Noninvasive LIVERFASt™ transition rate to liver fibrosis is similar to that estimated with liver biopsy in NAFLD patients

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ABSTRACT

Objective: The aim was to demonstrate that LF-Fib is an alternative to LB for the estimation of the transition rate to fibrosis [transition to stage F1 or more (TRF1) in T2D and noT2D, comparatively to other NITs [FIB-4, liver stiffness measurement (LSM) by Fibroscan].

Methods: TRF was evaluated using Cox-Mantel Hazard Ratio [HR(95%CI) and logrank comparison, p value=0.05] with a modeling of hazard from birth to age of LB or NIT in a prospectively collected NAFLD population evaluated for fibrosis with LB, and 3 concomitant NITs (LF-Fib, LSM, FIB4). NITs cut-offs with highest sensitivity for minimal fibrosis were used (0.28, 1.45 and 5.6kPa, respectively).

Results: N=583 pts were included, 52% T2D, 56% males, median (range) age 59.5 (18-85), HbA1c 6.6% (4.7-12), BMI 31.5 (20-54) kg/m2 (obesity 59%), mean (SE) time lapse between LB and NITs 1.7 (0.4) months. The estimation of TRF [HR (95%CI)] using LF-Fib was similar to that using LB in both T2D (0.67 (0.56-0.80) vs. 0.65 (0.54-0.79)), and noT2D [1.50 (1.26-1.78) vs. 1.54 (1.27-1.86)], respectively, with earlier TRF in noT2D compared to T2D (logrank p<0.0001). The TRF of TE and FIB4 were also similar to LB however, less fit in both T2D and noT2D groups for both TE [0.75 (0.63-0.89) vs 1.34 (1.12-1.60), p<0.001] and FIB4 [0.79 (0.63-0.99) vs 1.26 (1.01-1.59), p<0.05], respectively. In pts having ALT>30IU compared to those with ALT≤30IU, the TRF was faster in noT2D [2.13 (1.55-2.95) vs 0.47 (0.34-0.65), logrank p<0.001] and not significantly different in T2D [1.28 (0.93-1.75) vs 0.78 (0.57-1.07), p=ns]. In multivariate analysis, including NITs, arterial hypertension (AHT), HbA1c and BMI, only AHT, BMI≥35, male gender, FIB-4 and LF (Ste, Act and Fib) were significantly associated to TRF in T2D (all p<0.001) and the same with the exception of BMI and FIB-4 in noT2D (P<0.0001 for AHT and LF, P<0.05 for male gender).

Conclusion: Validated biomarkers such as LIVERFASt should allow a powerful analysis of fibrosis progression in NAFLD, similar to LB and better screening strategies for stratifying patients.

BACKGROUND

Liver biopsy is not adapted to routine diagnosis due to the high prevalence of NAFLD, 40% sample-related variability and poor acceptance

There is an urgent need for reliable non-invasive tools for differentiating NAFL from NASH and for disease staging.

Liver enzymes as are often normal despite advanced fibrosis in T2D and therefore, cannot be used to stage NASH fibrosis.

LIVERFASt™ (LF) is a serum AI based algorithm (CPT 0166U)Q for assessing liver fibrosis along with steatohepatitis that demonstrated prognostic value to predict overall and liver-related morbi-mortality.

AIMS

To demonstrate that LIVERFASt™ Fibrosis score (LF-Fib) is a surrogate of liver biopsy (LB) for the estimation of the transition rate to fibrosis F1 stage or more (TRF1), in type 2 diabetic (T2D) patients with better performances than liver stiffness measurement (LSM) by transient elastography and than FIB-4 index.

PATIENTS & METHODS

Patients:

- Prospectively collected NAFLD patients from a tertiary Liver Center (Bordeaux,France) (NCT01241227)
- Concomitant LB and LIVERFASt™, TE, FIB-4.

Transition rate to any fibrosis stage (TRF) was evaluated using modelling of hazard from birth to the age of the liver fibrosis estimator.

Cut-offs for minimal fibrosis, F1 stage:

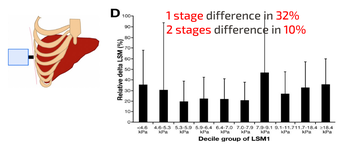
- LB SAF score: perisinusoidal zone 3 or portal fibrosis LIVERFASt™ -Fibrosis: 0.28; TE: 5.6 kPa; FIB-4: 1.45

Statistics:

- Cox Mantel Hazard Ratios [HR (95%CI), logrank comparison p value between groups]
- Logistic regression, Odds Ratios

Vibration Controlled Transient Elastography (TE) by Fibroscan (Echosens, Paris, France)

- **Quality criteria:** IQR/median, Success rate, 10 valid LSM
- **Variability** in 531 NAFLD patients paired measurements



- **Overestimation: Cytolysis** with ALT > 3x ULN, **non-fasting, MetS**: T2D, BMI>30, high-blood pressure

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LIVERFASt™ (Fibronostics, Orlando, Florida)

- **AI computer aided biomarkers** constructed **using SAF histological scoring** for assessing **noninvasively fibrosis, activity** and **steatosis**
- **Combines** 10 biomarkers including liver-specific fibrosis markers, lipid panel, liver enzymes, BMI, age, and gender.
- CPT 0166U

- **Underestimation risk**: inflammatory syndrome (e.g. ulcerated diabetic foot). **Overestimation risks**: hemolysis

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FIB-4 Index

- Algorithm **: platelet count, age, AST, and ALT**

$$\text{FIB-4} = \frac{\text{age(years)} \times \text{AS(IU/L)}}{\text{Platelet count } (10^9/\text{L}) \times \text{ALT(IU/L)}^{\frac{1}{2}}}$$

- **Dual cut-off** for advanced fibrosis (<1.45, >3.25)
- **Over or underestimation** : age range, cytolysis, normal ALT and AST (T2D)
- Lower diagnostic performance for cirrhosis in T2D

RESULTS

Cohort Flow Chart

N= 753 patients
From 2003 to 2020 in Liver Fibrosis Investigation Center, CHU Bordeaux, France

Excluded patients N= 170 missing data

N= 583 patients included with LIVERFAST ,TE and FIB-4

N=301 T2D patients | **N=282 Non T2D**

Characteristics of included patients

Characteristics N=583	Prevalence , median (SE or range)
Male Gender	56.4%
Age, years	59.5 (18-85)
BMI, Kg/m2	31.5 (20.1-54.0)
Obesity BMI≥30 , Kg/m2	59%
ALT, IU/L	55 (0.5)
AST, IU/L	59 (0.12)
HbA1c,%	6.6 (0.14)
Total cholesterol, mmol/l	5.14 (0.54)
Triglycerids, mmol/l	1.58 (0.43)

Non-invasive tests	Median (SE) scores
LIVERFASt™ Fibrosis score	0.48 (0.01)
LIVERFASt™ Activity score	0.41 (0.01)
LIVERFASt™ Steatosis score	0.74 (0.01)
FibroScan LSM CAP	9.6 (0.5) kPa 324 (2.6) dB/m
FIB-4	1.55 (0.08)
Time lapse between LB and NIT	1.7 (0.4) months.

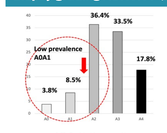
Prevalence of NAFLD features at liver biopsy

Liver Biopsy	
Biopsy length, mm	25 (11-95)mm
Biopsy no. fragments	3 (1-25)
NAS score (Kleiner)	
0-2	**8% (39)**
3-4	**33% (162)**
5-8	**59% (285)**

Biopsy staging of FIBROSIS

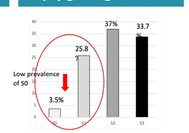
LB Fibrosis staging

Biopsy grading ACTIVITY (SAF)



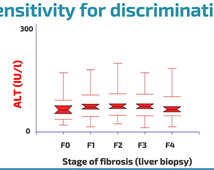
LB Activity grading

Biopsy grading STEATOSIS



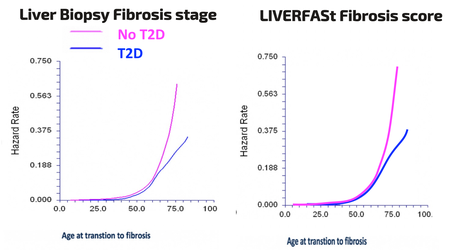
LB Steatosis grading

ALT lacks sensitivity for discriminating fibrosis staging



Stage of fibrosis (liver biopsy)

The Transition rate to fibrosis [HR Plot (95%CI), logrank] as per LIVERFASt™-Fibrosis score was similar to that of liver biopsy in both populations with or without T2D

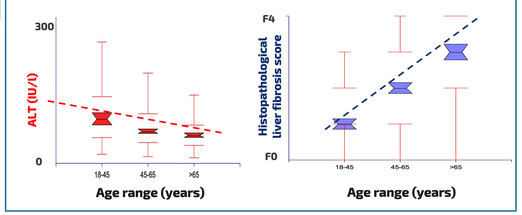


Cox Mantel HR (95%CI)
Non T2DM 1.50 (1.26-1.78)
T2DM 0.67 (0.56-0.80)
p<0.0001

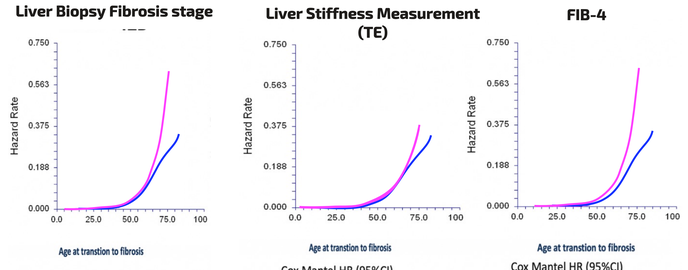
Cox Mantel HR (95%CI)
Non T2DM 1.54 (1.27-1.86)
T2DM 0.65 (0.54-0.79)
p<0.0001

Faster transition rate to F1 stage in patients without T2D compared to patients with T2D (logrank p<0.0001)

Opposite correlation of ALT and liver fibrosis with age ranges



The Transition rates to fibrosis [HR Plot (95%CI), logrank] as estimated using TE and FIB-4 were less similar to that of LB in both populations with or without T2D



Cox Mantel HR (95%CI)
Non T2DM 1.50 (1.26-1.78)
T2DM 0.67 (0.56-0.80)
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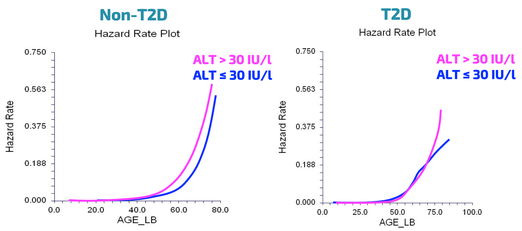
Cox Mantel HR (95%CI)
Non T2DM 1.34 (1.12-1.60)
T2DM 0.75 (0.63-0.89)
p<0.001

Cox Mantel HR (95%CI)
Non T2DM 1.26 (1.01-1.59)
T2DM 0.79 (0.63-0.99),
p=0.04

Abnormal ALT (>30IU/l) is driving the transition to fibrosis in patients without T2D and has no impact in T2D



Cox Mantel HR (95% CI)
ALT > 30 2.13 (1.55-2.95)
ALT ≤ 30 0.47 (0.34-0.65), logrank p<0.001

Cox Mantel HR (95% CI)
ALT > 30 1.28 (0.93-1.75)
ALT ≤ 30 0.78 (0.57-1.07), logrank p=ns

Multivariate analysis

In T2D NAFLD patients, LIVERFASt™ Fibrosis, Activity and Steatosis, high blood pressure and male gender were independently associated to the histological transition to fibrosis

Parameter	T2D patients	Non- T2D patients
LIVERFASt™ Fibrosis	**P<0.0001**	**P<0.0001**
LIVERFASt™ Activity	**P<0.0001**	**P<0.0001**
LIVERFASt™ Steatosis	**P<0.0001**	**P<0.0001**
LSM (TE by Fibroscan)	ns	ns
FIB-4	**P<0.0001**	ns
Blood Pressure (=high)	**P<0.0001**	**P<0.0001**
HbA1c	ns	ns
BMI≥35 Kg/m²	**P<0.0001**	ns
Gender (=male)	**P<0.01**	**P<0.05**

CONCLUSION

Liver-specific AI-based blood biomarkers, such as LIVERFASt™, allow:

- **Detection of progression from simple NAFL to NASH fibrosis, similar to liver histology**
- **Better and earlier screening strategy for stratifying high-risk patients for NASH, as T2D aged ≥45 years or having co-morbidities as obesity or arterial hypertension**
- **Improved estimation of elementary liver lesions with noninvasive standard-of-care**

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Disclosures

Fibronostics: RQ, MM, IA