Original research

Liver stiffness thresholds to predict disease progression and clinical outcomes in bridging fibrosis and cirrhosis

Rohit Loomba ,[1,2] Daniel Q. Huang ,[2,3,4] Arun J Sanyal,[5] Quentin Mark Anstee,[6] Michael Trauner ,[7] Eric J Lawitz,[8] Dora Ding,[9] Lily Ma,[9] Catherine Jia,[9] Andrew Billin,[9] Ryan S Huss,[9] Chuhan Chung,[9] Zachary Goodman,[10] Vincent Wai-Sun Wong ,[11] Takeshi Okanoue,[12] Manuel Romero-Gómez,[13] Manal F Abdelmalek,[14] Andrew Muir,[15] Nezam Afdhal,[16] Jaime Bosch,[17] Stephen Harrison,[18,19] Zobair M Younossi ,[20] Robert P Myers[9]

► Additional supplemental material is published online only. To view, please visit the journal online (http://dx.doi.org/10.1136/gutjnl-2022-327777).

For numbered affiliations see end of article.

Correspondence to
Professor Rohit Loomba, University of California San Diego, La Jolla, California, USA; roloomba@ucsd.edu

Received 3 May 2022
Accepted 8 August 2022

To cite: Loomba R, Huang DQ, Sanyal AJ, *et al*. *Gut* Epub ahead of print: [*please include* Day Month Year]. doi:10.1136/gutjnl-2022-327777

ABSTRACT

Objective In retrospective studies, liver stiffness (LS) by vibration-controlled transient elastography (VCTE) is associated with the risk of liver decompensation in patients with non-alcoholic steatohepatitis (NASH), but prospective data in biopsy-confirmed cohorts with advanced fibrosis are limited. We aimed to establish thresholds for LS by VCTE that predict progression to cirrhosis among patients with bridging fibrosis and hepatic decompensation among patients with cirrhosis due to NASH.

Design We used data from four randomised placebo-controlled trials of selonsertib and simtuzumab in participants with advanced fibrosis (F3–F4). The trials were discontinued due to lack of efficacy. Liver fibrosis was staged centrally at baseline and week 48 (selonsertib study) or week 96 (simtuzumab study). Associations between LS by VCTE with disease progression were determined using Cox proportional hazards regression analysis.

Results Progression to cirrhosis occurred in 16% (103/664) of participants with bridging fibrosis and adjudicated liver-related events occurred in 4% (27/734) of participants with baseline cirrhosis. The optimal baseline LS thresholds were ≥16.6 kPa for predicting progression to cirrhosis, and ≥30.7 kPa for predicting liver-related events. Baseline LS ≥16.6 kPa (adjusted HR 3.99; 95% CI 2.66 to 5.98, p<0.0001) and a ≥5 kPa (and ≥20%) increase (adjusted HR 1.98; 95% CI 1.20 to 3.26, p=0.008) were independent predictors of progression to cirrhosis in participants with bridging fibrosis, while baseline LS ≥30.7 kPa (adjusted HR 10.13, 95% CI 4.38 to 23.41, p<0.0001) predicted liver-related events in participants with cirrhosis.

Conclusion The LS thresholds identified in this study may be useful for risk stratification of NASH patients with advanced fibrosis.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Retrospective studies report that increasing liver stiffness as assessed by vibration-controlled transient elastography is associated with the increased risk of liver disease progression in patients with non-alcoholic fatty liver disease. However, there are limited prospective data in well-characterised patients with biopsy-confirmed non-alcoholic steatohepatitis (NASH) with bridging fibrosis and cirrhosis regarding the optimal cut-points associated with higher risk of progression to cirrhosis, and hepatic decompensation among patients with cirrhosis.

WHAT THIS STUDY ADDS

⇒ In this analysis of four large, prospective, international, multicentre, randomised placebo-controlled trials of participants with NASH and biopsy-proven advanced fibrosis (F3–F4), clinical disease progression was associated with higher liver stiffness by vibration-controlled transient elastography at baseline. We identified optimal liver stiffness thresholds for predicting progression to cirrhosis among patients with bridging fibrosis, and development of liver-related events among patients with compensated cirrhosis. In addition, we determined that a≥5 kPa (and ≥20%) increase in liver stiffness was associated with progression to cirrhosis, among participants with bridging fibrosis at baseline.

INTRODUCTION

Nearly one-third of the world's adult population has non-alcoholic fatty liver disease (NAFLD).[1 2] NAFLD encompasses both non-alcoholic fatty liver and non-alcoholic steatohepatitis (NASH), the inflammatory form of NAFLD that can progress to fibrosis, cirrhosis and subsequent decompensation.[3–7] The incidence of NASH cirrhosis and NAFLD-related hepatocellular carcinoma (HCC) are projected to increase rapidly in the next decade.[8–13]

The risk of liver-related mortality and decompensation in NASH increases in parallel with fibrosis stage.[14–17] Although histological staging of fibrosis is the reference standard, liver biopsy is limited

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ The liver stiffness thresholds identified in this study will be useful for risk stratification of patients with NASH in clinical trials and in clinical practice. High-risk patients identified using these thresholds could be offered increased clinical surveillance or targeted for enrolment in clinical trials for treatment of NASH-related fibrosis and cirrhosis. These data further support the use of non-invasive biomarkers as a surrogate to predict the risk of clinically meaningful outcomes.

by invasiveness, potential complications and sampling variability.[18 19] Non-invasive tests (NITs) of fibrosis, including serum markers such as NAFLD fibrosis score (NFS), fibrosis-4 (FIB-4) index, enhanced liver fibrosis (ELF) test and liver stiffness (LS) by vibration-controlled transient elastography (VCTE) are not prone to these limitations, and accurately predict histological fibrosis stage in patients with NASH.[20–28] Retrospective studies report that increasing baseline LS by VCTE[20 29 30] is associated with the risk of disease progression in patients with NAFLD, but prospective data in well-characterised NASH cohorts with biopsy-confirmed advanced fibrosis are limited. The optimal LS thresholds for prognostication of fibrosis progression and decompensation are unknown, however, a recent consensus report from the Baveno group has proposed incrementally increasing LS thresholds.[31]

With these considerations in mind, we analysed data from two recent phase three placebo-controlled trials of selonsertib, a selective inhibitor of apoptosis signal-regulating kinase 1 (ASK1) and two phase 2b placebo-controlled trials of simtuzumab, a humanised monoclonal antibody directed against lysyl oxidase-like 2.[32 33] While the studies were discontinued prematurely due to lack of efficacy, the prospectively collected data in these well characterised participants with serial liver biopsies provides a unique opportunity to study the association between baseline LS by VCTE and disease progression. The primary aim of this study was to establish thresholds of LS that prognosticate risk of clinical outcomes in participants with bridging fibrosis and cirrhosis due to NASH. The secondary aims were to examine the association between a change in LS and clinical outcomes, and to compare the prognostic ability of baseline LS versus baseline LS plus a combination of routine clinical parameters (age, sex, aspartate aminotransferase (AST), alanine aminotransferase (ALT), platelets and diabetes status), included within the Agile 3+ and Agile 4 scores, to define risk for fibrosis progression and decompensation.[34 35]

METHODS

Study population

This analysis used data from four large, randomised placebo-controlled trials of selonsertib and simtuzumab in participants with advanced (F3–F4) fibrosis due to NASH.[32 33] Preplanned analysis of the selonsertib studies at 48 weeks and the simtuzumab studies at 96 weeks concluded that these therapies were ineffective, and therefore, the trials were discontinued. At baseline, there were no differences between treatment groups; thus, treatment groups were combined for the present analysis. The primary findings of these studies, as well as the detailed methods, have been reported elsewhere.[32 33]

Briefly, the selonsertib STELLAR studies comprised of two randomised, double-blind, placebo-controlled, phase III trials conducted in Europe, North America, South America, Asia and the Pacific region. Eligible participants were 18–70 years of age with a histological diagnosis of NASH, available data for baseline LS by VCTE and advanced fibrosis (F3–F4). Patients with liver disease of other etiologies, a history of solid-organ transplantation, hepatic decompensation or HCC were excluded. Participants with bridging (F3) fibrosis (n=808; NCT03053050) and compensated cirrhosis (F4) (n=883; NCT03053063) were randomised 2:2:1 to receive selonsertib 18 mg, selonsertib 6 mg or placebo once daily for 48 weeks. The primary efficacy endpoint was the proportion of participants with ≥1 stage improvement in fibrosis without worsening of NASH at week 48.

The simtuzumab studies consisted of two phase 2b trials in North America and Europe. Eligible participants were 18–65 years of age with a body mass index (BMI) of at least 18 kg/m^2 with NASH and bridging fibrosis, NASH cirrhosis or cirrhosis with at least one clinical feature suggestive of NASH (such as diabetes, obesity or dyslipidaemia). Participants with liver disease of other aetiologies, a history of solid-organ transplantation, a history of malignancy other than non-melanomatous skin cancer and hepatic decompensation were excluded. Participants with bridging (F3) fibrosis (n=219; NCT01672866) were randomly assigned (1:1:1) to groups given weekly subcutaneous injections of simtuzumab (75 or 125 mg) or placebo for a planned duration of 240 weeks. The primary outcome was a decrease in hepatic collagen content by morphometry. Participants with compensated cirrhosis (F4) (n=258; NCT01672879) were randomly assigned (1:1:1) to receive intravenous infusions of simtuzumab (200 mg or 700 mg) or placebo every other week. The primary outcome was the change in hepatic venous pressure gradient.

Study assessments

Histology: Liver fibrosis was staged centrally at baseline (all patients), week 48 (all patients) and week 96 (simtuzumab studies only). All biopsies were read by a single central reader (ZG) who was blinded to treatment assignment but not biopsy sequence. Histological assessments included the adequacy of the biopsy specimen, confirmation of the diagnosis, fibrosis staged according to the NASH Clinical Research Network and modified Ishak fibrosis classifications, and grading of steatosis, lobular inflammation and hepatocellular ballooning according to the NAFLD activity score.

LS measurements: LS was measured by VCTE (FibroScan, Echosens, Paris, France) at baseline by trained operators, with participants in a fasting state and following standard reliability criteria, as previously described.[28] Data on type of VCTE probe (M vs XL) were available in the STELLAR studies only.

Serum markers: Fasting blood samples were obtained at baseline for clinical laboratory tests, including AST, ALT, platelets, glucose and ELF score (Siemens Healthcare, Erlangen Germany).

The Agile 3+and Agile 4 scores are novel non-invasive scores including LSM by VCTE and routine clinical parameters that were developed to identify advanced fibrosis (Agile 3+) and cirrhosis (Agile 4) among patients with NAFLD, and demonstrated better performance compared with FIB-4 and LS by VCTE.[34 35] The Agile 3+ and Agile 4 scores were calculated as follows: Agile 3+= $\frac{e^x}{1+e^x}$, where x=−3.92+2.30 ln (LS by VCTE)−0.01 (platelets)−0.99 (1/(AST/ALT))+1.09 (diabetes status)−0.39 (gender) +0.03 (age); Agile 4= $\frac{e^y}{1+e^y}$, where y=7.50–15.42 (1/√(LS by VCTE))−0.01 (platelets)−1.41 (1/(AST/ALT))−0.53 (gender) +0.42 (diabetes status).[34 35]

Objectives and outcome measures

The primary objective of this study was to determine the optimal thresholds of baseline LS by VCTE to predict progression to cirrhosis (F4) in participants with bridging (F3) fibrosis and liver-related clinical events in participants with cirrhosis. The secondary objectives were to determine if a ≥5 kPa (and ≥20%)[30] increase in LS predicts progression to cirrhosis (F4) in participants with bridging (F3) fibrosis and liver-related clinical events in participants with cirrhosis (F4). In addition, the performance of the Agile 3+ and Agile 4 scores as predictors for progression to cirrhosis and liver-related events were compared with baseline LS by VCTE.

At baseline, cirrhosis (F4) was defined by histology. Progression to cirrhosis from bridging (F3) fibrosis at baseline was defined by cirrhosis (F4) on a postbaseline biopsy, or the development of liver-related events. Liver-related events were adjudicated by a central committee of experts and defined as clinically apparent ascites requiring treatment, Grade ≥2 hepatic encephalopathy according to the West Haven criteria requiring treatment, and portal hypertension-related gastrointestinal bleeding, liver transplantation, qualification for transplantation (Model for End-Stage Liver Disease (MELD) ≥15) or mortality.

Statistical analysis

Baseline demographic and clinical characteristics are presented separately according to the presence of bridging fibrosis (F3) or cirrhosis (F4) at baseline. Associations between LS by VCTE and the Agile scores at baseline, and a ≥5 kPa (and ≥20%) increase of LS with disease progression through the end of follow-up were determined using Kaplan-Meier and Cox proportional hazards regression analysis. Discrimination of these measures for disease progression were described by c-statistics which are analogous to the area under a receiver operating characteristic curve estimated for logistic models.[36] The optimal baseline LS and Agile score thresholds were determined based on the maximal sum of sensitivity and specificity according to the logistic model, and the operating characteristics (sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV)) for disease progression at these thresholds were calculated. Statistical significance was defined as a two-tailed p value of ≤0.05. All statistical analyses were performed using SAS, V.9.4 (SAS Institute).

RESULTS

Characteristics of the study population

A total of 664 participants with bridging (F3) fibrosis and 734 participants with cirrhosis (F4) were included in this study (table 1). Among participants with bridging (F3) fibrosis, 56% were female, 72% were white, 70% had diabetes and the median (IQR) age and BMI were 58 years (51–64) and 32.7 kg/m^2 (29.0–37.0), respectively. At baseline, the median (IQR) LS by VCTE was 12.7 kPa (9.7–17.3). Out of 664 participants with bridging (F3) fibrosis had available data for LS prior to progression to cirrhosis, 14% had a ≥5 kPa (and ≥20%) increase in LS.

Among 734 participants with cirrhosis, 63% were female, 78% were white and 76% had diabetes; the median (IQR) age and BMI were 59 years (53–65) and 33.1 kg/m^2 (28.7–38.0), respectively (table 1). The median (IQR) platelet count was 160×1x10^9/L (124–206) and MELD score was 7 (6-8). At baseline, the median (IQR) LS by VCTE was 21.1 kPa (14.2, 29.3) and 63% (434/694) of participants were scanned with the XL probe. Out of 734 participants with cirrhosis (F4) had available data for LS prior to hepatic decompensation, 22% had a≥5 kPa (and ≥20%) increase in LS.

Table 1 Baseline demographics and clinical characteristics of patients with bridging fibrosis and cirrhosis due to non-alcoholic steatohepatitis

			Bridging fibrosis (F3): n=664	Cirrhosis (F4): n=734
Demographics	Age, years		58 (51, 64)	59 (53, 65)
	Women		371/664 (56)	461/734 (63)
	USA		336/664 (51)	432/734 (59)
	White		479/664 (72)	572/734 (78)
	Hispanic/Latino		94/664 (14)	95/734 (13)
	BMI, kg/m^2		32.7 (29.0, 37.0)	33.1 (28.7, 38.0)
	Diabetes		466/664 (70)	556/734 (76)
Liver biochemistry	ALT, U/L		53 (35, 80)	42 (31, 61)
	GGT, U/L		58 (38, 99)	81 (47, 144)
	Bilirubin, mg/dL		0.6 (0.4, 0.8)	0.6 (0.5, 0.9)
	MELD		6 (6, 7)	7 (6, 8)
	Platelets, x10^3/µL		206 (166, 257)	160 (124, 206)
Liver histology	NAS≥4		628/664 (95)	691/730 (95)
	Steatosis ≥2		60/664 (9)	33/730 (5)
	Lobular inflammation 3		332/664 (50)	396/730 (54)
	Ballooning 2		514/664 (77)	594/730 (81)
	Ishak fibrosis stage	F3	378/664 (57)	—
		F4	286/664 (43)	—
		F5	—	281/733 (38)
		F6	—	452/733 (62)
	Collagen content, %		4.4 (2.7, 6.4)	10.6 (7.4, 14.7)
	αSMA expression, %		6.0 (3.0, 9.2)	13.4 (8.9, 19.7)
Non-invasive tests	ELF		9.94 (9.34, 10.61)	10.60 (9.99, 11.26)
	FIB-4		1.66 (1.23, 2.50)	2.50 (1.73, 3.51)
	NAFLD fibrosis score		–0.227 (-1.053, 0.484)	0.615 (-0.215, 1.495)
	LS by VCTE, kPa		12.7 (9.7, 17.3)	21.1 (14.2, 29.3)
	XL probe, %		334/620 (54)	434/694 (63)

*Data are median (IQR) or n/n (%).
ALT, alanine aminotransferase; BMI, body mass index; ELF, enhanced liver fibrosis; GGT, γ-glutamyltransferase; FIB-4 index, fibrosis-4 index; LS, liver stiffness; MELD, model for end-Stage liver disease; NAFLD, non-alcoholic fatty liver disease; NAFLD Activity NAS, NAFLD activity score; VCTE, vibration-controlled transient elastography; αSMA, α-smooth muscle actin.

Progression to cirrhosis among participants with baseline bridging (F3) fibrosis

During a median follow-up of 16.6 months (IQR 15.0–19.4), 16% (103/664) of participants with bridging fibrosis progressed to cirrhosis. In total, 93.2% (n=96) of participants had cirrhosis diagnosed based on post-baseline histology, while 6.8% (n=7) developed liver-related events consistent with cirrhosis.

Optimal baseline LS threshold for predicting progression to cirrhosis

The risk of progression to cirrhosis was greater with higher LS by VCTE at baseline (HR per 3 kPa: 1.16; 95% CI 1.12 to 1.20). The optimal LS threshold at baseline was ≥16.6 kPa, with a c-statistic of 0.72 (95% CI 0.66 to 0.77) (figure 1). The sensitivity, specificity, PPV and NPV of this threshold for progression to cirrhosis were 58%, 76%, 31% and 91%, respectively (table 2). Progression to cirrhosis occurred in 31.1% (60/193) of participants with baseline LS ≥16.6 kPa compared with 9.1% (43/471) with LS <16.6 kPa (p<0.001; figures 1 and 2). Baseline LS by VCTE ≥16.6 kPa was associated with a nearly

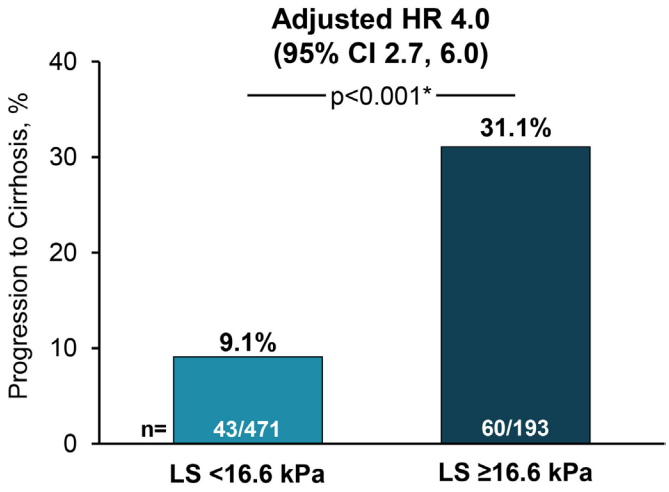


Figure 1 Liver stiffness (LS) by vibration-controlled transient elastography ≥16.6 kPa is associated with increased risk of progression to cirrhosis among patients with baseline bridging (F3) fibrosis secondary to non-alcoholic steatohepatitis. *Fisher's exact test.

fourfold risk (HR 3.86, 95% CI 2.61 to 5.72) of progression to cirrhosis in participants with bridging (F3) fibrosis. After adjustment for age, gender, ethnicity and BMI, baseline LS ≥16.6 kPa remained a strong and independent predictor for progression to cirrhosis (adjusted HR 3.99; 95% CI 2.66 to 5.98; p<0.0001) (online supplemental table 1).

A ≥5kPA (and ≥20%) increase in LS as a predictor of progression to cirrhosis

Progression to cirrhosis occurred in 22% (20/91) of participants with a≥5 kPA (and ≥20%) increase in LS compared with 14% (83/573) with <5 kPa (and ≥20%) increase in LS (p=0.051; online supplemental figures 1 and 2). A ≥5 kPA (and ≥20%) increase in LS was associated with a nearly 1.6-fold risk (HR 1.59, 95% CI 0.97 to 2.59) of progression to cirrhosis in participants with bridging (F3) fibrosis. After adjustment for baseline LS, age, gender, ethnicity and BMI, a ≥5 kPa (and ≥20%) increase in LS remained a strong and independent predictor for progression to cirrhosis (adjusted HR 1.98; 95% CI 1.20 to 3.26; p=0.008) (online supplemental table 2).

Table 2 Performance of liver stiffness by vibration-controlled transient elastography for predicting progression to cirrhosis among patients with baseline bridging (F3) fibrosis and liver-related clinical events among patients with cirrhosis (F4)

	Progression to cirrhosis (F4) in patients with bridging fibrosis (F3) (n=664)	Liver-related clinical events among patients with cirrhosis (F4) (n=734)
c-statistic (95% CI)	0.72 (0.66 to 0.77)	0.77 (0.67 to 0.87)
Optimal threshold	≥16.6 kPa	≥30.7 kPa
Sensitivity (95% CI)	58% (48 to 68) (60/103)	70% (50 to 86) (19/27)
Specificity (95% CI)	76% (73 to 80) (428/561)	79% (75 to 81) (555/707)
PPV (95% CI)	31% (25 to 38) (60/193)	11% (7 to 17) (19/171)
NPV (95% CI)	91% (88 to 93) (428/471)	99% (97 to 99) (555/563)

95% CI for sensitivity, specificity, PPV and NPV are based on exact limits.
NPV, negative predictive value; PPV, positive predictive value.

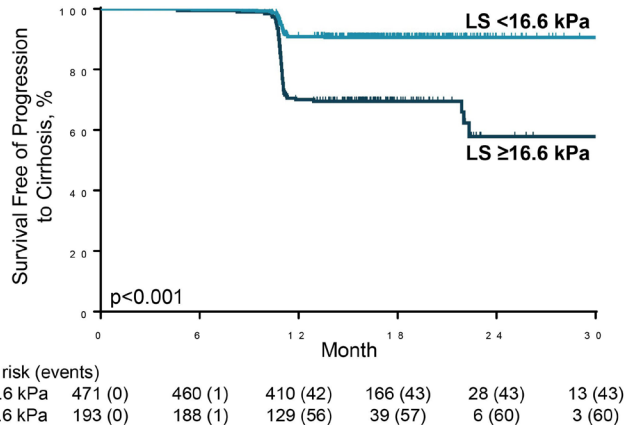


Figure 2 Progression to cirrhosis in patients with bridging (F3) fibrosis secondary to non-alcoholic steatohepatitis, stratified by liver stiffness (LS) by vibration-controlled transient elastography ≥16.6 kPa vs <16.6 kPa.

Optimal Agile 3+ threshold for predicting progression to cirrhosis

A subgroup of 629 participants with bridging (F3) fibrosis (95%) had complete data for calculation of the Agile 3+ score (online supplemental table 3). At baseline, the median (IQR) Agile 3+ score was 0.76 (0.53–0.89). In this subgroup, 15% (95/629) progressed to cirrhosis (89 on histology and 6 with clinical events) during a median follow-up of 16.6 months (IQR 15.0–19.4). The risk of disease progression was greater with higher baseline Agile 3+ score (HR per 0.1-units: 1.34; 95% CI 1.19 to 1.52; p<0.001). The median (IQR) baseline Agile 3+ score in participants with vs without progression to cirrhosis was 0.94 (0.82, 0.98) vs 0.88 (0.68, 0.97), respectively (p=0.001). The optimal threshold for baseline Agile 3+ score to predict progression to cirrhosis was ≥0.90 (online supplemental figure 3). After adjustment for age, gender, ethnicity and BMI, baseline Agile 3+≥0.90 was an independent predictor for progression to cirrhosis (adjusted HR 4.75, 95% CI 3.07 to 7.34; p<0.0001) (online supplemental table 4).

Comparison of LS versus Agile 3+ score for predicting progression to cirrhosis

Among participants with sufficient data (n=629) for calculation of the Agile 3+score, the performance of LS by VCTE and Agile 3+ for predicting progression to cirrhosis were similar (c-statistic 0.71 (95% CI 0.65 to 0.76) vs 0.70 (95% CI 0.64 to 0.76), p=0.88) (online supplemental table 5).

Liver-related events among participants with baseline cirrhosis (F4)

During a median follow-up of 16.2 months (IQR 13.9–18.7), 4% (27/734) of participants with cirrhosis at baseline had liver-related events: ascites (n=15), hepatic encephalopathy (n=5), portal hypertension-related upper gastrointestinal bleeding (n=3), qualification for liver transplantation (n=2), liver transplantation (n=1) and death (n=1).

Optimal baseline LS threshold for predicting liver-related events among participants with cirrhosis

The risk of liver-related events was greater with higher LS by VCTE at baseline (HR per 5 kPa: 1.29; 95% CI 1.18 to 1.41). The optimal LS threshold at baseline was ≥30.7

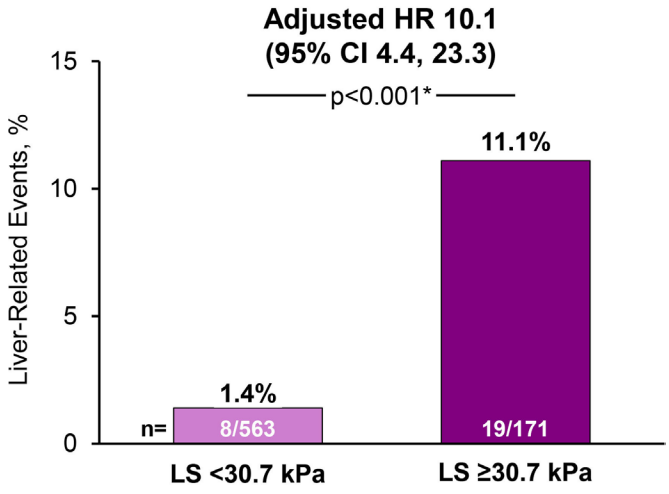


Figure 3 Liver stiffness (LS) by vibration-controlled transient elastography ≥30.7 kPa is associated with increased risk of liver-related events among patients with baseline cirrhosis (F4) secondary to non-alcoholic steatohepatitis. *Fisher exact test.

kPa, which had a c-statistic of 0.77 (95% CI 0.67 to 0.87) (figure 3). The sensitivity, specificity, PPV and NPV of this threshold for liver-related events were 70%, 79%, 11% and 99%, respectively (table 2). Liver-related events occurred in 11.1% (19/171) of cirrhotic participants with baseline LS ≥30.7 kPa compared with 1.4% (8/563) of participants with baseline LS <30.7 kPA (p<0.001; figures 3 and 4). Baseline LS by VCTE ≥30.7 kPa was associated with an 8-fold risk (HR 8.24; 95% CI 3.61 to 18.82) of clinical events in participants with cirrhosis (F4). LS by VCTE ≥30.7 kPa remained a strong and independent predictor of liver-related events after adjustment for age, gender, ethnicity and BMI (adjusted HR 10.13; 95% CI 4.38 to 23.41; p<0.0001) (online supplemental table 6), with a similar finding in another model incorporating weight changes (online supplemental table 7). A sensitivity analysis for classic clinical events related to portal hypertension (development of ascites, hepatic encephalopathy and portal hypertension-related bleeding) determined similar findings (online supplemental table 8).

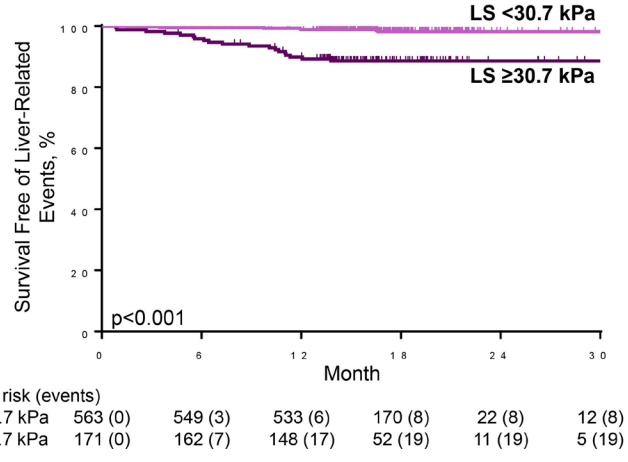


Figure 4 Liver-related events in patients with cirrhosis (F4) secondary to non-alcoholic steatohepatitis, stratified by liver stiffness (LS) by vibration-controlled elastography ≥30.7 kPa vs <30.7 kPa.

A ≥5kPA (and ≥20%) increase in LS as a predictor of liver-related events among participants with cirrhosis

Liver-related events occurred in 3% (4/160) of participants with a ≥5 kPa (and ≥20%) increase in LS compared with 4% (23/574) with <5 kPA (and ≥20%) increase in LS (p=0.36; online supplemental figures 4 and 5). Among participants with cirrhosis, a≥5 kPa (and ≥20%) increase in LS was not associated with liver-related events in univariable (HR 0.61, 95% CI 0.21 to 1.76; p=0.36) and multivariable analysis (adjusted HR 0.75; 95% CI 0.26 to 2.18; p=0.60) (online supplemental table 9).

Optimal Agile 4 threshold for predicting liver-related events among participants with baseline cirrhosis (F4)

A subgroup of 701 participants (96%) had data sufficient for calculation of the Agile four score (online supplemental table 1). The median (IQR) Agile four score at baseline was 0.63 (0.34–0.80). In this subgroup, 3% (23/701) had liver-related events during a median follow-up of 16.3 months (IQR 13.9–18.7). The risk of disease progression was greater with higher baseline Agile four score (HR per 0.1-units: 1.91; 95% CI 1.40 to 2.61; p<0.001). The median baseline Agile four scores in participants with versus without liver-related events were 0.53 (IQR 0.23–0.75) vs 0.37 (IQR 0.13–0.69), respectively (p=0.002). The optimal Agile four threshold for predicting liver-related events was ≥0.72 (online supplemental figure 6). After adjustment for age, gender, ethnicity and BMI, baseline Agile 4≥0.72 was an independent predictor for liver-related events (adjusted HR 11.84; 95% CI 3.51 to 39.99; p<0.0001) (online supplemental table 10).

Comparison of LS versus Agile 4 score and other NITs for predicting liver-related events

Among participants with sufficient data for analysis of the baseline Agile four score (n=701), the performance of LS by VCTE and Agile four for predicting liver-related events were similar (c-statistic 0.81 (95% CI 0.72 to 0.90) vs 0.82 (95% CI 0.74 to 0.90), p=0.97) (online supplemental table 5).

Baseline LS by VCTE had a similar performance compared with baseline NFS, ELF and FIB-4 for predicting liver-related events among those with baseline cirrhosis (F4) (online supplemental table 11).

Optimal baseline LS threshold for predicting liver-related events among participants with advanced (F3–F4) fibrosis

Among 664 participants with baseline advanced fibrosis (F3–F4), there were 34 incident liver-related events (seven from participants with baseline F3 and 27 from participants with baseline F4). The optimal LS threshold at baseline for predicting liver-related events remained ≥30.7 kPa, which had a c-statistic of 0.77 (95% CI 0.68 to 0.86). The sensitivity, specificity, PPV and NPV of this threshold for liver-related events were 62%, 87%, 10% and 99%, respectively. Baseline LS by VCTE ≥30.7 kPa was associated with an 11-fold risk (adjusted HR 10.52; 95% CI 5.15 to 21.48; p<0.0001) of liver-related events in participants with advanced fibrosis (F3–F4) after adjustment for age, gender, ethnicity, treatment arm and BMI (online supplemental table 12).

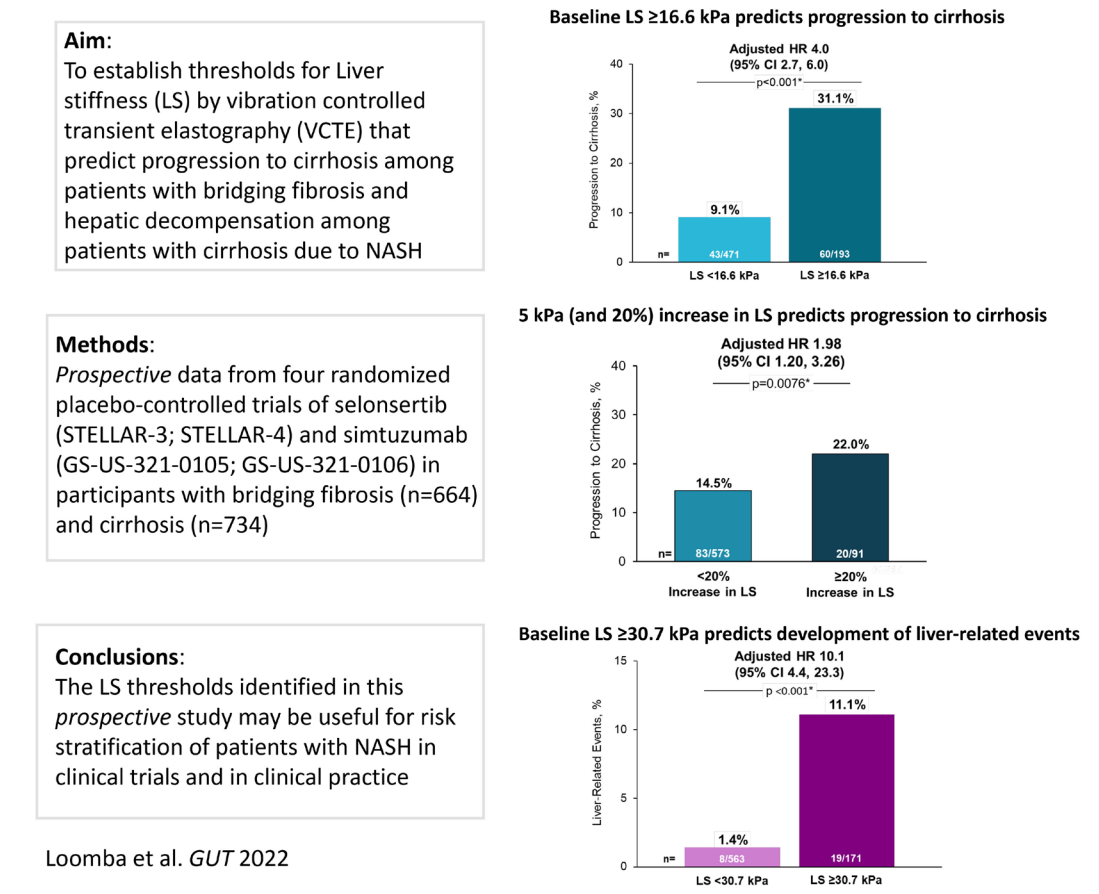


Figure 5 Graphical summary. NASH, non-alcoholic steatohepatitis.

DISCUSSION

Main findings

In this analysis of four large, randomised, placebo-controlled trials of selonsertib and simtuzumab in participants with NASH and biopsy-confirmed advanced fibrosis (F3–F4), baseline LS by VCTE was a strong and independent predictor of disease progression (Graphical summary enclosed as figure 5). Among participants with bridging fibrosis (F3), the optimal LS threshold at baseline LS to predict progression to cirrhosis was ≥16.6 kPa, which had a c-statistic of 0.72, consistent with good prognostic performance. Overall, 31% of participants with bridging fibrosis (F3) and LS ≥16.6 kPa at baseline progressed to cirrhosis, compared with only 9% with LS <16.6 kPa. After adjustment for age, gender, ethnicity and BMI, baseline LS ≥16.6 kPa was associated with a fourfold higher risk of progression to cirrhosis during a follow-up. A≥5 kPa (and ≥20%) increase in LS by VCTE was associated with an increased risk of progression to cirrhosis among participants with baseline bridging (F3) fibrosis.

Similar findings were observed among participants with cirrhosis (F4) at baseline, although a higher threshold for identifying patients at risk of liver-related complications was observed, as expected. Specifically, the optimal threshold of LS at baseline to predict liver-related events was ≥30.7 kPa, which had a c-statistic of 0.77. A total of 11% of participants with cirrhosis and baseline LS ≥30.7 kPa developed liver-related events vs only 1% of those with baseline LS <30.7 kPa. After adjustment for demographic factors and BMI, baseline LS by VCTE ≥30.7 kPa was associated with a 10-fold higher risk of liver-related events. In a sensitivity analysis of the F3 and F4 patient populations, the prognostic performance of VCTE did not differ based on the type of VCTE probe (M vs XL) used to measure LS (data not shown). In addition, we determined that the optimal LS threshold by VCTE (≥30.7 kPa) to predict development of liver-related events among participants with advanced fibrosis (F3-F4) was the same as the threshold for participants with cirrhosis (F4).

The Agile 3+and Agile four scores—which include LS by VCTE plus other clinical and demographic factors—and were designed to identify patients with bridging fibrosis and cirrhosis, respectively, were also significant predictors of disease progression in this cohort. However, their diagnostic performance was similar to LS measurement alone, suggesting that these additional parameters do not improve on the prognostic utility of LS by VCTE. Further studies are required to define the relationship between serum-based NITs and LS by transient elastography for the prediction of future clinical outcomes.

In context with current literature

Multiple studies have demonstrated a correlation between LS by VCTE with liver-related complications and mortality among patients with liver disease of various aetiologies, but

prospective data in patients with NASH and advanced fibrosis are limited.[37 38] A retrospective analysis of 1039 participants (53% with biopsy) with NAFLD and advanced fibrosis (F3 or F4) or with LS by VCTE >10 kPa, demonstrated that baseline LS was associated with liver-related events and mortality.[30] However, this study did not provide data for progression from bridging fibrosis to cirrhosis. Another study of 2251 patients with NAFLD demonstrated that baseline LS by VCTE was an independent predictor of survival, and liver-related and cardiovascular events, however, this study did not evaluate the utility of baseline LS for patients with F3 or F4 fibrosis due to the relatively small numbers with advanced fibrosis (13% had baseline LS >12 kPa).[29] The current prospective study of patients with biopsy-confirmed, advanced fibrosis (F3 or F4) provides clinical validation that baseline LS by VCTE can be used as a prognostic tool for progression to cirrhosis and development of liver-related events. In addition, the LS thresholds identified in this study may be useful for risk stratification of patients with NASH in clinical trials and in clinical practice to identify patients at increased risk of disease progression.[39] For example, high-risk patients could be offered increased clinical surveillance or targeted for enrolment in clinical trials of novel therapies. Finally, LS by VCTE, along with platelet count, may be useful to predict the development of clinically significant portal hypertension in patients with NASH and compensated advanced chronic liver disease, as suggested in a recent Baveno consensus report.[31 40] In this regard, the LS thresholds recommended by this group (eg, 15 and 30 kPa) are close to those of the optimal thresholds identified in our dataset (16.7 and 30.7 kPa) and have similar prognostic utility (online supplemental table 13).

Strengths and limitations

The novelty of this study includes its prospective design, well-phenotyped participants with serial, centrally read liver biopsies, adjudication of liver-related events by a committee of experts, and the establishment of thresholds for baseline LS by VCTE that predict disease progression in NAFLD with F3–F4 fibrosis. However, this study is not without limitations. First, all included participants were selected for clinical trials and it is unclear whether these data are generalisable to the broader population of NASH patients with advanced fibrosis. Therefore, these data require validation in clinical practice. Second, the histological definition of progression to cirrhosis in patients classified as having bridging fibrosis (F3) at baseline is susceptible to misclassification due to sampling variability of liver biopsy.[18] Third, the median follow-up duration was relatively short (~16–17 months) given the slow rate of disease progression in NASH, potentially contributing to a relatively low rate of clinical liver-related events; therefore, prospective studies with longer follow-up duration are required.[5] All liver biopsies were evaluated by a single pathologist, which may introduce an element of interpretation bias. Finally, although VCTEs were performed by trained operators, there was no quality control of the VCTE measurements, and further data are required to examine the variability of VCTE in NASH and advanced fibrosis. Nevertheless, our data demonstrate that LS by VCTE provides highly discriminant prognostic information even when performed under standards of usual clinical practice and lend further justification for the use of non-invasive surrogates in prognosticating clinically meaningful outcomes beyond ordinal histological staging of fibrosis alone.

CONCLUSION

In this analysis of four large, randomised placebo-controlled trials of participants with NASH and biopsy-proven advanced fibrosis (F3–F4), clinical disease progression was associated with higher LS by VCTE at baseline. The optimal LS thresholds for predicting progression to cirrhosis among patients with bridging fibrosis (F3) and development of liver-related events among patients with cirrhosis were ≥16.6 kPa and ≥30.7 kPa, respectively. A≥5 kPA (and ≥20%) increase in LS by VCTE was associated with an increased risk of progression to cirrhosis among participants with baseline bridging (F3) fibrosis. The LS thresholds identified in this study may be useful for risk stratification of patients with NASH in clinical trials and in clinical practice and lend further support to the use of non-invasive surrogates rather than liver histology to predict the risk of clinically meaningful outcomes.

Author affiliations
[1]Division of Epidemiology, Department of Family Medicine and Public Health, University of California at San Diego, University of California at San Diego, San Diego, California, USA
[2]NAFLD Research Center, University of California San Diego, La Jolla, California, USA
[3]Department of Medicine, Yong Loo Lin School of Medicine, National University of Singapore, Singapore
[4]Division of Gastroenterology and Hepatology, Department of Medicine, National University Health System, Singapore
[5]Division of Gastroenterology, Department of Internal Medicine, Virginia Commonwealth University School of Medicine, Richmond, Virginia, USA
[6]Translational and Clinical Research Institute, Faculty of Medical Sciences, Newcastle University, Newcastle upon Tyne, UK
[7]Division of Gastroenterology and Hepatology, Department of Medicine III, Medical University of Vienna, Vienna, Austria
[8]Texas Liver Institute, UT Health San Antonio, San Antonio, Texas, USA
[9]Gilead Sciences Inc, Foster City, California, USA
[10]Betty and Guy Beatty Center for Integrated Research, Inova Health System, Falls Church, Virginia, USA
[11]Department of Medicine and Therapeutics, Chinese University of Hong Kong, Hong Kong, China
[12]Hepatology Center, Saiseikai Suita Hospital, Suita, Japan
[13]Unit for The Clinical Management of Digestive Diseases and CIBERehd, Hospital Universitario de Valme, Sevilla, Spain
[14]Duke University, Durham, North Carolina, USA
[15]Duke Clinical Research Institute, Duke University Medical Center, Durham, North Carolina, USA
[16]Division of Gastroenterology and Hepatology, Beth Israel Medical Center, Harvard University Medical School, Boston, Massachusetts, USA
[17]Inselspital Universitätsspital Bern, Bern, Switzerland
[18]Radcliffe Department of Medicine, University of Oxford, Oxford, UK
[19]Pinnacle Clinical Research, San Antonio, Texas, USA
[20]Center for Liver Diseases, Inova Fairfax Hospital, Falls Church, Virginia, USA

Twitter Manuel Romero-Gómez @mromerogomez

Acknowledgements Editorial assistance was provided by Sandra Chen of Gilead Sciences.

Contributors Study design: RL and RPM. Data acquisition: RL, DD, CJ, AB, RSH, CC, ZG and RPM. Data analysis: RL, DH, DD, LM, CJ and RPM. Data interpretation and review/revision of the manuscript: all authors. Study concept and study supervision: RL and RPM. Writing of first draft: DH and RL. All authors approved the final draft of the manuscript as well as the authorship list. Gurantor of article: RL.

Funding This study was funded by Gilead Sciences (NCT03053050; NCT03053063; NCT01672866; NCT01672879)

Competing interests RL receives funding support from NIAAA (U01AA029019), NIEHS (5P42ES010337), NCATS (5UL1TR001442), NIDDK (U01DK130190, U01DK061734, R01DK106419, P30DK120515, R01DK121378, R01DK124318), NHLBI (P01HL147835), and DOD PRCRP (W81XWH-18-2-0026). RL serves as a consultant to Aardvark Therapeutics, Altimmune, Anylam/Regeneron, Amgen, Arrowhead Pharmaceuticals, AstraZeneca, Bristol-Myer Squibb, CohBar, Eli Lilly,

Galmed, Gilead, Glympse bio, Hightide, Inipharma, Intercept, Inventiva, Ionis, Janssen, Madrigal, Metacrine, NGM Biopharmaceuticals, Novartis, Novo Nordisk, Merck, Pfizer, Sagimet, Theratechnologies, 89 bio, Terns Pharmaceuticals and Viking Therapeutics. In addition his institutions received research grants from Arrowhead Pharmaceuticals, Astrazeneca, Boehringer-Ingelheim, Bristol-Myers Squibb, Eli Lilly, Galectin Therapeutics, Galmed Pharmaceuticals, Gilead, Intercept, Hanmi, Intercept, Inventiva, Ionis, Janssen, Madrigal Pharmaceuticals, Merck, NGM Biopharmaceuticals, Novo Nordisk, Merck, Pfizer, Sonic Incytes and Terns Pharmaceuticals. Co-founder of LipoNexus Inc. DH receives funding support from Singapore Ministry of Health's National Medical Research Council under its NMRC Research Training Fellowship (MOH-000595-01). In addition, he has served as an advisory board member for Eisai. MFA receives funding support from NIDDK (U01DK130177, U01DK061713, R01DK12137), NCI (UG1CA242643) and DOD (W81XWH2010514). MFA serves as a consultant/advisor to Bristol-Myer Squibb, CohBar, Inventiva, Madrigal, NGM Biopharmaceuticals, Novo Nordisk, Theratechnologies, 89 Bio, Somologics, and Hanmi Pharmaceuticals. In addition, her institutions received research grants from Allergan, Boehringer-Ingelheim, Bristol-Myers Squibb, Enyo, Enanta, Durect, Galectin Therapeutics, Gilead, Galmed, Intercept, Hanmi, Inventiva, Madrigal Pharmaceuticals, NGM Biopharmaceuticals, Novartis, Novo Nordisk, Poxel, TARGET PharmaSolutions, Celgene, Genentech. and Viking Pharmaceuticals. QMA is funded by the LITMUS (Liver Investigation: Testing Marker Utility in Steatohepatitis) consortium funded by the Innovative Medicines Initiative (IMI2) Program of the European Union under Grant Agreement 777377; this Joint Undertaking receives support from the European Union's Horizon 2020 research and innovation programme and EFPIA. He reports Research Grant Funding from Allergan/Tobira, AstraZeneca, Boehringer Ingelheim, GlaxoSmithKline, Glympse Bio, Intercept, Novartis Pharma AG, Pfizer Ltd; Consultancy from 89Bio, Abbvie/Allergan, Altimentiv, Altimmune, AstraZeneca, Axcella, Blade, BMS, BNN Cardio, Boehringer Ingelheim, Cirius, CymaBay, EcoR1, E3Bio, Eli Lilly & Company Ltd., Galmed, Genentech, Genfit SA, Gilead, Grunthal, HistoIndex, Indalo, Intercept Pharma Europe Ltd., Inventiva, IQVIA, Janssen, Johnson & Johnson, Madrigal, MedImmune, Medpace, Merck, Metacrine, NGMBio, North Sea Therapeutics, Novartis, Novo Nordisk A/S, PathAI, Pfizer, Poxel, ProSciento, Raptor Pharma, Roche, Servier, Shionogi, Terns, The Medicines Company, Viking Therapeutics; Speaker fees from Abbott Laboratories, Allergan/Tobira, BMS, Clinical Care Options, Falk, Fishawack, Genfit SA, Gilead, Integritas Communications, Kenes, Medscape; and Royalties from Elsevier. DD, LM, CJ, AB, RSH, CC and RPM are employees and shareholders of Gilead Sciences. VW served as a consultant or advisory board member for AbbVie, Boehringer Ingelheim, Echosens, Gilead Sciences, Inventiva, Merck, Novo Nordisk, Pfizer, ProSciento, Sagimet Biosciences, and TARGET PharmaSolutions; and a speaker for Abbott, AbbVie, Echosens, Gilead Sciences, Novo Nordisk. He has received a research grant from Gilead Sciences, and was a co-founder of Illuminatio Medical Technology Limited. JB was a consultant for Gilead Sciences, Actelion and Surrozen.

Patient consent for publication Not applicable.

Ethics approval This study involves human participants and was approved by (1) NAFLD Research Center, Division of Gastroenterology. University of California at San Diego, La Jolla, CA, USA. (2) Division of Gastroenterology and Hepatology, Department of Medicine, National University Health System, Singapore. (3) Virginia Commonwealth University, Richmond, Virginia, USA. (4) Translational and Clinical Research Institute, Faculty of Medical Sciences, Newcastle University, Newcastle upon Tyne, UK; Newcastle NIHR Biomedical Research Centre, Newcastle upon Tyne Hospitals NHS Trust, Newcastle upon Tyne, UK. (5) Medizinische Universität Wien, Austria. (6) Texas Liver Institute, UT Health San Antonio, Texas, USA. (7) Gilead Sciences, Foster City, California, USA. (8) Center for Liver Diseases, Department of Medicine, Inova Fairfax Hospital, Falls Church, Virginia, USA. (9) Institute of Digestive Disease, The Chinese University of Hong Kong. (10) Saiseikai Suita Hospital, Suita, Japan. (11) Instituto de Biomedicina de Sevilla, Hospital Universitario Virgen del Rocío, Universidad de Sevilla, Spain. (12) Duke Clinical Research Institute, Duke University, Durham, North Carolina, USA. (13) Division of Gastroenterology and Hepatology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts, USA. (14) Hospital Clinic-IDIBAPS and Ciberhed, University of Barcelona, Spain and Department of Visceral Surgery and Medicine, Bern University, Bern, Switzerland. (15) Pinnacle Clinical Research, San Antonio, Texas, USA.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement No data are available.

ORCID iDs
Rohit Loomba http://orcid.org/0000-0002-4845-9991
Daniel Q. Huang http://orcid.org/0000-0002-5165-5061
Michael Trauner http://orcid.org/0000-0002-1275-6425
Vincent Wai-Sun Wong http://orcid.org/0000-0003-2215-9410
Zobair M Younossi http://orcid.org/0000-0001-9313-577X

REFERENCES

1 Younossi ZM, Koenig AB, Abdelatif D, *et al*. Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology* 2016;64:73–84.
2 Le MH, Yeo YH, Li X, *et al*. 2019 global NAFLD prevalence: a systematic review and meta-analysis. *Clin Gastroenterol Hepatol* 2021;2021:2. doi:10.1016/j.cgh.2021.12.002
3 Loomba R, Sanyal AJ. The global NAFLD epidemic. *Nat Rev Gastroenterol Hepatol* 2013;10:686–90.
4 Loomba R, Friedman SL, Shulman GI. Mechanisms and disease consequences of nonalcoholic fatty liver disease. *Cell* 2021;184:2537–64.
5 Singh S, Allen AM, Wang Z, *et al*. Fibrosis progression in nonalcoholic fatty liver vs nonalcoholic steatohepatitis: a systematic review and meta-analysis of paired-biopsy studies. *Clin Gastroenterol Hepatol* 2015;13:643–54.
6 Dufour J-F, Anstee QM, Bugianesi E, *et al*. Current therapies and new developments in NASH. *Gut* 2022:326874. doi:10.1136/gutjnl-2021-326874
7 Anstee QM, Castera L, Loomba R. Impact of non-invasive biomarkers on hepatology practice: past, present and future. *J Hepatol* 2022;76:1362–78.
8 Estes C, Razavi H, Loomba R, *et al*. Modeling the epidemic of nonalcoholic fatty liver disease demonstrates an exponential increase in burden of disease. *Hepatology* 2018;67:123–33.
9 Estes C, Anstee QM, Arias-Loste MT, *et al*. Modeling NAFLD disease burden in China, France, Germany, Italy, Japan, Spain, United Kingdom, and United States for the period 2016-2030. *J Hepatol* 2018;69:896–904.
10 Tan DJH, Ng CH, Lin SY, *et al*. Clinical characteristics, surveillance, treatment allocation, and outcomes of non-alcoholic fatty liver disease-related hepatocellular carcinoma: a systematic review and meta-analysis. *Lancet Oncol* 2022;23:521–30.
11 Huang DQ, El-Serag HB, Loomba R. Global epidemiology of NAFLD-related HCC: trends, predictions, risk factors and prevention. *Nat Rev Gastroenterol Hepatol* 2021;18:223–38.
12 Huang DQ, Singal AG, Kono Y. Changing global epidemiology of liver cancer from 2010 to 2019: NASH is the fastest growing cause of liver cancer. *Cell Metab*;34:969–77.
13 Tan DJH, Setiawan VW, Ng CH, DJH T, CH N, *et al*. Global burden of liver cancer in males and females: changing etiological basis and the growing contribution of NASH. *Hepatology* 2022. doi:10.1002/hep.32758. [Epub ahead of print: 29 Aug 2022].
14 Dulai PS, Singh S, Patel J, *et al*. Increased risk of mortality by fibrosis stage in nonalcoholic fatty liver disease: systematic review and meta-analysis. *Hepatology* 2017;65:1557–65.
15 Sanyal AJ, Van Natta ML, Clark J, *et al*. Prospective study of outcomes in adults with nonalcoholic fatty liver disease. *N Engl J Med* 2021;385:1559–69.
16 Taylor RS, Taylor RJ, Bayliss S, *et al*. Association between fibrosis stage and outcomes of patients with nonalcoholic fatty liver disease: a systematic review and meta-analysis. *Gastroenterology* 2020;158:1611–25.
17 Ng CH, Lim WH, Hui Lim GE, *et al*. Mortality outcomes by fibrosis stage in nonalcoholic fatty liver disease: a systematic review and meta-analysis. *Clin Gastroenterol Hepatol* 2022:14. doi:10.1016/j.cgh.2022.04.014
18 Ratziu V, Charlotte F, Heurtier A, *et al*. Sampling variability of liver biopsy in nonalcoholic fatty liver disease. *Gastroenterology* 2005;128:1898–906.
19 Rockey DC, Caldwell SH, Goodman ZD, *et al*. Liver biopsy. *Hepatology* 2009;49:1017–44.
20 Boursier J, Vergniol J, Guillet A, *et al*. Diagnostic accuracy and prognostic significance of blood fibrosis tests and liver stiffness measurement by FibroScan in non-alcoholic fatty liver disease. *J Hepatol* 2016;65:570–8.
21 Eddowes PJ, Sasso M, Allison M, *et al*. Accuracy of FibroScan controlled attenuation parameter and liver stiffness measurement in assessing steatosis and fibrosis in patients with nonalcoholic fatty liver disease. *Gastroenterology* 2019;156:1717–30.
22 Wong VW-S, Vergniol J, Wong GL-H, *et al*. Diagnosis of fibrosis and cirrhosis using liver stiffness measurement in nonalcoholic fatty liver disease. *Hepatology* 2010;51:454–62.
23 Jung J, Loomba RR, Imajo K, *et al*. MRE combined with FIB-4 (MEFIB) index in detection of candidates for pharmacological treatment of NASH-related fibrosis. *Gut* 2021;70:1946–53.
24 Angulo P, Bugianesi E, Bjornsson ES, *et al*. Simple noninvasive systems predict long-term outcomes of patients with nonalcoholic fatty liver disease. *Gastroenterology* 2013;145:782–9.
25 Loomba R, Adams LA. Advances in non-invasive assessment of hepatic fibrosis. *Gut* 2020;69:1343–52.
26 Tamaki N, Ajmera V, Loomba R. Non-invasive methods for imaging hepatic steatosis and their clinical importance in NAFLD. *Nat Rev Endocrinol* 2022;18:55–66.

27 Tamaki N, Imajo K, Sharpton S. MRE plus FIB-4 (MEFIB) versus FAST in detection of candidates for pharmacological treatment of NASH-related fibrosis. *Hepatology*2021.
28 Anstee QM, Lawitz EJ, Alkhouri N, *et al*. Noninvasive tests accurately identify advanced fibrosis due to NASH: baseline data from the stellar trials. *Hepatology* 2019;70:1521–30.
29 Shili-Masmoudi S, Wong GL-H, Hiriart J-B, *et al*. Liver stiffness measurement predicts long-term survival and complications in non-alcoholic fatty liver disease. *Liver Int* 2020;40:581–9.
30 Petta S, Sebastiani G, Viganò M, *et al*. Monitoring occurrence of liver-related events and survival by transient elastography in patients with nonalcoholic fatty liver disease and compensated advanced chronic liver disease. *Clin Gastroenterol Hepatol* 2021;19:806–15.
31 de Franchis R, Bosch J, Garcia-Tsao G. BAVENO VII - renewing consensus in portal hypertension: report of the Baveno VII consensus workshop: personalized care in portal hypertension. *J Hepatol*;76:959–74.
32 Harrison SA, Wong VW-S, Okanoue T, *et al*. Selonsertib for patients with bridging fibrosis or compensated cirrhosis due to NASH: Results from randomized phase III STELLAR trials. *J Hepatol* 2020;73:26–39.
33 Harrison SA, Abdelmalek MF, Caldwell S, *et al*. Simtuzumab is ineffective for patients with bridging fibrosis or compensated cirrhosis caused by nonalcoholic steatohepatitis. *Gastroenterology* 2018;155:1140–53.
34 Younossi ZS, Newsome PN, Chan W-K. Development and validation of Agile 3+: novel FibroScan based score for the diagnosis of advanced fibrosis in patients with nonalcoholic fatty liver disease. *J Hepatol* 2021;75:S205–93.
35 Newsome PN, Sasso M, Deeks JJ, *et al*. Independent validation of Agile 4: novel FibroScan based score for the diagnosis of cirrhosis in patients with non-alcoholic fatty liver disease. *J Hepatol* 2021;75:S205–93.
36 Heagerty PJ, Zheng Y. Survival model predictive accuracy and ROC curves. *Biometrics* 2005;61:92–105.
37 Pang JXQ, Zimmer S, Niu S, *et al*. Liver stiffness by transient elastography predicts liver-related complications and mortality in patients with chronic liver disease. *PLoS One* 2014;9:e95776.
38 Vergniol J, Foucher J, Terrebonne E, *et al*. Noninvasive tests for fibrosis and liver stiffness predict 5-year outcomes of patients with chronic hepatitis C. *Gastroenterology* 2011;140:9.e1-3.:1970–9.
39 Manns MP, Burra P, Sargent J, *et al*. The Lancet–EASL Commission on liver diseases in Europe: overcoming unmet needs, stigma, and inequities. *The Lancet* 2018;392:621–2.
40 Hsieh Y-C, Lee K-C, Wang Y-W. Correlation between noninvasive markers of fibrosis and hepatic venous pressure gradient in patients with compensated cirrhosis due to nonalcoholic steatohepatitis. *Hepatology* 2015;62:10.1371/journal.pone. 0208903:33A–92.

Supplemental Table 1. Univariable and multivariable predictors of progression to cirrhosis among patients with baseline bridging (F3) fibrosis secondary to non-alcoholic steatohepatitis

	Univariable		**Multivariable**	
	Hazard Ratio (95% CI)	**p-value**	**Hazard Ratio (95% CI)**	**p-value**
Age, per yr	1.01 (0.99, 1.03)	0.3483	1.01 (0.99, 1.03)	0.4083
Sex (Female vs. Male)	0.98 (0.67, 1.45)	0.9355	1.12 (0.75, 1.66)	0.5879
Ethnicity (Non-hispanic/Latino vs. Hispanic/Latino)	0.68 (0.41, 1.12)	0.1320	0.63 (0.38, 1.04)	0.0714
BMI, per kg/m^2	1.02 (0.99, 1.05)	0.2178	1.00 (0.97, 1.03)	0.8561
LS by VCTE ≥16.6 kPa	3.86 (2.61, 5.72)	<0.001	3.99 (2.66, 5.98)	<.0001

BMI, body mass index; LS by VCTE, liver stiffness by vibration-controlled transient elastography.

Supplemental Table 2. Univariable and multivariable predictors of progression to cirrhosis among patients with baseline bridging (F3) fibrosis secondary to non-alcoholic steatohepatitis

	Univariable		**Multivariable**	
	Hazard Ratio (95% CI)	**p-value**	**Hazard Ratio (95% CI)**	**p-value**
Baseline LS by VCTE >=16.6 kPa vs. <16.6 kPa	3.86 (2.61, 5.72)	<0.001	4.26 (2.83, 6.40)	<0.001
Age, per yr	1.01 (0.99, 1.03)	0.3483	1.01 (0.99, 1.03)	0.4092
Sex (Female vs. Male)	0.98 (0.67, 1.45)	0.9355	1.08 (0.73, 1.61)	0.6871
Ethnicity (Non-hispanic/Latino vs. Hispanic/Latino)	0.68 (0.41, 1.12)	0.1320	0.65 (0.39, 1.07)	0.0927
BMI, per kg/m^2	1.02 (0.99, 1.05)	0.2178	0.99 (0.96, 1.02)	0.6179
≥20% increase in LS by VCTE vs <20% increase	1.59 (0.97, 2.59)	0.0638	1.98 (1.20, 3.26)	0.0076

BMI, body mass index; LS by VCTE, liver stiffness by vibration-controlled transient elastography.

Supplemental Table 3. Baseline demographics and clinical characteristics of the subgroup of patients with sufficient data for determination of the Agile 3+ and 4 scores

		Bridging Fibrosis (F3) n=629	**Compensated Cirrhosis (F4) n=701**
Demographics	Age, y	58 (51, 64)	59 (54, 65)
	Women, n (%)	350 (56)	442 (63)
	Diabetes, n (%)	444 (71)	531 (76)
Liver biochemistry	ALT, U/L	53 (36, 80)	43 (32, 61)
	AST, U/L	46 (33, 65)	45 (34, 61)
	AST/ALT ratio	0.86 (0.72, 1.04)	1.02 (0.84, 1.24)
	Platelets, $10^3/\mu L$	206 (166, 256)	161 (125, 206)
Liver histology	NAS ≥4, n (%)	594 (94)	662 (95)[†]
	Steatosis Grades 2–3, n (%)	53 (8)	33 (5)[†]
	Lobular inflammation Grade 3, n (%)	317 (50)	377 (54)[†]
	Hepatocellular ballooning Grade 2, n (%)	489 (78)	570 (82)[†]
Biopsy quality	Length, cm	2.2 (1.6, 3.0)	2.0 (1.5, 2.8)
	Length ≥2 cm, n (%)	383 (61)	388 (55)
Noninvasive tests	NFS	-0.192 (-1.016, 0.500)	0.619 (-0.212, 1.493)
	FIB-4	1.68 (1.23, 2.50)	2.49 (1.73, 3.49)
	ELF	9.94 (9.34, 10.61)	10.60 (9.97, 11.27)
	LS by VCTE, kPa	12.7 (9.7, 17.3)	21.1 (14.3, 28.9)
	Agile 3+	0.76 (0.53, 0.89)	0.95 (0.86, 0.98)
	Agile 4	0.20 (0.08, 0.39)	0.63 (0.34, 0.80)

Data are median (interquartile range) or n/n (%).
ALT, alanine aminotransferase; αSMA, α-smooth muscle actin; BMI, body mass index; ELF, Enhanced Liver Fibrosis score (Siemens Healthcare GmbH, Erlangen Germany); FIB-4, fibrosis-4 index; GGT, γ-glutamyltransferase; LS by VCTE, liver stiffness by vibration-controlled transient elastography; NAFLD Activity Score, NAS.
[†] Total n=699.

Supplemental Table 4. Univariable and multivariable predictors of progression to cirrhosis among patients with bridging (F3) fibrosis secondary to non-alcoholic steatohepatitis

	Univariable		**Multivariable**	
	Hazard Ratio (95% CI)	**p-value**	**Hazard Ratio (95% CI)**	**p-value**
Age, per yr	1.01 (0.99, 1.03)	0.5227	0.99 (0.97, 1.01)	0.3738
Sex (Female vs. Male)	0.93 (0.62, 1.39)	0.7270	0.84 (0.56, 1.26)	0.3958
Ethnicity (Non-hispanic/Latino vs. Hispanic/Latino)	0.68 (0.40, 1.13)	0.1351	0.61 (0.36, 1.03)	0.0619
BMI, per kg/m^2	1.02 (0.99, 1.05)	0.1715	1.00 (0.97, 1.03)	0.8734
Agile 3+ score ≥ 0.90	4.34 (2.89, 6.50)	<.0001	4.75 (3.07, 7.34)	<.0001

BMI, body mass index.

Supplemental Table 5. Performance of liver stiffness by vibration-controlled transient elastography versus the Agile scores for predicting disease progression*

	Progression to Cirrhosis (F4) in Patients with Bridging Fibrosis (F3) (n=629)		**Liver-Related Clinical Events Among Patients with Cirrhosis (F4) (n=701)**	
	Liver stiffness by VCTE	**Agile 3+**	**Liver stiffness by VCTE**	**Agile 4**
c-statistic (95% CI)	0.71 (0.65, 0.76)	0.70 (0.64, 0.76)	0.81 (0.72, 0.90)	0.82 (0.74, 0.90)
Optimal threshold	≥ 16.6 kPa	≥ 0.90	≥ 30.7 kPa	≥ 0.72
Sensitivity	58% (55/95)	55% (52/95)	74% (17/23)	87% (20/23)
Specificity	76% (407/534)	81% (431/534)	79% (535/678)	64% (433/678)
PPV	30% (55/182)	34% (52/155)	11% (17/160)	8% (20/265)
NPV	91% (407/447)	91% (431/474)	99% (535/541)	99% (433/436)

NPV, negative predictive value; PPV, positive predictive value; VCTE, vibration-controlled transient elastography.
* Analysis restricted to patients with complete data for calculation of the Agile scores.

Supplemental Table 6. Univariable and multivariable predictors for the development of liver-related events among patients with baseline cirrhosis (F4) secondary to non-alcoholic steatohepatitis

	Univariable		**Multivariable**	
	Hazard Ratio (95% CI)	**p-value**	**Hazard Ratio (95% CI)**	**p-value**
Age, per yr	1.05 (1.00, 1.11)	0.0644	1.06 (1.00, 1.13)	0.0533
Sex (Female vs. Male)	2.08 (0.84, 5.15)	0.1142	2.39 (0.95, 5.98)	0.0637
Ethnicity (Non-hispanic/Latino vs. Hispanic/Latino)	0.86 (0.30, 2.48)	0.7772	0.82 (0.28, 2.39)	0.7175
BMI, per kg/m^2	0.98 (0.92, 1.03)	0.4082	0.97 (0.91, 1.03)	0.3431
LS by VCTE ≥30.7 kPa	8.24 (3.61, 18.8)	<0.001	10.13 (4.38, 23.41)	<.0001

BMI, body mass index; LS by VCTE, liver stiffness by vibration-controlled transient elastography.

Supplemental Table 7. Univariable and multivariable predictors for the development of liver-related events among patients with baseline cirrhosis (F4) secondary to non-alcoholic steatohepatitis

	Univariable		**Multivariable**	
	Hazard Ratio (95% CI)	**p-value**	**Hazard Ratio (95% CI)**	**p-value**
Age, per yr	1.05 (1.00, 1.11)	0.0644	1.06 (1.00, 1.13)	0.0512
Sex (Female vs. Male)	2.08 (0.84, 5.15)	0.1142	2.37 (0.94, 5.96)	0.0661
Ethnicity (Non-hispanic/Latino vs. Hispanic/Latino)	0.86 (0.30, 2.48)	0.7772	0.80 (0.27, 2.35)	0.6911
BMI, per kg/m^2	0.98 (0.92, 1.03)	0.4082	0.97 (0.92, 1.03)	0.3642
LS by VCTE ≥30.7 kPa	8.24 (3.61, 18.8)	<0.001	10.13 (4.38, 23.41)	<0.001
Weight loss ≥ 5% (yes vs no)	0.46 (0.11, 1.93)	0.2852	0.45 (0.11, 1.89)	0.2729

BMI, body mass index; LS by VCTE, liver stiffness by vibration-controlled transient elastography.

Supplemental Table 8. Univariable and multivariable predictors for the development of ascites, hepatic encephalopathy and portal hypertension-related bleeding among patients with baseline cirrhosis (F4) secondary to non-alcoholic steatohepatitis

	Univariable		**Multivariable**	
	Hazard Ratio (95% CI)	**p-value**	**Hazard Ratio (95% CI)**	**p-value**
Age, per yr	1.06 (1.00, 1.12)	0.0655	1.06 (1.00, 1.14)	0.0603
Sex (Female vs. Male)	2.13 (0.79, 5.75)	0.1338	2.46 (0.90, 6.72)	0.0780
Ethnicity (Non-hispanic/Latino vs. Hispanic/Latino)	0.99 (0.30, 3.34)	0.9920	0.96 (0.28, 3.25)	0.9446
BMI, per kg/m^2	0.97 (0.91, 1.04)	0.3773	0.97 (0.91, 1.04)	0.3505
LS by VCTE ≥30.7 kPa	7.90 (3.25, 19.20)	<.0001	9.77 (3.97, 24.05)	<.0001

BMI, body mass index; LS by VCTE, liver stiffness by vibration-controlled transient elastography.

Supplemental Table 9. Univariable and multivariable predictors for the development of liver-related events among patients with baseline cirrhosis (F4) secondary to non-alcoholic steatohepatitis

	Univariable		**Multivariable**	
	Hazard Ratio (95% CI)	**p-value**	**Hazard Ratio (95% CI)**	**p-value**
Baseline LS by VCTE >=30.7 kPa vs. <30.7 kPa	8.24 (3.61, 18.82)	<0.001	10.04 (4.34, 23.23)	<0.001
Age, per yr	1.05 (1.00, 1.11)	0.0644	1.06 (1.00, 1.13)	0.0508
Sex (Female vs. Male)	2.08 (0.84, 5.15)	0.1142	2.35 (0.94, 5.91)	0.0686
Ethnicity (Non-hispanic/Latino vs. Hispanic/Latino)	0.86 (0.30, 2.48)	0.7772	0.84 (0.29, 2.45)	0.7446
BMI, per kg/m^2	0.98 (0.92, 1.03)	0.4082	0.97 (0.91, 1.03)	0.3511
≥20% increase in LS by VCTE vs <20% increase	0.61 (0.21, 1.76)	0.3618	0.75 (0.26, 2.18)	0.5959

BMI, body mass index; LS by VCTE, liver stiffness by vibration-controlled transient elastography.

Supplemental Table 10. Univariable and multivariable predictors of liver-related events among patients with cirrhosis (F4) secondary to non-alcoholic steatohepatitis

	Univariable		**Multivariable**	
	Hazard Ratio (95% CI)	**p-value**	**Hazard Ratio (95% CI)**	**p-value**
Age, per yr	1.03 (0.98, 1.09)	0.2379	1.02 (0.96, 1.08)	0.5509
Sex (Female vs. Male)	2.11 (0.78, 5.69)	0.1390	1.90 (0.70, 5.18)	0.2094
Ethnicity (Non-hispanic/Latino vs. Hispanic/Latino)	1.55 (0.36, 6.62)	0.5523	1.75 (0.41, 7.49)	0.4532
BMI, per kg/m^2	0.96 (0.90, 1.03)	0.2405	0.95 (0.89, 1.01)	0.1054
Agile 4 score ≥ 0.72	11.48 (3.41, 38.65)	<.0001	11.84 (3.51, 39.99)	<.0001

BMI, body mass index.

Supplemental Table 11. Concordance statistics by logistic regression for baseline LS by VCTE and baseline NITs in predicting liver-related events

NIT	Number of participants	c-statistic (95% CI) of NIT	c-statistic (95% CI) of LS by VCTE	Difference	p-value
FIB-4	728	0.76 (0.66, 0.86)	0.76 (0.65, 0.87)	0.00 (-0.15, 0.15)	0.9685
NFS	732	0.70 (0.60, 0.80)	0.77 (0.66, 0.87)	-0.07 (-0.23, 0.10)	0.4336
ELF	733	0.80 (0.71, 0.89)	0.77 (0.67, 0.87)	0.03 (-0.07, 0.14)	0.5340

LS, liver stiffness; VCTE, vibration-controlled transient elastography; NIT, non-invasive tests; Fibrosis-4 index, FIB-4, NAFLD fibrosis score, NFS; Enhanced Liver Fibrosis test, ELF

Supplemental Table 12. Multivariable predictors of liver-related events among patients with advanced fibrosis (F3-F4) secondary to non-alcoholic steatohepatitis

	Multivariable	
	Adjusted HR (95% CI)	**p-value**
Age	1.05 (1.00, 1.11)	0.0466
Sex (Female vs. Male)	1.63 (0.77, 3.46)	0.2027
Ethnicity (Non-hispanic/ Latino vs. Hispanic/Latino	0.68 (0.26, 1.81)	0.4417
BMI	0.98 (0.93, 1.04)	0.5266
LS by VCTE >=30.7 kPa vs. <30.7 kPa	10.52 (5.15, 21.48)	<.0001
Treatment arm Selonsertib 6 mg vs Placebo Selonsertib 18 mg vs Placebo Simtuzumab low dose vs Placebo Simtuzumab high dose vs Placebo	 2.34 (0.67, 8.15) 1.35 (0.36, 5.01) 3.77 (0.38, 37.12) 2.53 (0.26, 24.47)	 0.1815 0.6574 0.2556 0.4213

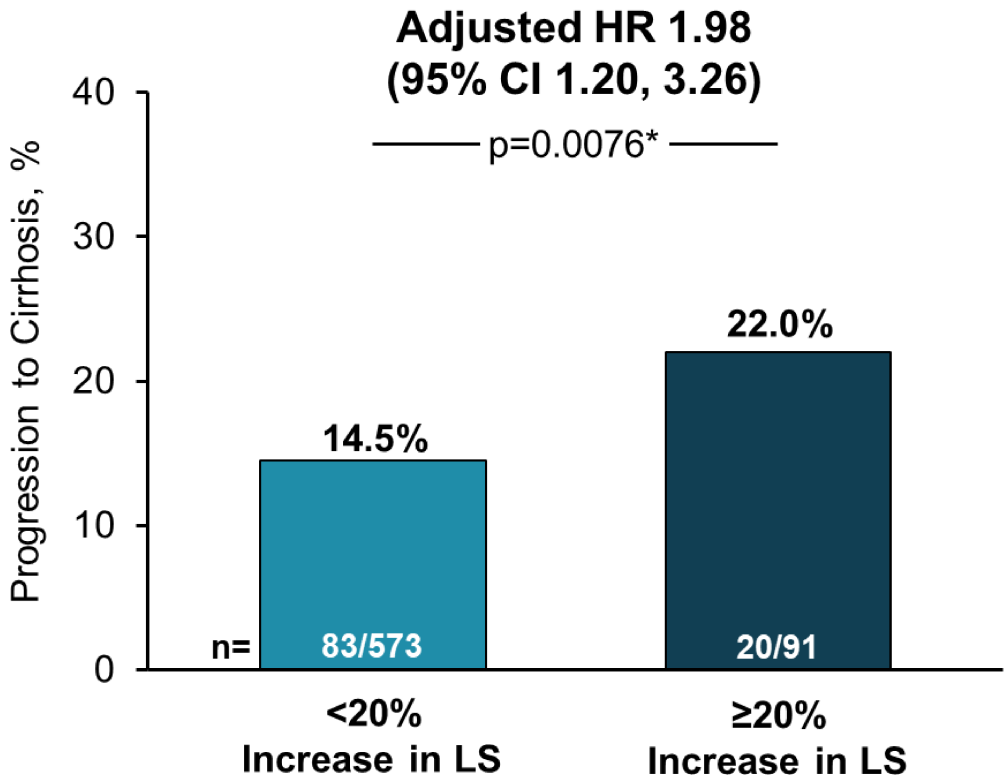
BMI, body mass index; LS, liver stiffness; VCTE, vibration-controlled transient elastography

Supplemental Table 13. Performance of liver stiffness by vibration-controlled transient elastography according to thresholds recommended by the Baveno VII consensus for identification of patients with advanced chronic liver disease [25]

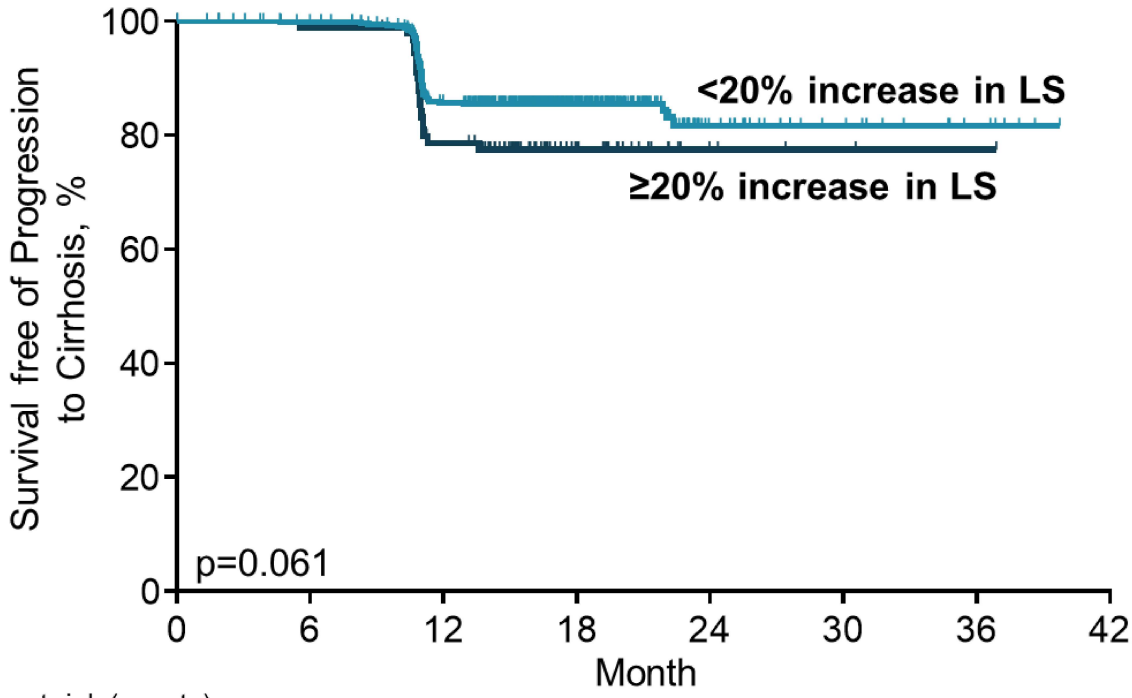
Threshold	Proportion with Event	Sensitivity, % (95% CI)	Specificity, % (95% CI)	PPV, % (95% CI)	NPV, % (95% CI)	Hazard Ratio (95% CI)
Progression to Cirrhosis (F4) in Patients with Bridging Fibrosis (F3) (n=664)						
LS ≥ 10 kPa	91/483 (19%)	88 (81, 94)	30 (26, 34)	19 (15, 23)	93 (89, 97)	3.01 (1.65, 5.50)
LS < 10 kPa	12/181 (7%)					
LS ≥ 15 kPa*	64/240 (27%)	62 (52, 72)	69 (65, 72)	27 (21, 33)	91 (88, 93)	3.21 (2.16, 4.79)
LS < 15 kPa	39/424 (9%)					
LS ≥ 20 kPa	34/112 (30%)	33 (24, 43)	86 (83, 89)	30 (22, 40)	88 (84, 90)	2.70 (1.79, 4.08)
LS < 20 kPa	69/552 (13%)					
LS ≥ 25 kPa	25/52 (48%)	24 (16, 34)	95 (93, 97)	48 (34, 62)	87 (84, 90)	4.52 (2.88, 7.10)
LS < 25 kPa	78/612 (13%)					
LS ≥ 30 kPa	17/32 (53%)	17 (10, 25)	97 (96, 98)	53 (35, 71)	86 (83, 89)	4.54 (2.69, 7.64)
LS < 30 kPa	86/632 (14%)					
LS ≥ 35 kPa	9/20 (45%)	9 (4, 16)	98 (97, 99)	45 (23, 68)	85 (82, 88)	3.45 (1.74, 6.83)
LS < 35 kPa	94/644 (15%)					
Liver-Related Events in Patients with Cirrhosis (F4) (n=734)						
LS ≥ 10 kPa	26/672 (4%)	96 (81, 100)	9 (7, 11)	4 (3, 6)	98 (91, 100)	2.46 (0.33, 18.10)
LS < 10 kPa	1/62 (2%)					
LS ≥ 15 kPa	25/532 (5%)	93 (76, 99)	28 (25, 32)	5 (3, 7)	99 (96, 100)	4.92 (1.17, 20.78)
LS < 15 kPa	2/202 (1%)					
LS ≥ 20 kPa	22/400 (6%)	81 (62, 94)	47 (43, 50)	6 (3, 8)	99 (97, 100)	3.83 (1.45, 10.12)
LS < 20 kPa	5/334 (1%)					
LS ≥ 25 kPa	21/268 (8%)	78 (58, 91)	65 (0.61, 0.69)	8 (5, 12)	99 (97, 100)	6.43 (2.60, 15.94)
LS < 25 kPa	6/466 (1%)					
LS ≥ 30 kPa*	19/177 (11%)	70 (50, 86)	78 (74, 81)	11 (7, 16)	99 (97, 99)	7.87 (3.44, 17.98)
LS < 30 kPa	8/557 (1%)					
LS ≥ 35 kPa	16/127 (13%)	59 (39, 78)	84 (81, 87)	13 (7, 20)	98 (97, 99)	7.44 (3.45, 16.03)
LS < 35 kPa	11/607 (2%)					

NPV, negative predictive value; PPV, positive predictive value; LS, liver stiffness by vibration-controlled transient elastography. 95% CI for sensitivity, specificity, PPV, and NPV are based on exact limits. * Optimal threshold based on maximal sum of sensitivity and specificity.

Supplemental Figure 1. ≥5kPA (and ≥20%) increase in Liver stiffness (LS) by vibration-controlled transient elastography (VCTE) is associated with increased risk of progression to cirrhosis among patients with baseline bridging (F3) fibrosis secondary to non-alcoholic steatohepatitis

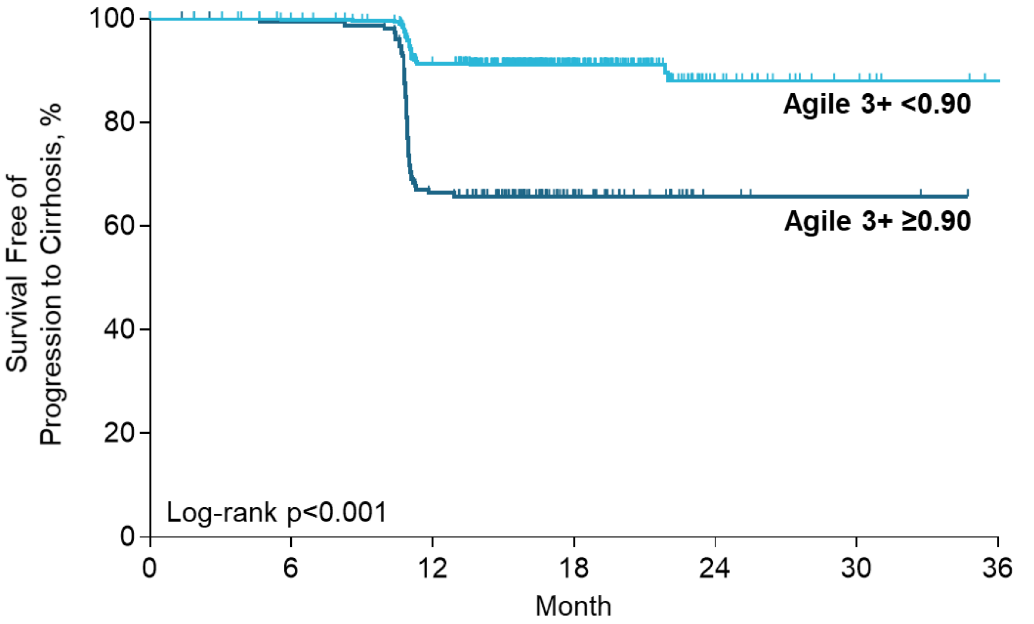


Supplemental Figure 2. Progression to cirrhosis in patients with bridging (F3) fibrosis secondary to non-alcoholic steatohepatitis, stratified by ≥5kPA (and ≥20%) increase in Liver stiffness (LS) by vibration-controlled transient elastography (VCTE)



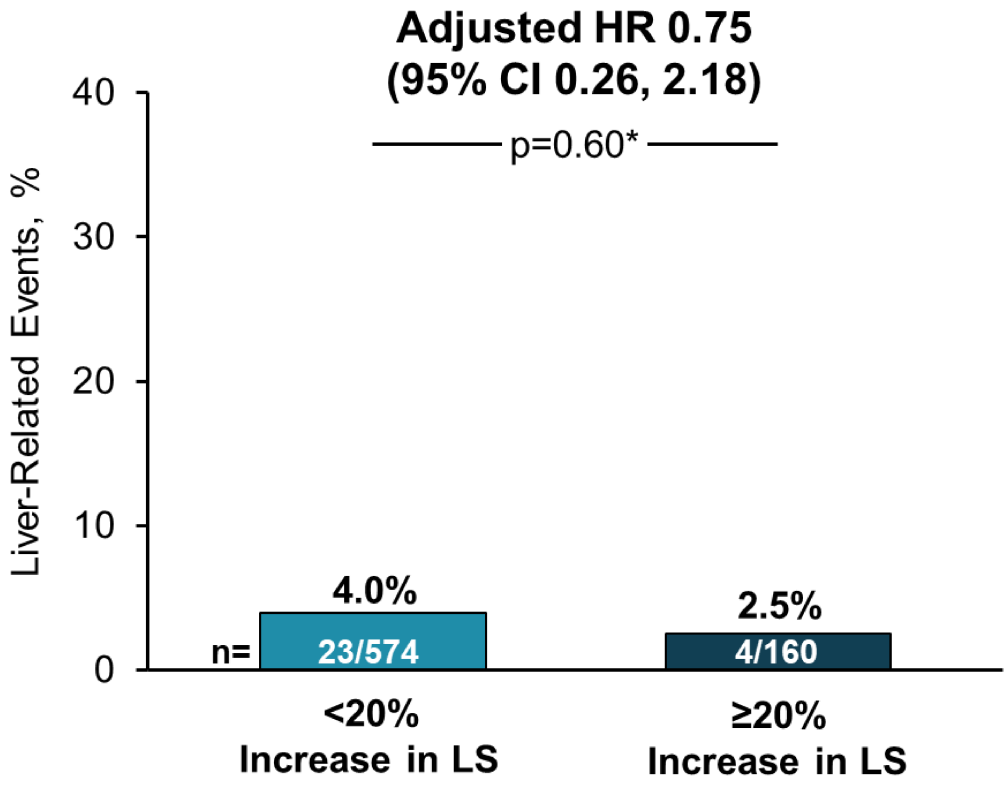
n at risk (events)								
<20%	573 (0)	559 (1)	470 (79)	178 (80)	30 (83)	14 (83)	6 (83)	0 (83)
≥20%	91 (0)	89 (1)	69 (19)	27 (20)	4 (20)	2 (20)	1 (20)	0 (20)

Supplemental Figure 3. Progression to cirrhosis in patients with bridging (F3) fibrosis secondary to non-alcoholic steatohepatitis, stratified by Agile 3+ score ≥ 0.90 versus < 0.90



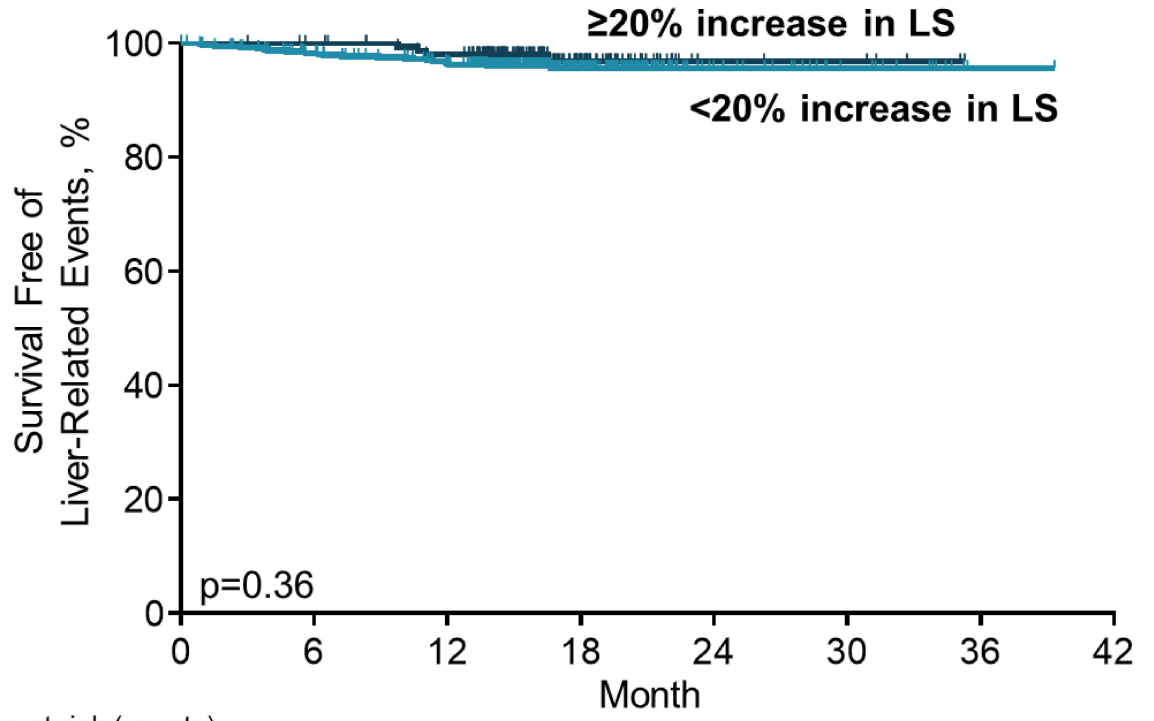
n at risk (events)							
<0.90	474 (0)	465 (1)	414 (40)	158 (41)	27 (43)	11 (43)	5 (43)
≥0.90	155 (0)	151 (1)	100 (51)	34 (52)	4 (52)	2 (52)	0 (52)

Supplemental Figure 4. Liver-related events in patients with cirrhosis (F4) secondary to non-alcoholic steatohepatitis, stratified by≥5kPA (and ≥20%) increase in Liver stiffness (LS) by vibration-controlled transient elastography (VCTE



*Fisher exact test.

Supplemental Figure 5. Liver-related events in patients with cirrhosis (F4) secondary to non-alcoholic steatohepatitis, stratified by≥5kPA (and ≥20%) increase in Liver stiffness (LS) by vibration-controlled transient elastography (VCTE



n at risk (events)								
<20%	574 (0)	554 (10)	531 (20)	170 (23)	27 (23)	12 (23)	1 (23)	0 (23)
≥20%	160 (0)	157 (0)	150 (3)	52 (4)	6 (4)	5 (4)	0 (4)	0 (4)

Supplemental Figure 6. Liver-related events in patients with cirrhosis (F4) secondary to non-alcoholic steatohepatitis, stratified by Agile 4 ≥ 0.72 versus < 0.72