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## **Non-invasive tests accurately stratify patients with NAFLD based on their risk of liver-related events**

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#### Author contributions

	Study design	Data acquisition	Analysis	Drafting/ critical revision
J. Boursier	X	X	X	X
H Hagström	X	X		X
M Ekstedt	X	X		X
C Moreau			X	
M Bonacci				X
S Cure	X			X
J Ampuero		X		
P Nasr		X		
L Tallab		X		
CM Canivet		X		
S Kechagias		X		
Y Sánchez		X		
E Dincuff		X		
A Lucena		X		
M Roux			X	X
J Riou			X	
A Trylesinski	X			X
M Romero-Gomez	X	X		X

## ABSTRACT

**Background and Aims:** Previous works on the prognostic significance of non-invasive liver fibrosis tests in non-alcoholic fatty liver disease (NAFLD) lack direct comparison to liver biopsy. We aimed to evaluate the prognostic accuracy of FIB4 and vibration-controlled transient elastography (VCTE), with comparison to liver biopsy, for the prediction of liver-related events (LREs) in NAFLD.

**Methods:** 1,057 NAFLD patients with baseline FIB4 and VCTE were included in a multicenter cohort. Of these, 594 patients had also baseline liver biopsy. The main study outcome during follow-up was LREs, a composite endpoint combining cirrhosis complications or hepatocellular carcinoma. Discriminative ability was evaluated using Harrell's C-index.

**Results:** FIB4 and VCTE showed good accuracy for the prediction of LREs with Harrell's C-indexes  $>0.80$  (respectively: 0.817 [0.768-0.866] vs 0.878 [0.835-0.921],  $p=0.059$ ). In the biopsy subgroup, Harrell's C-indexes of histological fibrosis staging and VCTE were not significantly different (respectively: 0.932 [0.910-0.955] vs 0.881 [0.832-0.931],  $p=0.164$ ), while both significantly outperformed FIB4 for the prediction of LREs. FIB4 and VCTE were independent predictors of LREs in the whole study cohort. The stepwise FIB4-VCTE algorithm accurately stratified the risk of LREs: compared to patients with "FIB4  $<1.30$ ", those with "FIB4  $\geq 1.30$  then VCTE  $<8.0$  kPa" had similar risk of LREs (aHR: 1.3 [95%CI: 0.3-6.8]), whereas LREs risk significantly increased in patients with "FIB4  $\geq 1.30$  then VCTE 8.0-12.0 kPa" (aHR: 3.8 [95%CI: 1.3-10.9]), and even more for those with "FIB4  $\geq 1.30$  then VCTE  $>12.0$  kPa" (aHR: 12.4 [95%CI: 5.1-30.2]).

**Conclusion:** VCTE and FIB4 accurately stratify the risk of LREs in NAFLD. These non-invasive tests are alternatives to liver biopsy for the identification of patients in need for specialized management.

## **LAY SUMMARY**

The amount of fibrosis in the liver is closely associated with the risk of liver-related complications in non-alcoholic fatty liver disease (NAFLD). Liver biopsy currently remains the reference for the evaluation of liver fibrosis, but it cannot be performed in all NAFLD patients because of its invasiveness. Therefore, we evaluated the ability of non-invasive liver fibrosis tests to predict liver-related complications in NAFLD. Our results show that the blood test FIB4 and transient elastography stratify the risk of liver-related complications in NAFLD, and that transient elastography provides similar prognostic accuracy when compared to liver biopsy. These results argue for the use of non-invasive liver fibrosis tests instead of liver biopsy for the management of patients with NAFLD.

## INTRODUCTION

Among all liver histology features observed in non-alcoholic fatty liver disease (NAFLD), liver fibrosis is the main determinant of patient prognosis (1). Longitudinal studies have demonstrated that the risk of liver-related events (LREs) significantly increases at fibrosis stage 2 (F2, significant fibrosis) and becomes exponentially higher when transitioning to stage F3 (bridging fibrosis) and later F4 (cirrhosis) (1). Therefore, liver fibrosis should be evaluated in all patients with NAFLD to determine the stage of the disease and guide patient management, including therapy allocation once available (2-4).

Liver biopsy remains the reference for evaluating disease severity in NAFLD, but its invasive nature, several pragmatic issues (e.g., associated costs), limitations to scalability, and finally the patient's reluctance to undergo the procedure limit its widespread use for that purpose. Furthermore, liver biopsy remains hampered by sample bias and significant limitations in inter-observer reproducibility (5, 6). Several non-invasive tests, mainly elastography devices and blood marker-based algorithms, are now available to estimate liver fibrosis. These tests have demonstrated good accuracy for the diagnosis of advanced fibrosis in NAFLD (7-9). However, diagnostic studies use liver biopsy as gold standard, when in reality it is an imperfect reference leading to underestimation of the true performance of non-invasive tests (10). Moreover, reaching perfect diagnostic accuracy for the non-invasive staging of liver fibrosis is probably an unattainable goal as a liver architecture semi-quantitative classification (histology) cannot perfectly correlate with a quantitative measurement such as blood protein levels (blood tests) or a physical signal such as the speed of a shear wave in the liver (elastography).

An interesting concept has recently emerged though. Considering that non-invasive tests correlate to liver fibrosis, and fibrosis predicts prognosis, non-invasive tests may work as prognostic markers in patients with NAFLD (11-14). This approach could change the paradigm in NAFLD management, moving from the diagnosis of liver lesions to the prediction of prognosis. Resulting from this concept, a strong argument for the use of non-invasive tests instead of liver biopsy when discussing NAFLD management in clinical practice would be to demonstrate that these different modalities bring similar information regarding the prognosis assessment.

In the study presented here, we aimed to evaluate the prognostic significance of non-invasive liver fibrosis tests with comparison to histological fibrosis staging in a multicenter cohort of patients with NAFLD.

## **PATIENTS AND METHODS**

### **Patients**

Local cohorts of patients with NAFLD from five tertiary centers (Angers in France, Linköping and Stockholm in Sweden, Virgen del Rocio and Valme Hospitals in Seville) were pooled for the present study (details about the cohorts are presented in the **Supplementary Material**). NAFLD was defined as the presence of liver steatosis on imaging or histology, without any of the following conditions: concomitant steatosis-inducing drugs, excessive alcohol consumption (>210 g/week in men or >140 g/week in women), or other causes of chronic liver disease (chronic viral hepatitis, hemochromatosis, cholestatic chronic liver disease, auto-immune hepatitis, alpha1 antitrypsin deficiency). Patients with baseline FIB4 blood test and/or vibration controlled transient elastography (VCTE) were included in the present work. Those with a history of bariatric surgery, a history of LREs before baseline, and/or follow-up <3 months were excluded. The study protocol conformed to the ethical guidelines of the current Declaration of Helsinki and received approval by the local Ethics Committees.

### **Non-invasive tests of liver fibrosis**

Clinical data, biology, and liver stiffness results were collected at baseline and follow-up visits. Recorded clinical data and blood parameters were age, sex, antidiabetic treatment, antihypertensive treatment, lipid-lowering treatment, aspartate aminotransferase (AST, IU/L), alanine aminotransferase (ALT, IU/L), gamma-glutamyl transferase (IU/L) and platelets (Giga/l). Diabetes was defined as the use of antidiabetic medications. The blood fibrosis test FIB4 was calculated according to the published formula:  $[\text{age} * \text{AST [IU/L]}] / [\text{platelets (G/L)} * \text{ALT (IU/L)}^{1/2}]$  (15). FIB4 results were categorized into subgroups according to the published cut-offs 1.30 and 3.25 (15, 16). To avoid unreliable results linked to extra-hepatic affections, FIB4 results were considered invalid and excluded from the statistical analysis if any of the following conditions were met: AST  $\geq 400$  UI/L, ALT  $\geq 400$  UI/L, platelets  $\leq 20$  Giga/L or  $\geq 500$  Giga/L.

Liver stiffness measurements were performed using VCTE technology (FibroScan device; Echosens, Paris, France). The examinations were performed by experienced observers according to the manufacturer's recommendations (17). Ten valid measurements were required and the VCTE results were expressed in kiloPascals (kPa) as the median of those valid measurements. Ninety-two percent of the VCTE examinations were reliable according to the criteria derived from diagnostic studies (8). VCTE results were categorized into three groups according to cut-offs recently published in a large multicenter study (18) and a meta-analysis (19): <8.0 kPa (low risk of advanced fibrosis), 8.0–12.0 kPa (intermediate risk of advanced fibrosis), and >12.0 kPa (high risk of advanced fibrosis).

The blood test FIB4 and VCTE were combined in the FIB4-VCTE algorithm (**Figure s1**). Such combinatorial stepwise approach was initially developed for the non-invasive diagnosis of advanced liver fibrosis (8, 20), and has been recently proposed as a pathway for patient referral to the liver specialist (21).

### **Liver biopsy**

Data on liver biopsy were collected when available at baseline. Liver histological lesions were evaluated in each center by a senior expert specialized in hepatology as per the NASH CRN classification (22). Inter-observer agreement for liver fibrosis staging in NAFLD has been shown to be very good to excellent between expert pathologists from tertiary centers (22–24). Non-alcoholic steatohepatitis (NASH) was defined as the presence of all three of the following conditions: steatosis grade  $\geq 1$ , lobular inflammation grade  $\geq 1$ , and ballooning grade  $\geq 1$ . Fibrosis was staged as follows: F0 = no fibrosis, F1 = perisinusoidal or portal/periportal fibrosis, F2 = perisinusoidal and portal/periportal fibrosis, F3 = bridging fibrosis and F4 = cirrhosis. “Advanced fibrosis” was defined as fibrosis stage  $F \geq 3$ , and “no/mild fibrosis” as F0–2.

### **Follow-up and study outcomes**

Clinical events were retrieved from patient files according to a predefined procedure to ensure that data collection was conducted in a similar manner across participating centers. Clinical events recorded during follow-up were cirrhosis complications (i.e., clinical ascites, spontaneous bacterial peritonitis, hepato-renal syndrome, gastrointestinal varices, variceal bleeding, liver failure, and encephalopathy [absent / present regardless of the severity

stage]), hepatocellular carcinoma, and death. The primary outcome of the study was LREs, a composite endpoint defined by the first occurrence of a cirrhosis complication or hepatocellular carcinoma. Patients were followed until the first LRE, death, or the last available follow-up visit. In cases of bariatric surgery, patients were censored as without LREs at the date of that surgery.

## Statistics

Missing data were handled using multiple imputation by random forests with the R package *missForest* (25). Continuous variables were expressed as medians with 1<sup>st</sup> and 3<sup>rd</sup> quartiles, and categorical data were expressed as total numbers and percentages. The ability of the fibrosis tests to discriminate between patients with and without future LREs was evaluated using Harrell's C-index (26). Harrell's C-indexes were computed and compared using the R package *compareC*, according to methodology previously published (27). Multiple testing was corrected using the Bonferroni method. Survival curves were determined using the Kaplan-Meier method and compared with the log-rank test. To evaluate the prognostic significance of the non-invasive tests used with their published cut-offs, we performed multivariable Cox models including FIB4 or VCTE with adjustment for age, sex, antidiabetic treatment, antihypertensive treatment, and lipid-lowering treatment. For these analyses, the non-invasive test was introduced as a categorical variable, i.e., three groups defined by the published cut-offs (FIB4: <1.30, 1.30-3.25, >3.25; VCTE: <8.0 kPa, 8.0-12.0 kPa; >12.0 kPa), with calculation of the corresponding adjusted hazard ratios (aHR) for LREs.

A competing risk regression considering death as the competing event for LREs was performed using the Fine-Gray method (28). Survival free of liver-related events with the Kaplan Meier method and with the cumulative incidence function considering death as competing risk (R package *cmprsk*) were computed on the same graph. Survival curves with both methods were perfectly superimposed (**Figure s2**), which showed that death as competing risk did not influence the results in our study. Therefore, results from the most parsimonious analyses (standard Cox model) are presented in the main manuscript, and those from the competing risk analyses in Supplementary Material. All statistical analyses were performed using R version 3.6.2.

## RESULTS

### Patients

In all, 1,376 patients with NAFLD were screened for the study. After removing those with exclusion criteria (**Figure s3**), 1,057 patients with baseline FIB4 and VCTE were included. Of these, 594 had liver biopsy available within one year from baseline (biopsy group). Baseline characteristics of the 1,057 patients included are presented in **Table 1**. Median age was 55 years, 62% of the patients were male, 37% had diabetes, 44% were under antihypertensive treatment, and 27% were under lipid-lowering treatment. Liver-related events occurred in 62 patients during the 3.1 years median follow-up (**Table 2**). The first occurrence was hepatocellular carcinoma in 14 patients and cirrhosis complication in the 48 others (ascites: n=26, gastrointestinal varices: n=18, liver failure/encephalopathy: n=4). Among the 32 deaths observed during the patient follow-up, 17 occurred after liver-related events.

### Comparison of prognostic accuracy between fibrosis tests

FIB4 and VCTE (both as continuous variables) showed good accuracy for the prediction of LREs in the full study cohort, with Harrell's C-indexes above 0.80 (**Table 3**). VCTE had higher Harrell's C-index than FIB4, but the difference did not reach statistical significance (0.878 [0.835-0.921] vs 0.817 [0.768-0.866], p=0.059). In the biopsy subgroup, Harrell's C-indexes of liver biopsy and VCTE were not significantly different; however, both significantly outperformed FIB4 for the prediction of LREs (**Table 3**). With regards to the prediction of all-cause mortality in the whole study cohort, FIB4 showed better discrimination than VCTE (0.895 [0.858-0.932] vs 0.717 [0.590-0.844], p=0.003).

### Prognostic validation of the published diagnostic cut-offs for FIB4 and VCTE

Baseline FIB4 and VCTE results were categorized into three subgroups according to published cut-offs. LRE-free survival according to baseline non-invasive tests in the whole study cohort is depicted in **Figure 1**. Univariate analysis showed that subgroups defined by the non-invasive tests had significantly different prognoses (**Table 4**). Patients with intermediate FIB4 results between 1.30 and 3.25 had a seven-fold higher risk of LREs, while aHR for the intermediate VCTE results (8-12 kPa) reached borderline significance (aHR: 3.9,

95% CI: 0.9-18.0). FIB4 results  $>3.25$  were found in 6.5% of the patients and associated with a 30-fold increased risk of LREs (aHR: 29.5, 95% CI: 10.6-82.3). VCTE results  $>12.0$  kPa were seen in 28.1% of the patients and associated with a 21-fold increased risk of LREs (aHR: 20.5, 95% CI: 4.9-86.5). **Figure s4** shows the risk stratification in the biopsy group according to the subgroups defined by FIB4, VCTE, and histology. Liver biopsy and VCTE showed a similar pattern, both of which distinguishing two subgroups of patients with clearly different prognoses, whereas FIB4 individualized three subgroups including one with intermediate prognosis.

### **Impact of diabetes on the risk of liver-related events**

Diabetes was an independent predictor of LREs in both multivariable analyses we performed for FIB4 and VCTE. We therefore further explored the potential impact of diabetes in patients with FIB4  $<1.30$ , as guidelines indicate these patients should be kept at the primary care level with no need for referral to the liver specialist. In patients having baseline FIB4  $<1.30$ , Kaplan-Meier curves showed that prognosis was impaired in patients with diabetes compared to those without (**Figure s5**). Eight patients experienced LREs during follow-up despite a baseline FIB4  $<1.30$  (**Table s1**). Interestingly, six of these patients had diabetes, and all VCTE results were increased since baseline. For patients with available FIB4 monitoring, the blood test result progressively increased, reaching FIB4  $\geq 1.30$  before LREs occurrence.

The scatter plot in **Figure s6** shows the correlation between FIB4 and VCTE results at baseline. Fifty percent of the patients with baseline FIB4  $<1.30$  had an elevated VCTE result  $>8.0$  kPa, and this rate was higher in patients with diabetes compared to those without (77% vs 40%,  $p<0.001$ ; **Figure s7a**). Fifty-five percent of patients with baseline FIB4  $<1.30$  had a liver biopsy available within one year. In this subgroup, the proportion of patients with advanced fibrosis despite FIB4  $<1.30$  was eight times higher in patients with diabetes than it was in those without: 34% vs 4%, respectively ( $p<0.001$ ). Among patients with diabetes and advanced fibrosis despite baseline FIB4  $<1.30$ , VCTE results were  $\geq 8.0$  kPa in 88% of the cases (**Figure s7b**). These results suggest that a FIB4 result  $<1.30$  should be considered with caution in patients with diabetes, and that VCTE could help to refine the evaluation in this situation.

### **Prognostic accuracy of the FIB4-VCTE algorithm**

We finally studied the prognostic significance of the stepwise FIB4-VCTE algorithm (**Figure s1**). The aHRs for LREs were not significantly different between the first two categories of the algorithm (“FIB4 <1.30” and “FIB4 ≥1.30 then VCTE <8.0 kPa”; **Table 5**). These two categories included 63% of the patients who therefore do not require referral to the liver specialist due to a good prognosis. The aHRs for LREs were significantly higher in the “Increased risk” and “High risk” categories of the FIB4-VCTE algorithm, demonstrating these patients need specialized management (**Figure 2**). The competing risk regression showed results similar to those of the main analysis (**Table s2**). LRE-free survival according to the four categories of the FIB4-VCTE algorithm is depicted in **Figure 3**.

Fibrosis stages as a function of the FIB4-VCTE algorithm categories in the biopsy group are shown in **Table s3**. The rate of patients correctly classified for the diagnosis of advanced fibrosis in the three non-invasive categories “FIB4 <1.30”, “FIB4 ≥1.30 then VCTE <8.0 kPa”, and “FIB4 ≥1.30 then VCTE >12.0 kPa” was, respectively: 89%, 85%, and 70%.

## DISCUSSION

NAFLD now affects 25% of the worldwide population and makes up a significant part of the burden of chronic liver diseases (29). Proportionally, only a minority of patients with NAFLD develop end-stage liver disease or hepatocellular carcinoma. The prevalence of NAFLD is projected to increase even further in the upcoming years mainly due to the rising prevalence of obesity and metabolic syndrome (30). Therefore, the challenge in current clinical practice is the accurate identification of patients at risk of developing LREs, especially considering the likely upcoming arrival of new pharmacological treatments. Thus, one key question would be whether non-invasive tests can be used instead of liver biopsy to predict NAFLD patients who are at a higher risk of liver-related morbidity and mortality over time. Our study shows that the blood test FIB4 and VCTE stratify the risk of LREs in patients with NAFLD, and that VCTE provides similar prognostic accuracy when compared to liver biopsy. Such results suggest that these tests can be an acceptable alternative to liver biopsy for prognostication, and possibly guide decisions on treatment allocation.

The optimal thresholds of non-invasive tests for the diagnosis of advanced fibrosis in NAFLD are still being debated. Indeed, depending on the sample studied, published cut-offs show different results for sensitivity, specificity, and predictive values (7), which brings into question their generalizability to daily clinical practice, especially in primary care. The aHRs from our multivariable analyses demonstrate that, beyond their diagnostic accuracy, the thresholds for FIB4 (1.30 and 3.25) and VCTE (8.0 and 12.0 kPa) delineate patient subgroups with different prognoses. In our study, the risk for LREs was very low for values below the lower cut-off, whereas that risk was somewhat increased between the two cut-offs, and the highest above the higher cut-off. Taken together, these results suggest that FIB4, elastography, and histology should no longer be put into competition in diagnostic studies but rather be considered as three different approaches (respectively functional, physical, and architectural), each able to evaluate NAFLD severity and to stratify the prognosis of the disease.

Blood tests and elastography are notable for their non-invasive nature and the advantages each brings to the table: the former can be easily prescribed by any physician, while the latter gives an immediate result during the consultation, and, when included in ultrasonography devices, additional information on liver morphology. It has been suggested to combine these advantages by using these non-invasive tests in a sequential algorithm (8, 20). This manifests by the first line use of a blood test such as FIB4 that, when low, will identify patients with a low risk of advanced fibrosis and a favorable prognosis. Should FIB4 yield intermediate or high results, second line VCTE can be used to improve identification of advanced fibrosis (31). Such an approach has been shown to discriminate well between patients with and without advanced fibrosis, while reducing the need for liver biopsy (8, 20). Our results provide prognostic validation of this FIB4-VCTE sequential approach by showing that patients with  $FIB4 < 1.30$  and those with  $FIB4 \geq 1.30$  and  $VCTE < 8.0$  kPa have excellent prognoses, while those with  $FIB4 \geq 1.30$  and  $VCTE \geq 8.0$  kPa have an increased risk of LREs. Our results here are important because they validate the recent proposal of using the sequential FIB4-VCTE algorithm as a pathway between primary care physicians and liver specialists (21). Indeed, our results demonstrate that using this pathway can retain patients with good prognoses in the primary care setting, while those in need of specialized management because of an impaired prognosis can be referred to a liver specialist. This

approach has been suggested to be cost-effective in the UK and the US (32, 33), and such evaluations should now be performed in other countries.

Whatever the fibrosis test examined in the multivariable analysis, we consistently identified diabetes as an independent predictor of LREs. Accordingly, in the subgroup with FIB4 <1.30, patients with diabetes showed an impaired prognosis compared to those without. This is problematic, as current guidelines indicate that all patients with FIB4 <1.30 should not be referred to a liver specialist.

A first simple option to circumvent this limitation would be to repeat the FIB4 measurement during follow-up. Indeed, a second FIB4 evaluation within five years improves the identification of subjects with increased liver-related risk in the general population (34). In our work, most of the patients who experienced a LRE despite baseline FIB4 <1.30 had at least one additional FIB4 during their follow-up. In almost all these patients, FIB4 became  $\geq 1.30$  several years before the liver-related event, suggesting repeated measurements of FIB4 are useful to monitor liver-related risks in patients with NAFLD. Another option would be to perform another fibrosis test. Indeed, most of the patients with advanced fibrosis despite baseline FIB4 <1.30 had diabetes and elevated liver stiffness. This suggests that in patients with diabetes and FIB4 <1.30, VCTE may be useful in identifying the subset with impaired prognosis.

These results coming from post-hoc analyses and a small subset of the study cohort are exploratory, so they should be considered with caution and need validation. Previous works have suggested that advanced fibrosis is associated with the duration of diabetes (35), and the association with diabetes control remains debated. Finally, further works are required to better define the profile of patients with diabetes who could benefit from an additional VCTE examination despite FIB4 results <1.30.

We acknowledge some limitations in the current work. Follow-up was rather short, and this limited our ability to ascertain LREs and resulted in wide confidence intervals. However, the size of our cohorts ensured enough outcomes for a meaningful analysis. Further studies with longer follow-up duration and sample sizes conducted in real-life cohorts are required to validate our findings. As expected, patients from the biopsy group had more advanced liver disease than those without (higher serum transaminases, higher results for non-invasive

tests of liver fibrosis), reflecting the indications for liver biopsy according to international practice guidelines. Slight differences were consequently observed between the whole study cohort and the biopsy group for the FIB4 and VCTE Harrell C-indexes. The prognostic accuracies reported for FIB4, VCTE, and the FIB4-VCTE algorithm in the whole study cohort are more prone to generalization than those observed in the biopsy group, as the latter corresponds to a subset of selected patients. In a perfect study, liver biopsy would have been available in all patients, but such work is impossible given it is not ethical to perform this invasive procedure when there is no argument for significant liver disease.

In conclusion, FIB4 and VCTE allow for an accurate prediction of LREs in NAFLD, and therefore position as pertinent tools in place of liver biopsy for the identification of at-risk patients in need of specialized management. The sequential FIB4-VCTE algorithm accurately stratifies LRE risk and should be evaluated in real-life conditions as a referral pathway between primary care physicians and liver specialists.

## **ABBREVIATIONS**

aHR: adjusted hazard ratio

ALT: alanine aminotransferase

AST: aspartate aminotransferase

kPa: kiloPascals

LREs: liver-related events

NAFLD: non-alcoholic fatty liver disease

NASH: non-alcoholic steatohepatitis

VCTE: vibration controlled transient elastography

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## **DATA AVAILABILITY STATEMENT**

Data can be available upon reasonable request to the Corresponding Author

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## TABLES

**Table 1: Baseline characteristics of the study cohort**

	<b>All (n=1,057)</b>	<b>Liver biopsy available (n=594)</b>	<b>No liver biopsy (n=463)</b>	<b>p</b>
Age (years)	55.4 [45.5, 62.5]	54.5 [45.9, 62.2]	56.2 [44.9, 63.0]	0.562
Male sex (%)	659 (62.3)	365 (61.4)	294 (63.5)	0.523
Antidiabetic treatment (%)	387 (36.6)	223 (37.5)	164 (35.4)	0.480
Antihypertensive treatment (%)	469 (44.4)	256 (43.1)	213 (46.0)	0.350
Lipid lowering treatment (%)	284 (26.9)	145 (24.4)	139 (30.0)	0.042
AST (IU/L)	40 [30, 56]	42 [31, 60]	38 [29, 50]	<0.001
ALT (IU/L)	62 [42, 88]	65 [46, 90]	59 [38, 85]	0.003
Gamma GT (IU/L)	73 [41, 146]	81 [45, 156]	66 [38, 136]	0.004
Platelets (G/L)	222 [185, 262]	219 [183, 257]	224 [188, 265]	0.117
FIB4	1.27 [0.83, 1.93]	1.30 [0.86, 2.04]	1.22 [0.81, 1.86]	0.027
VCTE (kPa)	9.4 [6.9, 12.6]	10.4 [7.6, 14.0]	8.3 [6.3, 11.4]	<0.001
Biopsy length (mm)	-	20 [15, 30]	-	-
NASH (%)	-	310 (52.2)	-	-
Fibrosis stage (%):				-
- F0	-	166 (28.0)	-	
- F1	-	142 (23.9)	-	
- F2	-	106 (17.8)	-	
- F3	-	138 (23.2)	-	
- F4	-	42 (7.1)	-	

Continuous variables are expressed as medians with 1<sup>st</sup> and 3<sup>rd</sup> quartiles; categorical variables are presented as numbers with percentages into bracket

AST: aspartate aminotransferase; ALT: alanine aminotransferase; VCTE: vibration controlled transient elastography; kPa: kilo Pascal; NASH: non-alcoholic steatohepatitis

<sup>a</sup> Liver-related event: composite endpoint combining cirrhosis complications and/or hepatocellular carcinoma

<sup>b</sup> Cirrhosis complications: ascites, spontaneous bacterial peritonitis, hepatorenal syndrome, gastrointestinal varices, variceal bleeding, liver failure, encephalopathy

**Table 2: Liver-related events during the follow-up**

	<b>All (n=1,057)</b>	<b>Liver biopsy available (n=594)</b>	<b>No liver biopsy (n=463)</b>
Follow-up (years)	3.1 [1.3, 6.4]	2.2 [0.7, 5.6]	3.9 [2.3, 6.9]
<b>Events during follow-up (n, %):</b>			
- Bariatric surgery	16 (1.5)	9 (1.5)	7 (1.5)
- Liver-related event <sup>a</sup>	62 (5.9)	42 (7.1)	20 (4.3)
- Cirrhosis complication <sup>b</sup>	52 (4.9)	38 (6.4)	14 (3.0)
- Hepatocellular carcinoma	20 (1.9)	8 (1.3)	12 (2.6)
- Death	32 (3.0)	12 (2.0)	20 (4.3)
- Liver-related death	12 (1.1)	5 (0.8)	7 (1.5)

Continuous variables are expressed as medians with 1<sup>st</sup> and 3<sup>rd</sup> quartiles; categorical variables are presented as number with percentages into brackets

<sup>a</sup> Liver-related event: composite endpoint combining cirrhosis complications and/or hepatocellular carcinoma

<sup>b</sup> Cirrhosis complications: ascites, spontaneous bacterial peritonitis, hepatorenal syndrome, gastrointestinal varices, variceal bleeding, liver failure, encephalopathy

**Table 3: Comparison of Harrell C-indexes for the prediction of liver-related events**

<b>Fibrosis test</b>	<b>Whole study cohort</b>	<b>Biopsy group</b>
FIB4	0.817 (0.768 - 0.866)	0.775 (0.712 - 0.837)
VCTE	0.878 (0.835 - 0.921)	0.881 (0.832 - 0.931)
Biopsy	-	0.932 (0.910 - 0.955)
<hr/>		
<b>Comparison (p)</b>		
FIB4 vs VCTE	0.059	0.015
FIB4 vs biopsy	-	<0.001
VCTE vs biopsy	-	0.164

VCTE: vibration-controlled transient elastography

**Table 4: Unadjusted (HR) and adjusted (aHR) hazard ratios for the prediction of liver-related events according to baseline FIB4 or VCTE results**

Fibrosis test	Stratification	Patients (n)	Events (n)	Incidence rate <sup>b</sup>	Univariate analysis		Multivariable analysis <sup>a</sup>	
					HR	[95% CI]	aHR	[95% CI]
FIB4	<1.30	545	8	2.6	1.0	(ref)	1.0	(ref)
	1.30 - 3.25	443	37	20.8	8.9	[3.9 - 20.2]	6.7	[2.7 - 16.5]
	>3.25	69	17	90.8	36.3	[14.8 - 89.5]	29.5	[10.6 - 82.3]
VCTE	<8.0 kPa	392	2	1.3	1.0	(ref)	1.0	(ref)
	8.0 - 12.0 kPa	368	11	5.2	5.0	[1.1 - 22.9]	3.9	[0.9 - 18.0]
	>12.0 kPa	297	49	37.5	32.8	[8.0 - 135.3]	20.5	[4.9 - 86.5]

VCTE: vibration-controlled transient elastography

<sup>a</sup> Multivariable Cox Model adjusted for age, sex, antidiabetic treatment, antihypertensive treatment, and lipid-lowering treatment

<sup>b</sup> per 1000 person/years

**Table 5: Unadjusted (HR) and adjusted (aHR) hazard ratios for the prediction of liver-related events according to the groups defined by the FIB4-VCTE algorithm**

Stratification	Patients (n)	Events (n)	Incidence rate <sup>b</sup>	Univariate analysis		Multivariable analysis <sup>a</sup>	
				HR	[95% CI]	aHR	[95% CI]
FIB4 <1.30	545	8	2.6	1.0	(ref)	1.0	(ref)
FIB4 ≥1.30 & VCTE < 8.0	119	2	4.4	1.7	[0.4 - 8.4]	1.3	[0.3 - 6.8]
FIB4 ≥1.30 & VCTE 8.0 - 12.0	163	10	14.9	6.2	[2.3 - 16.3]	3.8	[1.3 - 10.9]
FIB4 ≥1.30 & VCTE >12.0	230	42	50.3	20.3	[9.1 - 45.4]	12.4	[5.1 - 30.2]

VCTE: vibration-controlled transient elastography

<sup>a</sup> Multivariable Cox Model adjusted on age, sex, antidiabetic treatment, antihypertensive treatment, and lipid-lowering treatment

<sup>b</sup> per 1000 person/years

## FIGURE LEGENDS

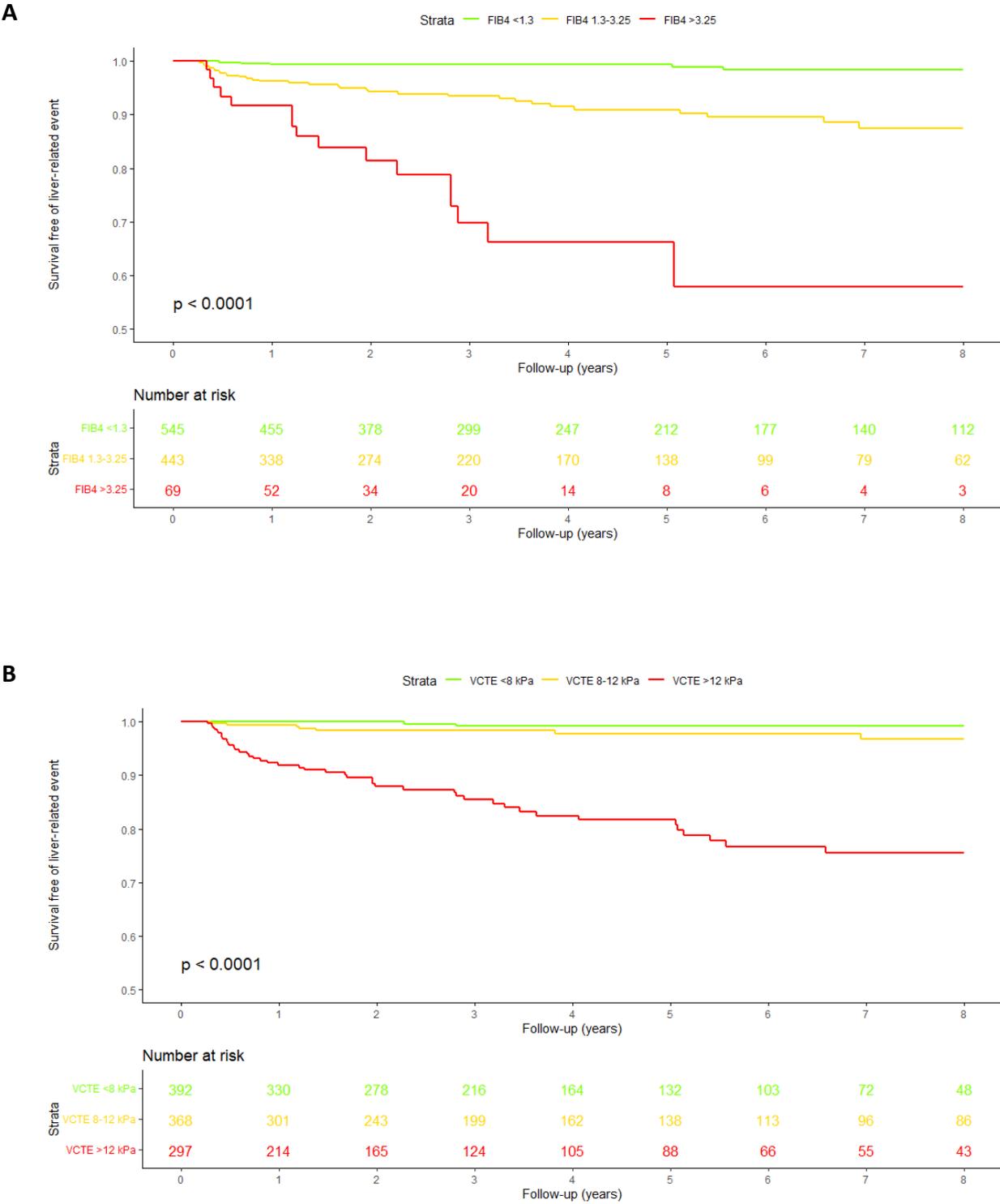
**Figure 1: Survival free of liver-related events according to the groups defined by the FIB4 (panel 1a) or the VCTE (panel 1b) thresholds**

**Figure 2: Prognostic significance of the stepwise FIB4-VCTE algorithm**

The stepwise FIB4-VCTE algorithm was initially developed for the non-invasive diagnosis of advanced liver fibrosis and has been recently proposed as a pathway for patient referral to the liver specialist. Our results provide prognostic validation in NAFLD of this pathway based on non-invasive tests. Patients diagnosed with no/mild liver fibrosis (F0-2) according to the algorithm have a low risk of liver-related events and therefore do not need to be referred to the liver specialist. In contrast, patients in the grey zone of the algorithm and, even more so, those diagnosed with advanced fibrosis (F3-4) have an increased risk of liver-related events and therefore require specialized management of their liver disease.

**Figure 3: Survival free of liver-related events according to the four groups defined by the FIB4-VCTE algorithm**

**Figure 1: Survival free of liver-related events according to the groups defined by the FIB4 (panel 1a) or the VCTE (panel 1b) thresholds**



**Figure 2: Prognostic significance of the stepwise FIB4-VCTE algorithm**

The stepwise FIB4-VCTE algorithm was initially developed for the non-invasive diagnosis of advanced liver fibrosis and has been recently proposed as a pathway for patient referral to the liver specialist. Our results provide prognostic validation in NAFLD of this pathway based on non-invasive tests. Patients diagnosed with no/mild liver fibrosis (F0-2) according to the algorithm have a low risk of liver-related events and therefore do not need to be referred to the liver specialist. In contrast, patients in the grey zone of the algorithm and, even more so, those diagnosed with advanced fibrosis (F3-4) have an increased risk of liver-related events and therefore require specialized management of their liver disease.

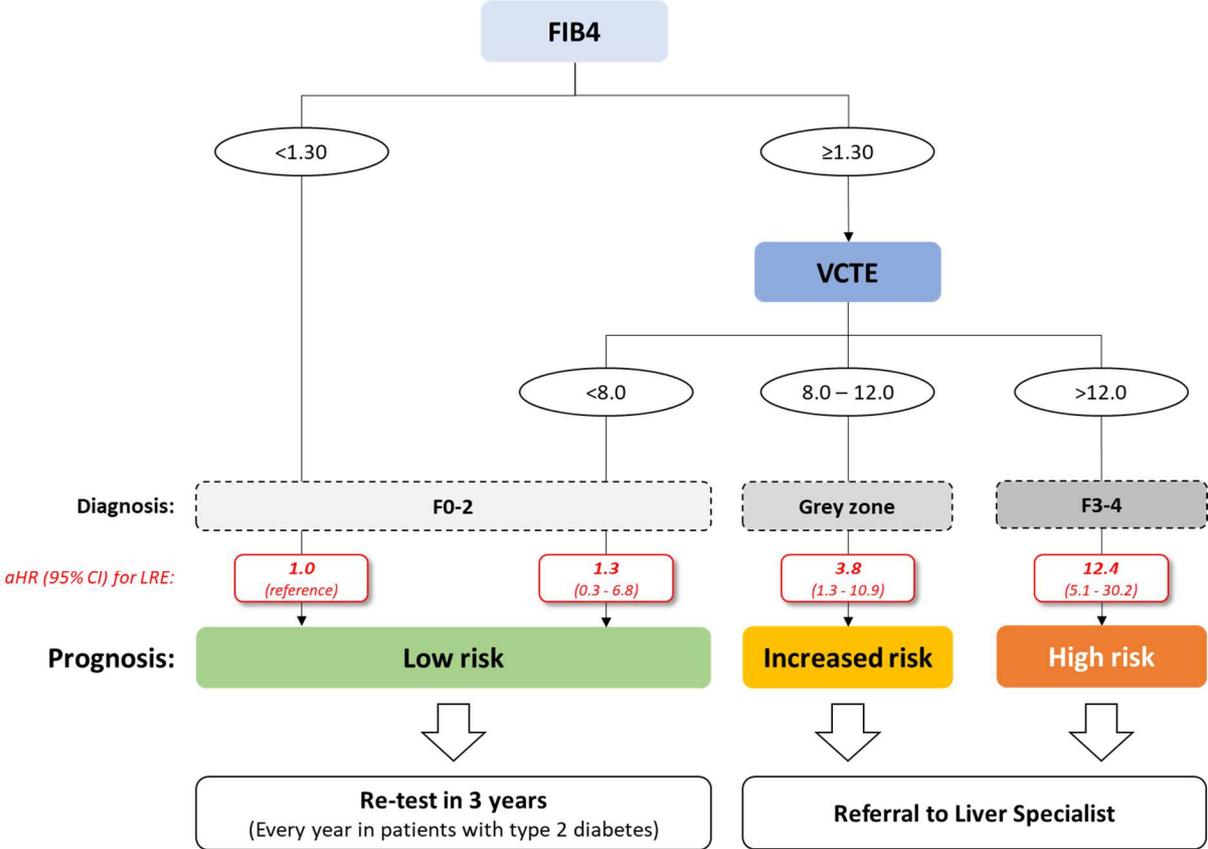


Figure 3: Survival free of liver-related events according to the four groups defined by the FIB4-VCTE algorithm

