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1 **Hepatocyte-derived biomarkers predict liver-related events at 2 years in Child-Pugh**
2 **class A alcohol-related cirrhosis**
3 **CLEAN VERSION**
4

5 Laure Elkrief^{1,2}, Nathalie Ganne-Carrié^{3,4,5}, Hana Manceau^{1,6}, Marion Tanguy¹, Shantha Ram
6 Valainathan^{1,7}, Alix Riescher-Tuczkiwicz¹, Louise Biquard¹, Nathalie Barget⁸ Cendrine
7 Chaffaut⁹, Alexandre Louvet¹⁰, Valérie Paradis^{1,11}, Marianne Ziol^{4,12}, Rikke Bæk¹³, Malene
8 Møller Jørgensen^{13,14}, Guillaume Van Niel¹⁵, Pierre-Michael Coly¹⁵, Adel Hammoutène¹,
9 Fanny Dujardin¹⁶, Katell Peoc'h⁵, Thierry Poynard^{17,18}, Sylvie Chevret⁹ and Pierre-Emmanuel
10 Rautou^{1,7}, CIRRAL group

- 11
12
13 1 Université Paris-Cité, Inserm, Centre de recherche sur l'inflammation, UMR 1149, Paris,
14 France
15 2 Service d'hépatogastroentérologie, Hôpital Trousseau, CHRU de Tours, France
16 3 AP-HP, Service d'Hépatologie, Hôpital Avicenne, Bobigny, France
17 4 Sorbonne Paris Nord, UFR SMBH, Bobigny, France
18 5 INSERM UMR 1138, centre des cordeliers, Université Paris-Cité, Paris, France
19 6 AP-HP, Hôpital Beaujon, service de Biochimie et biologie moléculaire, Paris, France
20 7 AP-HP, Service d'Hépatologie, Hôpital Beaujon, DMU DIGEST, Centre de Référence des
21 Maladies Vasculaires du Foie, FILFOIE, ERN RARE-LIVER, Clichy, France
22 8 APHP, Centre de ressources biologiques (BB0033-00027) des Hôpitaux Universitaires
23 Paris-Seine-Saint-Denis, Bobigny, France
24 9. Service de Biostatistique et Information médicale, Hôpital Saint-Louis, AP-HP and Inserm,
25 UMR-1153, ECSTRRA Team, Paris, France
26 10 Service d'Hépatogastroentérologie, Hôpital Huriez, CHRU de Lille, France
27 11 AP-HP, Hôpital Beaujon, Service d'anatomopathologie, DMU DIGEST, Clichy, France
28 12 AP-HP, Service d'anatomopathologie, Hôpital Avicenne, Bobigny, France
29 13 Department of Clinical Immunology, Aalborg University Hospital, Aalborg, Denmark
30 14 Department of Clinical Medicine, Aalborg University, Aalborg, Denmark
31 15 Institute for Psychiatry and Neurosciences of Paris, Hopital Saint-Anne, Université de
32 Paris, Institut national de la santé et de la recherche médicale, U1266, Paris, France
33 16 Service d'anatomopathologie, Hôpital Trousseau, CHRU de Tours, France
34 17 Biopredictive, Paris, France
35 18. Sorbonne University, Paris France
36
37

1 **Conflict of interest**

2 TP is the inventor of FibroTest.

3 P-E.R. has received research funding from Terrafirma and acted as consultant for Hemostod,
4 Mursla, Genfit, Boehringer Ingelheim and Abbelight, and received speaker fees from Tillots
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15 **Authors' contribution**

16 Conception and design of the study: LE, PER, NG, SC

17 Generation, collection, assembly, analysis and/or interpretation of data: LE, NG, HM,
18 MT, AR, LB, NB, CC, AL, VP, MZ, RB, MM, FD, KP, AH, PMC, GVN

19 Drafting or revision of the manuscript: LE, PER, NG, MT, TP, SC

20 Approval of the final version of the manuscript: NG, HM, MT, AR, LB, NB, CC, AL,
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1 Contact information

2 Prof. Pierre-Emmanuel Rautou
3 Service d'Hépatologie, Hôpital Beaujon
4 100 boulevard du General Leclerc,
5 92100 Clichy, France
6 Tel: +331 40 87 50 91, Fax +331 40 87 44 35
7 pierre-emmanuel.rautou@inserm.fr

8

9 List of abbreviations

10 IEVs, large extracellular vesicles; MELD, Model for End Stage Liver Disease; HVPG, Hepatic
11 venous pressure gradient; EVs, Extracellular vesicles; HR, hazards ratio; CI, confidence
12 interval ; ROC, receiver operating characteristic; AUC, area under the curve; PCLS, precision
13 cut liver slices;

14

15 Data Availability Statement

16 Participating centres provided pseudonymised patient datasets for this study. They are
17 available from the corresponding author upon request.

18

1 ABSTRACT

2 In patients with compensated alcohol-related cirrhosis, reliable prognostic biomarkers are
3 lacking. Keratin-18 and hepatocyte-derived large extracellular vesicles (IEVs) concentrations
4 reflect disease activity, but their ability to predict liver-related events is unknown.

5 **Methods:** We measured plasma keratin-18 and hepatocyte IEVs concentrations in 500
6 patients with Child-Pugh class A alcohol-related cirrhosis. Ability of these hepatocyte-derived
7 biomarkers, alone or combined with MELD and FibroTest, to predict liver-related events at 2
8 years was analyzed, taking into account the alcohol consumption at inclusion and during the
9 follow-up.

10 **Results:** Keratin-18 and hepatocyte IEVs concentrations increased with alcohol
11 consumption. In patients without active alcohol consumption at enrollment (n=419), keratin-
12 18 concentration predicted liver-related events at 2 years, independently of FibroTest and
13 MELD. Patients with both keratin-18 concentration >285 U/L and FibroTest >0.74 had a 24%
14 cumulative incidence of liver-related events at 2 years, versus 5% to 14% in other groups of
15 patients. Similar results were obtained when combining keratin-18 concentration >285 U/L
16 with MELD >10. In patients with active alcohol consumption at enrollment (n=81), hepatocyte
17 IEVs predicted liver-related events at 2 years, independently of FibroTest and MELD.
18 Patients with both hepatocyte IEVs concentration >50 U/L and FibroTest>0.74 had a 62%
19 cumulative incidence of liver-related events at 2 years, versus 8% to 13% in other groups of
20 patients. Combining hepatocyte IEVs concentration >50 U/L with MELD >10 had a lower
21 discrimination ability. Similar results were obtained using as endpoint decompensation of
22 cirrhosis defined according Baveno VII criteria.

23 **Conclusion:** In patients with Child-Pugh class A alcohol-related cirrhosis, combining
24 hepatocyte-derived biomarkers with FibroTest or MELD score identifies patients at high-risk
25 of liver-related events, and could be used for risk stratification and patient selection in clinical
26 trials.

27

28 **Keywords**

1 liver cirrhosis, alcoholic liver disease, biomarkers, prognosis, decompensation of cirrhosis,

2 FibroTest

3

1 **IMPACT AND IMPLICATIONS**

- 2 • In patients with compensated alcohol-related cirrhosis, reliable predictors of outcome are
3 lacking.
- 4 • In patients with Child-Pugh class A alcohol-related cirrhosis, combining hepatocyte-
5 derived biomarkers (keratin-18 and hepatocyte-large extracellular vesicles) with
6 FibroTest or MELD score identifies those at high-risk of liver-related events at 2 years
- 7 • The identified patients at high-risk of liver-related events are the target-of-choice
8 population for intensive surveillance (*e.g.*, referral to tertiary care centers; intensive
9 control of risk factors) and inclusion in clinical trials.

10

11

12

1 **Introduction**

2 Excessive alcohol consumption is one of the most common causes of cirrhosis worldwide
3 (1,2). In patients with alcohol-related cirrhosis, decompensation of cirrhosis is a turning point
4 in terms of risk of mortality (3), quality of life (4) and costs for the healthcare systems (5).
5 Patients at high-risk of decompensation are the target-of-choice population for intensive
6 surveillance (*e.g.*, referral to tertiary care centers; intensive control of risk factors) and
7 inclusion in clinical trials. Hepatic venous pressure gradient (HVPG) predicts
8 decompensation of cirrhosis, since patients with HVPG ≥ 10 mm Hg have a 2-year risk of
9 decompensation of 25%, *vs.* $< 10\%$ in patients below this threshold (6,7). However,
10 invasiveness – although minimal – and limited availability of HVPG measurement outside
11 expert centers hinder the use of this procedure in routine clinical practice (8). Non-invasive
12 tools have been proposed to substitute for HVPG in its prediction of clinical outcomes,
13 including transient elastography; unfortunately, these tools lack availability for first-line risk
14 assessment of disease severity. Therefore, blood noninvasive biomarkers able to accurately
15 predict decompensation in patients with alcohol-related cirrhosis are needed.

16 In patients with alcohol-related cirrhosis, various prognostic indicators have been identified
17 reflecting (i) disease activity, (ii) liver function, (iii) amount of fibrosis, and (iv) alcohol
18 consumption (9–11). Disease activity can be estimated on liver biopsy, and refers to the
19 presence of hepatocyte injury and inflammation (9). Histological features of disease activity
20 have been associated with a poor outcome in patients with alcohol-related liver disease (12).
21 Accordingly, plasma keratin-18 fragments concentrations, reflecting hepatocyte death, have
22 been associated with mortality in patients with alcohol-related liver disease (13,14).
23 Extracellular vesicles (EVs) are vesicles released in the extracellular space by all cell types
24 upon exposure to stress. EVs can be found in all biological fluids including blood.
25 Composition of EVs reflects the type of stress their mother cell was exposed to. Therefore,
26 EVs content holds great potential for predicting disease trajectory (15). In patients with
27 alcohol-related cirrhosis, concentrations of hepatocyte-derived large EVs (IEVs) are
28 significantly higher in patients with alcoholic hepatitis than in those without (16,17); in

1 addition, hepatocyte IEVs have been associated with short-term mortality in a cohort of
2 patients with compensated and decompensated cirrhosis, independently of MELD score (18).
3 However, the usefulness of keratin-18 and hepatocyte IEVs concentrations to predict liver-
4 related events at 2 years in patients with Child-Pugh class A alcohol-related cirrhosis has
5 never been evaluated.

6 The Model for End-Stage Liver Disease (MELD), reflecting liver dysfunction, is currently
7 widely used to allocate organs to patients with severe cirrhosis (19,20). In patients with
8 compensated cirrhosis, MELD ≥ 10 has been associated with an increased risk of cirrhosis
9 decompensation (11). However, prognostic value of MELD is limited in patients with
10 compensated cirrhosis, since MELD ≥ 10 has only 61% sensitivity and 17% positive
11 predictive value for the prediction of 2 year-decompensation or death (3). The amount of liver
12 fibrosis, measured on liver biopsies using collagen proportionate area, has been related to
13 HVG and decompensation or liver-related death in patients with cirrhosis (21,22).
14 FibroTest, estimating the amount of fibrosis, has been related to liver-related events and
15 mortality in large cohorts of patients with alcohol-related liver disease of various fibrosis
16 stage, mixing together patients without and with cirrhosis (23). Interest of FibroTest to predict
17 cirrhosis decompensation among patients with Child-Pugh class A alcohol-related cirrhosis
18 has not been evaluated. Finally, even at the stage of cirrhosis, the course of the disease is
19 largely influenced by alcohol consumption (9,24), abstinent patients having a much lower risk
20 of decompensation than patients with active alcohol consumption (24,25). Alcohol
21 consumption status was usually not taken into account in the studies assessing the ability of
22 biomarkers to predict decompensation in patients with alcohol-related cirrhosis.

23 Therefore, the aim of the present study was to evaluate the ability of hepatocyte-derived
24 biomarkers, alone or combined with MELD and FibroTest, to predict liver-related events in
25 patients with Child-Pugh class A alcohol-related cirrhosis, taking into account the alcohol
26 consumption status.

27

1 **PATIENTS AND METHODS**

2 We used STROBE (26) as a reporting guideline to ensure a clear and complete report of the
3 study design, conduct and findings.

4 5 **Design**

6 This study was an ancillary study of the institutional CIRRAL multicenter cohort study (27),
7 promoted by Assistance Publique - Hôpitaux de Paris. This study was approved by the local
8 institutional review board (Comité de Protection des Personnes, Aulnay-sous-Bois, France).
9 Written informed consent was obtained from each patient before enrollment.

10

11 **Patients**

12 Patients with Child-Pugh class A alcohol-related cirrhosis were included from the French
13 multicentric prospective CIRRAL cohort between October, 2010 and April, 2016 (27). The
14 primary endpoint of CIRRAL study was to assess the incidence of hepatocellular carcinoma
15 in patients with alcohol-related cirrhosis. All patients had histologically proven cirrhosis –
16 whatever the duration between liver biopsy and inclusion in CIRRAL and the indication for
17 liver biopsy- with a history of excessive alcohol consumption according to the 2003 World
18 Health Organization criteria (more than 21 units per week for female and more than 28 units
19 per week for male) (28) that occurred for at least ten years and was considered the main
20 cause of chronic liver disease. Patients did not have associated hepatitis B virus (HBV) or
21 hepatitis C virus (HCV) infection. All patients had Child-Pugh class A cirrhosis at enrollment,
22 but some had a previous history of decompensated cirrhosis before enrollment. Patients with
23 either a history of primary liver cancer or detectable liver nodule at enrollment were excluded.
24 From this cohort, we specifically selected patients fulfilling the following criteria: (i) available
25 plasma samples for Keratin-18, hepatocyte IEVs and FibroTest™ measurements; (ii) Child-
26 Pugh class A at enrollment, and (iii) no history of transjugular intrahepatic portosystemic
27 shunt at enrollment (Figure 1). Active alcohol consumption at enrollment was defined as
28 either hazardous or harmful of alcohol use, *i.e.* a consumption of 7 units per week or more at

1 enrollment (29). Metabolic syndrome was defined as the presence of at least 3 of the
2 following features: abdominal obesity with increased waist circumference, serum lipids
3 triglycerides ≥ 150 mg/dL (1.7 mmol/L), serum HDL-Cholesterol < 40 mg/dL (1.03 mmol/L) in
4 men or < 50 mg/dL (1.29 mmol/L) in women, blood pressure ≥ 130 mm Hg systolic or ≥ 85 mm
5 Hg diastolic, fasting serum glucose ≥ 100 mg/dL (5.6 mmol/L) (30). MELD was calculated
6 according to UNOS (31). Date of entry was the date of enrollment in the CIRRAL study.
7 Patients were then followed until the reference date of January 11th 2017. Besides the usual
8 clinical and laboratory data, self-reported alcohol consumption was recorded at each
9 scheduled visit as the average number of units per week since the last visit.

10

11 **Study endpoints**

12 All the following outcomes were considered from the date of enrollment in the CIRRAL study,
13 and were prospectively collected as part of the case report form. All extrahepatic events
14 occurring during follow-up were also recorded. Likely cause(s) of death were established.

15 The primary endpoint of the present study was liver-related events at 2 years, defined as a
16 composite endpoint including at least one liver-related event among clinical ascites, overt
17 hepatic encephalopathy, variceal bleeding, spontaneous bacterial peritonitis, acute kidney
18 injury, increase in Child-Pugh score ≥ 2 points, liver transplantation in the absence of
19 hepatocellular carcinoma and/or liver-related deaths in the absence of hepatocellular
20 carcinoma. With this endpoint, we aimed to measure the occurrence of liver deterioration.
21 This composite endpoint was derived from previous studies (6,32).

22 Secondary endpoints included (i) liver-related death and liver transplantation in the absence
23 of hepatocellular carcinoma within 2 years, (ii) bacterial infection (including spontaneous
24 bacterial peritonitis, bacteriemia, pneumonia, urinary tract infection and skin infection), within
25 2 years; and (iii) hepatocellular carcinoma, within 2 years. Since Baveno VII guidelines
26 recently refined definition of cirrhosis decompensation (33), we secondarily added cirrhosis
27 decompensation as a post-hoc composite endpoint, defined as time to occurrence of ascites,
28 overt hepatic encephalopathy, or variceal bleeding, whichever occurred first.

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Plasma preparation, and Culture and treatment of HepG2 cells and of Precision cut liver slices (PCLS)

Detailed methods are presented in Supplementary methods.

Measurement of hepatocyte-derived biomarkers concentrations and of FibroTest

We determined concentrations of hepatocyte-derived IEVs in plasma samples and in culture medium of HepG2 and of PCLS from human samples using a technique previously described (16,18,34). EV characterization was performed according MISEV guidelines (35). General characterization was performed using CD9, HSC70 and albumin western blots. Single EV characterization was performed using transmission electronic microscopy and super resolution microscopy (35) (Supplementary Figure 1). Finally, quantitative assessment of EVs was performed using tunable resistive pulse sensing.

FibroTest (Biopredictive, Paris, France) was determined as previously published (36,37). Details are provided in Supplementary methods and Supplementary Figure 1.

Statistical analysis

Results are presented by summary statistics, namely median (interquartile range) or absolute frequency (percentage) unless specified otherwise, with comparisons between baseline groups based on the Wilcoxon rank-sum test, or the exact Fisher exact, respectively.

Correlations between laboratory values and hepatocyte-derived biomarkers were assessed using nonparametric Spearman correlation coefficient.

Cumulative incidence of liver-related events was estimated using the nonparametric cumulative incidence function, considering hepatocellular carcinoma and liver-unrelated deaths as competing events; in patients without active alcohol consumption, alcohol relapse was considered as an additional competing event. Cumulative incidence of liver-related death was estimated using the cumulative incidence function, considering hepatocellular carcinoma and liver-unrelated deaths as competing events and liver transplantation as a

1 liver-related death. Cumulative incidence of hepatocellular carcinoma and bacterial infections
2 were estimated using the cumulative incidence function, considering death and liver
3 transplantation as competing events. Cumulative incidence of cirrhosis decompensation was
4 estimated using the cumulative incidence function, considering hepatocellular carcinoma,
5 death and liver transplantation as competing events. Since alcohol consumption is a main
6 driver for a poor outcome in patients with alcohol-related cirrhosis (24,25), all analyses were
7 stratified on alcohol consumption, distinguishing patients without (0 to 6 units per week) from
8 those with an active (7 units per week or more) alcohol consumption at enrollment,
9 separately.

10 To assess the ability of hepatocyte-derived biomarkers, including keratin-18 and hepatocyte
11 IEV concentrations, to predict outcome at 2 years, cause-specific Cox models were fitted,
12 with effects quantified by hazards ratio (HR) with 95% confidence interval (95% CI).
13 Proportional hazards were assessed using the Grambsch and Therneau test (38).
14 Univariable models were fitted, then multivariable models were performed with the Cox
15 proportional hazards model, including hepatocyte-derived biomarkers, MELD and FibroTest.
16 To assess the discriminatory performance of biomarkers to predict liver-related events,
17 taking into account competing events, time-dependent receiver operating characteristic
18 (ROC) curves using the cumulative sensitivity and dynamic specificity definitions measured
19 at time $t=24$ months, and resulting area under the curve (AUC), were used (39). This allowed
20 to select the best cut-off value of keratin-18 and hepatocyte IEV concentrations according the
21 Youden index. The previously reported cutoff of 65 U/L for hepatocyte IEVs was also
22 considered (18). For MELD and FibroTest, 10 and 0.74 were chosen as cutoff values,
23 respectively, based on Youden index (Supplementary Table 1), but also because these
24 thresholds have been previously reported as predicting patients' outcome (11,40–42). Given
25 the low prevalence of missing data, simple imputation of missing data was performed, based
26 on the mean of continuous variables and the mode of discrete or qualitative variables. Last,
27 the net reclassification improvement (NRI) was used as a performance measure in
28 reclassifying patients in terms of liver-related events, when FibroTest or MELD together with

1 hepatocyte derived biomarkers are compared to MELD or FibroTest alone. More details on
2 statistical analyses are in the Supplementary methods. All tests were two-sided with p-values
3 <0.05 considered significant. Analyses were performed using open-source R software
4 version 4.0.3 (<http://www.R-project.org/>), and LATEX. More details are provided in
5 Supplementary methods.

6

1 **RESULTS**

2 **Patients**

3 Between October 7 2010 and April 30 2016, 650 patients were included in the CIRRAL
4 cohort in 22 centers. Among them, 500 fulfilled the inclusion criteria, including 419 without
5 and 81 patients with active alcohol consumption at enrollment (Figure 1). The main
6 characteristics of the patients as well as plasma biomarkers concentrations are summarized
7 in Table 1. Among the 343 patients completely abstinent from alcohol at enrollment, median
8 duration of alcohol abstinence was 24 (9-69) months. Among the 320 patients with a history
9 of decompensation, median duration between last decompensation and inclusion into the
10 study was 32 (14-68) months and 40 (IQR 17-130) months in patients without and with active
11 alcohol consumption at inclusion, respectively. Only 163 out of 500 patients had reliable liver
12 stiffness measurement performed within one year before or after inclusion.

13 **Hepatocyte biomarker concentrations according to clinical and laboratory features**

14 Keratin-18 concentrations were significantly higher in patients with active alcohol
15 consumption than in those without, and also higher in patients who declared a consumption
16 of 1-6 glasses than in fully abstinent patients (Table 1 and Figure 2A). Clinical and laboratory
17 features associated with keratin-18 concentrations are shown in Supplementary Tables 2
18 and 3. Out of metabolic syndrome features, only diabetes was associated with keratin-18
19 concentrations (Supplementary Table 2 and 3, and Supplementary Figure 2).

20 Hepatocyte IEVs concentrations were significantly higher in patients with active alcohol
21 consumption than in those without (Table 1 and Figure 2B). Clinical and laboratory features
22 associated with IEVs concentrations are shown in Supplementary Tables 4 and 5. In patients
23 with active alcohol consumption, hepatocyte IEVs concentrations were lower in patients with
24 features of the metabolic syndrome than in those without (Supplementary Table 4). Plasma
25 total IEV concentrations were also measured using TRPS in a subgroup of 23 patients of the
26 CIRRAL cohort and found higher in patients with active alcohol consumption than in those
27 without (Figure 2C and 2D).

1 The fraction of Keratin-18 unbound to IEVs also increased with alcohol consumption (data
2 not shown).

3

4 **Ability of hepatocyte biomarkers concentrations to predict liver-related events at 2** 5 **years**

6 Median follow-up was 48 months (IQR, 34 to 71) and 52 months (IQR, 34 to 74) in patients
7 without and with active alcohol consumption at enrollment, respectively. Events occurring
8 during the 2 years follow-up period after enrollment are detailed in Figure 1 and in
9 Supplementary Table 6. A total of 120 patients experienced a total of 222 liver-related events
10 that accounted for the primary endpoint at 24 months, including 95 patients without any
11 active alcohol consumption at baseline who developed a total of 182 events and 25 patients
12 with active alcohol consumption who developed 40 events. Cumulative incidence (95% CI) of
13 liver-related events at 2 years was 11.7% (8.8-15.1) and 19.9% (11.8-29.7) in patients
14 without and with active alcohol consumption, respectively. Patients' characteristics as well as
15 plasma biomarkers concentrations at enrollment, associated with liver-related events, are
16 shown in Table 2. Of note, history of previous decompensation was not associated with liver-
17 related events at 2 years, neither in patients without nor in those with active alcohol
18 consumption at inclusion (Table 2).

19

20 *Keratin-18 concentrations*

21 Keratin-18 concentrations were highly predictive of liver-related events in patients without
22 active alcohol consumption, but not in those with active alcohol consumption (Table 2 and
23 Figure 3). In patients without alcohol consumption, time-dependent AUC analysis found that
24 a cut-off value of keratin-18 concentration >285 U/L yielded the most accurate sensitivity and
25 specificity (50% and 75%, respectively) to predict liver-related events at 2 years
26 (Supplementary Table 1 and 7). Sensitivity and specificity of MELD ≥ 10 were 43% and 77%,
27 respectively. Sensitivity and specificity of FibroTest >0.74 were 72% and 57%, respectively
28 (Supplementary Table 7). The NRI (95% CI) of adding keratin-18 concentration >285 U/L to

1 FibroTest or MELD in patients with a liver-related event at 24 months was estimated at 0.42
2 (0.00; 0.57) over FibroTest alone and 0.41 (-0.09; 0.60) over MELD alone.

3 In patients without active alcohol consumption, incidence of liver-related events at 2 years
4 was 20.1% (13.3-28.0) in patients with keratin-18 concentrations >285 U/L versus 8.3% (5.4-
5 11.9) in patients having keratin-18 concentrations below this threshold (Supplementary
6 Figure 3A). After adjustment on MELD and FibroTest, total keratin-18 concentrations >285
7 U/L still predicted liver-related events at 2 years (Figure 3 and Supplementary Table 8). As
8 shown in Figure 4A and 4B, patients with both high keratin-18 and either high FibroTest or
9 high MELD had a higher risk of liver-related events at 2 years (24.2%, 95%CI, 15.1 to 34.5
10 and 28.7%, 95%CI, 16.6 to 41.9, respectively) than other groups of patients. Figure 5A and
11 5B display the predicted probability of liver-related event at 2 years according to keratin-18
12 concentrations and FibroTest or MELD in patients without active alcohol consumption. Total
13 keratin-18 concentrations >285 U/L still predicted liver-related events at 2 years after
14 adjustment on MELD, FibroTest and alcohol consumption (HR 1.72 (1.11-2.68, p=0.012)
15 (Supplementary Table 9). When restricting the analysis to the 343 patients with null alcohol
16 consumption at enrollment, keratin-18 concentrations >285 U/L still predicted liver-related
17 events at 2 years (HR 1.70 (95% CI 1.04-2.77), p=0.03), after adjustment on MELD and
18 FibroTest (data not shown).

19 During the 2 years follow-up, 63 (15%) cirrhosis decompensations occurred in patients
20 without active alcohol consumption, according to recent Baveno VII criteria. Patients'
21 characteristics at enrollment associated with cirrhosis decompensation are shown in
22 Supplementary Table 10. By multivariate analysis, MELD and keratin-18 concentrations were
23 independent predictors of cirrhosis decompensation at 2 years (Fig 3 and Supplementary
24 Table 11).

25

26 *Hepatocyte IEV concentrations*

27 Hepatocyte IEVs concentrations were highly predictive of liver-related events in patients with
28 active alcohol consumption, but not in patients without. In patients with active alcohol

1 consumption, time-dependent AUC analysis found that a cut-off value of hepatocyte IEVs
2 >50 U/L yielded the most accurate sensitivity and specificity (72% and 73%, respectively) to
3 predict liver-related events at 2 years (Supplementary Table 1 and 7). Sensitivity and
4 specificity of MELD ≥ 10 were 33% and 76%, respectively. Sensitivity and specificity of
5 FibroTest >0.74 were 66% and 65%, respectively (Supplementary Table 7).

6 In patients with active alcohol consumption, incidence of liver-related events at 2 years was
7 39.2% (95% CI 21.2-56.8) in patients with hepatocyte IEV concentrations >50 U/L versus
8 8.5% (95% CI 2.7-18.7) in patients having hepatocyte IEVs concentrations below this
9 threshold (Supplementary Figure 3B). After adjustment on MELD and FibroTest, hepatocyte
10 EV >50 U/L still predicted liver-related events at 2 years (Figure 3 and Supplementary Table
11 8). We did similar analyses using hepatocyte IEVs >65 U/L threshold. After adjustment on
12 MELD and FibroTest, hepatocyte IEV >65 U/L still predicted liver-related events, but the
13 predictive accuracy was less obvious (Supplementary Table 8). As shown in Figure 4C and
14 4D, patients with both high hepatocyte IEVs concentrations and either high FibroTest or high
15 MELD had a higher risk of liver-related events at 2 years (62.4% (33.0-81.8) and 56.2%
16 (11.6-85.8), respectively) than other groups of patients. Figure 5C and 5D display the
17 predicted probability of liver-related event at 2 years hepatocyte IEVs concentrations and
18 FibroTest or MELD in patients with active alcohol consumption. Hepatocyte IEVs
19 concentrations >50 U/L still predicted liver-related events at 2 years after adjustment on
20 MELD, FibroTest and alcohol consumption (HR 4.42 (1.80-10.88), $p = 0.0012$)
21 (Supplementary Table 9). The NRI (95% CI) of adding hepatocyte IEVs >50 U/L to FibroTest
22 or MELD in patients with liver-related event at 24 months was 0.62 (0.00; 0.88) over
23 FibroTest alone, and 0.30 (0.00; 0.78) over MELD alone.

24 During the 2 years follow-up, 14 (17%) cirrhosis decompensations occurred in patients with
25 active alcohol consumption, according to recent Baveno VII criteria. Patients' characteristics
26 at enrollment associated with cirrhosis decompensation are shown in Supplementary Table
27 10. By multivariate analysis, only hepatocyte IEVs concentrations tended to be independent
28 predictors of cirrhosis decompensation at 2 years (Figure 3, Supplementary Table 11).

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Ability of hepatocyte biomarkers to predict other events at 2 years after enrollment

Death or liver transplantation

During the 2 years follow-up, 51 (12%) and 11 (14%) liver-related death or liver transplantation without hepatocellular carcinoma occurred in patients without and with active alcohol consumption, respectively. Patients' characteristics at enrollment associated with liver-related death or liver transplantation are shown in Supplementary Table 12. Independent predictors of death or liver transplantation at 2 years were MELD and FibroTest – but not keratin-18 concentrations – in patients without active alcohol consumption, and MELD and hepatocyte IEVs concentrations in patients with active alcohol consumption (Figure 3 and Supplementary Table 13).

Bacterial infections

Thirteen (3%) and 7 (9%) bacterial infections occurred in patients without and with active alcohol consumption, respectively. Patients' characteristics at enrollment associated with bacterial infection, considering death or liver transplantation as competing events, are shown in Supplementary Table 14. In patients without active alcohol consumption, no independent predictor of bacterial infection was identified. In patients with active alcohol consumption, only hepatocyte IEVs concentrations independently predicted bacterial infection within 2 years (Figure 3 and Supplementary Table 15).

Hepatocellular carcinoma

Thirty-nine (9%) and 10 (12%) patients developed hepatocellular carcinoma in patients without and in those with active alcohol consumption, respectively. Patients' characteristics at enrollment associated with hepatocellular carcinoma, considering death or liver transplantation as competing events, are shown in Supplementary Table 16. In patients without or with active alcohol consumption, FibroTest was the only independent predictor of hepatocellular carcinoma (Figure 3 and Supplementary Table 17). Hepatocyte biomarkers were not independent predictors of hepatocellular carcinoma.

1 **Effect of alcohol on keratin-18 and hepatocyte IEVs release by cultured hepatocytes**

2 To get insight into the differences observed in the ability of keratin-18 and hepatocyte IEVs to
3 predict outcome, we incubated a hepatocyte cell line, HepG2, with DMEM with or without
4 various concentrations of ethanol. After 24 hours, exposure to alcohol resulted in increased
5 concentration of hepatocyte IEVs, but not of the fraction of keratin-18 unbound to IEVs
6 (Figure 6A). Similar results were observed when PCLS from human livers were treated with
7 increasing concentrations of ethanol (Figure 6B). EVs release increased over time
8 (Supplementary Figure 4).

9

10 **DISCUSSION**

11 Patients with compensated alcohol-related cirrhosis are the target-of choice population for an
12 intensive surveillance, intensive management of alcohol use disorders, or new drugs aiming
13 at preventing liver-related events. Previous studies evaluating the interest of hepatocyte
14 derived biomarkers in patients with cirrhosis focused on the estimation of portal hypertension
15 (18,34), on the diagnosis of alcoholic hepatitis (14,16), or on short-term mortality in patients
16 with all-stage severity of cirrhosis (13,14,18). The present study demonstrates, in a large
17 prospective cohort of patients with Child-Pugh class A alcohol-related cirrhosis, that
18 combining hepatocyte-derived biomarkers to usual biomarkers, namely MELD or FibroTest,
19 allows estimating the individual risk of liver-related events at 2 years (Figure 5). Importantly,
20 these results took into account alcohol consumption at inclusion, *i.e.* separating patients
21 without and with active alcohol consumption at inclusion, but also during follow-up. Indeed, in
22 patients without active alcohol consumption at inclusion, alcohol relapse was treated as a
23 competing event. Although alcohol withdrawal is a cornerstone of the management of
24 patients with alcohol-related cirrhosis, with a high impact on long-term prognosis (25,43),
25 most studies evaluating biomarkers of diagnosis and prognosis in this setting did not take
26 into account the alcohol consumption status nor its changes over time (23,44).

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1 The first major finding of the present study is that circulating concentrations of the hepatocyte
2 biomarkers keratin-18 and hepatocyte-IEVs improve ability of FibroTest and of MELD to
3 predict the risk of liver-related events at 2-year and allow the identification of patients at high
4 risk. Indeed, in patients without active alcohol consumption, those with both keratin-18
5 concentration >285 U/L and MELD ≥ 10 had a 28.7% cumulative incidence of liver-related
6 events at 2 years vs. 5 to 14% in patients without this combination. In patients with active
7 alcohol consumption, those with both total hepatocyte-EVs concentration >50 U/L and
8 FibroTest >0.74 had a 62% cumulative incidence of liver-related events at 2 years, vs. 8 to
9 13% in patients without this combination. This synergy between hepatocyte biomarkers and
10 FibroTest or MELD is likely accounted for by the different pathophysiological processes they
11 reflect, namely liver cell stress or injury, liver fibrosis and liver function, respectively. A
12 limitation of the current study is the lack of a validation cohort to confirm our results.
13 However, the large number of patients prospectively included and followed-up in multiple
14 centers strengthens our results. Even though no study assessed so far the ability of
15 hepatocyte-derived biomarkers to predict liver-related events in patients with Child-Pugh
16 class A cirrhosis, our results are in line with previous studies. Keratin-18 fragments
17 concentrations predicted mortality in a cohort of 175 patients (39% with ascites) with alcohol-
18 related cirrhosis followed for more than 2 years (13), as well as 90-day mortality in patients
19 with alcoholic hepatitis in the STOPAH trial (14). Hepatocyte-derived IEVs concentrations
20 also predicted 6-month mortality independently of Child-Pugh and MELD scores in a
21 previous study including patients with cirrhosis related to various causes (49% related
22 hepatitis C virus infection), with different severity stages (61% Child Pugh B or C) (18). Of
23 note, although keratin 18 concentration has been commonly used as diagnostic or prognostic
24 biomarkers of acute or chronic liver diseases, it is not specific for liver damage, as keratin 18
25 can also be released in case of injury or inflammation of non-hepatic epithelial tissues,
26 including gastrointestinal, lung, or kidney epithelia (45). In this regard, serum keratin 18
27 concentrations can be elevated in various malignancies including lung, colorectal,
28 esophageal, gastric, head and neck cancers (46–50). Clinician should thus be aware of the

1 risk of false positive of keratin 18 concentrations in these settings. Similar data on the
2 specificity of keratin-18 carried by IEVs are lacking so far and will need to be explored.

3 In the present study, the primary composite endpoint, namely the occurrence of at least one
4 liver-related event at 2 years, had many components, as the liver events denoting liver
5 deterioration are numerous. Even if there could be some difference in individual effects
6 across each of those components, we do think that the relevance of such a grouping is
7 meaningful for both the patients (that is, in terms of morbidity) and the physicians (that is, in
8 terms of clinical decisions) points of view. Furthermore, similar results were observed when
9 addressing the ability of keratin-18 and hepatocyte IEVs concentration to predict
10 decompensation of cirrhosis, according to the Baveno VII consensus conference. Moreover,
11 hepatocyte IEVs concentrations not only predicted liver-related events, but also bacterial
12 infection and 2-year risk of death or liver transplantation.

13 Liver stiffness measurement ≥ 20 or 25 kPa using transient or shearwave elastography is
14 increasingly used to predict decompensation and death in patients with compensated
15 cirrhosis(11,51). Unfortunately, liver stiffness measurement was available at inclusion only in
16 a minority of the patients in the CIRRAL cohort (52). We could thus not compare the
17 accuracy of hepatocyte-derived biomarkers to that of liver stiffness measurement, nor
18 combine those hepatocyte-derived biomarkers with liver stiffness measurement to try to
19 improve prediction of patients' outcome. Dedicated studies addressing this question are
20 needed and should include a medico-economic evaluation. Although measurement of
21 hepatocyte-derived biomarkers concentration relies on commercially available and affordable
22 immune-assay based tests, assessing the economic impact of including these tests would be
23 useful for healthy authorities.

24

25 The second major result of the present study is that the choice of the hepatocyte biomarker
26 to be used should take into account alcohol consumption, a finding allowed by the
27 prospective design of the CIRRAL trial including careful alcohol consumption analysis. The

1 different prediction ability of Keratin-18 and hepatocyte-IEVs according to alcohol
2 consumption status suggests that these hepatocyte biomarkers reflect different
3 pathophysiological processes driving patients' outcome. Several lines of evidence suggest
4 that hepatocyte IEVs capture individual susceptibility of hepatocytes to alcohol by reflecting
5 hepatocyte apoptosis in patients with active alcohol consumption. Indeed, alcohol induces
6 hepatocytes apoptosis following caspase-dependent and caspase-independent pathways
7 (53,54). Apoptosis is a well-known stimulus for IEVs release and our results (Figures 2 and
8 6) – as well as those from several studies- showed that alcohol stimulates the release of
9 extracellular vesicles from hepatocytes *in vitro* and *in vivo* (55,56). Furthermore, circulating
10 concentrations of hepatocyte-IEVs were associated with apoptotic hepatocytes on liver
11 biopsies of patients with cirrhosis of various causes (18). In the CIRRAL cohort, although all
12 included patients had biopsy proven alcohol-related cirrhosis, liver biopsies were not
13 performed at enrollment in the majority of the patients, so that we were not able to evaluate
14 the association between hepatocyte IEV concentrations and histological lesions. Keratin-18
15 concentrations predicted 2-years liver-related events in patients without active alcohol
16 consumption better than hepatocyte-IEVs. Keratin-18 is a member of the intermediate
17 filament family of cytoskeletal proteins (57). The M65 fragment of keratin-18 is a marker of
18 overall cell death, including both apoptosis and other forms of cell death (45). These results
19 suggest that, in patients without active alcohol consumption, other types of cell stress or cell
20 death than apoptosis drive patients' outcome. Our observation that alcohol does not directly
21 induce the release by hepatocytes of Keratin-18 unbound to IEV *in vitro* (Figure 6) by HepG2
22 cells and *ex vivo* by PCLS from human samples suggests that the higher plasma
23 concentration of Keratin-18 unbound to IEVs observed in patients with active alcohol
24 consumption does not result from a direct action of alcohol on hepatocytes, but rather from
25 an indirect effect of alcohol on hepatocytes (*e.g.* via gut microbiota or via inflammation). Of
26 note, although both plasma keratin 18 and hepatocyte IEVs concentrations were higher in
27 patients with alcohol consumption exceeding 21 glasses/week than in abstinent patients or in
28 patients drinking less than 6 glasses per week, we did not find a clear dose-dependent

1 relationship between alcohol consumption and hepatocyte biomarkers concentrations.
2 Although similar results were observed *in vitro*, with a sensitivity to alcohol different between
3 HepG2 cells and PCLS from human samples (Figure 6), caution is needed in the
4 interpretation of these data. This absence of difference might be due to a lack of power, with
5 a lower number of patients in the groups drinking more than 7 glasses per week. Another
6 explanation might be that self-reported alcohol consumption was not a perfect reflection of
7 alcohol intake, as objective tools, such as phosphatidylethanol or ethylglucuronide, were not
8 available in the CIRRAL trial.

9
10 In the present study, neither keratin-18 nor hepatocyte IEVs concentrations predicted
11 hepatocellular carcinoma at 2 years. This result is in line with a previous study of 175
12 patients with alcohol-related cirrhosis, where keratin-18 concentrations did not predict
13 hepatocellular carcinoma-related death (13). Conversely, the present study further validates
14 accuracy of FibroTest in patients with compensated alcohol-related cirrhosis to predict
15 hepatocellular carcinoma development (40). Furthermore, this study shows that using
16 FibroTest or its derived tests (such as the LCR 1 – LCR 2 algorithm) could be useful for risk -
17 stratification and personalized strategies of hepatocellular carcinoma screening (59). In
18 addition, our study further validates that a MELD over 10 predicts decompensation of
19 cirrhosis in patients with compensated disease, while it is known that MELD above 15
20 predicts liver-related death in patients with already decompensated cirrhosis (11,60).
21 Interestingly, in our study, previous history of decompensation of cirrhosis was not
22 associated with liver-related events at 2 years. The long time lapse between diagnosis of
23 cirrhosis and inclusion in the present cohort might account for this result.

24
25 In conclusion, hepatocyte-derived biomarkers combined with FibroTest or MELD allow the
26 identification of a population of patient with Child-Pugh A cirrhosis at high risk of liver-related
27 events at 2 years. The usefulness of these hepatocyte-derived biomarkers to predict liver-

1 related events in patients with non-alcohol-related cirrhosis should be investigated in further
2 studies.
3

1 FIGURES' LEGEND

2 **Figure 1. Flow chart and number of events**

3

4 **Figure 2. Distribution of keratin-18 concentrations (Fig 2A) and hepatocyte large**
5 **extracellular vesicles (IEVs) concentrations (Fig 2B) according to alcohol**
6 **consumption at enrollment** (Wilcoxon rank-sum test). Red lines indicate median values
7 and orange lines indicate interquartile range. **Plasma total IEV concentrations measured**
8 **using tunable resistive pulse sensing (IEV/mL) (Fig 2C; Wilcoxon rank-sum test) and size**
9 **distribution (Fig 2D; analysis of variance for repeated measures), in 23 patients with Child-**
10 **Pugh class A alcohol-related cirrhosis selected among the included patients (11 abstinent, 12**
11 **with active alcohol consumption).**

12

13 **Figure 3. Forrest plots.** Multivariate cause-specific Cox regression analyses of the ability of
14 keratin-18 concentration to predict primary and secondary endpoints in patients without
15 active alcohol consumption (panel A), and of hepatocyte large extracellular vesicles (IEVs)
16 concentration to predict primary and secondary endpoints in patients with active alcohol
17 consumption (panel B), adjusted on MELD and FibroTest.

18 Filled circles indicate Hazard ratio (HR). Horizontal lines indicate 95% confidence intervals
19 (CI).

20

21 **Figure 4. Cumulative incidence of liver-related events within 2 years according to**
22 **hepatocyte biomarkers concentrations and FibroTest or MELD.** Cumulative incidence of
23 liver-related events within 2 years in patients without active alcohol consumption according to
24 keratin-18 concentrations and FibroTest are displayed in Fig 4A, and according to keratin-18
25 and MELD in Fig 4B. Cumulative incidence of liver-related events within 2 years in patients
26 with active alcohol consumption according to hepatocyte large extracellular vesicles (IEVs)
27 concentrations and FibroTest are displayed in Fig 4C, and according to hepatocyte large
28 extracellular vesicles (IEVs) concentrations and MELD in Fig 4D. Gray's test has been used.

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Figure 5. Stratification model for prediction of liver-related events at 2 years in patients with Child-Pugh class A alcohol-related cirrhosis.

The proposed stratification is based on either FibroTest ≤ 0.74 vs. >0.74 (Fig 5A and C) or MELD <10 vs. ≥ 10 (Fig 5 B and D) and either keratin-18 concentrations ≤ 285 U/L vs. >285 U/L in patients without active alcohol consumption (Fig 5A and B) or hepatocyte large extracellular vesicle concentrations ≤ 50 U/L vs. >50 U/L in patients with active alcohol consumption (Fig 5C and D). IEV, large extracellular vesicles
Abbreviations: MELD, model for end stage liver disease; IEVs, large extracellular vesicles

Figure 6. Release of Keratin-18 and hepatocyte large extracellular vesicles (IEVs) by HepG2 cells and in patient-derived precision cut live slices (PCLS).

Concentrations of the fraction of Keratin-18 unbound to large extracellular vesicles (IEVs) and of hepatocyte IEVs measured in the supernatant of HepG2 cells (A; n=8) and of PCLS isolated from patients (B; 3 slices from 3 independent human livers) exposed for 24 hrs either to cell culture medium (blue) or to alcohol (1, 10 and 100 mmol/L). Only significant differences are shown (Wilcoxon rank-sum test).

1 REFERENCES

- 2 1. Pimpin L, Cortez-Pinto H, Negro F, Corbould E, Lazarus JV, Webber L, *et al.* Burden
3 of liver disease in Europe: Epidemiology and analysis of risk factors to identify prevention
4 policies. *J Hepatol.* 2018;69:718–735.
- 5 2. Jepsen P, Younossi ZM. The global burden of cirrhosis: A review of disability-
6 adjusted life-years lost and unmet needs. *J Hepatol.* 2021;75 Suppl 1:S3–S13.
- 7 3. Trebicka J, Fernandez J, Papp M, Caraceni P, Laleman W, Gambino C, *et al.*
8 PREDICT identifies precipitating events associated with the clinical course of acutely
9 decompensated cirrhosis. *J Hepatol.* 2021;74:1097–1108.
- 10 4. Labenz C, Toenges G, Schattenberg JM, Nagel M, Huber Y, Marquardt JU, *et al.*
11 Health-related quality of life in patients with compensated and decompensated liver cirrhosis.
12 *Eur J Intern Med.* 2019;70:54–59.
- 13 5. Desai AP, Mohan P, Nokes B, Sheth D, Knapp S, Boustani M, *et al.* Increasing
14 Economic Burden in Hospitalized Patients With Cirrhosis: Analysis of a National Database.
15 *Clin Transl Gastroenterol.* 2019;10:e00062.
- 16 6. Villanueva C, Albillos A, Genescà J, Garcia-Pagan JC, Calleja JL, Aracil C, *et al.* β
17 blockers to prevent decompensation of cirrhosis in patients with clinically significant portal
18 hypertension (PREDESCI): a randomised, double-blind, placebo-controlled, multicentre trial.
19 *Lancet.* 2019;393:1597–1608.
- 20 7. Ripoll C, Groszmann R, Garcia-Tsao G, Grace N, Burroughs A, Planas R, *et al.*
21 Hepatic venous pressure gradient predicts clinical decompensation in patients with
22 compensated cirrhosis. *Gastroenterology.* 2007;133:481–488.
- 23 8. de Franchis R, Baveno VI Faculty. Expanding consensus in portal hypertension:
24 Report of the Baveno VI Consensus Workshop: Stratifying risk and individualizing care for
25 portal hypertension. *J. Hepatol.* 2015;63:743–752.
- 26 9. Lackner C, Spindelboeck W, Haybaeck J, Douschan P, Rainer F, Terracciano L, *et al.*
27 Histological parameters and alcohol abstinence determine long-term prognosis in patients
28 with alcoholic liver disease. *J Hepatol.* 2017;66:610–618.
- 29 10. D’Amico G, Garcia-Tsao G, Pagliaro L. Natural history and prognostic indicators of
30 survival in cirrhosis: a systematic review of 118 studies. *J. Hepatol.* 2006;44:217–231.
- 31 11. Trebicka J, Gu W, de Ledinghen V, Aubé C, Krag A, Praktijnjo M, *et al.* Two-
32 dimensional shear wave elastography predicts survival in advanced chronic liver disease.
33 *Gut.* 2021;
- 34 12. Lackner C, Stauber RE, Davies S, Denk H, Dienes HP, Gnemmi V, *et al.*
35 Development and prognostic relevance of a histologic grading and staging system for
36 alcohol-related liver disease. *J Hepatol.* 2021;75:810–819.
- 37 13. Mueller S, Nahon P, Rausch V, Peccerella T, Silva I, Yagmur E, *et al.* Caspase-

- 1 cleaved keratin-18 fragments increase during alcohol withdrawal and predict liver-related
2 death in patients with alcoholic liver disease. *Hepatology*. 2017;66:96–107.
- 3 14. Atkinson SR, Grove JI, Liebig S, Astbury S, Vergis N, Goldin R, *et al.* In Severe
4 Alcoholic Hepatitis, Serum Keratin-18 Fragments Are Diagnostic, Prognostic, and
5 Theragnostic Biomarkers. *Am J Gastroenterol*. 2020;115:1857–1868.
- 6 15. Thietart S, Rautou P-E. Extracellular vesicles as biomarkers in liver diseases: A
7 clinician's point of view. *J Hepatol*. 2020;73:1507–1525.
- 8 16. Bissonnette J, Altamirano J, Devue C, Roux O, Payancé A, Lebrech D, *et al.* A
9 prospective study of the utility of plasma biomarkers to diagnose alcoholic hepatitis.
10 *Hepatology*. 2017;66:555–563.
- 11 17. Sukriti S, Maras JS, Bihari C, Das S, Vyas AK, Sharma S, *et al.* Microvesicles in
12 hepatic and peripheral vein can predict nonresponse to corticosteroid therapy in severe
13 alcoholic hepatitis. *Alimentary Pharmacology & Therapeutics*. 2018;47:1151–1161.
- 14 18. Payancé A, Silva-Junior G, Bissonnette J, Tanguy M, Pasquet B, Levi C, *et al.*
15 Hepatocyte microvesicle levels improve prediction of mortality in patients with cirrhosis.
16 *Hepatology*. 2018;68:1508–1518.
- 17 19. Kamath PS, Wiesner RH, Malinchoc M, Kremers W, Therneau TM, Kosberg CL, *et al.*
18 A model to predict survival in patients with end-stage liver disease. *Hepatology*.
19 2001;33:464–470.
- 20 20. Durand F, Valla D. Assessment of the prognosis of cirrhosis: Child-Pugh versus
21 MELD. *J Hepatol*. 2005;42 Suppl:S100-107.
- 22 21. Tsochatzis E, Bruno S, Isgro G, Hall A, Theocharidou E, Manousou P, *et al.* Collagen
23 proportionate area is superior to other histological methods for sub-classifying cirrhosis and
24 determining prognosis. *J Hepatol*. 2014;60:948–954.
- 25 22. Israelsen M, Guerrero Misas M, Koutsoumourakis A, Huang Y, Thiele M, Hall A, *et al.*
26 Collagen proportionate area predicts clinical outcomes in patients with alcohol-related liver
27 disease. *Aliment Pharmacol Ther*. 2020;52:1728–1739.
- 28 23. Rasmussen DN, Thiele M, Johansen S, Kjærgaard M, Lindvig KP, Israelsen M, *et al.*
29 Prognostic performance of seven biomarkers compared to liver biopsy in early alcohol-
30 related liver disease. *J Hepatol*. 2021;S0168-8278(21)00411–6.
- 31 24. Pessione F, Ramond MJ, Peters L, Pham BN, Batel P, Rueff B, *et al.* Five-year
32 survival predictive factors in patients with excessive alcohol intake and cirrhosis. Effect of
33 alcoholic hepatitis, smoking and abstinence. *Liver Int*. 2003;23:45–53.
- 34 25. Louvet A, Labreuche J, Artru F, Bouthors A, Rolland B, Saffers P, *et al.* Main drivers
35 of outcome differ between short term and long term in severe alcoholic hepatitis: A
36 prospective study. *Hepatology*. 2017;66:1464–1473.
- 37 26. von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP. The

- 1 Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement:
2 Guidelines for reporting observational studies. *International Journal of Surgery*.
3 2014;12:1495–1499.
- 4 27. Ganne-Carrié N, Chaffaut C, Bourcier V, Archambeaud I, Perarnau J-M, Oberti F, et
5 al. Estimate of hepatocellular carcinoma incidence in patients with alcoholic cirrhosis. *J*.
6 *Hepatol*. 2018;69:1274–1283.
- 7 28. World Health Organization. Regional Office for Europe. (2003). Food-based dietary
8 guidelines in the WHO European Region. Copenhagen : WHO Regional Office for Europe.
9 [Internet]. Available from: <https://apps.who.int/iris/handle/10665/107490>
- 10 29. Reid MC, Fiellin DA, O'Connor PG. Hazardous and Harmful Alcohol Consumption in
11 Primary Care. *Archives of Internal Medicine*. 1999;159:1681–1689.
- 12 30. Grundy SM, Brewer HB, Cleeman JI, Smith SC, Lenfant C. Definition of Metabolic
13 Syndrome. *Circulation*. 2004;109:433–438.
- 14 31. Wiesner R, Edwards E, Freeman R, Harper A, Kim R, Kamath P, et al. Model for end-
15 stage liver disease (MELD) and allocation of donor livers. *Gastroenterology*. 2003;124:91–
16 96.
- 17 32. Villa E, Cammà C, Marietta M, Luongo M, Critelli R, Colopi S, et al. Enoxaparin
18 prevents portal vein thrombosis and liver decompensation in patients with advanced
19 cirrhosis. *Gastroenterology*. 2012;143:1253-1260.e1–4.
- 20 33. de Franchis R, Bosch J, Garcia-Tsao G, Reiberger T, Ripoll C, Abraldes JG, et al.
21 BAVENO VII - RENEWING CONSENSUS IN PORTAL HYPERTENSION. *Journal of*
22 *Hepatology*. 2021;S0168827821022996.
- 23 34. Rautou P-E, Bresson J, Sainte-Marie Y, Vion A-C, Paradis V, Renard J-M, et al.
24 Abnormal plasma microparticles impair vasoconstrictor responses in patients with cirrhosis.
25 *Gastroenterology*. 2012;143:166-176.e6.
- 26 35. Théry C, Witwer KW, Aikawa E, Alcaraz MJ, Anderson JD, Andriantsitohaina R, et al.
27 Minimal information for studies of extracellular vesicles 2018 (MISEV2018): a position
28 statement of the International Society for Extracellular Vesicles and update of the
29 MISEV2014 guidelines. *Journal of Extracellular Vesicles*. 2018;7:1535750.
- 30 36. Halfon P, Munteanu M, Poynard T. FibroTest-ActiTest as a non-invasive marker of
31 liver fibrosis. *Gastroenterol Clin Biol*. 2008;32:22–39.
- 32 37. Poynard T, Munteanu M, Deckmyn O, Ngo Y, Drane F, Messous D, et al. Applicability
33 and precautions of use of liver injury biomarker FibroTest. A reappraisal at 7 years of age.
34 *BMC Gastroenterol*. 2011;11:39.
- 35 38. Grambsch PM, Therneau TM. Proportional Hazards Tests and Diagnostics Based on
36 Weighted Residuals. *Biometrika*. 1994;81:515–526.
- 37 39. Blanche P, Dartigues J-F, Jacqmin-Gadda H. Estimating and comparing time-

- 1 dependent areas under receiver operating characteristic curves for censored event times
2 with competing risks. *Stat Med.* 2013;32:5381–5397.
- 3 40. Poynard T, Vergniol J, Ngo Y, Foucher J, Munteanu M, Merrouche W, et al. Staging
4 chronic hepatitis C in seven categories using fibrosis biomarker (FibroTest™) and transient
5 elastography (FibroScan®). *J Hepatol.* 2014;60:706–714.
- 6 41. Poynard T, Vergniol J, Ngo Y, Foucher J, Thibault V, Munteanu M, et al. Staging
7 chronic hepatitis B into seven categories, defining inactive carriers and assessing treatment
8 impact using a fibrosis biomarker (FibroTest®) and elastography (FibroScan®). *J Hepatol.*
9 2014;61:994–1003.
- 10 42. Ripoll C, Lastra P, Rincón D, Catalina V, Bañares R. Comparison of MELD, HVPG,
11 and their changes to predict clinically relevant endpoints in cirrhosis. *Scandinavian Journal of*
12 *Gastroenterology.* 2012;47:204–211.
- 13 43. **Altamirano J, López-Pelayo H,** Michelena J, Jones PD, Ortega L, Ginès P, et al.
14 Alcohol abstinence in patients surviving an episode of alcoholic hepatitis: Prediction and
15 impact on long-term survival. *Hepatology.* 2017;66:1842–1853.
- 16 44. Rhodes FA, Trembling P, Panovska-Griffiths J, Tanwar S, Westbrook RH, Rodger A,
17 et al. Systematic review: Investigating the prognostic performance of four non-invasive tests
18 in alcohol-related liver disease. *Journal of Gastroenterology and Hepatology.* 2021;36:1435–
19 1449.
- 20 45. Ku N-O, Strnad P, Bantel H, Omary MB. Keratins: Biomarkers and modulators of
21 apoptotic and necrotic cell death in the liver. *Hepatology.* 2016;64:966–976.
- 22 46. Ozturk B, Coskun U, Sancak B, Yaman E, Buyukberber S, Benekli M. Elevated serum
23 levels of M30 and M65 in patients with locally advanced head and neck tumors. *Int*
24 *Immunopharmacol.* 2009;9:645–648.
- 25 47. Ulukaya E, Yilmaztepe A, Akgoz S, Linder S, Karadag M. The levels of caspase-
26 cleaved cytokeratin 18 are elevated in serum from patients with lung cancer and helpful to
27 predict the survival. *Lung Cancer.* 2007;56:399–404.
- 28 48. Yaman E, Coskun U, Sancak B, Buyukberber S, Ozturk B, Benekli M. Serum M30
29 levels are associated with survival in advanced gastric carcinoma patients. *Int*
30 *Immunopharmacol.* 2010;10:719–722.
- 31 49. Scott LC, Evans TRJ, Cassidy J, Harden S, Paul J, Ullah R, et al. Cytokeratin 18 in
32 plasma of patients with gastrointestinal adenocarcinoma as a biomarker of tumour response.
33 *Br J Cancer.* 2009;101:410–417.
- 34 50. Greystoke A, Dean E, Saunders MP, Cummings J, Hughes A, Ranson M, et al. Multi-
35 level evidence that circulating CK18 is a biomarker of tumour burden in colorectal cancer. *Br*
36 *J Cancer.* 2012;107:1518–1524.
- 37 51. Semmler G, Binter T, Kozbial K, Schwabl P, Hametner-Schreil S, Zanetto A, et al.

- 1 Noninvasive Risk Stratification After HCV Eradication in Patients With Advanced Chronic
2 Liver Disease. *Hepatology*. 2021;73:1275–1289.
- 3 52. **Elkrief L, Lazareth M**, Chevret S, Paradis V, Magaz M, Blaise L, et al. Liver stiffness
4 by transient elastography to detect porto-sinusoidal vascular liver disease with portal
5 hypertension. *Hepatology*. 2020;
- 6 53. Wang S, Pacher P, De Lisle RC, Huang H, Ding W-X. A Mechanistic Review of Cell
7 Death in Alcohol-Induced Liver Injury. *Alcoholism: Clinical and Experimental Research*.
8 2016;40:1215–1223.
- 9 54. Seitz HK, Bataller R, Cortez-Pinto H, Gao B, Gual A, Lackner C, et al. Alcoholic liver
10 disease. *Nat Rev Dis Primers*. 2018;4:16.
- 11 55. Kostallari E, Valainathan S, Biquard L, Shah VH, Rautou P-E. Role of extracellular
12 vesicles in liver diseases and their therapeutic potential. *Adv Drug Deliv Rev*.
13 2021;175:113816.
- 14 56. Nagy LE, Ding W-X, Cresci G, Saikia P, Shah VH. Linking Pathogenic Mechanisms of
15 Alcoholic Liver Disease With Clinical Phenotypes. *Gastroenterology*. 2016;150:1756–1768.
- 16 57. Romano V, Hatzfeld M, Magin TM, Zimbelmann R, Franke WW, Maier G, et al.
17 Cytokeratin expression in simple epithelia. I. Identification of mRNA coding for human
18 cytokeratin no. 18 by a cDNA clone. *Differentiation*. 1986;30:244–253.
- 19 58. Louvet A, Bourcier V, Archambeaud I, d'Alteroche L, Chaffaut C, Oberti F, et al. Low
20 alcohol consumption influences outcomes in individuals with alcohol-related compensated
21 cirrhosis in a French multicenter cohort. *J Hepatol*. 2023;78:501–512.
- 22 59. Poynard T, Peta V, Deckmyn O, Munteanu M, Moussalli J, Ngo Y, et al. LCR1 and
23 LCR2, two multi-analyte blood tests to assess liver cancer risk in patients without or with
24 cirrhosis. *Aliment Pharmacol Ther*. 2019;49:308–320.
- 25 60. Merion RM, Schaubel DE, Dykstra DM, Freeman RB, Port FK, Wolfe RA. The survival
26 benefit of liver transplantation. *Am J Transplant*. 2005;5:307–313.

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28

1 **Table 1. Characteristics and plasma biomarkers levels of the included patients**
 2 **according to the alcohol status**

	No active alcohol consumption N=419	Active alcohol consumption N=81
Patients' characteristics		
Age, years	58 (51-64)	61 (54-67)
Male gender	281 (67)	59 (73)
Body mass index, kg/m ²	27.5 (24.3-31.2)	27.4 (23.5-30.5)
Interval between diagnostic liver biopsy and enrollment, months	24 (9-69)	35 (9-75)
Alcohol consumption, number of units per week		
0	343 (82)	0
1-6	76 (18)	0
7-20	0	33 (43)
≥21	0	44 (57)
Metabolic syndrome	71 (19)	20 (27)
Diabetes	89 (21)	24 (30)
Arterial hypertension	163 (39)	43 (53)
Hypertriglyceridemia	35 (8)	11 (14)
History of decompensation	282 (69)	38 (47)
Ascites	207 (50)	26 (33)
Variceal bleeding	80 (19)	11 (14)
Encephalopathy	39 (9)	6 (8)
Jaundice	121 (29)	16 (20)
Esophageal varices		
Absent	195 (49)	50 (67)
Small	124 (31)	8 (10)
Medium or large or history of Bleeding or band ligation	83 (21)	17 (22)
Prothrombin time, %	78 (69-91)	81 (65-93)

Serum albumin, g/L	40 (37-43)	41 (37-43)
Platelet count, x10 ⁹ /L	141 (101-189)	137 (95-172)
Serum bilirubin, µmol/L	14 (9-20)	13 (10-19)
Serum AST, IU/L	32 (26-48)	42 (31-72)
Serum ALT, IU/L	26 (19.5-36)	31 (21-48)
Serum GGT, IU/L	99 (51-189)	232 (108-442)
Serum creatinine, µmol/L	69 (61-81)	69 (57-84)
Plasma biomarkers		
MELD*	9 (8-10)	9 (8-10)
MELD* ≥10	103 (27)	23 (29)
Keratin 18	183 (121-308)	270 (165-725)
Keratin 18 >285 U/L	121 (29)	39 (48)
Hepatocyte IEVs	13 (0-46)	30 (0-98)
Hepatocyte IEVs >50 U/L	99 (24)	30 (37)
Hepatocyte IEVs >65 U/L	86 (20)	26 (32)
FibroTest	0.72 (0.48-0.87)	0.69 (0.43-0.86)
FibroTest >0.74	198 (47)	34 (42)

1 Abbreviations: AST, aspartate amino transferase, ALT, alanine amino transferase, GGT,
2 gammaglutamyl transpeptidase, MELD, model for end stage liver disease; IEVs, large
3 extracellular vesicles
4 * MELD was available in 386/419 patients with past alcohol consumption and in 78/81
5 patients with active alcohol consumption
6 Data are presented as median (interquartile range) or absolute number (percentage)

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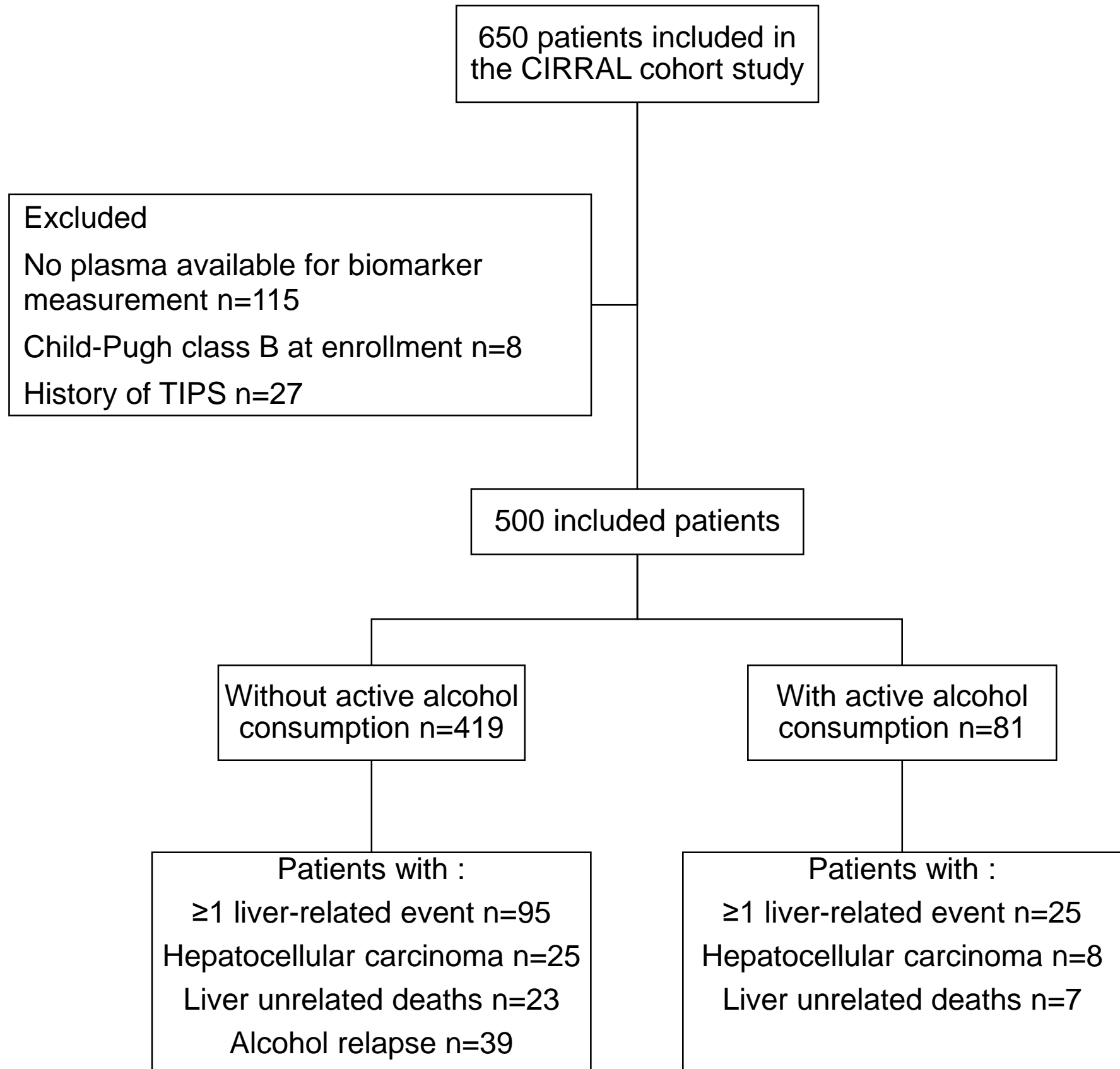
1 **Table 2. Univariable cause-specific Cox regression analysis of the variables**
 2 **associated with liver-related events at 2 years according to alcohol status at**
 3 **enrollment**

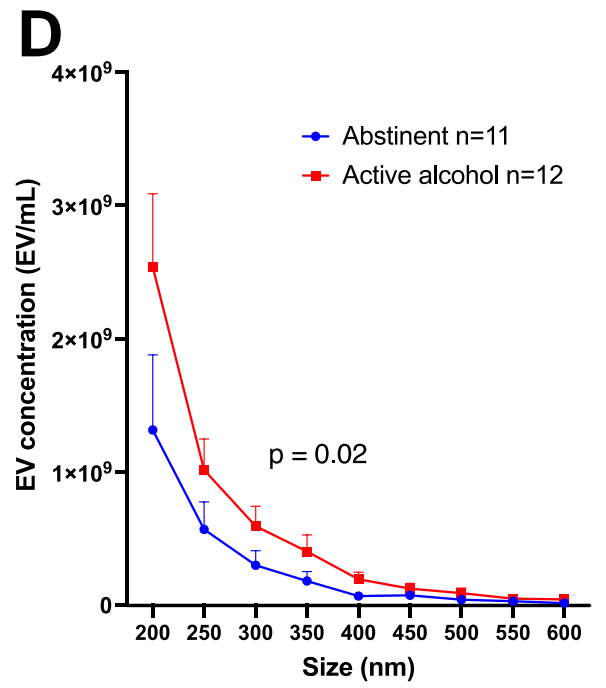
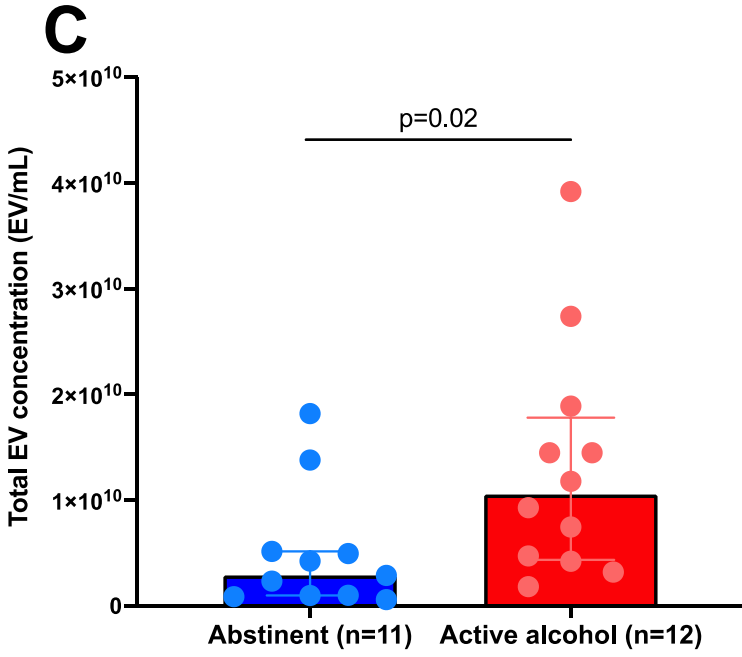
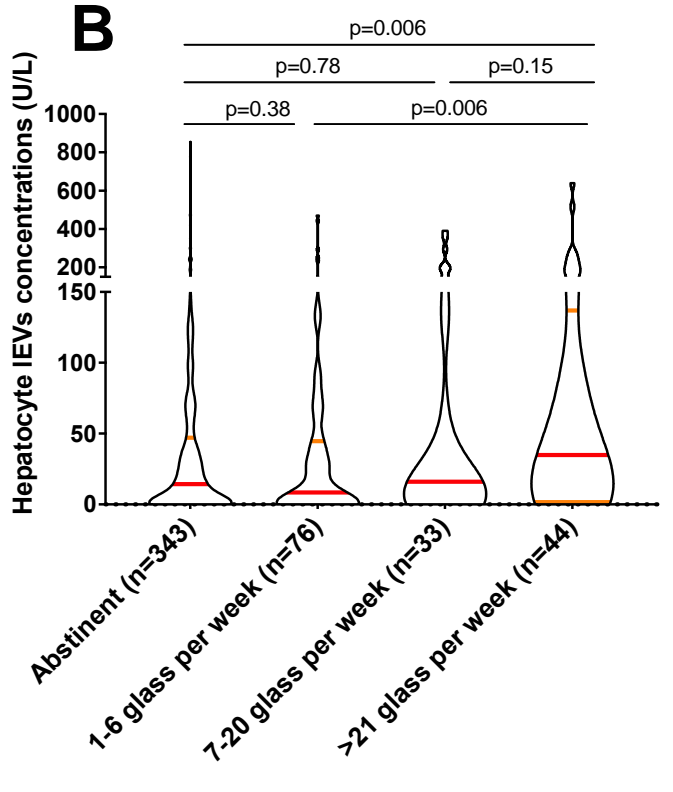
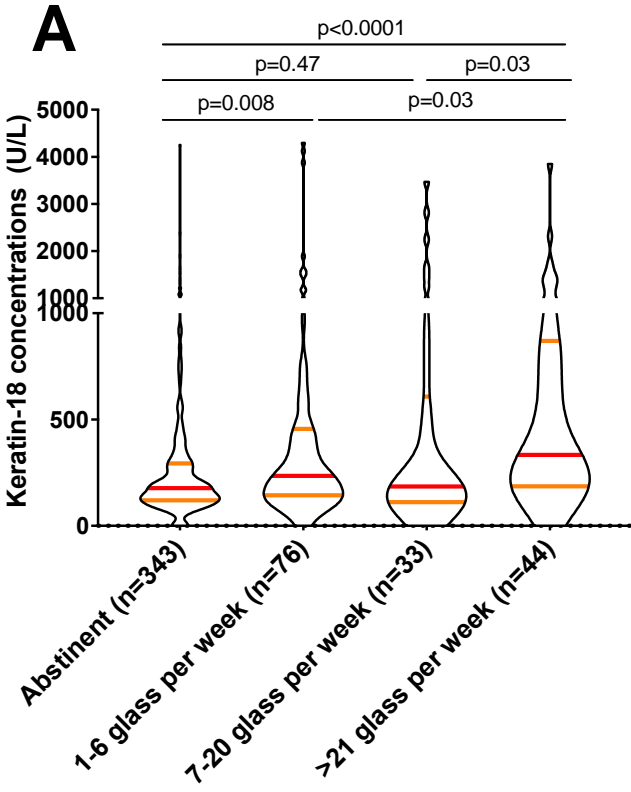
4 In patients with past alcohol consumption, hepatocellular carcinoma, liver-unrelated deaths
 5 and alcohol relapse were treated as competing events; In patients with active alcohol
 6 consumption, hepatocellular carcinoma and liver-unrelated deaths were treated as
 7 competing events

	No active alcohol consumption N=419			Active alcohol consumption N=81			
	HR	(95% CI)	P value	HR	(95% CI)	P value	
Patients' characteristics							
Age, /10 years	1.02	0.82-1.26	0.87	0.79	0.50-1.25	0.31	
Male gender	1.18	0.77-1.81	0.45	0.41	0.19-0.90	0.027	
Body mass index, /10 kg/m ²	1.01	0.98-1.05	0.47	1.03	0.95-1.11	0.50	
Number of units per week							
0	1.00		0.068				
1-6	1.60	0.97-2.65					
7-20				1.00			
≥21				1.31	0.57-3.00	0.52	
Metabolic syndrome	0.78		0.44-1.39	0.40	0.35	0.10-1.19	0.093
Diabetes	1.26	0.79-2.01	0.33	0.44	0.17-1.18	0.10	
Arterial hypertension	1.11	0.74-1.67	0.63	0.65	0.30-1.44	0.29	
Hypertriglyceridemia	1.19	0.6-2.37	0.62	1.56	0.59-4.17	0.37	
Past history of decompensation	1.42	0.88-2.28	0.15	1.73	0.78-3.82	0.18	
Esophageal varices							
Absent	1.00		0.008	1.00		0.36	
Small	1.92	1.19-3.10		1.64	0.57-4.74		
Medium or large or history of bleeding or band ligation	2.43	1.43-4.12		8.35	2.97-23.4		<0.0001
Prothrombin time, /10%	0.71	0.62-0.80	<0.0001	0.76	0.63-0.93	0.006	
Serum albumin, /10g/L	0.26	0.15-0.42	<0.0001	0.42	0.16-1.11	0.080	
Platelet count, /100Giga/L	0.47	0.33-0.69	0.001	0.41	0.19-0.89	0.024	
Serum bilirubin, /10 μmol/L	1.83	1.47-2.27	<0.0001	2.17	1.30-3.62	0.003	
Serum AST, /10IU/L	1.06	1.03-1.10	0.0007	1.11	1.02-1.22	0.020	

Serum ALT, /10IU/L	1.06	0.97-1.16	0.17	1.04	0.89-1.20	0.65
Serum GGT, /10IU/L	1.01	1.01-1.02	0.024	1.01	1.00-1.02	0.007
Serum creatinine, /10 μ mol/L	0.97	0.87-1.09	0.61	0.75	0.61-0.93	0.008
Plasma biomarkers						
MELD***	1.37	1.24-1.52	<0.0001	1.19	1-1.42	0.051
MELD \geq10***	2.64	1.71-4.09	<0.0001	1.45	0.62-3.36	0.39
Plasma keratin 18 x 10^{-2} U/L	1.06	1.04-1.09	<0.0001	1.03	0.99-1.07	0.12
Plasma keratin 18 >285 U/L	2.31	1.54-3.48	<0.0001	2.28	1.00-5.16	0.049
Hepatocyte IEVs x 10^{-1} U/L	1.00	0.98-1.02	0.78	1.003	1.001-1.006	0.003
Hepatocyte IEVs >50 U/L	1.23	0.77-1.95	0.38	5.17	2.22-12.02	0.0001
Hepatocyte IEVs >65 U/L	1.28	0.79-2.06	0.32	12.46	1.67-93.0	0.009
FibroTest	10.15	3.54-29.09	<0.0001	12.46	1.67-92.99	0.014
FibroTest >0.74	2.57	1.68-3.92	<0.0001	3.79	1.64-8.75	0.002

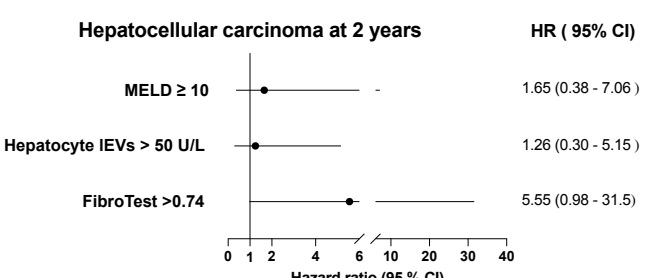
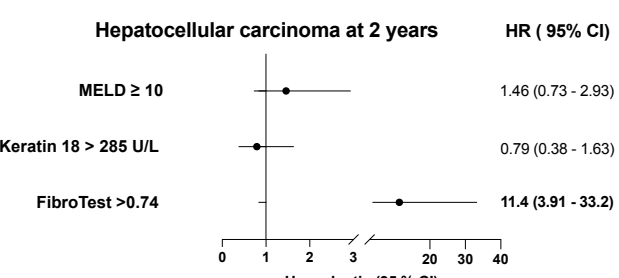
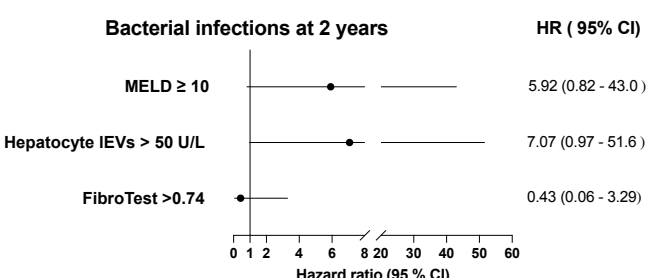
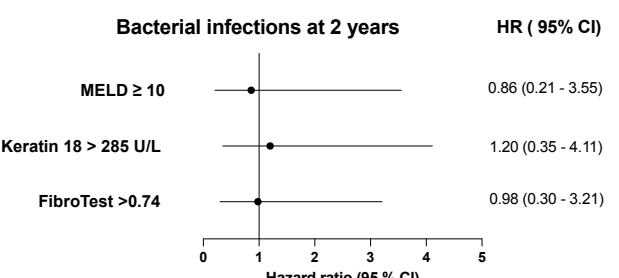
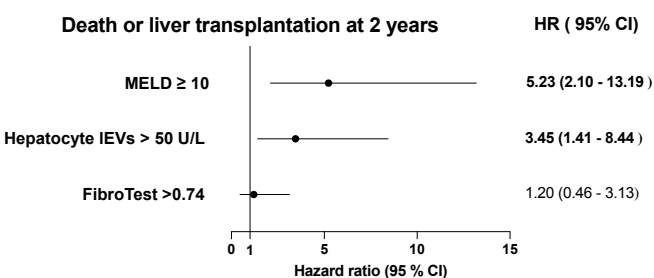
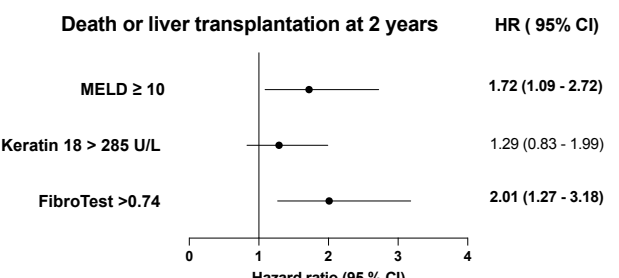
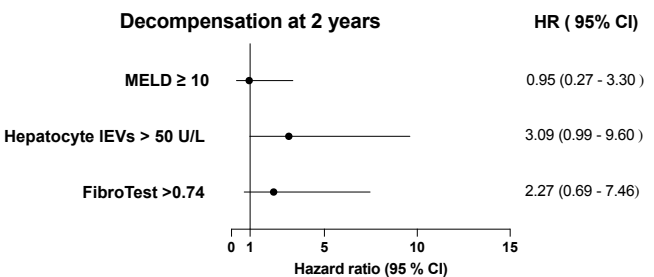
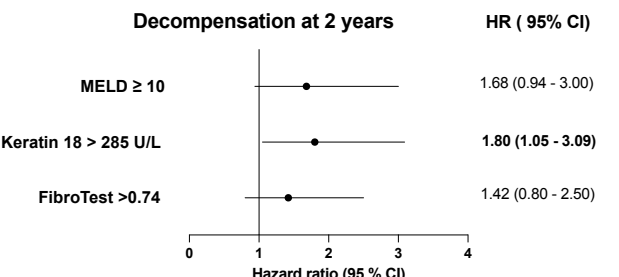
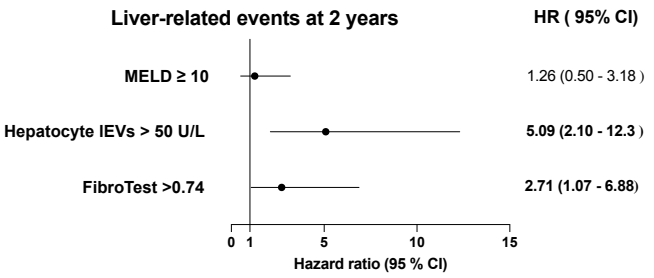
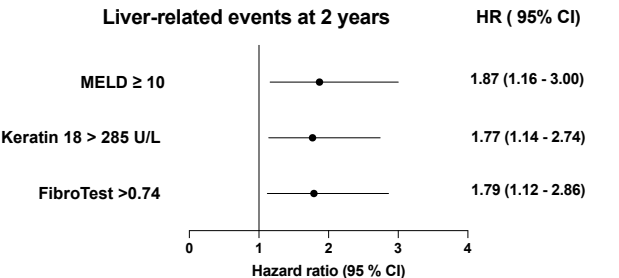
- 1 Abbreviations : AST, aspartate amino transferase, ALT, alanine amino transferase, GGT,
- 2 gammaglutamyl transpeptidase, MELD, model for end stage liver disease; IEVs, large
- 3 extracellular vesicles
- 4 *** MELD was available in 386/419 patients with past alcohol consumption and in 78/81
- 5 patients with active alcohol consumption
- 6 Bold indicate statistically significant results
- 7

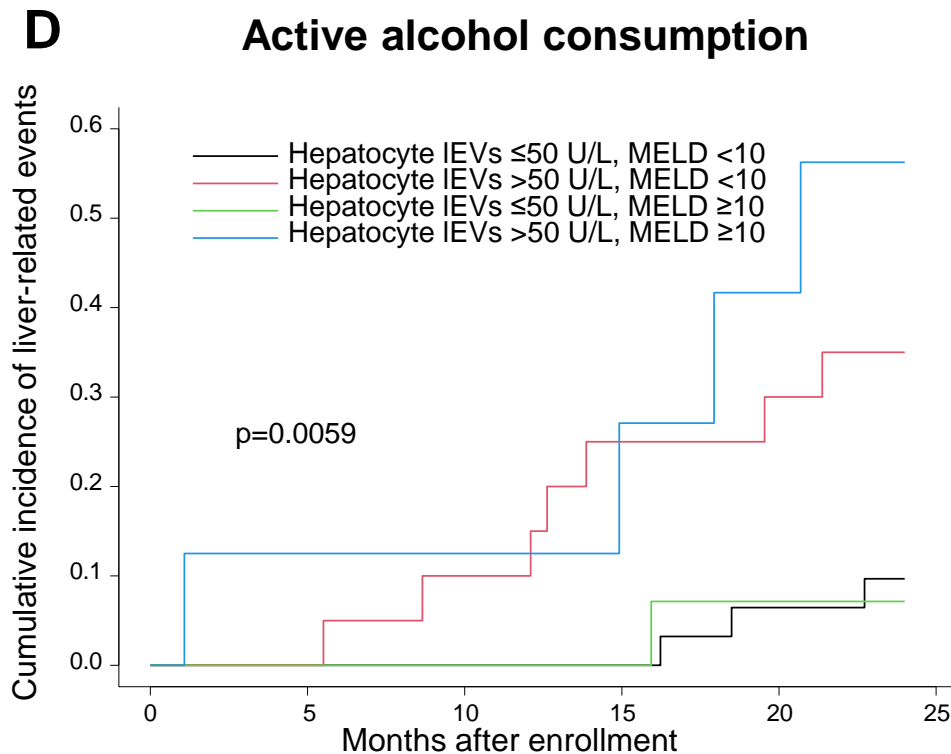
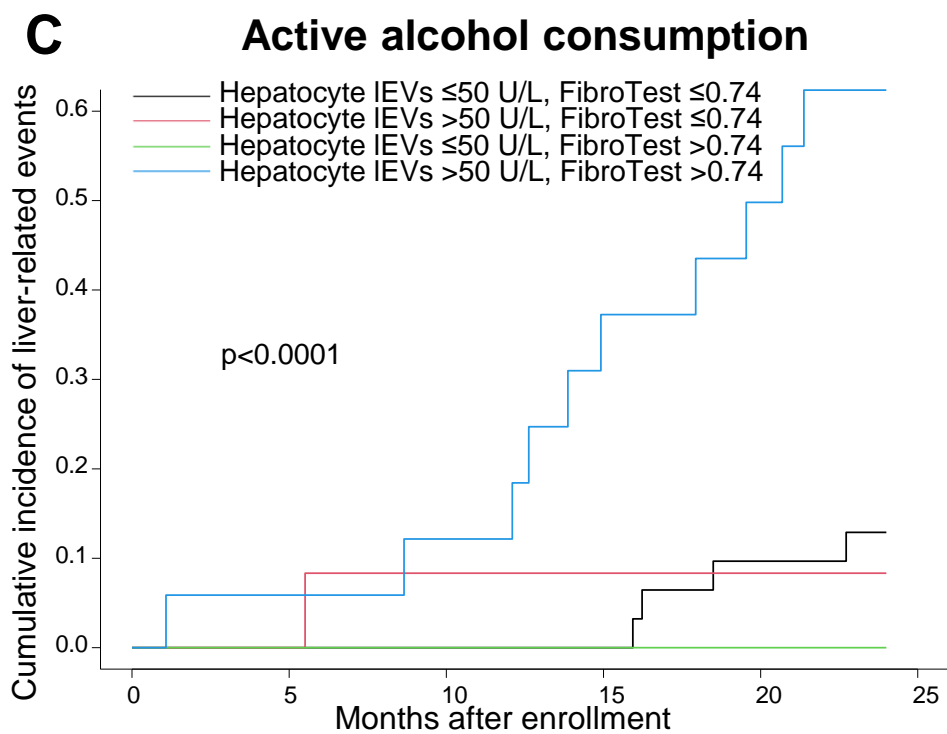
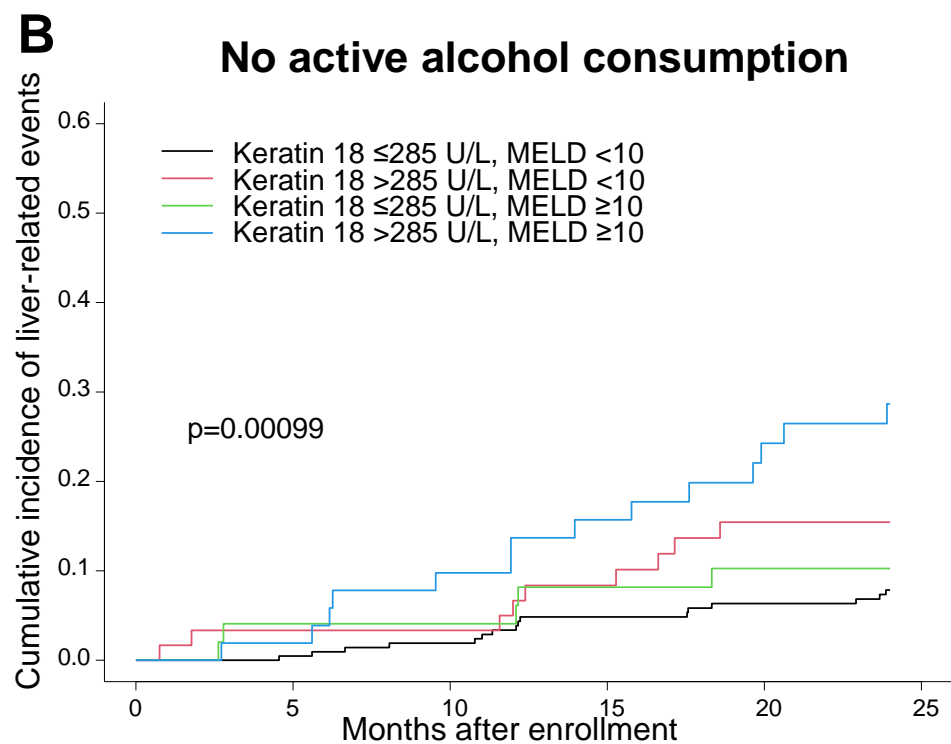
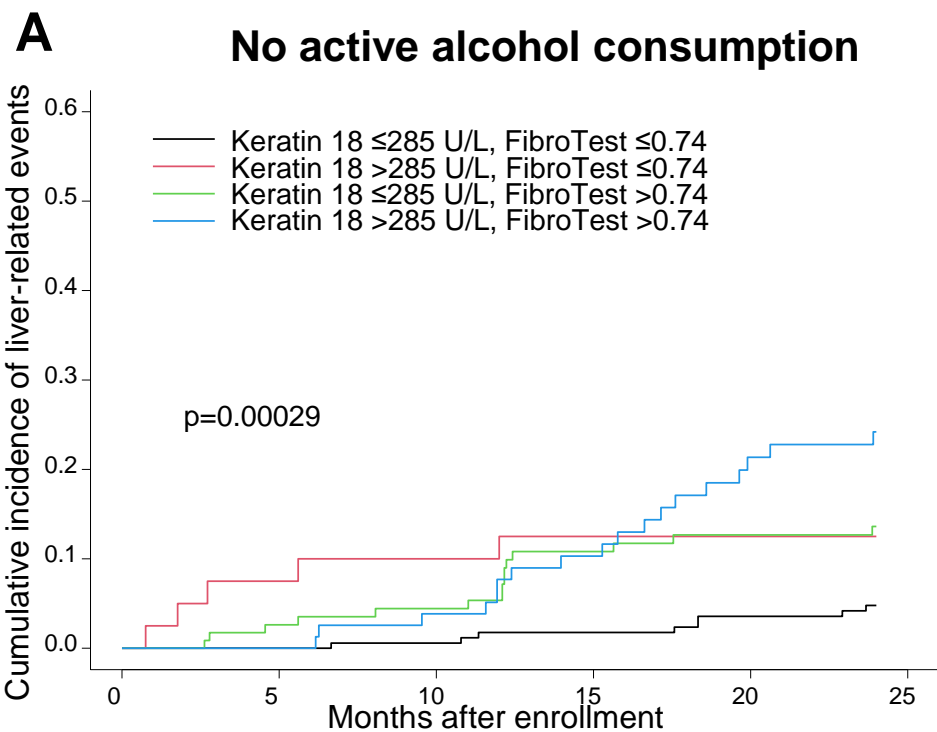




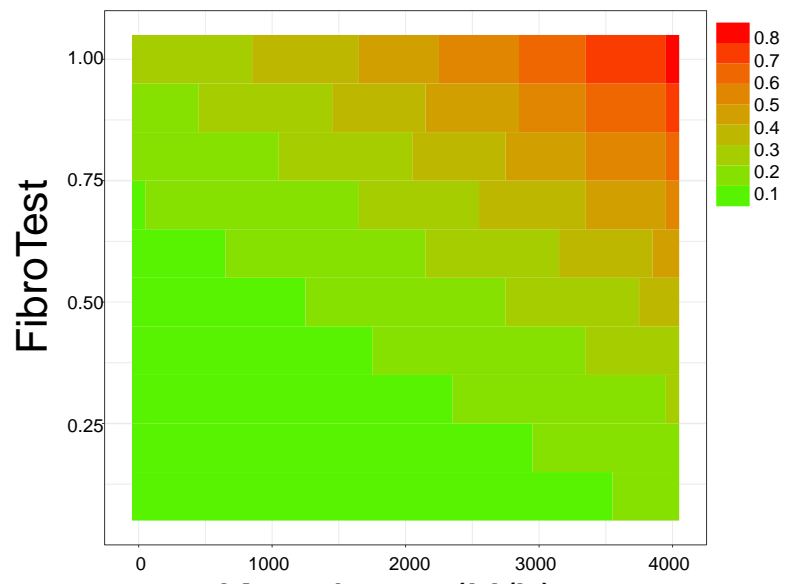
Without active alcohol consumption

With active alcohol consumption





A No active alcohol consumption



Without active alcohol consumption (n=419)

FibroTest

FibroTest ≤ 0.74

FibroTest > 0.74

Keratin-18 ≤ 285 U/L

Keratin-18 > 285 U/L

Keratin-18 ≤ 285 U/L

Keratin-18 > 285 U/L

N=181

N=40

N=117

N=81

2 years liver-related events

2 years liver-related events

2 years liver-related events

2 years liver-related events

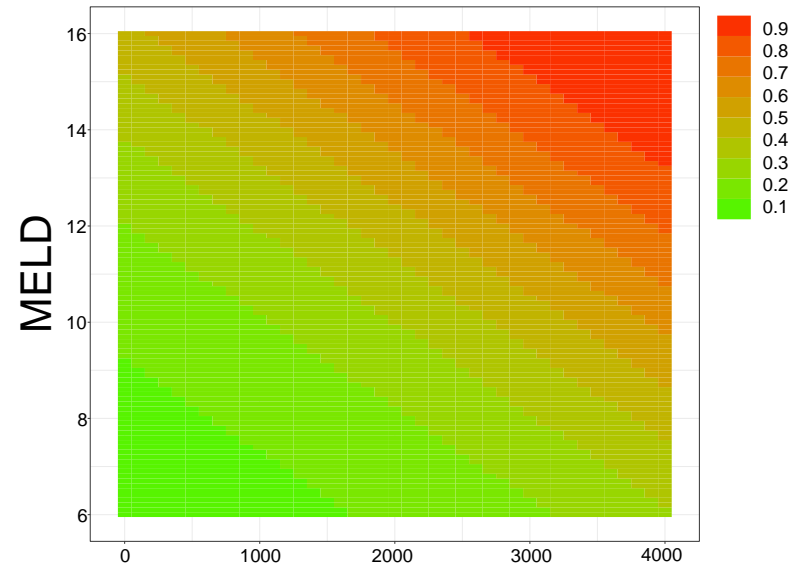
5.2% (1.6-8.7)

12.7% (1.6-22.6)

14.4% (7.3-20.9)

26.6% (15.2-36.4)

B No active alcohol consumption



Keratin 18 (U/L)

Without active alcohol consumption (n=419)

MELD

MELD < 10

MELD ≥ 10

Keratin-18 ≤ 285 U/L

Keratin-18 > 285 U/L

Keratin-18 ≤ 285 U/L

Keratin-18 > 285 U/L

N=223

N=60

N=49

N=54

2 years liver-related events

2 years liver-related events

2 years liver-related events

2 years liver-related events

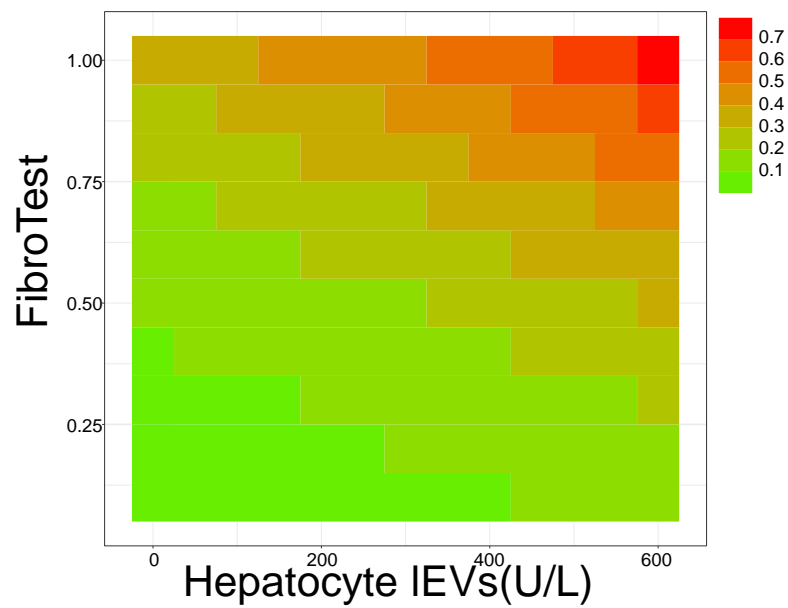
8.4% (4.4 - 12.3)

16.2% (5.9- 25.4)

11.0% (1.4 - 19.7)

31.4% (16.1- 44.0)

C Active alcohol consumption



With active alcohol consumption (n=81)

FibroTest

FibroTest ≤ 0.74

FibroTest > 0.74

Hepatocyte IELs ≤ 50 U/L

Hepatocyte IELs > 50 U/L

Hepatocyte IELs ≤ 50 U/L

Hepatocyte IELs > 50 U/L

N=34

N=13

N=17

N=17

2 years liver-related events

2 years liver-related events

2 years liver-related events

2 years liver-related events

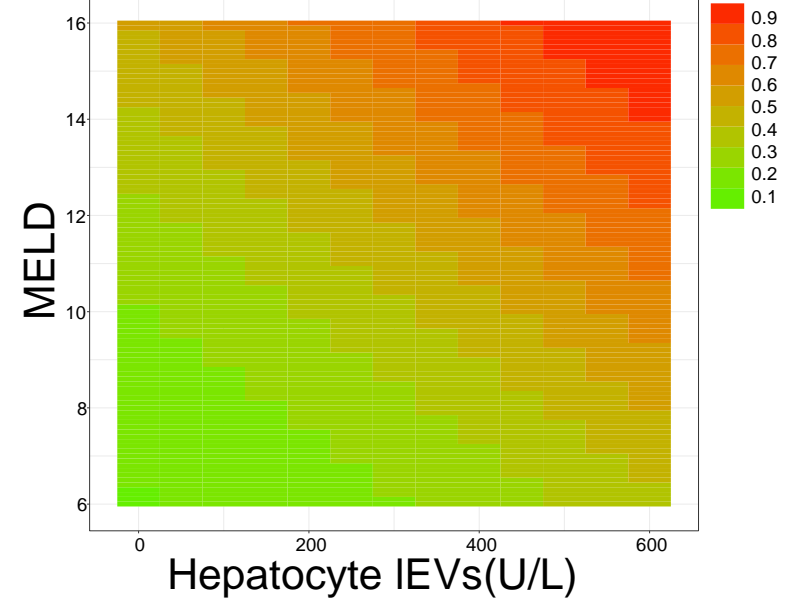
13.2% (0.7-24.5)

8.3% (0-22.77)

0%

62.4% (29.2-80)

D Active alcohol consumption



With active alcohol consumption (n=81)

MELD

MELD < 10

MELD ≥ 10

Hepatocyte IELs ≤ 50 U/L

Hepatocyte IELs > 50 U/L

Hepatocyte IELs ≤ 50 U/L

Hepatocyte IELs > 50 U/L

N=34

N=21

N=15

N=8

2 years liver-related events

2 years liver-related events

2 years liver-related events

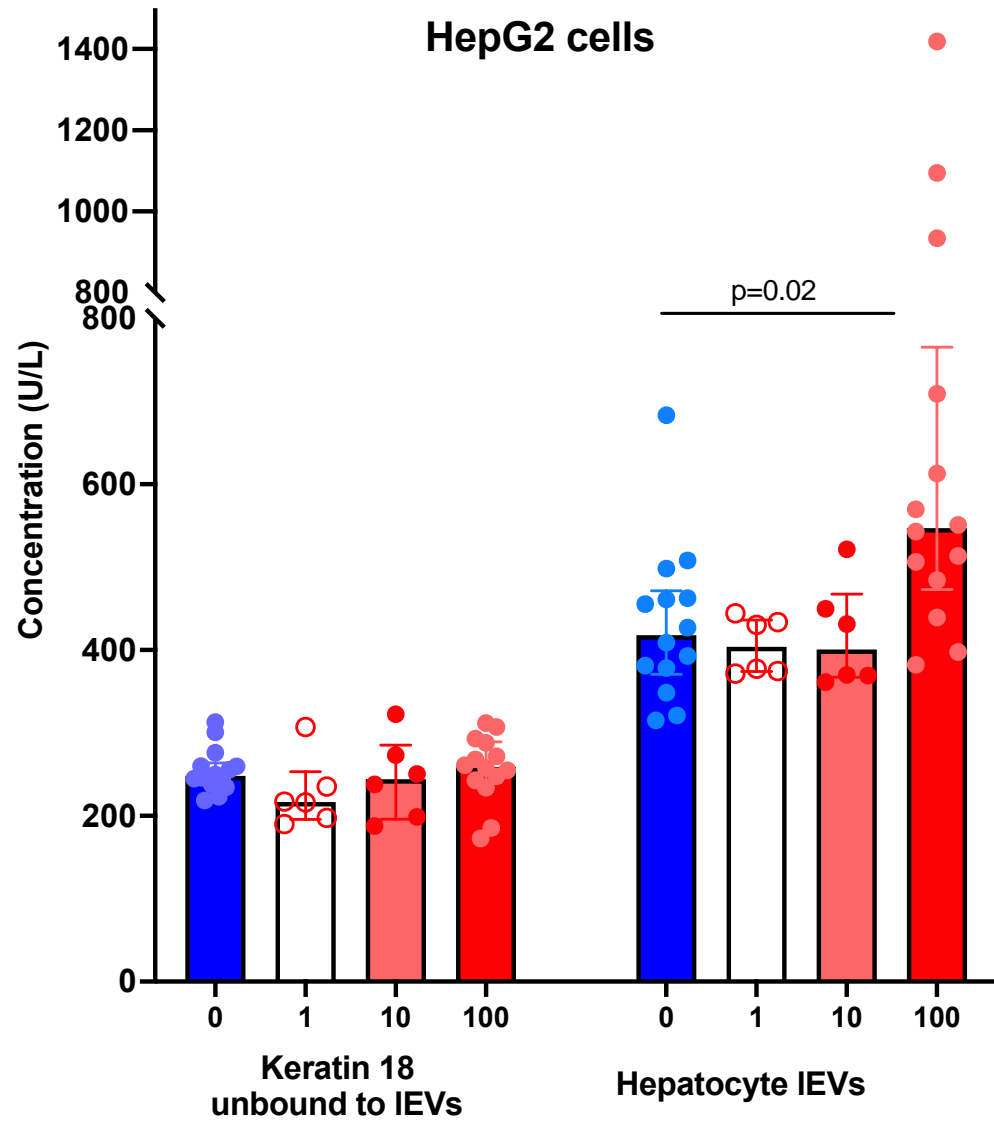
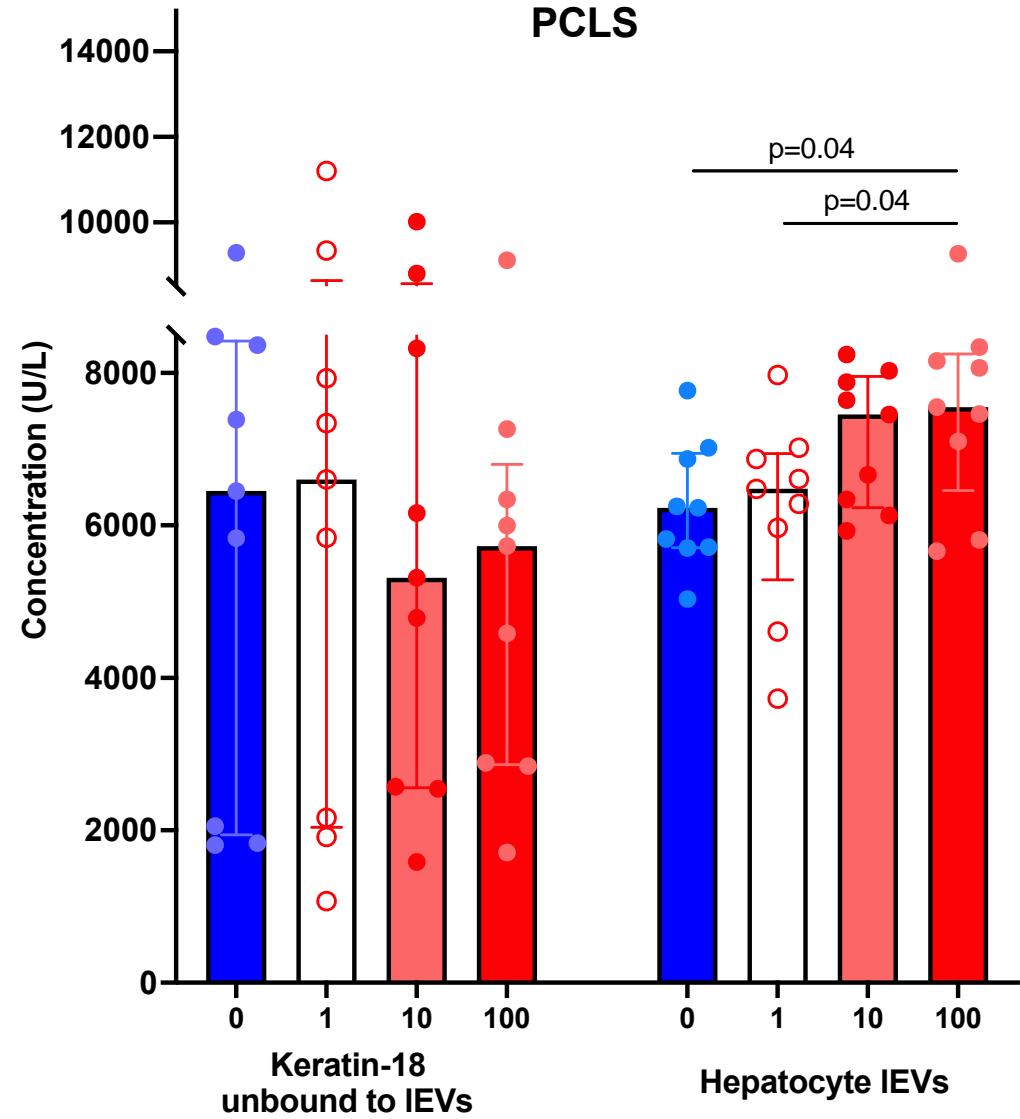
2 years liver-related events

9.9% (0-20)

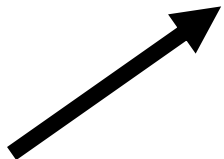
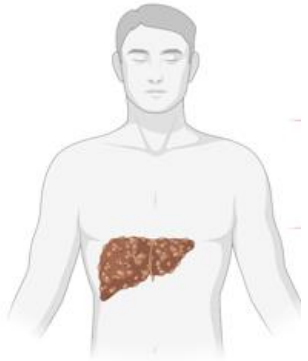
36.2% (10.7-54.5)

7.7% (0-21.1)

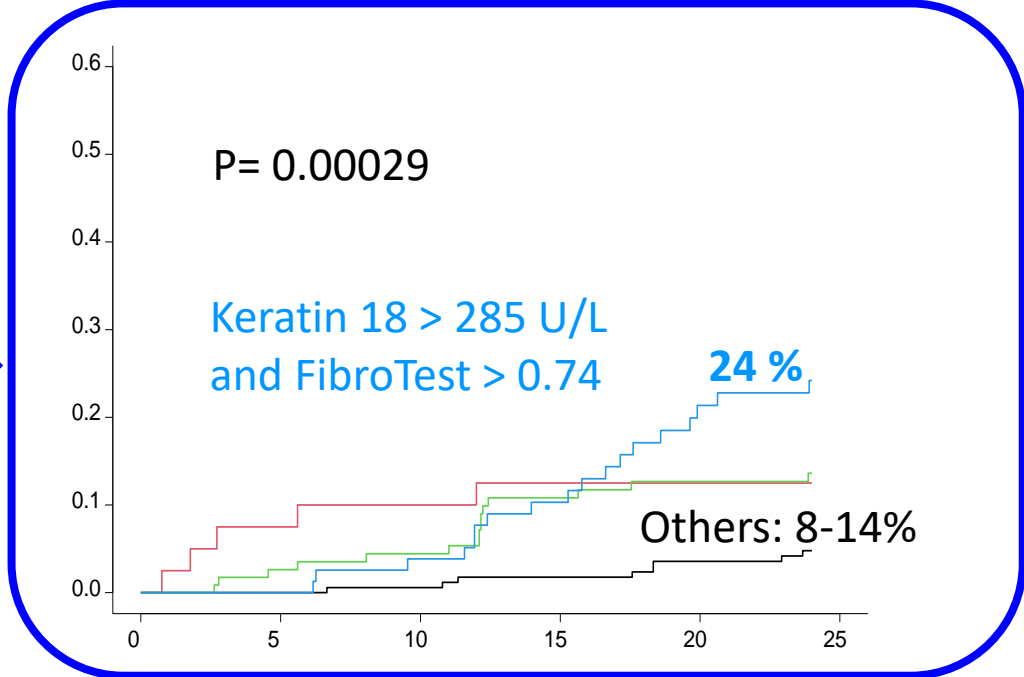
65.0% (0-88.4)

A**B**

At enrollment
MELD, FibroTest
Keratin 18
Hepatocyte large extracellular vesicles (IEVs)

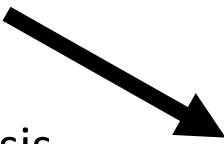



Without active alcohol
(n=419)

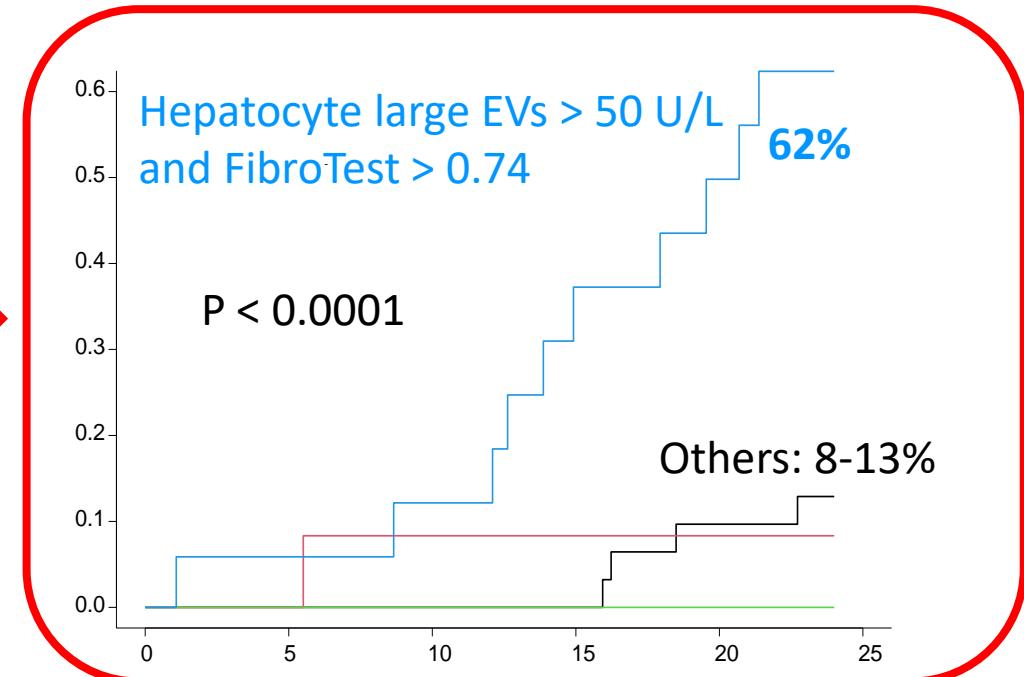


2 groups

500 patients
Child-Pugh A
alcohol-related cirrhosis
Prospective inclusion




With active alcohol
(n=81)



Primary endpoint
Liver-related event at 2 years
Competitive events: Liver-unrelated death, hepatocellular carcinoma