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Monitoring Occurrence of Liver-Related Events and Survival by Transient Elastography in Patients With Nonalcoholic Fatty Liver Disease and Compensated Advanced Chronic Liver Disease

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BACKGROUND & AIMS: Patients with advanced fibrosis related to nonalcoholic fatty liver disease (NAFLD) are at risk of developing hepatic and extrahepatic complications. We investigated whether, in a large cohort of patients with NAFLD and compensated advanced chronic liver disease, baseline liver stiffness measurements (LSMs) and their changes can be used to identify patients at risk for liver-related and extrahepatic events. **METHODS:** We performed a retrospective analysis of consecutive patients with NAFLD (n [1039) with a histologic diagnosis of F3-F4 fibrosis and/or LSMs >10 kPa, followed for at least 6 months, from medical centers in 6 countries. LSMs were made by FibroScan using the M or XL probe and recorded at baseline and within 1 year from the last follow-up examination. Differences between follow-up and baseline LSMs were categorized as: improvement (reduction of more than 20%), stable (reduction of 20% to an increase of 20%), impairment (an increase of 20% or more). We recorded hepatic events (such as liver decompensation, ascites, encephalopathy, variceal bleeding, jaundice, or hepatocellular carcinoma [HCC]) and overall and liver-related mortality during a median follow-up time of 35 months (interquartile range, 19-63 months).

RESULTS: Based on Cox regression analysis, baseline LSM was independently associated with occurrence of hepatic decompensation (hazard ratio [HR], 1.03; 95% CI, 1.02-1.04; P < .001), HCC (HR, 1.03; 95% CI, 1.00-1.04; P [.003), and liver-related death (HR, 1.02; 95% CI, 1.02-1.03; P [.005). In 533 patients with available LSMs during the follow-up period, change in LSM was independently

associated with hepatic decompensation (HR, 1.56; 95% CI, 1.05-2.51; P [.04), HCC (HR, 1.72; 95% CI, 1.01-3.02; P [.04), overall mortality (HR, 1.73; 95% CI, 1.11-2.69; P [.01), and liver-related mortality (HR, 1.96; 95% CI, 1.10-3.38; P [.02).

CONCLUSIONS: In patients with NAFLD and compensated advanced chronic liver disease, baseline LSM and change in LSM are associated with risk of liver-related events and mortality.

Key words: NASH; Steatohepatitis; cACLD; Prognostic Factor.

Nonalcoholic fatty liver disease (NAFLD) is the leading cause of chronic liver disease worldwide with a prevalence of about 25% in general population.^{1,2} The clinical relevance of NAFLD arises from the increased risk of developing both liver-related and extrahepatic complications.³⁻⁵

Recent long-term natural history studies and a meta-analysis pooling available evidence demonstrated that the severity of liver fibrosis and especially the presence of advanced fibrosis—defined as stage F3 or F4 fibrosis—is the main driver of prognosis in NAFLD, being the main risk factor for developing not only liver-related events but also extrahepatic complications.⁶⁻⁸ Along this line, noninvasive markers that can predict liver disease severity and outcomes in patients with NAFLD and advanced fibrosis are a major unmet need.

Liver stiffness measurement by FibroScan (EchoSens, Paris, France) is a noninvasive and widely available tool with validated diagnostic accuracy for advanced fibrosis in patients with NAFLD,⁹ which is also used in identifying patients at low risk for esophageal varices saving endoscopic screening¹⁰ as well as increases over time of LSM predicted liver-related events in patients with chronic hepatic C.¹¹

Data about the accuracy of LSM in the prediction of events in NAFLD, and especially in patients with NAFLD and F3-F4 fibrosis, are scarce. With this in mind, we investigated whether, in a large cohort of patients with NAFLD and compensated advanced chronic liver disease (cACLD), LSM at baseline and its changes during follow-up, are accurate for the prediction of liver-related and extrahepatic events.

Patients and Methods

Patient Selection

Data from 1039 patients and prospectively recruited at the first diagnosis of NAFLD with cACLD in 10 centers were retrospectively reviewed and analyzed. Inclusion and exclusion criteria were reported in supplemental material.

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 hospital Internal Review Boards and their Ethics Committees, and

written informed consent for the study was obtained from all patients.

Patient Evaluation

Clinical, anthropometric, biochemical and histological data were collected at the time of enrollment (more data are available in Supplementary Materials).

Follow-up visits, laboratory tests, ultrasound examination, esophageal gastroscopy, and management of both esophageal varices and HCC were performed as for guidelines.¹²⁻¹⁴

During follow-up, liver-related and extrahepatic events were recorded. Liver-related events were categorized as either liver decompensation (occurrence of ascites and/or bleeding varices and/or encephalopathy and/or jaundice) or development of HCC. They were also evaluated for liver transplantation, as were patients who experienced LD, when indicated.¹⁴ Extrahepatic events were categorized as either cardiovascular events (stroke, transient ischemic attack, myocardial infarction, unstable angina) or extrahepatic cancers. Evidence of extrahepatic events was provided by clinical charts from emergency areas or hospitalization. Death was also recorded and classified according to associated events (liver related, including liver transplantation, or unrelated).

Transient elastography was performed with the FibroScan (Echosens, Paris, France) medical device, using the M or XL probes. In each center,¹⁵ LSM was recorded within 3 months from blood tests and within 1 year from the last follow-up.

Statistics

To evaluate the occurrence of liver decompensation, HCC, cardiovascular events, extrahepatic cancers, and death, we included all consecutive patients who had at least 6 months of follow-up. Patients lost at follow-up (12% of the total population) were censored at the time of the last visit (more data are available in Supplementary Materials).

Continuous variables were summarized as mean SD, with categorical variables as frequency and percentage. D-LSM was defined as the difference between follow-up and baseline LSM and was categorized as <-20% (improvement), -20% to +20% (stable), and >+20% (impairment). This last criterion was used

because values above and below 15% were considered as a normal variability of the procedure (as defined per the interquartile to median ratio of 30%). Covariates used for the multivariate Cox model were chosen based on their significance in univariate analysis ($P < .10$). Variables in the final model with a P value of $< .05$ were considered statistically significant. In order to take into account the between-center heterogeneity, we fitted a random-effects (frailty) Cox model.

Analyses were performed using SPSS version 18 (IBM, Armonk, NY), and IDE software RStudio (version 3.4.1; RStudio, Boston, MA) for the R (version 2.1; R Foundation for Statistical Computing, Vienna, Austria) using the packages “timeROC” and “survival.”

Results

Clinical and Features and Liver Stiffness

Baseline characteristics of the 1039 patients with NAFLD and cACLD are shown in Table 1. The diagnosis of NAFLD was supported by histology in 550 (52.9%) cases, and 7.2% of the population had Child-Pugh class A6.

Baseline median LSM value was 17.6 kPa. LSM was obtained by using an M probe in 776 patients and an XL probe in 263 patients; as expected, mean body mass index (BMI) (34.4 ± 6.5 kg/m² vs 31.9 ± 5.8 kg/m²; $P < .001$) and the prevalence of obesity (75.2% vs 60.2%; $P < .001$) were significantly higher in patients with LSM by the M probe compared with those with LSM by the XL probe.

In a subgroup of 533 patients LSM within 1 year from the last follow-up and obtained by using the same probe used at baseline was available. These patients were older and had higher length of follow-up compared with those without LSM available at follow-up (Supplementary Table 1). Median delay between baseline and follow-up LSM was 37 months. In this group of patients, 53.3% experienced an improvement in follow-up LSM ($< 20\%$ from baseline), 27.2% had stable values, and 19.5% had an impairment $> 20\%$ in LSM values from baseline. Notably, among these 3 classes of patients, the presence of diabetes at baseline significantly predicted follow-up changes in LSM (56.8%, 68.2% and 71.1%, respectively; $P \leq .01$).

Liver-Related and Extrahepatic Outcomes

Absolute numbers and the actuarial incidence rates for hepatic and extrahepatic events are reported in Supplementary Table 2.

Prediction of Liver Decompensation by LSM

Independent variables predicting liver decompensation by Cox multivariate analysis included: age (hazard ratio [HR], 1.06; 95% confidence interval [CI], 1.02–1.09;

What You Need to Know

Background

It is not clear whether, in patients with nonalcoholic fatty liver disease (NAFLD) and compensated advanced chronic liver disease, baseline liver stiffness measurements (LSMs) or their changes can be used to identify patients at risk for liver-related and extrahepatic events.

Findings

In patients with NAFLD and compensated advanced chronic liver disease, baseline LSM and change in LSM are associated with risk of liver-related events and mortality.

Implications for patient care

LSMs should be made at multiple timepoints in patients with NAFLD and compensated cirrhosis to monitor disease progression.

$P \leq .001$), presence of Child-Pugh class A6 (HR, 3.04; 95% CI, 1.69–5.44; $P < .001$), platelet (PLT) count (HR, 0.98; 95% CI, 0.97–0.98; $P < .001$), and baseline LSM (HR, 1.03; 95% CI, 1.02–1.04; $P < .001$) (Table 2). When including in the model PLT count $< 150 \times 10^3/\text{mm}^3$ as categorical variable instead of PLT count as a continuous variable, similar results were observed for LSM, and PLT count $< 150 \times 10^3/\text{mm}^3$ remained significantly associated with liver decompensation (HR, 7.83; 95% CI, 2.51–21.3; $P < .001$). The time-dependent receiver-operating characteristic of baseline LSM in predicting liver decompensation was 0.76 (95% CI 0.68–0.83). The threshold of 21 kPa indicating clinically significant portal hypertension (CSPH)¹³ was confirmed independently associated with higher occurrence of liver decompensation (HR, 3.71; 95% CI, 1.89–6.78; $P < .001$) (Figure 1).

In patients with LSM available at follow-up, D-LSM (HR, 1.56; 95% CI, 1.05–2.51; $P \leq .04$) (Figure 2A), together with baseline LSM (HR, 1.03; 95% CI, 1.00–1.05; $P \leq .01$), significantly predicted the occurrence of liver decompensation (Table 2). Notably, the model including D-LSM better predicted decompensation than the model without (Harrell's C-index of 0.86 vs 0.83; $P \leq .03$). Figure 3A shows the crude rate of liver decompensation at the end of follow-up among D-LSM risk classes. When assessing the risk for liver decompensation in patients with or without CSPH by LSM, we found that D-LSM significantly predicted liver decompensation in patients without CSPH (HR, 3.85; 95% CI, 1.38–9.5; $P \leq .003$) (Figure 4A and B) but not in those with CSPH (HR, 1.45; 95% CI, 0.93–2.21; $P \leq .07$). Moreover, in patients without baseline CSPH (LSM < 21 kPa), the rate of liver decompensation occurrence was 6.5% in those who reached at follow-up an LSM value suggestive of CSPH,

Table 1. Baseline Demographic, Metabolic, Laboratory, and Instrumental Features of Patients With NAFLD and cACLD (N = 1039)

Age, y	60.3	10.7
Male, %	56.3	
BMI, kg/m ²	32.4	6.1
Obesity (BMI ≥ 30 kg/m ²)	66.3	
ALT, IU/L	62.8	50.3
PLT count, ×10 ³ /mm ³	186.6	74.3
Total bilirubin, mg/dL	0.7	0.4
INR	1.0	0.2
Albumin, g/L	4.2	0.4
Blood glucose, mg/dL	128.0	80.4
Total cholesterol, mg/dL	171.4	53.5
Triglycerides, mg/dL	150.5	99.4
Type 2 diabetes	60.8	
Arterial hypertension	68.2	
LSM, kPa	17.6	(13.1–26.1)
Child-Pugh class A5/A6	92.8	7.2
Time to follow-up, mo	35	(19–63)

NOTE. Values are mean ± SD, %, or median (interquartile range).

ALT, alanine aminotransferase; BMI, body mass index; cACLD, compensated advanced chronic liver disease; INR, international normalized ratio; LSM, liver stiffness measurement; NAFLD, nonalcoholic fatty liver disease; PLT, platelet.

and 2.3% in those in whom LSM did not reach this threshold ($P = .07$).

Monitoring LSM Does Predict HCC Occurrence

Female sex (HR, 0.30; 95% CI, 0.13–0.69; $P = .005$), age (HR, 1.06; 95% CI, 1.01–1.09; $P = .007$), and baseline LSM (HR, 1.03; 95% CI, 1.00–1.04; $P = .003$) were independent variables by Cox regression associated with the development of HCC (Table 2). When including in the model PLT count $<150 \times 10^3/\text{mm}^3$ as categorical variable instead of PLT count as a continuous variable, similar results were observed for LSM, and PLT count $<150 \times 10^3/\text{mm}^3$ was confirmed to be not significantly associated with HCC (HR, 0.99; 95% CI, 0.35–2.72; $P = .95$). The time-dependent area under the receiver-operating characteristic curve of baseline LSM in predicting HCC was clinically not acceptable (area under the receiver-operating characteristic curve, 0.66; 95% CI, 0.49–0.83).

In patients with LSM available at follow-up, D-LSM (HR, 1.72; 95% CI, 1.01–3.02; $P = .04$) (Figure 2B) but not baseline LSM (HR, 1.02; 95% CI, 0.98–1.05; $P = .27$) significantly predicted the occurrence of HCC (Table 2). Notably, the model including D-LSM better predicted decompensation than the model without (Harrell's C-

index of 0.84 vs 0.79; $P = .002$). Figure 3B shows the crude rate of HCC at the end of follow-up among D-LSM risk classes.

LSM Does Not Predict Extrahepatic Events Occurrence

Baseline LSM (HR, 1.01; 95% CI, 0.99–1.03; $P = .15$) and D-LSM (HR, 1.42; 95% CI, 0.78–2.59; $P = .24$) were not associated with occurrence of cardiovascular events at univariate Cox regression analysis.

Baseline LSM was associated with occurrence of extrahepatic neoplasm (HR, 1.02; 95% CI, 1.00–1.04; $P = .03$) in the univariate analysis but not in the multivariate Cox regression analysis (HR, 1.02; 95% CI, 0.99–1.04; $P = .42$). D-LSM was also not associated with the development of extrahepatic cancers (HR, 0.78; 95% CI, 0.42–1.45; $P = .44$) (Table 2).

D-LSM Predicted Overall and Liver-Related Mortality

Baseline LSM was not associated with overall mortality (HR, 1.01; 95% CI, 0.99–1.03; $P = .48$) (Table 2). In patients with LSM available at follow-up, D-LSM (HR, 1.73; 95% CI, 1.11–2.69; $P = .01$) (Figure 2C) and Child-Pugh class A6 vs A5 (HR, 4.09; 95% CI, 1.01–16.4; $P = .04$) but not baseline LSM (HR, 1.01; 95% CI, 0.97–1.04; $P = .46$) were independently associated with overall mortality (Table 2). Figure 3C shows the crude rate of overall death among D-LSM risk classes.

Age (HR, 1.06; 95% CI, 1.02–1.11; $P = .005$), PLT count (HR, 0.99; 95% CI, 0.98–0.99; $P = .01$), and baseline LSM (HR, 1.02; 95% CI, 1.00–1.03; $P = .005$) (time-dependent receiver-operating characteristic, 0.76; 95% CI, 0.60–0.91) were significant risk factors for liver-related death (Table 2). In patients with available D-LSM, age (HR, 1.06; 95% CI, 1.00–1.16; $P = .02$) and D-LSM (HR, 1.96; 95% CI, 1.10–3.38; $P = .02$) (Figure 2D) but not baseline LSM (HR, 1.02; 95% CI, 0.98–1.06; $P = .18$) were independent variables predicting liver-related death (Table 2). Notably, the model including D-LSM better predicted liver-related death than the model without (Harrell's C-index of 0.80 vs 0.77; $P = .03$). Figure 3D shows the crude rate of liver-related death among D-LSM risk classes.

Finally, neither baseline LSM (HR, 1.00; 95% CI, 0.97–1.03; $P = .75$) nor D-LSM (HR, 1.28; 95% CI, 0.59–2.75; $P = .52$) was associated with extrahepatic death at univariate Cox regression analysis.

Discussion

In the current study carried out in a large multicenter cohort of individuals with NAFLD and cACLD, and prospectively followed for a median time of 3 years, we found that baseline LSM accurately predicts liver

Table 2. Cox Regression Analysis of Factors Associated With Liver Events and Liver-Related Death in the Entire Cohort of NAFLD and cACLD

Group	Variable	Entire Cohort (N ¼ 1039)	Cohort With Availability of Follow-up LSM (n ¼ 533)
Liver decompensation	Age	1.06 (1.02–1.09), .001	1.06 (1.00–1.11), .02
	Child-Pugh class A6	3.04 (1.69–5.44), <.001	1.63 (0.49–5.28), .42
	PLT count	0.98 (0.97–0.98), <.001	0.98 (0.97–0.98), <.001
	Baseline LSM	1.03 (1.02–1.04), <.001	1.03 (1.00–1.05), .01
	D-LSM	—	1.56 (1.05–2.51), .04
Hepatocellular carcinoma	Female	0.30 (0.13–0.69), .005	0.28 (0.08–0.85), .02
	Age	1.06 (1.01–1.09), .007	1.04 (0.98–1.10), .13
	PLT count	1.00 (0.99–1.00), .25	1.00 (0.99–1.00), .73
	Child-Pugh class A6	0.80 (0.25–2.49), .71	3.25 (0.80–13.1), .09
	Baseline LSM	1.03 (1.00–1.04), .003	1.02 (0.98–1.05), .27
	D-LSM	—	1.72 (1.01–3.02), .04
Cardiovascular event	Female	0.46 (0.21–0.96), .04	0.18 (0.03–0.78), .02
	Age	1.03 (0.99–1.07), .08	1.06 (0.99–1.13), .07
	Arterial hypertension	2.16 (0.81–5.72), .12	3.03 (0.67–13.6), .15
Extrahepatic cancer	Age	1.04 (0.99–1.08), .06	1.04(0.98–1.09), .19
	Child-Pugh class A6	1.78 (0.51–6.07), .36	1.12 (0.13–9.46), .92
	Baseline LSM	1.02 (0.99–1.04), .12	1.01 (0.97–1.04), .756
Overall death	Female	0.62 (0.33–1.14), .13	0.60 (0.27–1.33), .21
	Age	1.04 (1.01–1.08), .01	1.04 (0.99–1.08), .09
	BMI	0.91 (0.84–0.97), .006	0.93 (0.85–1.02), .12
	Child-Pugh class A6	4.22 (1.83–9.71), <.001	4.09 (1.01–16.4), .04
	PLT count	1.00 (0.99–1.00), .21	1.00 (0.99–1.00), .78
	Baseline LSM	1.01 (0.99–1.03), .18	1.01 (0.97–1.04), .46
	D-LSM	—	1.73 (1.11–2.69), .01
Liver-related death	Age	1.06 (1.02–1.11), .005	1.06 (1.00–1.16), .02
	Child-Pugh class A6	1.71 (0.60–4.13), .36	2.12 (0.31–11.5), .49
	PLT count	0.99 (0.98–0.99), .01	0.99 (0.98–1.00), .34
	Baseline LSM	1.02 (1.00–1.03), .005	1.02 (0.98–1.06), .18
	D-LSM	—	1.96 (1.10–3.38), .02

NOTE. Values are hazard ratio (95% confidence interval), *P* value.

BMI, body mass index; cACLD, compensated advanced chronic liver disease; LSM, liver stiffness measurement; NAFLD, nonalcoholic fatty liver disease; PLT, platelet.

decompensation and liver-related death, while changes over time in LSM (D-LSM) can further stratify the risk of development of liver-related complications.

In our study, liver-related events were the most frequently observed complications (6.8% liver decompensation, 3.4% HCC), followed by cardiovascular events (3.4%) and extrahepatic cancers (2.4%). Moreover, we observed an overall death rate of 5.4%, mostly due to liver-related causes (3.2%). Long-term studies investigating the natural history of patients with biopsy-proven

NAFLD reported cardiovascular events and extrahepatic cancers as the 2 most frequent causes of death, even if the observed higher increase in the relative risk of death was showed for liver-related causes.^{16,17} The occurrence rates of hepatic and extrahepatic outcomes that we reported differ with respect to other studies,^{16,17} perhaps due to the selection of a population with cACLD, already committed for a higher risk of liver-related complications. Baseline LSM values accurately predicted the occurrence of liver decompensation. This result was

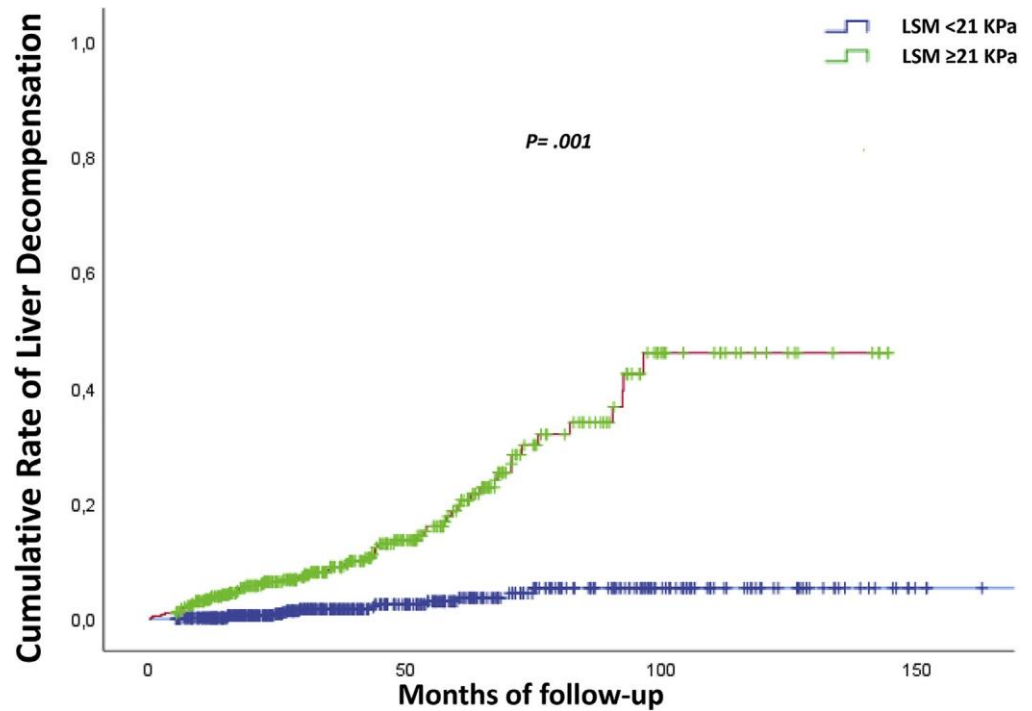


Figure 1. Occurrence of liver decompensation in the entire cohort of NAFLD patients with cACLD according to LSM value of 21 kPa indicating a high risk of CSPH. *P* value by log-rank test.

maintained after adjusting for the severity of liver disease (Child-Pugh class A5 vs A6) and for surrogate markers of portal hypertension (PLT count). Notably, we found that when using the LSM threshold of 21 kPa, validated as indicating a high risk for CSPH,¹³ also in a

setting of patients at risk for decompensation because of with cACLD, we identified 2 different populations, one at low (2%) and another at high (14%) risk of hepatic decompensation. Our study agrees with recent evidence that higher baseline LSM values can predict

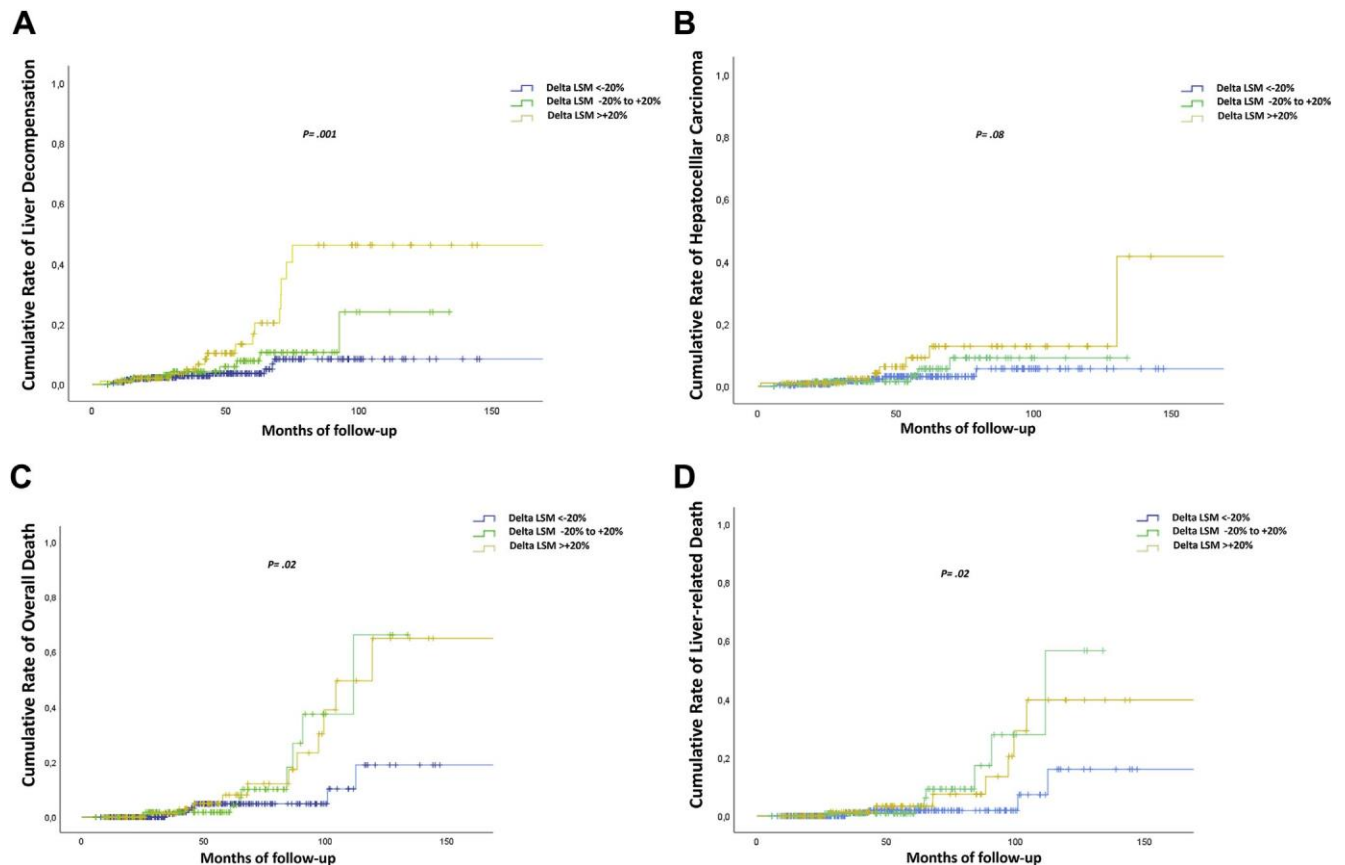


Figure 2. D-LSM risk classes and occurrence of liver-related events and death in the entire cohort of NAFLD patients with cACLD. (A) Liver decompensation, (B) HCC, (C) overall death, (D) liver-related death. *P* value by log-rank test.

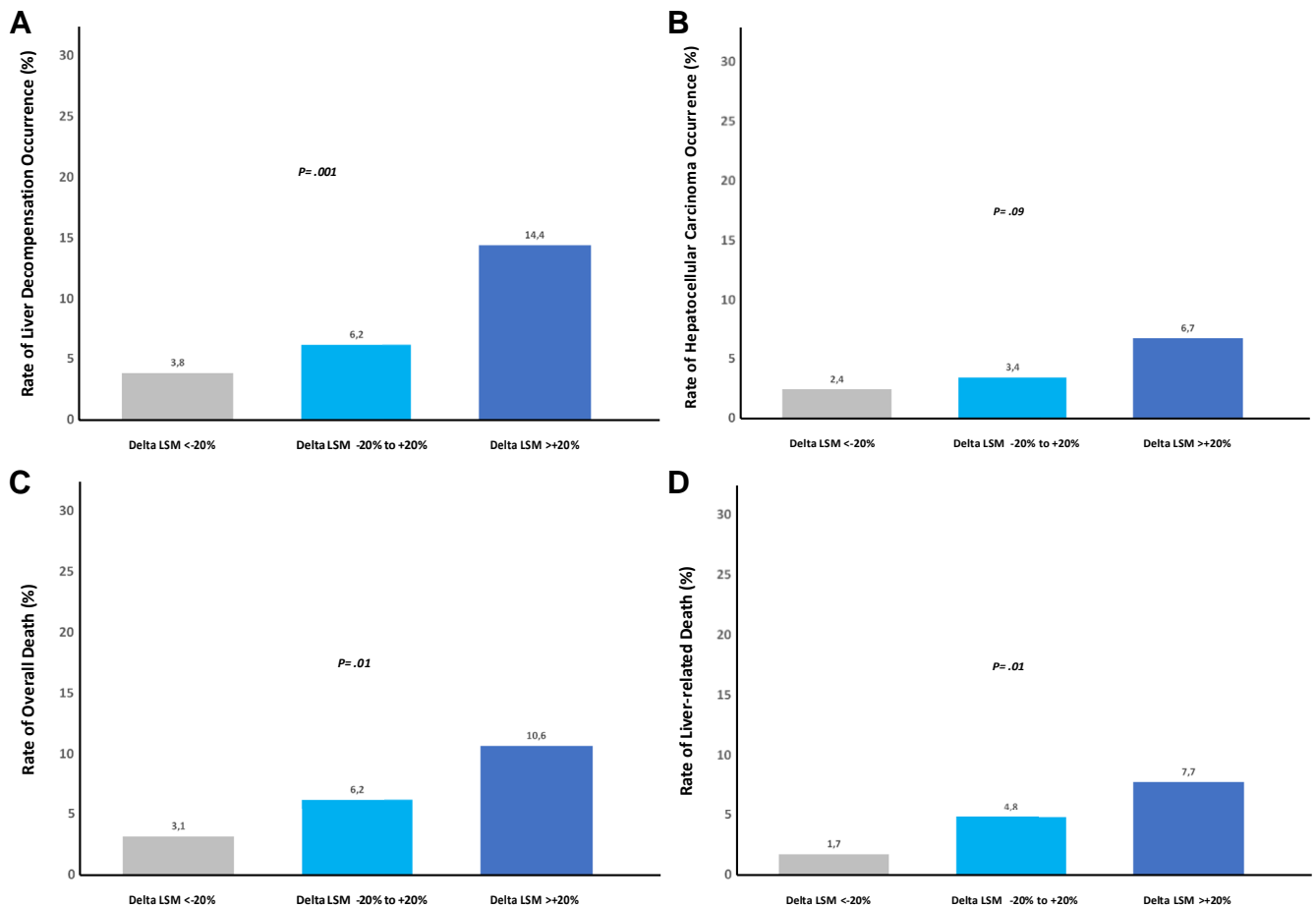


Figure 3. Crude rate of liver-related events and death at the end of follow-up according to D-LSM risk classes in the entire cohort of NAFLD patients with cACLD. (A) Liver decompensation, (B) HCC, (C) overall death, (D) liver-related death. *P* value by log-rank test.

the development of liver-related events in NAFLD.¹⁸ However this last study included a smaller cohort of patients with NAFLD and advanced liver disease, did not consider separately liver decompensation and HCC, and did not explore the clinical utility of LSM in the at high-risk setting of patients with severe fibrosis or compensated cirrhosis.¹⁹ Another relevant finding of our study is that D-LSM can further stratify the risk for liver decompensation. We demonstrated a progressive increase in the probability of hepatic decompensation from 3.8% in patients with improved KPa of at least 20%, to 6.2% in stable kPa -20% to 20%, and further to 14.4% in those with impaired LSM >20% from baseline. Notably, when stratifying patients according to the risk of CSPH, we showed that while in patients at high CSPH risk the D-LSM no longer predicted hepatic decompensation, its predictability was maintained in patients at low risk of CSPH at baseline, and indeed, LSM improvement was associated with no hepatic decompensation, while the risk progressively increased to 3.2% in stable stiffness, and further to 10% when LSM was impaired.

Baseline LSM values were independently associated with the occurrence of HCC, even if the overall accuracy was not clinically acceptable. Consistent with our results,

a recent study in NAFLD patients at any stage of liver fibrosis showed a significant link between HCC risk and LSM values, but the authors could not find accurate specific cutoffs to predict HCC occurrence.¹⁸ D-LSM but not baseline LSM showed an independent association with the risk of developing HCC: from 2.4% in improvement to 3.4% in stable and further to 6.7% when there was impaired stiffness.

After adjusting for confounders, we found an independent association between baseline LSM and liver-related mortality but not overall mortality. The good prediction ability of baseline LSM for liver-related mortality was also demonstrated in 2 independent studies focusing on patients with clinical diagnosis of NAFLD at any stage of liver fibrosis.^{19,20} Regarding the association between overall mortality and baseline LSM, one study reported a lower diagnostic performance of baseline LSM with respect to the prediction of liver-related mortality,¹⁹ while another study showed good performance in predicting overall mortality.¹⁸ Differences in the baseline prevalence of liver disease severity and, consequently in the incidence of hepatic and extrahepatic events leading to mortality, can explain the observed differences among studies. Notably, when in our cohort we considered D-LSM, we found that it could significantly stratify the risk

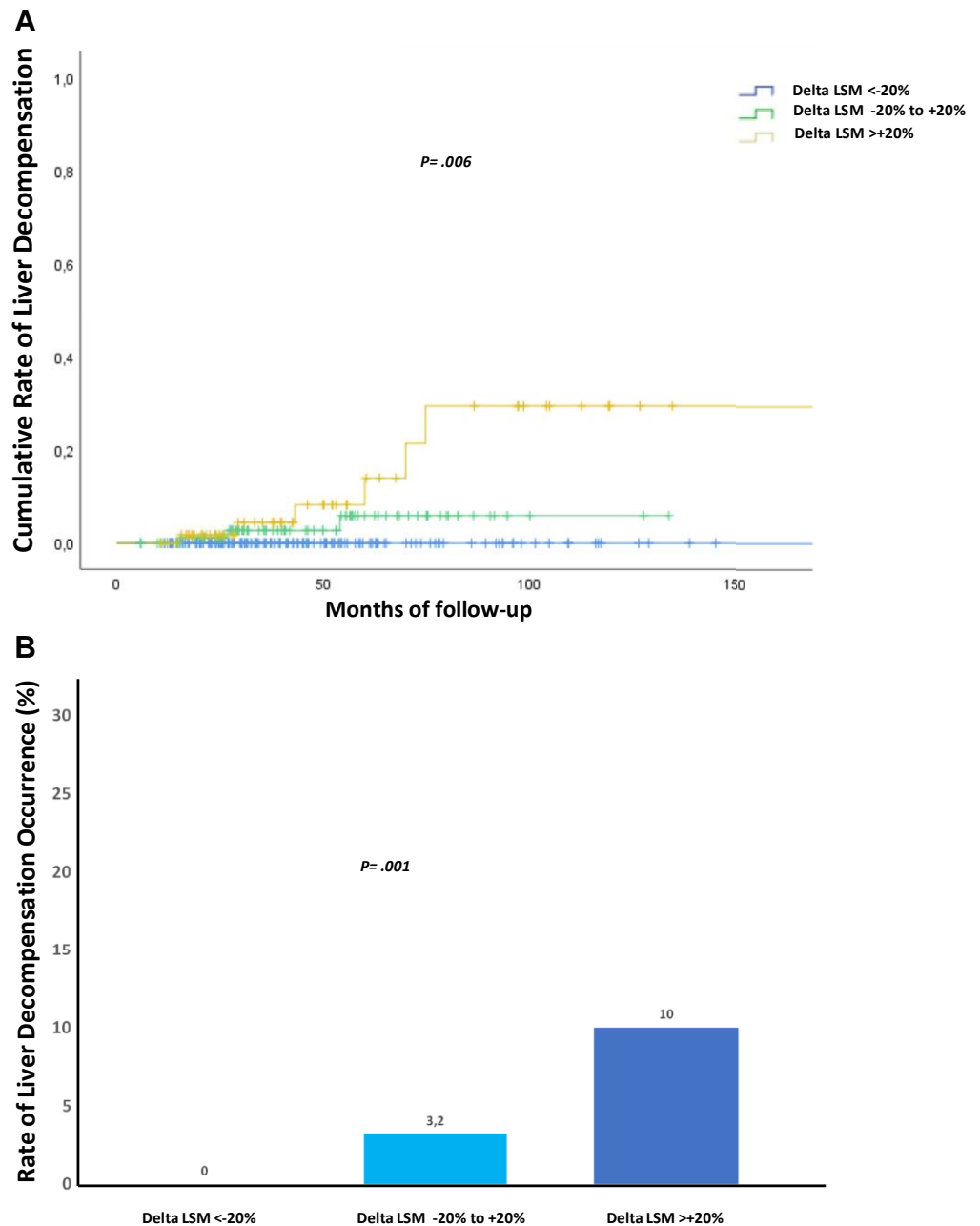


Figure 4. (A) Occurrence and (B) crude rate of liver decompensation in the subgroup of patients with NAFLD and without CSPH by LSM (LSM <21 kPa).

of both overall and hepatic death, suggesting that impairment in liver disease severity can also increase the risk for extrahepatic mortality, as also suggested in a recent meta-analysis.⁶

We observed that 53% of patients with paired LSM had LSM improvement defined as LSM reduction >20% from baseline, this percentage being higher than that reported in literature for at least 1-stage fibrosis regression in patients with paired liver biopsies.²¹ However, it is well known in the literature that LSM in NASH not only is an expression of hepatic fibrosis, but also is directly associated with alanine aminotransferase (ALT) levels—as an expression of liver inflammation—and BMI.²² Consistently, the reduction of at least 20% that we observed in about half of NASH patients

with paired LSM can be considered as a surrogate of improvement in liver damage (fibrosis and/or inflammation) or in its risk factors like obesity. Unfortunately, this is only a plausible hypothesis because data on ALT and BMI at follow-up were not available.

From a clinical point of view, our study suggests that in a setting of patients with NAFLD at high risk of hepatic complications because of cACLD, a dynamic and integrated evaluation of baseline LSM together with D-LSM can help in stratifying the risk of liver decompensation, while D-LSM alone, not baseline LSM, could better stratify the risk of HCC occurrence and of both hepatic and extrahepatic death (Supplementary Figure 1). We can hypothesize that LSM impairment over time can be expression of an impairment in liver disease severity in

terms of fibrosis, inflammation, steatosis, and portal hypertension.^{10,22,23} Notably, we found that the presence of diabetes at baseline indicates a higher risk of D-LSM impairment. These data agree with the available literature identifying diabetes as a risk factor for liver disease progression and liver-related complications.^{24–26}

In our study, we did not find any significant independent association between baseline LSM or D-LSM and the occurrence of cardiovascular events and extrahepatic cancers. Our results agree with data reported in a cohort of NAFLD patients at any stage of liver damage, in which baseline LSM was not associated with extrahepatic cancers while showing a statistically significant association but not clinically acceptable accuracy for cardiovascular event development.¹⁸

The main limitation of this study lies in the potentially limited external validity of the results for different populations and settings. Our study included a large cohort of patients with NAFLD and advanced liver fibrosis followed at tertiary care centers. Another relevant limitation is the retrospective design of the study, and the not standardized protocol of LSM follow-up potentially leading to a selection bias. The lack of data about follow-up clinical variables including biochemical tests like ALT—expression of liver inflammation—and BMI could further limit the interpretation of our results. In particular, weight loss leading to BMI reduction is known to be associated with NASH resolution and fibrosis improvement in NAFLD patients,²⁷ and ALT normalization has been identified as a predictor of histological improvement in NASH²⁸; consistently, the lack of data about the effect of ALT and BMI changes on liver-related outcomes can limit the strength of our results about LSM changes and prognosis in NAFLD population. In fact, D-LSM could be expression of factors also influencing the natural history of liver disease such as weight changes, transaminase fluctuations, or reflecting progression of liver disease such as changes in PLT count and in liver function indexes. Finally, hidden alcohol intake at baseline and during follow-up, and lack of data about baseline and follow-up use of nonselective beta-blockers, could further affect the observed results.

In conclusion, this study conducted in a multicenter cohort of patients with NAFLD and cACLD showed that an integrated assessment of baseline LSM or D-LSM can help in stratifying the risk of development of liver-related complications and of both hepatic and overall mortality. These data, if further validated, could help personalize prognosis and follow-up in NAFLD with cACLD.

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Supplementary Material

Patient Selection

Inclusion criteria were presence of a reliable liver stiffness measurement (LSM) within 6 months of nonalcoholic fatty liver disease (NAFLD) diagnosis, NAFLD with F3 or F4 fibrosis by histology,¹ or LSM >10 kPa obtained by FibroScan machine (Echosens, Paris, France) by using an M or XL probe.² In patients without histology, diagnosis of NAFLD required detection of liver hyperechogenicity by ultrasound plus at least 1 criterion of the metabolic syndrome (obesity, diabetes, arterial hypertension, dyslipidemia). Other causes of liver disease were ruled out, including alcohol intake >20 g/d during the previous year (evaluated by interview of patients on amount, frequency and type, and confirmed by at least 1 family member), viral (hepatitis B surface antigen, anti-hepatitis C virus, and anti-HIV negativity), and autoimmune hepatitis, hereditary hemochromatosis, and alpha-1 antitrypsin deficiency. Patients included in pharmacological trials for nonalcoholic steatohepatitis treatment, or with advanced (Child-Pugh class B or C) cirrhosis, hepatocellular carcinoma, liver transplantation, esophageal varices banding as secondary prophylaxis, portal or splenic vein thrombosis, and splenectomy, were excluded. The study cohort finally included 269 patients (recruitment March 2004 to October 2018) from the Centre d'Investigation de la Fibrose Hépatique, Bordeaux University Hospital; 146 patients (recruitment February 2008 to January 2019) from the Division of Gastroenterology and Hepatology, McGill University Health Centre of Montreal; 124 patients (recruitment September 2010 to October 2018) from the Hepatology Unit, Ospedale San Giuseppe University of Milan; 122 patients (recruitment July 2007 to December 2018) from the Section of Gastroenterology and Hepatology, University of Palermo; 102 patients (recruitment September 2010 to October 2018) from the Hospital Universitario Virgen del Rocío de Sevilla; 90 patients (recruitment July 2006 to November 2017) from the Department of Medicine and Therapeutics, The Chinese University of Hong Kong; 79 patients (recruitment April 2004 to October 2018) from the Hepato-Gastroenterology Department of Angers University Hospital; 57 patients (recruitment January 2008 to July 2018) from the Swiss Liver Center; 25 patients (recruitment September 2008 to February 2019) from the Division of Gastroenterology, Department of Medical Sciences, University of Torino; and 18 patients (recruitment July 2008 to October 2018) from the Department of Pathophysiology and Transplantation, Ca' Granda IRCCS Foundation, Policlinico Hospital, University of Milan. A proportion of patients (n=348) was already included in a published study assessing LSM as predictor of events in patients with NAFLD at any stage of liver disease.³

Patient Evaluation

Clinical, anthropometric, biochemical, and histological data were collected at the time of enrollment.

Body mass index (BMI) was calculated. Obesity was defined as BMI ≥ 30 kg/m². The diagnosis of type 2 diabetes was based on the revised criteria of the American Diabetes Association, using values of fasting blood glucose ≥ 126 mg/dL.⁴ In patients with a previous diagnosis of type 2 diabetes, current therapy with insulin or oral hypoglycemic agents was documented. After a 12-hour overnight fasting blood sample was drawn to determine serum levels of aspartate aminotransferase, alanine aminotransferase, platelet count, albumin, total bilirubin, international normalized ratio, total cholesterol, triglycerides, and plasma glucose concentration. The Kleiner classification¹ was used to stage fibrosis from 0 to 4.

Follow-up visits and laboratory tests were done at baseline and repeated at 6-month intervals. Ultrasound examination was carried out every 6 months according to international guidelines.⁵ In the presence of cirrhosis, esophageal gastroscopy was performed at baseline and repeated as recommended by clinical guidelines.⁶ Patients with progression to medium or large (F2 or F3) esophageal varices were treated with b-blockers or underwent elastic banding, while no prophylaxis was scheduled for patients with small (F1) varices.⁶

During follow-up, liver-related and extrahepatic events were recorded. Liver-related events were categorized as either liver decompensation (occurrence of ascites and/or bleeding varices and/or encephalopathy and/or jaundice) or development of HCC. Patients who had a diagnosis of HCC during the follow-up were evaluated for available therapies (surgical resection, radiofrequency ablation, transarterial chemoembolization, or treatment with sorafenib starting in 2007), as indicated in the guidelines.⁵ They were also evaluated for liver transplantation, as were patients who experienced liver disease, when indicated.⁷ Extrahepatic events were categorized as either cardiovascular events (stroke, transient ischemic attack, myocardial infarction, unstable angina) or extrahepatic cancers. Evidence of extrahepatic events was provided by clinical charts from emergency areas or hospitalization. Death was also recorded and classified according to associated events (liver related, including liver transplantation, or unrelated).

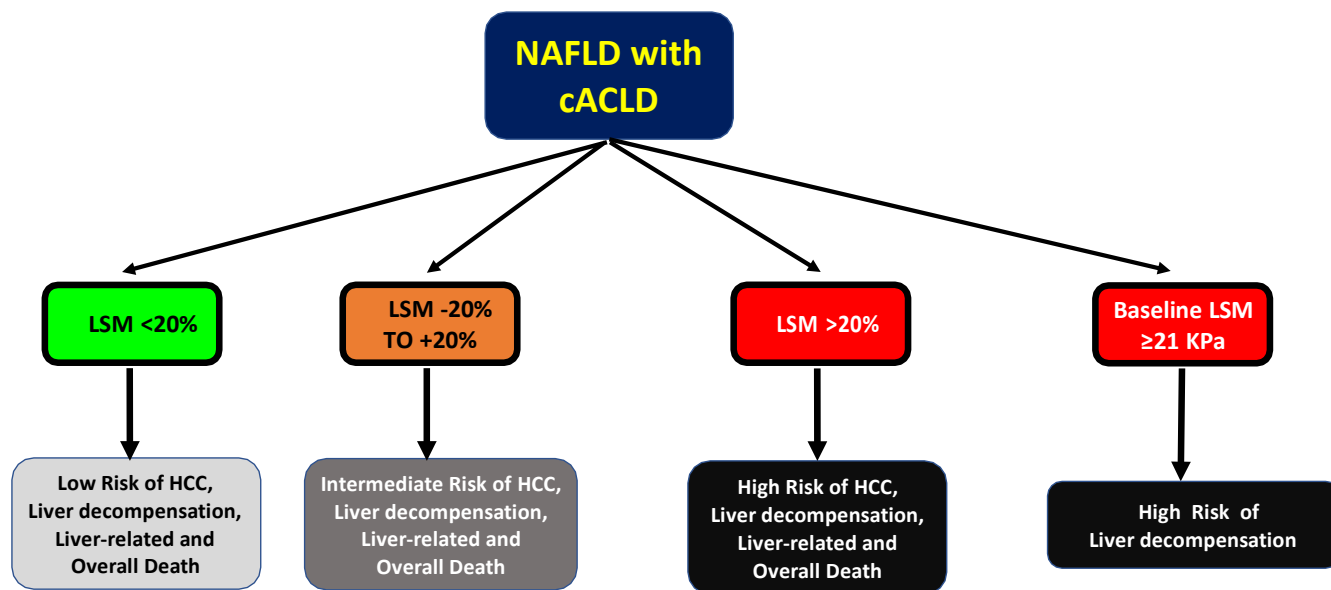
Transient elastography was performed with the FibroScan (EchoSens, Paris, France) medical device, using the M or XL probes. In each center, LSM was assessed after at least 4 hour fasting, by a trained operator who had previously performed at least 300 determinations in patients with chronic liver disease. Only patients with 10 valid measurements and with reliable results according to published criteria were enrolled.⁸ LSM was recorded within 3 months from blood tests and within 1 year from the last follow-up.

Statistics

To evaluate the occurrence of liver decompensation, HCC, cardiovascular events, extrahepatic cancers, and death, we included all consecutive patients who had at least 6 months of follow-up. Patients lost at follow-up (12% of the total population) were censored at the time of the last visit.

Continuous variables were summarized as mean SD, and categorical variables as frequency and percentage. The time-dependent receiver-operating characteristic curve was used to estimate the area under the receiver-operating characteristic curve, which expresses the diagnostic power of the LSM variable associated with the occurrence of events. Covariates used for the multivariate Cox model were sex, age, obesity, diabetes, arterial hypertension, platelet count, albumin, Child-Pugh class, baseline LSM, and D-LSM, which was defined as the difference between follow-up and baseline LSM and was categorized as $<-20\%$ (improvement), -20% to $+20\%$ (stable), and $>+20\%$ (impairment). This last criterion was used because values above and below 15% were considered as a normal variability of the procedure (as defined per the interquartile range-to-median ratio of 30%). Child-Pugh class and albumin were not included in the same models to avoid collinearity. They were chosen based on their significance in univariate analysis ($P < .10$). Variables in the final model with a P value of $<.05$ were considered statistically significant. In order to take into account the between-center heterogeneity we fitted a random-effects (frailty) Cox model. The results are expressed as adjusted hazard ratios and their 95% confidence intervals. The concordance between an observed response and multivariate predictor was calculated by Harrell's C-index with 95% confidence interval.

Analyses were performed using SPSS version 18.0 (IBM, Armonk, NY), and IDE software RStudio (version 3.4.1; RStudio, Boston, MA) for R software (version 2.1; R Foundation for Statistical Computing, Vienna, Austria) using the packages "timeROC" and "survival."



Supplementary Figure 1. Proposed algorithm to stratify the risk of complications in patients with compensated advanced chronic liver disease (cACLD) by using baseline and delta liver stiffness measurement (D-LSM). HCC, hepatocellular carcinoma; NAFLD, nonalcoholic fatty liver disease.

Supplementary Table 1. Baseline Demographic, Metabolic, Laboratory, and Instrumental Features of Patients With NAFLD and cACLD With and Without Availability of Follow-Up LSM

	NAFLD with cACLD and available LSM at follow-up (n = 533)	NAFLD with cACLD and not available LSM at follow-up (n = 506)	P value
Age, y	61.1 9.8	59.5 11.5	.01
Male	56.1	56.5	.89
BMI, kg/m ²	32.5 6.0	32.4 6.1	.87
Obesity (BMI ≥30 kg/m ²)	65.2	61.7	.23
ALT, IU/L	62.8 56.6	62.8 42.6	.98
PLT count, 10 ³ /mm ³	182.4 75.7	191.5 72.6	.04
Total bilirubin, mg/dL	0.7 0.4	0.7 0.4	.70
INR	1.1 0.2	1.0 0.2	.11
Albumin, g/L	4.2 0.4	4.2 0.4	.46
Blood glucose, mg/dL	118.7 37.4	138.7 27.4	.43
Total cholesterol, mg/dL	178.8 40.2	166.3 60.8	.002
Triglycerides, mg/dL	154.6 90.3	147.83 105.3	.28
Type 2 diabetes	62.9	58.9	.21
Arterial hypertension	67.0	69.5	.36
Baseline LSM, kPa	18.4 (13.8–26.3)	17 (12.4–25.7)	.95
Follow-up LSM, kPa	14.3 (9.4–23.6)	—	—
Child-Pugh class A5–A6	96.2/3.8	89.4/10.6	<.001
Time of follow-up, mo	42 (26–64)	26 (14–61)	<.001
Liver decompensation occurrence	35 (6.5)	36 (7.1)	.72
Hepatocellular carcinoma occurrence	18 (3.3)	17 (3.3)	.98
Cardiovascular event occurrence	15 (2.8)	20 (3.9)	.30
Extrahepatic cancer occurrence	18 (3.4)	7 (1.4)	.03
Overall death	29 (5.4)	27 (5.3)	.94
Liver-related death	20 (3.8)	13 (2.5)	.27

NOTE. Values are mean (SD, %, or median (interquartile range)).

ALT, alanine aminotransferase; BMI, body mass index; cACLD, compensated advanced chronic liver disease; INR, international normalized ratio; LSM, liver stiffness measurement; NAFLD, nonalcoholic fatty liver disease; PLT, platelet.

Supplementary Table 2. Hepatic and Extrahepatic Events Recorded During Follow-Up in NAFLD Patients With cACLD (N = 1039)

Liver events	
Liver decompensation	71 (6.8)
Liver decompensation rate	
1 y	1.5
2 y	2.8
3 y	4.5
5 y	8.9
10 y	18.2
Hepatocellular carcinoma	35 (3.4)
Hepatocellular carcinoma rate	
1 y	0.7
2 y	1.2
3 y	2.0
5 y	4.6
10 y	9.4
Extrahepatic events	
Cardiovascular events	35 (3.4)
Cardiovascular event rate	
1 y	0.9
2 y	1.3
3 y	2.0
5 y	4.4
10 y	12.2
Extrahepatic cancer	25 (2.4)
Extrahepatic cancer rate	
1 y	0.5
2 y	1.2
3 y	1.7
5 y	3.1
10 y	5.4
Death	
Overall death	56 (5.4)
Overall death rate	
1 y	0
2 y	1
3 y	2.9
5 y	5.1
10 y	26.3
Liver-related death	33 (3.2)
Liver-related death rate	
1 y	0
2 y	0.2
3 y	1.6
5 y	2.4
10 y	19.8

NOTE. Values are n (%) or %.
cACLD, compensated advanced chronic liver disease; NAFLD, nonalcoholic fatty liver disease.