypsin deficiency. In the training cohort, the Kleiner scoring system21 was used for histological assessment of NAFLD. Fibrosis stages were assigned from 0 to 4. Liver stiffness measurement (LSM) was used to assess fibrosis non-invasively in the replication cohort. LSM was evaluated with transient elastography by FibroScan (Echosens, Paris, France), using the M and the XL probe when appropriate. LSM was assessed after overnight fasting. LSM < 7.9 kPa was defined as indicating a low risk of F3-F4 fibrosis; LSM 7.9-9.6 kPa as an intermediate risk; LSM ≥ 9.6 kPa as a high risk.22,23 The study was carried out in accordance with the principles of the Helsinki Declaration and with local and national laws. Approval was obtained from the AOUP "Paolo Giaccone" of Palermo.

2.2 | Patient evaluation

Clinical and metabolic data were collected at the time of enrolment. Body mass index (BMI) was calculated in kilograms for weight and in meters for height. Obesity was defined as BMI ≥ 30 kg/m2 . The diagnosis of T2D was made according to the American Diabetes Association,24 using a value of fasting blood glucose ≥126 mg/dl. In patients with a previous diagnosis of T2D, current medications and their changes were documented and used to meet a case definition of T2D. Arterial hypertension was defined by systolic blood pressure ≥140 mm Hg and/or diastolic blood pressure ≥90 mm Hg, or use of blood pressure lowering agents.25 A 12-hour overnight fasting blood sample was drawn to determine serum levels of ALT, GGT, PLT, total cholesterol and triglycerides.

2.3 | LRE and EHE assessment

Incident LRE and EHE were recorded during the entire follow-up period in both groups. LRE was defined as LD (occurrence of either ascites, variceal haemorrhage, encephalopathy, jaundice or HCC). EHE were defined as either CVE (stroke, transient ischaemic attack, myocardial infarction and unstable angina) or EHC not including non-melanomatous skin cancers. Clinical, biochemical and ultrasound examinations were conducted annually in patients with F0-F2 fibrosis and, for stricter surveillance for HCC and LD, every 6 months in patients with F3 fibrosis or cirrhosis, according to international guidelines.26 In the presence of cirrhosis, oesophageal gastroscopy was performed at baseline and repeated as recommended by clinical guidelines.27 Patients with progression to medium or large (F2 or F3) oesophageal varices were treated with bblockers or underwent elastic banding, whereas no prophylaxis was provided to patients with small (F1) varices. Patients developing LRE during follow-up were evaluated for available therapies and/or for liver transplantation, if appropriate. Evidence of both LRE and EHE was provided by inpatient and outpatient medical records. Patients were censored at the last available visit or in case of death; patients who underwent liver transplantation were censored as dead.

2.4 | Statistics

Observed counts and incidence rates of hepatic and extra-hepatic events were documented. Patient characteristics were reported within strata: mean ± standard deviation for covariates with symmetrical continuous distributions; median and interquartile range for variables with skewed continuous distributions; and percentage of cases for binary variables. Pairwise comparisons among fibrosis strata, that is F0-F1, F2 and F3-F4, were performed by using t-tests for covariates with symmetrical continuous distributions; MannWhitney tests for variables with skewed continuous distributions; and chi-square tests to compare percentages. In all cases, a correction factor for multiple tests was applied.28 Univariate analysis was conducted by estimating several univariate models as covariates. All variables that we considered were measured at baseline; consequently, no time-dependent covariates were evaluated. The variables that we considered were: hypertension; T2D; gender; age in years, as well as its categorised version using the median as cut point, 50 for F0-F2 patients, 55 for F3-F4; BMI, as well as the binary version "BMI30" (≤30/>30); platelet counts, as well as Plt150 (platelets > 150.000/Platelets ≤ 150.000); ALT and log(ALT); GGT and log(GGT); cholesterol level; triglyceride level and log(triglyceride level). Robust estimates of the standard errors were used to take into account heterogeneity due to the multicentre study design.

The multivariate model included all the covariates resulting significant (P < 5%) in the univariate models. These candidate covariates were then included in the multivariate model one at time, according to a forward selection based on p-value and AIC. Moreover, the selected number of covariates in both the final models for LREs and EHEs agree with the principle of parsimony that avoids overfitting. Both univariate and multivariate analyses were performed by stratified analyses according to the severity of liver fibrosis. Observed CIFs and competing risks models were used to model the risk of occurrence of LRE and EHE considered as competitive. In particular, CSC and subdistribution hazards (FG)29 models were fitted. For each of the CSC and FG models, two types of models were employed: the first was a competing risks model fitted on the subset with fibrosis < F3, but using fibrosis with levels F0-F1 and F2 as stratification covariate, with the remaining covariates assumed the same for both strata. The model thus assumes different cause-specific (or subdistribution) baseline hazards by fibrosis strata. The second competing risks model was fitted on the fibrosis stratum F3-F4, by employing a different set of covariates, as a result of the selection process based on P-value, AIC (forward selection), and on clinical criteria. To take into account heterogeneity due to inter-hospital variations, a generalised estimating equation (GEE) approach was used, and robust standard errors were obtained. Predicted CIFs were provided for both hepatic and EHE occurrences by each model. The fitted models were replicated externally by using a replication cohort of NAFLD patients with non-invasive stratification of liver fibrosis, by comparing the predicted CIFs in the training and in the replication sets. All analyses were performed by using R (version 4.0.2) and the "survival" and "mstate" statistical packages.

3 | RESULTS

3.1 | Baseline patient characteristics

Baseline characteristics of the 2135 patients with a histological diagnosis of NAFLD stratified according to the severity of liver fibrosis (F0-F1 = 1136; F2 = 362 and F3-F4 = 637) are shown in Table 1. As expected, mean age was significantly higher in patients with F3- F4 compared to F0-F1 and F2 fibrosis. The prevalence of metabolic comorbidities (obesity, T2D and arterial hypertension) progressively increased according to the severity of liver fibrosis. Previous CVE was more frequent in patients with F3-F4 than in patients with F0- F1 or F2 fibrosis, while the prevalence of previous EHC was not significantly different between the groups. Similar demographic, metabolic and clinical features were found in the replication cohort (n = 2790, 1365 at low, 286 at intermediate and 1139 at high risk of advanced liver fibrosis by LSM) (Table S1).

3.2 | Incident LRE and EHE

F0-F1, F2 and F3-F4 patients had median follow-up horizons of 97.0, 79.6 and 65.6 months, respectively. F0-F1 patients experienced 8 LRE and 66 EHE (2.1% and 5.8%, respectively), F2 patients developed 7 LRE and 36 EHE (4.4% and 9.9%, respectively) and F3-F4 patients suffered 81 LRE and 64 EHE (18.8% and 14.6%). In the entire cohort, 30 LRE (2 in F0-F1, 4 in F2 and 24 in F3-F4 patients) and 44 EHE-related (19 in F0-F1, 6 in F2 and 19 in F3-F4 patients) deaths were recorded. Table 2 shows the crude probability of LRE or EHE as first event, followed by EHE or LRE, respectively, as second event, stratified for fibrosis severity. Notably, patients with F0-F1 and F2 fibrosis had a clinically relevant probability of EHE as first event (5.8% and 9.9%, respectively), despite a very low or low probability of first LRE (0.6% and 1.6%, respectively). Also, in patients with F0-F1 and F2 fibrosis with a first EHE, we confirmed the low probability of LRE as second event (1.5% and 2.7%, respectively). On the other hand, patients with F3-F4 fibrosis had a comparably high probability of both LRE and EHE as first event (12% and 9.4%, respectively), and a similarly high risk of EHE and LRE as second event (5.2% and 6.6%, respectively). Similar data, summarised in Table S2, were found in the replication cohort.

3.3 | Competing risk analysis of LRE and EHE

To estimate the competing risk occurrence of LRE and EHE, 10-year CIFs were calculated. When considered as competitors, the cumulative incidence of not only LRE but also EHE progressively increased over time according to the severity of liver fibrosis. In F0-F1 patients, the probabilities of LRE and EHE were 0.1% and 0.4% at 12 months, 0.2% and 1.9% at 36 months, 0.2% and 3% at 60 months, 0.4% and 6.6% at 120 months, respectively (Figure 1A) (Table 3). In F2 patients, the probabilities of LRE and EHE were 0.6% and 2.3% at 12 months, 1.6% and 2.6% at 36 months, 2% and 3.8% at 60 months, 4.4% and 11.6% at 120 months, respectively (Figure 1B) (Table 3). Finally, in F3-F4 patients, the probabilities of LRE and EHE were 2.1% and 1.1% at 12 months, 5.7% and 4.3% at 36 months, 9.7% and 6.4% at 60 months, 21% and 10.8% at 120 months, respectively (Figure 1C) (Table 3). Rates of LRE and EHE in the subgroup of patients without histories of previous CVE and/or extrahepatic cancers stratified according to the severity of liver fibrosis are shown in Table 3. Finally, in the subgroup of patients with F3-F4 fibrosis, the risk of developing LRE with respect to EHE was lower in F3 while higher in F4 patients. Specifically, in F3 patients, the probabilities of HCC, LD, and EHE were 0.2%, 0.2% and 1.7% at 12 months; 2%, 0.2% and 5.1% at 36 months; 3.3%, 1.9% and 8% at 60 months and 5.4%, 4.8% and 12.8% at 120 months, respectively (Figure S1). Conversely, in F4 patients, the probabilities of HCC, LD and EHE were 2.2%, 1.5% and 0.4% at 12 months; 4.8%, 5.2% and 2.8% at 36 months; 6.4%, 8.3% and 3.8% at 60 months and 10.7%, 20.9% and 8.1% at 120 months, respectively (Figure S1). Similar cumulative incidences of LRE and EHE stratified for the risk of fibrosis severity were observed in the replication cohort (Figure 1) (Table 3).

3.4 | Predictors of LRE and EHE

The observed and previously described link between the severity of liver fibrosis and increased competing cumulative incidence of not only LRE but also EHE was underscored by using the cause-specific proportional CSC, that, after adjusting for age, confirmed F2 (HR 5.08, SE 0.42, $P < 0.001$), F3 (HR 12.91, SE 0.50, $P < 0.001$) and F4 (HR 39.23, SE 0.39, $P < 0.001$) fibrosis as independent risk factors for LRE, and F2 (HR 1.6, SE 0.14, $P < 0.001$) and F3 (HR 1.87, SE 0.19, $P < 0.001$) fibrosis as independent predictors of EHE. Similar results were obtained by using the FG model (data not shown). We conducted further analyses by stratifying the population according to liver fibrosis severity. Results of univariate CSC models are reported in Table S3. Using multivariate CSC (Table 4) for LRE and EHE in F0-F1 and F2 patients, age > 50 years (HR 2.7, beta 0.99, $P = 0.001$) was the only independent predictor of developing LRE; while age > 50 years (HR 2.96, beta 1.08, $P < 0.001$); previous CVE (HR 2.07, beta 0.73, $P = 0.03$) and previous EHC (HR 2.36, beta 0.86, $P = 0.003$) were independent risk factors for incident EHE. When excluding patients with histories of previous CVE and/or extrahepatic cancer, age > 50 years was the only predictor of developing both LRE (HR 2.09, SE 0.32, $P = 0.02$) and EHE (HR 3.13, SE 0.31, $P < 0.001$). The CSC model (Table 4) disclosed that in F3-F4 patients, age > 55 years (HR 1.73, beta 0.55, $P = 0.03$); obesity (HR 1.52, beta 0.42, $P = 0.03$); PLT < 150 000/mmc (HR 3.66, beta 1.30, $P < 0.001$) and log(GGT) (HR 1.77, beta 0.57, $P < 0.001$) were significantly associated with LRE, while, age > 55 years (HR 1.74, beta 0.55, $P = 0.006$) and previous CVE (HR 2.51, beta 0.92, $P < 0.001$) were independent predictors of EHE. In the subgroup of patients without histories of previous CVE and/or EHC, age > 55 years (HR 1.91, SE 0.31, $P = 0.03$); obesity (HR 1.69, SE 0.20, $P = 0.01$); PLT < 150 000/ mmc (HR 3.86, SE 1.18, $P < 0.001$) and log(GGT) (HR 1.85, SE 0.75, $P < 0.001$) were confirmed independent predictors of LRE, while age > 55 years (HR 1.59, SE 0.20, $P = 0.02$) and serum triglycerides (HR 1.92, SE 0.26, $P = 0.01$) were independently associated with a higher risk of EHE. Predicted CIFs for LRE and EHE in F0-F1, F2 and F3-F4 patients were obtained from the models, and classes of patients at high or low risk of events according to presence or absence of all independent risk factors were considered. Specifically, in F0-F1 patients the probability of EHE ranged from 0.18%, 0.96%, 1.55% and 3.56% at 12, 36, 60 and 120 months, respectively, in the lowrisk class; to 2.61%, 13.06%, 20.24% and 40.89% at 12, 36, 60 and 120 months, respectively, in the high-risk class (Figure 2A). In F2 patients, the probability of EHE ranged from 1.03%, 1.18%, 1.75% and 5.69% at 12, 36, 60 and 120 months, respectively, in the lowrisk class, to 13.96%, 15.80%, 22.61% and 56.83% at 12, 36, 60 and 120 months, respectively, in the high-risk class (Figure 2B). LRE risk also increased from the low-risk to the high-risk class in both F0-F1

and F2 patients but remained negligible in F0-F1 (Figure 2A) and low in F2 (Figure 2B). In patients with F3-F4 fibrosis, the probability of LRE ranged from 0.39%, 1.10%, 1.94% and 4.69% at 12, 36, 60 and 120 months, respectively, in the low-risk class; to 7.10%, 18.31%, 29.01% and 51.97% at 12, 36, 60 and 120 months, respectively, in the high-risk class (Figure 2C). On the other hand, the probability of EHE ranged from 0.69%, 2.57%, 4.09% and 7.24% at 12, 36, 60 and 120 months, respectively, in the low-risk class, to 2.90%, 9.78%, 14.33% and 21.04% at 12, 36, 60 and 120 months, respectively, in the high-risk class (Figure 2C). Similar results were obtained when replacing the CSC model with the FG model (Table 4 and Figure S2). Notably, probabilities of events predicted by classical Cox models were slightly (for F0-F2) to moderately (for F3-F4 patients) biased, compared to results obtained by CSC and FG models (Figures S3-S5). Finally, both the CSC and FG models yielded congruous results in the replication cohort, as summarised in Table S4. Specifically, by using the CSC (Table S4) for LRE and EHE in patients at low/intermediate risk of advanced fibrosis, age > 50 years (HR 1.3, beta 0.26, P = 0.01) was the only independent predictor of LRE, while age > 50 years (HR 2.13, beta 0.76, P < 0.001), and previous CVE (HR 2.48, beta 0.91, P < 0.001) were independent risk factors for EHE. The CSC model (Table S4) disclosed that for F3-F4 patients, age > 55 years (HR 1.95, beta 0.67, P < 0.001), PLT < 150 000/ mmc (HR 5.02, beta 1.61, P < 0.001) and log(GGT) (HR 1.31, beta 0.27, P = 0.003) were significantly associated with LRE, while, age > 55 years (HR 2.06, beta 0.72, P = 0.005) and previous CVE (HR 2.37, beta 0.86, P = 0.004) were independent predictors of EHE. The obtained predicted CIFs for LRE and EHE in patients at low, intermediate and high risk of advanced fibrosis are reported in Figure 2. Similar results were obtained when replacing the CSC model with the FG model (Table S4 and Figure S2). Subgroup analyses were performed in patients without histories of previous CVE and/or EHC. In this sub-cohort, in patients at low-intermedium risk of advanced fibrosis by LSM, age > 50 years (HR 1.49, SE 0.52, P < 0.001) was independent predictor of LRE, and age > 50 years (HR 1.47, SE 0.32, P < 0.001) and higher log(GGT) serum levels (HR 1.21, SE 0.15, P < 0.001) were independent predictors of EHE. In patients at high risk of advanced fibrosis by LSM, age > 55 years (HR 1.80, SE 0.27, P = 0.001) and PLT < 150 000 mmc (HR 5.27, SE 0.21, P < 0.001) were independent predictors of LRE, and age > 55 years (HR 1.89, SE 0.33, P = 0.01) was an independent predictor of EHE.

4 | DISCUSSION

We applied competing risks modelling to a large cohort of individuals with a histological diagnosis of NAFLD that was prospectively followed for a median follow-up time of 84 months and found that the probability of developing LRE was negligible in F0-F1, low in F2, and clinically relevant in F3-F4 patients. Furthermore, the risk of incident EHE persisted among all three groups and increased according to the baseline severity of liver fibrosis. Notably, these results were replicated in a large cohort of patients with NAFLD diagnosed by non-invasive assessment of liver fibrosis. Patients with NAFLD are characterised by a high risk of developing not only hepatic but also EHE3-13 that are associated with the severity of liver fibrosis.15,16 In the context of the heterogeneous burden of hepatic and non-hepatic outcomes and the potential differential effects of fibrosis on their development, available evidence on the natural history of NAFLD is limited by the lack of conclusive data when LRE and EHE are considered as competing risks. In the present study, the application of competitive risk modelling on the full spectrum of NAFLD patients confirmed that the severity of liver fibrosis is an independent risk factor for both LRE and EHE. Notably, after stratifying for liver fibrosis severity, we observed that in F0- F1 patients the probability of developing LRE was negligible over time. However, these patients experienced a low but progressive increase in the risk of EHE (Table 3). Patients with F2 fibrosis were characterised by an LRE risk that was low but higher than in the F0- F1 group, risk of HE (Table 3), mostly due to the incidence of HCC; and also exhibited an increasing incidence of EHE (Table 3). Finally, patients with F3-F4 fibrosis had a higher and clinically relevant probability of developing both LRE and EHE (Table 3). Subgroup analyses in patients without previous CVE and/or previous extrahepatic cancers, as expected, demonstrated a slightly lower risk of developing EHE. Our data indicate that at a horizon of 60 months, the probability of developing LRE is negligible in F0-F1 patients, low in F2 patients, and clinically relevant in F3-F4 patients, while the risk of EHE is present in all NAFLD patients but increased

according to the severity of liver fibrosis. Notably, our data from F2 patients add further insights regarding the risk of developing HCC in patients with NASH but without cirrhosis,30-33 even if systematic screening in these patients cannot be suggested.34-36 A recent multicentre study18 used a competitive risks model to evaluate the occurrence of hepatic and extrahepatic events in a cohort of 458 patients with histological diagnosis of NAFLD with bridging fibrosis or cirrhosis, including patients with Child A6 cirrhosis. The probability of LRE increased progressively from F3 to F4 Child-Pugh A5, and further to F4 Child-Pugh A6 patients. In contrast, an inverse trend for EHE was observed.18 A subgroup analysis of our cohort that split F3 from F4 patients showed a high incidence of LRE and EHE in both subgroups, and further confirmed the higher incidence of EHE in respect to LRE in F3 patients and an inverse relationship in F4 patients. Our analysis also showed a lower HCC incidence in F3 compared to F4 patients, thus raising doubts regarding the cost-effectiveness of HCC screening in F3 patients, in whom clusters at higher risk for HCC should be identified. Our competitive cause-specific risks model in F0-F1 and F2 patients identified older age and previous history of EHE as risk factors for incident EHE, and older age as an independent predictor of LRE. On the other hand, in patients with F3-F4 fibrosis, older age, obesity, thrombocytopenia, and higher log(GGT) levels were risk factors for LRE, while older age and previous CVE were predictors of EHE. Notably, when combining these risk factors (presence or absence of all the independent predictors), we identified classes of patients with high or low probabilities of developing events. Specifically, from the low- to the high-risk class, the 60-month probability of LRE increased between F2 and F3-F4 patients. Similarly, the 60-month risk of EHE increased between F0-F1, F2, F3-F4 patients (Figure 2, Table 4, Figure S2). Collectively, these data could inform risk stratification in NAFLD patients to further refine follow-up and resource allocation. It is especially notable that all results were confirmed when replacing the CSC with the FG model. The present study addresses another unresolved question of NAFLD natural history, especially in patients with advanced fibrosis; that is whether first LRE or EHE in NAFLD patients are definitively committed to the liver or not liver-related way. We showed that among F0-F1 or F2 patients developing a first EHE, the risk for a second LRE remains low, especially in the F0-F1 group. Most notably, F3-F4 patients who had a first LRE maintained a clinically relevant risk of EHE, while the inverse was observed for those experiencing a first EHE. These findings suggest that patients with NAFLD and advanced fibrosis should receive follow-up for both hepatic and extrahepatic complications after developing a first event. Our findings in the histological cohort were replicated in a large multicentre cohort of NAFLD patients in whom liver fibrosis was assessed non-invasively by using LSM. This is a crucial point because, despite the limited accuracy of LSM for staging fibrosis in NAFLD,37,38 we demonstrated that similar results regarding natural history and risk stratification were yielded by histological and LSM assessments. Consequently, the reproducibility of our results will allow us to confidently apply our conclusions to large clinical populations in whom NAFLD severity may be evaluated non-invasively, even if our tertiary cohort is different from NAFLD individuals from the general population. The clinical applicability of our findings is further strengthened because competitive risk modelling is a more appropriate statistical method to assess the impact of clinical decision making in complex epidemiological and clinical contexts such as NAFLD, that feature independent major clinical endpoints.19 A Competing risk is an event that either precludes or modifies the risk of an event of interest. Time-to-event analysis using either the naive Kaplan-Meier estimator or the PH Cox model to estimate the probability of the event of interest in the presence of competing risks could provide biased estimates, as also partially observed in the present study. Consequently, CSC and/or FG models should be applied and the predicted CIFs reported instead. Although CSC and FG often yield similar results, the interpretation and the conclusions drawn from these models may differ. The CSC model estimates risk factor effects on the hazard of a specific event. Although CSC estimates may be identical to those obtained from a PH Cox model, the probability of the event of interest from the Cox model could be overestimated. However, caution should be exercised when interpreting the effects of risk factors on the CIFs from a CSC model, as an increase in the hazard due to increased predictor values does not necessarily correspond to an increased CIF.20 The FG model overcomes this problem as it can be viewed as a model for the CIF. Unlike in the CSC, where a predictor acts on the hazard (instantaneous rate

of occurrence of the event of primary interest is estimated in subjects who are event-free), in the FG the predictor acts on the subdistribution hazard, that is the instantaneous rate of occurrence of the event for subjects who are event-free or who have experienced a competing event, which in turn acts on the predicted CIF proportionally. In the FG model, an increase in the covariate is related to an increase in the corresponding CIF, but the magnitude of the CIF increase cannot be quantified. A drawback of the FG model is that the sum of the CIFs of the two events may exceed 1 for some covariate patterns.39 This drawback is obviated by the CSC model. The primary limitation of this study is the lack of timedependent variables and data capturing liver disease progression over time. Another notable limitation is the lack of data regarding common risk factors for EHE such as smoking and family histories of CVE and extrahepatic cancers. The multicentre study design could also affect our results; however, a GEE approach was used, and robust standard errors were obtained. Another potential limitation is that the competing risks and classical PH Cox models may yield similar predicted probabilities when the proportion of events is low (<10%). Such a low proportion was present in our F0-F1 and F2 strata, but not in F3-F4. Consequently, the results of the competing risks model can be viewed as a refinement of the PH Cox analysis in the F0-F1 and F2 strata, while providing optimal results in F3-F4, as it produces unbiased predicted CIFs. Other limitations are the use of BMI ≥ 30 kg/m2 instead of ethnic-specific BMIs to diagnose obesity, the potentially hidden alcohol abuse during follow-up, the lack of baseline and follow-up data on high-risk oesophageal varices and of time-dependent variables, and selection bias in the liver biopsy cohort. Regarding this last point, we observed a lower LRE incidence rates in all fibrosis strata compared to the findings of a recent meta-analysis.40 This difference, probably related to the heterogeneous follow-up among studies, could potentially affect the applicability of our results. Finally, the aggregation of HCC and LD together as LRE and cardiovascular events and extrahepatic cancers as EHE further limits the interpretation of our results. In conclusion, this study of NAFLD patients stratified for baseline severity of liver fibrosis showed that over a 60-month horizon the probability of EHE is consistent and increases according to the severity of fibrosis, while the risk of LRE is negligible in F0-F1 patients, low in F2 patients, and significant in F3-F4 patients. The latter require ongoing follow-up for both LRE and EHE. These data can inform personalised prognosis and follow-up in patients with NAFLD.

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Tables

TABLE 1 Baseline demographic, laboratory, metabolic and clinical features of histological cohort of NAFLD (n = 2135), stratified for fibrosis severity (F0-F1 vs F2 vs F3-F4).

Note: Data are given as: (#) mean ± standard deviations or (*) median (1st quartile-3rd quartile) or (§) percentage of cases (%). Abbreviations: ALT, alanine aminotransferase; BMI, body mass index; CVE, cardiovascular event; EHC, extrahepatic cancer; GGT, gamma glutamyl trasferase; PLT, platelets.
a P value = comparison between F2 and F3-F4.
b P value = comparison between F2 and F0-F1.
c P value = comparison between F3-F4 and F0-F1.

TABLE 2 Liver-related events and extrahepatic events occurrence as first or second events, stratified for fibrosis severity assessed by biopsy (F0-F1 vs F2 vs F3-F4), in NAFLD histological cohort (n = 2135).
Abbreviations: CVE, cardiovascular event; E, events; EHC, extrahepatic cancer; HCC, hepatocellular carcinoma; LD, liver decompensation.

TABLE 3 LRE and EHE in histological cohort (derivation set) and clinical cohort (replication set) of patients with NAFLD stratified for severity of fibrosis.
Abbreviations: EHE, extrahepatic events; LRE, liver-related events; LSM, liver stiffness measurement.

TABLE 4 Variables associated with LRE and EHE in the cause-specific Cox and Fine-Gray models, in histological cohort of patients with NAFLD, stratified for severity of fibrosis (F0-F1-F2 vs F3-F4).
Abbreviations: BMI, body mass index; CI, confidence interval; CVE, cardiovascular event; EHC, extrahepatic cancer; EHE, extrahepatic events; GGT, gamma glutamyl transferase; HR, hazard ratio; LRE, liver-related events; PLT, platelets; SE, standard error.

Figures

FIGURE 1 Observed cumulative incidence functions of hepatic and extrahepatic events in patients with F0-F1 fibrosis or at low risk of developing advanced fibrosis (A), F2 fibrosis or at intermediate risk of developing advanced fibrosis (B), and F3-F4 fibrosis or at high risk of developing advanced fibrosis (C).

FIGURE 2 Predicted cumulative incidence functions of hepatic and extrahepatic events by cause-specific Cox hazard model in patients with F0- F1 fibrosis or at low risk of developing advanced fibrosis (A), F2 fibrosis or at intermediate risk of developing advanced fibrosis (B), and F3-F4 fibrosis or at high risk of developing advanced fibrosis (C).