

## **Supplementary material**

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## QUID NASH protocol

<https://clinicaltrials.gov/ct2/show/NCT03634098>

### Synopsis

Complete title	Identification and validation of non-invasive biomarkers (virtual biopsy) of the diagnosis and severity of NASH in type 2 diabetics: a cross-sectional study. Collection of tissue for the identification of new signalling pathways.
Acronym	QUID-NASH
Coordinating Investigator	Prof Laurent Castera
Scientific Manager	Prof Valérie Paradis
Sponsor	Assistance Publique – Hôpitaux de Paris
Scientific justification	<p>Metabolic liver diseases are silent disorders with significant morbidity. Around 70% of type 2 diabetes (DT2) patients are affected. Of these 50% develop clinically significant lesions (including non-alcoholic steatohepatitis or NASH) as they are accompanied by an increased risk of complications; and 15% develop into severe fibrosis or cirrhosis. These diseases have a slow and asymptomatic course. Their pathophysiology is poorly understood. Their management contends with the absence of a specific diagnostic marker, the necessity for invasive diagnostic procedures (liver biopsy), and the absence of an established treatment. Non-invasive methods (“<b>first generation tests</b>”) have recently experienced significant growth: marketing of the FibroTest as a marker of fibrosis; marketing of the FibroTest, the Fibrometer and the FibroScan, for the initial assessment of chronic hepatitis C in adults; marketing of the FibroTest, Fibrometer and ELF-test for the diagnosis of metabolic liver disease and the diagnosis of fibrosis; marketing of the SteatoTest (AP-HP patent) for the diagnosis of steatosis. The ActiTest (AP-HP patent) is much used to evaluate the necroinflammatory activity of chronic viral hepatitis C and B. Only the ActiTest is validated for the diagnosis of NASH. The NashTest (AP-HP patent) is little used. Several imaging biomarkers (liver ultrasound, FibroScan CAP, elastography and nuclear magnetic resonance) are very widely used for the diagnosis of steatosis. Two new “<b>second generation</b>” <b>blood tests</b> (AP-HP patents) are currently under development, NIT-NASHr, and NIT-A2F2. NIT-NASHr is a new combination of the components of the SteatoTest and the NASH-Test intended to assess the severity of the NASH. NIT-A2F2 is a combination of the NIT-NASHr and the FibroTest, intended to diagnose clinically significant metabolic liver disease. In the project, these tests will be subjected to a performance validation in the context in which they will be used (DT2 without other liver disease). At the same time, significant progress has been observed in the incorporation of omics data enabling the characterization of diverse pathologies and identification of their mechanisms. The transcriptomics and metabolomics of biological fluids are particularly promising for the construction of “<b>third generation</b>” tests.</p> <p>QUID-NASH aims to develop a virtual liver biopsy in DT2 subjects, based on the identification of simple or combined, multimodal, non-invasive biomarkers, obtained by new quantitative imaging techniques (magnetic resonance and ultra-fast ultrasound UFUS); and/or detailed clinical-biological phenotyping data; and/or data obtained by different omics approaches (metabolomics, targeted genetics, transcriptomics). The profiling of extracellular vesicles and immune cells will supplement these data. This approach will also allow us to improve knowledge of the disease (new signalling pathways, new therapeutic targets).</p>
Objective and main assessment criterion	To study in type 2 diabetes patients with a liver biopsy, the performance in the diagnosis of <b>NASH</b> of a simple or composite biomarker (3rd generation test) constructed from clinical-biological data, quantitative imaging data (magnetic resonance and ultra-fast ultrasound UFUS), and blood omics data (metabolomics, targeted genetics, transcriptomics), supplemented by the profiling of extracellular vesicles and the phenotyping of immune cells. A population sample will be used to develop the biomarker; validation will be carried out in an independent sample. <u>Judgement criterion</u> : histological diagnosis of NASH (as established by the centralized review of liver biopsy slides), blind to omics and imaging results.
Secondary objectives and assessment criteria	To study in type 2 diabetes patients with a liver biopsy, the performance in the diagnosis of <b>clinically significant metabolic liver diseases</b> of a simple or composite biomarker (3rd generation test) constructed from clinical-biological data, quantitative imaging data (magnetic resonance and ultra-fast ultrasound UFUS), and blood omics data (metabolomics, targeted genetics, transcriptomics), supplemented by the profiling of extracellular vesicles and the phenotyping of immune cells. A population sample will be used to develop the biomarker; validation will be carried out in an independent sample. <u>Judgement criterion</u> : histological diagnosis of clinically significant metabolic liver disease (SAF-Score $\geq A2$ or $\geq F2$ )

	<p>To study in type 2 diabetes patients with a liver biopsy, the performance in the diagnosis of <b>primary NASH lesions</b> of a simple or composite biomarker (3rd generation test) constructed from clinical-biological data, quantitative imaging data (magnetic resonance and ultra-fast ultrasound UFUS), and blood omics data (metabolomics, targeted genetics, transcriptomics), supplemented by the profiling of extracellular vesicles and the phenotyping of immune cells. A population sample will be used to develop the biomarker; validation will be carried out in an independent sample. <u>Judgement criterion:</u> histological diagnosis of primary NASH lesions (lobular inflammation, ballooning, steatosis).</p> <p>To validate the performance of NIT-NASHr and NIT-A2F2 second generation tests for the diagnosis and assessment of the severity of <b>metabolic liver disease</b> in DT2 patients. <u>Judgement criterion:</u> metabolic liver disease (adjudicated by an independent committee)</p> <p>To identify pathophysiological mechanisms that may lead to therapeutic targets from omics data obtained from liver tissue in association with non-invasive NASH biomarkers previously identified. <u>Judgement criterion:</u> markers - blood and liver tissue omics, and radiological markers</p> <p>Construction of a biocollection (stools, urine, plasma, serum, PBMC, liver biopsy fragment)</p> <p>To study the inter-centre and intra-patient reproducibility of the imaging measurements. <u>Judgement criterion:</u> for the MRI: steatosis, biomechanical properties, T1, diffusivity; for the Aixplorer ultrasound: steatosis, biomechanical properties, vascular properties</p>
Experimental outline	Pilot intra-subject and inter-centre reproducibility study, followed by a multicentre cross-sectional study
Population concerned	<p>Volunteers (for the reproducibility study)</p> <p>DT2 patients with liver function test and liver biopsy abnormalities (for the performance of the 3rd generation tests that will constitute the virtual biopsy)</p> <p>DT2 patients (for the performance of 2nd generation tests)</p>
Main inclusion criteria	<p><u>Pilot phase:</u> adult volunteers</p> <p><u>Performance of 3rd generation tests (virtual biopsy):</u> adult DT2 patients, with liver biopsy performed as part of care, and haemoglobin &gt;7g/L (or &gt;10g/L in the event of cardiovascular or respiratory pathology)</p> <p><u>Performance of 2nd generation tests:</u> adult DT2 patients</p>
Main exclusion criteria	<p><u>Exclusion criteria common to different populations:</u> absence of consent, vulnerable person, protected adult, absence of social security, pregnancy or breastfeeding</p> <p><u>Pilot phase:</u> contraindication to MRI, corpulence incompatible with the performance of an MRI</p> <p><u>Performance of the virtual biopsy:</u> contraindication to MRI, corpulence incompatible with the performance of an MRI, liver diseases already known or linked to other aetiologies</p> <p><u>Performance of 2nd generation tests:</u> liver diseases already known or linked to other aetiologies</p>
Actions or Product that are the subject of the research	<p><u>Pilot phase:</u> Quantitative MRI (with morphometry, elastography, T1 relaxometry, diffusion sequence and multi-echo gradient echo sequence)</p> <p>Aixplorer Ultrasound (Shear Wave Elastography - SWE, Supersonic Imagine) and quantitative Doppler</p> <p><u>Performance of the virtual biopsy:</u> Quantitative MRI (with morphometry, elastography, T1 relaxometry, diffusion sequence and multi-echo gradient echo sequence)</p> <p>Aixplorer Ultrasound (Supersonic Imagine) with morphometry, elastography, determination of steatosis and quantitative Doppler</p> <p><u>Performance of 2nd generation tests:</u> None</p>
Comparative group	Nil
Other actions or procedures added by the research	<p><u>Pilot phase:</u> None</p> <p><u>Performance of the virtual biopsy:</u> "first generation" blood tests, not performed in the month preceding enrolment, targeted genetics, transcriptomics, metabolomics analyses and 2nd generation blood tests (12 additional tubes of blood in total: 67 ml: sample of urine and stools), biocollection (liver tissue, peripheral blood mononuclear cells PBMC, plasma, urine, stools)</p> <p><u>Performance of 2nd generation tests:</u> None</p>
Benefits expected for the participants and for society	Definition of new non-invasive methods for detecting NASH making possible the facilitation and extension of its management and the assessment of the efficacy of specific treatments
Risks added by the research	Specify the risk level added by the research: B
Practical conduct	<p><u>Pilot phase:</u> recruitment of volunteers in Radiology Departments, procurement of consent and execution of imaging examinations on fasting subjects. 1 day of participation</p> <p><u>Performance of the virtual biopsy:</u> recruitment in Diabetology or Hepatology Departments, inclusion in the Hepatology Day Hospital of subjects fasting since midnight the previous day. Imaging examinations performed before the biopsy, specific samples for the research, then performance of the liver biopsy as part of care. If the imaging examinations cannot be performed on the day of the biopsy, they will be done on</p>

	<p>another day before, or afterwards provided an interval of one week after the biopsy is observed. 3 months of participation at most (between information and imaging). No follow-up planned</p> <p><u>Performance of 2nd generation tests:</u> recruitment in Diabetology Departments. 1 day of participation</p>
Number of subjects selected	<p><u>Pilot phase:</u> 20 volunteers</p> <p><u>Performance of the virtual biopsy:</u> 600 patients (2 samples of 300 patients)</p> <p><u>Performance of 2nd generation tests:</u> 200 patients</p>
Provisional number of centres	<p><u>Pilot phase:</u> 2 centres in France (Beaujon Radiology Department, Beaujon, and Necker Radiology Department)</p> <p><u>Clinical phase:</u> 5 centres in France (Cochin Hepatology Department, Cochin Diabetology Department, Beaujon Hepatology Department, Lariboisière Diabetology Department, HEGP [Georges Pompidou European Hospital] Nutrition Department)</p>
Duration of the research	<p><u>Pilot phase:</u></p> <ul style="list-style-type: none"> <li>- duration of enrolment: 10 weeks</li> <li>- duration of participation (treatment and follow-up): 1 month maximum (the time to perform the examinations in the 2 centres)</li> <li>- total duration 14 weeks</li> </ul> <p><u>Performance of the virtual biopsy and performance of 2nd generation tests:</u></p> <ul style="list-style-type: none"> <li>- duration of enrolment: 36 months</li> <li>- duration of participation: 1 month</li> <li>- total duration: 37 months</li> </ul>
Number of enrolments planned per centre and per month	<p><u>Pilot phase:</u> 5 volunteers/centre/month</p> <p><u>Performance of the virtual biopsy:</u> 4 patients/centre/month</p> <p><u>Performance of 2nd generation tests:</u> 3 to 4 patients/centre/month</p>
Statistical analysis	<p>Intra-patient and inter-centre reproducibility of MRI measurements, using a Bland-Altman plot. The following analyses will involve the learning sample and the validation sample. Descriptive analysis of clinical, biological, omics data, profiling of circulating cells and microvesicles, and quantitative imaging in the form of the mean (standard deviation) and categorical variables with frequencies of each modality and percentage in relation to the sample. Principal component analysis to identify any aberrant data (markers and/or patients). On the learning sample, association between the set of potential explanatory variables derived from data providers and the diagnosis of NASH, then the diagnosis of clinically significant metabolic liver disease and finally the diagnosis of primary NASH lesions using univariate logistic regression. Construction of a parsimonious set of variables whose combination is predictive of the diagnosis of NASH using multivariate logistic regression with L1 penalization (LASSO). A multiple imputation method will be used to take account of missing data. The diagnostic performance of this model will be described by the area under the curve AUC, its 95% confidence interval, and the sensitivity, specificity and predictive values for different levels of linear prediction and disease prevalence. Cross-validation by Bootstrap. In order to guarantee robustness with respect to overfitting, the same assessment will be performed on the validation sample.</p> <p>The learning sample and the patients not biopsied will be used to support the validation of NIT-NASHr and NIT-A2F2 second generation serum markers for the diagnosis and staging of metabolic liver disease. In order to identify the main biological signalling pathways characterising NASH pathology, a detailed analysis of the transcriptomics data collected via the liver biopsy will be performed. The first stage will consist in the identification of the differential signature of NASH patients. The signalling pathways and biological functions enriched in this signature will then be identified. Finally methods without a priori could be used to integrate hepatic transcriptomics data and non-invasive markers in order to identify gene networks impacting on the pathology and key genes. These analyses could therefore identify potential pharmacological targets.</p>
Source of funding	RHU (University Hospital Health Research)
Independent Oversight Committee envisaged	No

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### **Clinical and biological assessment**

Body mass index (BMI) was calculated as body weight (kg) divided by squared body height ( $m^2$ ). Waist circumference (WC) was measured at a level midway between the lower rib margin and iliac crest with the tape all around the body standing up. Blood pressure was measured on right arm in the sitting position after resting for at least 15 min.

For each patient, a 12-hour fasting blood collection was performed locally on fresh samples for assessment of the following laboratory parameters: platelet count, international normalized ratio (INR), aspartate transaminase (AST), alanine transaminase (ALT), gamma-glutamyl-transferase, total bilirubin, albumin, ferritin, total cholesterol, high-density lipoprotein (HDL)-cholesterol, low-density lipoprotein (LDL)-cholesterol, triglycerides, fasting blood glucose, A1C hemoglobin (HbA1C), creatinine, estimated glomerular filtration rate (eGFR) using CKD-EPI formula. Urine albumin and creatinine were measured to calculate the urine albumin-to-creatinine ratio (UACR).

**Histopathologic assessment**

Biopsy specimens were fixed in formalin, embedded in paraffin, and stained with hematoxylin and eosin and Sirius Red for fibrosis evaluation. The length and the number of fragments were assessed, and the quality scored according to a 3 classes classification (adequate, marginal, and inadequate). Interpretability for biopsy was based on the standard criteria of length, width, and lack of major fragmentation. If inadequate, the cause was specified: length, fragmentation, or technical issues i.e., inadequate coloration, or granuloma or other diagnosis when no NAFLD but other histological features suggestive of another diagnostic were observed (e.g. granulomatous hepatitis, biliary disease, autoimmune hepatitis).

**Suppl. Table 1. Comparison of patients with adequate and inadequate liver biopsies**

Characteristics	Inadequate LB (N=30)	Adequate LB (N=330)	P value
Sex			0.24
Male	15 (50%)	207 (62.7%)	
Female	15 (50%)	123 (37.3%)	
Age - years	62 (56-65)	59 (52-66)	0.32
WC - cm	109 (103-120)	109* (101-120)	0.55
BMI - kg/m <sup>2</sup>	32 (28-36)	32 (28-36)	0.58
LB route			0.10
Percutaneous	15 (50%)	220 (66.7%)	
Transjugular	15 (50%)	110 (33.3%)	
Liver specimen length (mm)	10 (6-16.5)	17 (12-22)	<0.0001
Liver specimen fragments number	4 (1-6)	2 (1-3)	0.0008

Footnote: P-values were computed with Chi-square tests for qualitative data and with Welch tests for quantitative data. Abbreviations: BMI, body-mass index; LB, liver biopsy; WC, waist circumference. \* Available in 323 patients.



**Suppl. Table 2. Comparison among the 330 patients with liver biopsy between those with ALT  $\leq$  40 IU/L and those with ALT > 40 IU/L**

Characteristics	ALT $\leq$ 40IU/L, (n=120)	ALT> 40IU/L, (n=210)	OR [IC95%]	p (Wald)
Sex, F	62/120 (51.7%)	61/210 (29%)	0.38 [0.24;0.61]	<0.0001
Age, years	61 [53;67]	59 [51;65]	0.98 [0.96;1]	0.078
Body-mass index, kg/m <sup>2</sup>	32 [28;36]	32 [28;35]	0.99 [0.95;1.03]	0.58
Waist circumference, cm	09 [101.25;119.75]	109 [101;120]	1 [0.98;1.02]	0.90
Waist circumference (M: >102cm; F: >88cm)	95/118 (80.5%)	165/205 (80.5%)	1 [0.56;1.76]	>0.99
Hypertension	83/120 (69.2%)	139/209 (66.5%)	0.89 [0.54;1.43]	0.62
HDL-cholesterol (M: <1.03mmo/L; F: <1.29mmol/L)	66/120 (55%)	129/210 (61.4%)	1.3 [0.83;2.05]	0.25
Triglyceride >1.7mmol/L	58/120 (48.3%)	109/210 (51.9%)	1.15 [0.74;1.81]	0.53
Known duration of diabetes, years	10 [5.75;16.25]	9 [4;15]	0.98 [0.95;1]	0.079
Retinopathy	30/117 (25.6%)	32/209 (15.3%)	0.52 [0.3;0.92]	0.024
Neuropathy	30/116 (25.9%)	38/206 (18.4%)	0.65 [0.38;1.12]	0.12
Nephropathy	51/120 (42.5%)	90/209 (43.1%)	1.02 [0.65;1.61]	0.92
At least one microvascular complication	77/118 (65.3%)	119/208 (57.2%)	0.71 [0.44;1.13]	0.15
Cerebrovascular disease	4/120 (3.3%)	8/210 (3.8%)	1.15 [0.35;4.38]	0.82
Peripheral arterial disease	4/120 (3.3%)	6/209 (2.9%)	0.86 [0.24;3.41]	0.81
Ischemic heart disease	26/120 (21.7%)	24/209 (11.5%)	0.47 [0.25;0.86]	0.015
At least one macrovascular complication	30/120 (25%)	36/209 (17.2%)	0.62 [0.36;1.08]	0.092
Oral antidiabetic drugs	116/120 (96.7%)	204/210 (97.1%)	1.17 [0.29;4.19]	0.81
Insulin without GLP1	15/120 (12.5%)	14/210 (6.7%)	0.5 [0.23;1.08]	0.076
GLP1 without insulin	22/120 (18.3%)	53/210 (25.2%)	1.5 [0.87;2.67]	0.15
GLP1 & Insulin	51/120 (42.5%)	62/210 (29.5%)	0.57 [0.35;0.91]	0.017
Cardiovascular drugs	90/120 (75%)	150/210 (71.4%)	0.83 [0.5;1.38]	0.48
Lipid-lowering drugs	85/120 (70.8%)	128/210 (61%)	0.64 [0.39;1.03]	0.072
Platelet count, G/L	248.5 [201;304]	236.5 [194;285.75]	1 [0.99;1]	0.16
INR	1 [1;1.03]	1 [1;1.04]	1.98 [0.23;21.55]	0.55
AST, IU/L	26 [23;32]	42 [33;53]	1.16 [1.12;1.21]	<0.0001
GGT, IU/L	42.5 [27;64]	61 [40;100.5]	1.01 [1;1.01]	0.002
Total bilirubin, $\mu$ mol/L	7 [5.6;10]	9.7 [6.8;14]	1.14 [1.08;1.21]	<0.0001
Serum albumin, g/L	42.8 [39;45]	44 [41;46]	1.13 [1.06;1.21]	0.0003
Total cholesterol, mmol/L	3.81 [3.34;4.66]	4.11 [3.52;4.91]	1.15 [0.92;1.44]	0.22
LDL-cholesterol, mmol/L	1.89 [1.53;2.4]	2.16 [1.56;2.86]	1.17 [0.9;1.53]	0.25
HDL-cholesterol, mmol/L	1.07 [0.91;1.29]	1.03 [0.88;1.22]	0.96 [0.57;1.66]	0.87
Triglycerides, mmol/L	1.62 [1.06;2.33]	1.76 [1.32;2.49]	1.13 [0.94;1.39]	0.21
Fasting blood glucose, g/L	8.03 [6.63;10.39]	8.47 [7.05;10.39]	1.02 [0.94;1.11]	0.60
HbA1C, %	7.4 [6.8;8.38]	7.6 [6.8;8.4]	1.07 [0.9;1.28]	0.45
Serum creatinine, $\mu$ mol/L	69.7 [56.45;83.5]	72 [61;85]	1 [0.99;1]	0.16
Serum ferritin, $\mu$ g/L	85 [42.75;156]	134.9 [65.47;234.75]	1.003 [1.002;1.005]	0.0005
eGFR, mL/mn	3.08 [81.22;103.91]	96.14 [81.87;104.26]	1.01 [1;1.02]	0.096
UACR, mg/mmol	2.01 [1;5.34]	1.95 [0.92;5.84]	1 [1;1.01]	0.78
FIB-4	1.15 [0.88;1.67]	1.24 [0.91;1.72]	1.15 [0.88;1.56]	0.35
NAFLD fibrosis score	-0.5 [-1.4;0.55]	-0.8 [-1.6;0.1]	0.81 [0.67;0.97]	0.021
CAP, dB/m	329 [292;370]	342.5 [312.25;372]	1 [1;1.01]	0.075
LSM, kPa	7.5 [5.9;11.45]	8.5 [6.3;12]	1 [0.98;1.03]	0.84

**Suppl. Table 3. Factors associated with advanced fibrosis in univariate analysis**

Characteristics	Missing Data (n)	F0-F2 (N=206)	F3-F4 (N=124)	Bivariate analysis	
				OR (95%CI)	p
Age - years		57 (51-64)	61 (55-66)	1.04 (1.01-1.06)	0.003
Female sex		74 (36%)	49 (40%)	1.17 (0.73-1.84)	0.51
BMI - kg/m <sup>2</sup>		31 (28-35)	33 (29-36)	1.05 (1.01-1.09)	0.03
WC >102 cm in M or >88 cm in F	7	156 (77%)	104 (87%)	1.96 (1.07-3.73)	0.03
Hypertension	1	136 (66%)	86 (70%)	1.20 (0.74-1.95)	0.47
HDL-C <1.03 mmol/L in M or <1.29 mmol/L in F		112 (54%)	83 (67%)	1.70 (1.07-2.72)	0.03
Triglyceride >1.7 mmol/L		100 (49%)	67 (54%)	1.25 (0.8-1.95)	0.33
Duration of diabetes - years	1	9 (4-15)	10 (5-17)	1.02 (1-1.05)	0.08
Retinopathy	4	37 (18%)	25 (20%)	1.14 (0.64-2.01)	0.64
Neuropathy	8	44 (22%)	24 (20%)	0.90 (0.51-1.56)	0.70
Nephropathy	1	83 (40%)	58 (47%)	1.32 (0.84-2.08)	0.22
Stroke		8 (4%)	4 (3%)	0.83 (0.22-2.68)	0.76
Peripheral arterial disease	1	7 (3%)	3 (2%)	0.71 (0.15-2.61)	0.63
Ischemic heart disease	1	31 (15%)	19 (15%)	1.03 (0.55-1.9)	0.92
Insulin without GLP1		16 (8%)	13 (11%)	1.39 (0.64-3.00)	0.40
GLP1 & Insulin		71 (35%)	42 (34%)	0.97 (0.61-1.55)	0.91
Cardiovascular drugs		144 (70%)	96 (77%)	1.48 (0.89-2.5)	0.14
Lipid-lowering drugs		130 (63%)	83 (67%)	1.18 (0.74-1.9)	0.48
Platelet count - g/L		248 (204-304)	221 (183-268)	0.994 (0.991-0.998)	0.0009
AST - IU/L	1	31 (25-42)	40 (33-54)	1.03 (1.02-1.05)	<0.0001
ALT - IU/L		45 (33-61)	54 (39-80)	1.01 (1.01-1.02)	0.001
GGT - IU/L		47 (30-67)	75 (44-129)	1.008 (1.004-1.012)	<0.0001
Total bilirubin - µmol/L		8.6 (6.03-11.8)	9.4 (6.3-13.8)	1.03 (0.99-1.07)	0.19
Serum albumin - g/L	8	44 (41-46)	43 (40-45)	0.94 (0.89-1.00)	0.06
Serum ferritin - µg/L	5	100 (56-197)	117 (52-221)	1.00 (1.00-1.00)	0.96
Total cholesterol - g/L		1.55 (1.30-1.90)	1.50 (1.32-1.83)	0.77 (0.43-1.35)	0.36
LDL-C - mmol/L	15	2.07 (1.58-2.79)	2.07 (1.50-2.63)	0.86 (0.66-1.11)	0.25
HDL-C - mmol/L		1.03 (0.91-1.29)	1.01 (0.88-1.2)	0.79 (0.43-1.35)	0.41
Triglycerides - mmol/L		1.66 (1.18-2.36)	1.77 (1.39-2.56)	1.10 (0.91-1.32)	0.32
Fasting blood glucose - mmol/L	1	8.3 (6.93-10.34)	8.3 (7.04-11.01)	1.00 (0.93-1.09)	0.91
HbA1C - %	3	7.5 (6.8-8.4)	7.6 (6.8-8.4)	1.06 (0.89-1.26)	0.54
Serum creatinine - µmol/L	2	70 (60.3-84.1)	72.5 (59.3-83.8)	1.00 (0.99-1.00)	0.31
eGFR - ml/min	2	96.8 (84.7-105.2)	92.7 (77.6-101.7)	0.99 (0.98-1.00)	0.27
UACR - mg/mmol	70	1.94 (0.89-5.50)	2.4 (1.08-6.43)	1.00 (0.99-1.00)	0.52
FIB-4	1	1.13 (0.81-1.47)	1.5 (1.09-2.08)	2.92 (2.01-4.39)	<0.0001
NAFLD fibrosis score	8	-0.9 (-1.7- -0.1)	-0.3 (-1.2-0.7)	1.60 (1.32-1.96)	<0.0001
CAP - dB/m	13	334 (301.75-364)	351 (313-379)	1.007(1.002-1.012)	0.01
Liver stiffness - kPa	3	6.9 (5.5-9.1)	12.0 (9.2-16.7)	1.24 (1.17-1.32)	<0.0001
Activity 3-4 vs. 0-2 (SAF)		31 (15%)	81 (65%)	10.63 [6.32;18.34]	<0.0001

Footnote: Qualitative data were described with absolute and relative frequencies and quantitative data were described with median and interquartile interval. Abbreviations: OR corresponds to odd's ratio; aOR: adjusted odd's ratio; 95%CI : 95% confidence interval; p: the p-value of Wald test for bivariate analysis, respectively the adjusted p-value for multivariate analysis; ALT: alanine aminotransferase; AST: aspartate aminotransferase; BMI: body mass index; CAP: controlled attenuation parameter; GGT: gamma glutamyl transferase; eGRF: glomular filtration rate; HbA1C: Haemoglobin A1C; HDL-C: cholesterol high-density lipoprotein; LDL-C: cholesterol low-density lipoprotein; UACR: Urine albumin creatinine ratio ; WC: waist circumference.

**Suppl. Table 4. Comparison of diagnostic performance for NASH and AF between multivariate models (with and without VCTE) and LSM, FIB-4, AST and ALT**

Model	AUROC (95%CI)	Rule-out Cut-off	n (%)	Se	Sp	PPV	NPV	Grey zone % (n)	Rule-in Cut-off	n (%)	Se	Sp	PPV	NPV	CC (%)
<b>NASH (N=300)</b>															
<b>Without VCTE</b>	0.81 (0.76-0.86)	< 0.397	n=77 (26%)	0.9 (156/173)	0.47 (60/127)	0.7 (156/223)	0.78 (60/77)	49% (n=146)	> 0.775	n=77 (26%)	0.38 (65/173)	0.91 (115/127)	0.84 (65/77)	0.52 (115/223)	(TP:65 + TN:60) 42%
<b>With VCTE</b>	0.82 (0.77-0.87)	< 0.413	n=91 (30%)	0.9 (156/173)	0.58 (74/127)	0.75 (156/209)	0.81 (74/91)	46% (n=139)	> 0.806	n=70 (23%)	0.34 (58/173)	0.91 (115/127)	0.83 (58/70)	0.5 (115/230)	(TP:58 +TN:74) 44%
<b>P* value</b>	0.58														
<b>LSM, kPa</b>	0.70 (0.64-0.76)	< 5.5	n=49 (16%)	0.91 (157/173)	0.26 (33/127)	0.63 (157/251)	0.67 (33/49)	64% (n=192)	> 13.2	n=59 (20%)	0.28 (48/173)	0.91 (116/127)	0.81 (48/59)	0.48 (116/241)	(TP: 48 + TN: 33) 27%
<b>FIB4</b>	0.62 (0.55-0.68)	< 0.8	n=50 (17%)	0.9 (155/173)	0.25 (32/127)	0.62 (155/250)	0.64 (32/50)	70% (n=209)	> 2.07	n=41 (14%)	0.17 (29/173)	0.91 (115/127)	0.71 (29/41)	0.44 (115/259)	(TP: 29 + TN: 32) 20%
<b>ALT, IU/L</b>	0.68 (0.62-0.74)	< 31	n=48 (16%)	0.9 (156/173)	0.24 (31/127)	0.62 (156/252)	0.65 (31/48)	66% (n=198)	> 75	n=54 (18%)	0.25 (43/173)	0.91 (116/127)	0.8 (43/54)	0.47 (116/246)	(TP: 43 + TN: 31) 25%
<b>AST, IU/L</b>	0.74 (0.69-0.80)	< 26	n=62 (21%)	0.89 (154/173)	0.34 (43/127)	0.65 (154/238)	0.69 (43/62)	57% (n=171)	> 47	n=67 (22%)	0.33 (57/173)	0.92 (117/127)	0.85 (57/67)	0.5 (117/233)	(TP: 57 +TN: 43) 33%
<b>F3F4 (N=319)</b>															
<b>Without VCTE</b>	0.78 (0.73-0.83)	< 0.252	n=114 (36%)	0.9 0 (104/116)	0.5 (102/203)	0.51 (104/205)	0.89 (102/114)	45% (n=144)	> 0.53	n=61 (19%)	0.36 (42/116)	0.91 (184/203)	0.69 (42/61)	0.71 (184/258)	(TP:42 + TN:102) 45%
<b>With VCTE</b>	0.85 (0.81-0.89)	< 0.228	n=137 (43%)	0.90 (104/116)	0.62 (125/203)	0.57 (104/182)	0.91 (125/137)	32% (n=101)	> 0.477	n=81 (25%)	0.53 (62/116)	0.91 (184/203)	0.77 (62/81)	0.77 (184/238)	(TP:62 + TN:125) 59%
<b>P** value</b>	0.0007														
<b>LSM, kPa</b>	0.84 (0.79-0.88)	< 7.5	n=135 (42%)	0.91 (105/116)	0.61 (124/203)	0.57 (105/184)	0.92 (124/135)	33% (n=106)	> 11.9	n=78 (24%)	0.52 (60/116)	0.91 (185/203)	0.77 (60/78)	0.77 (185/241)	(TP: 60 + TN: 124) 58%
<b>FIB4</b>	0.71 (0.65-0.76)	< 0.91	n=81 (25%)	0.89 (103/116)	0.33 (68/203)	0.43 (103/238)	0.84 (68/81)	57% (n=181)	> 1.93	n=57 (18%)	0.33 (38/116)	0.91 (184/203)	0.67 (38/57)	0.7 (184/262)	(TP: 38 + TN: 68) 33%
<b>ALT, IU/L</b>	0.61 (0.54-0.68)	< 28	n=37 (12%)	0.9 (104/116)	0.12 (25/203)	0.37 (104/282)	0.68 (25/37)	76% (n=244)	> 85	n=38 (12%)	0.16 (19/116)	0.91 (184/203)	0.5 (19/38)	0.65 (184/281)	(TP: 19 + TN: 25) 14%
<b>AST, IU/L</b>	0.70 (0.64-0.76)	< 27	n=74 (23%)	0.9 (104/116)	0.31 (62/203)	0.42 (104/245)	0.84 (62/74)	60% (n=192)	> 52	n=53 (17%)	0.29 (34/116)	0.91 (184/203)	0.64 (34/53)	0.69 (184/266)	(TP: 34 + TN: 62) 30%

Footnote: Se sensitivity, Sp specificity, PPV positive predictive value, NPV negative predictive value, CC correctly classified, TP true positive, TN true negative; Grey zone corresponds to patients with results between the two cutoffs. P\* comparison of AUROCs with Delong's test for paired data, between multivariate models for NASH with and without VCTE, P\*\* comparison of AUROC with Delong's test for paired data, between multivariate models for AF with and without VCTE. Sample sizes were dependent on the availability of all the variables studied in the table to ensure comparability of the reported estimates. Thus, the sample sizes and AUROC estimates differ from the results reported in the main manuscript.

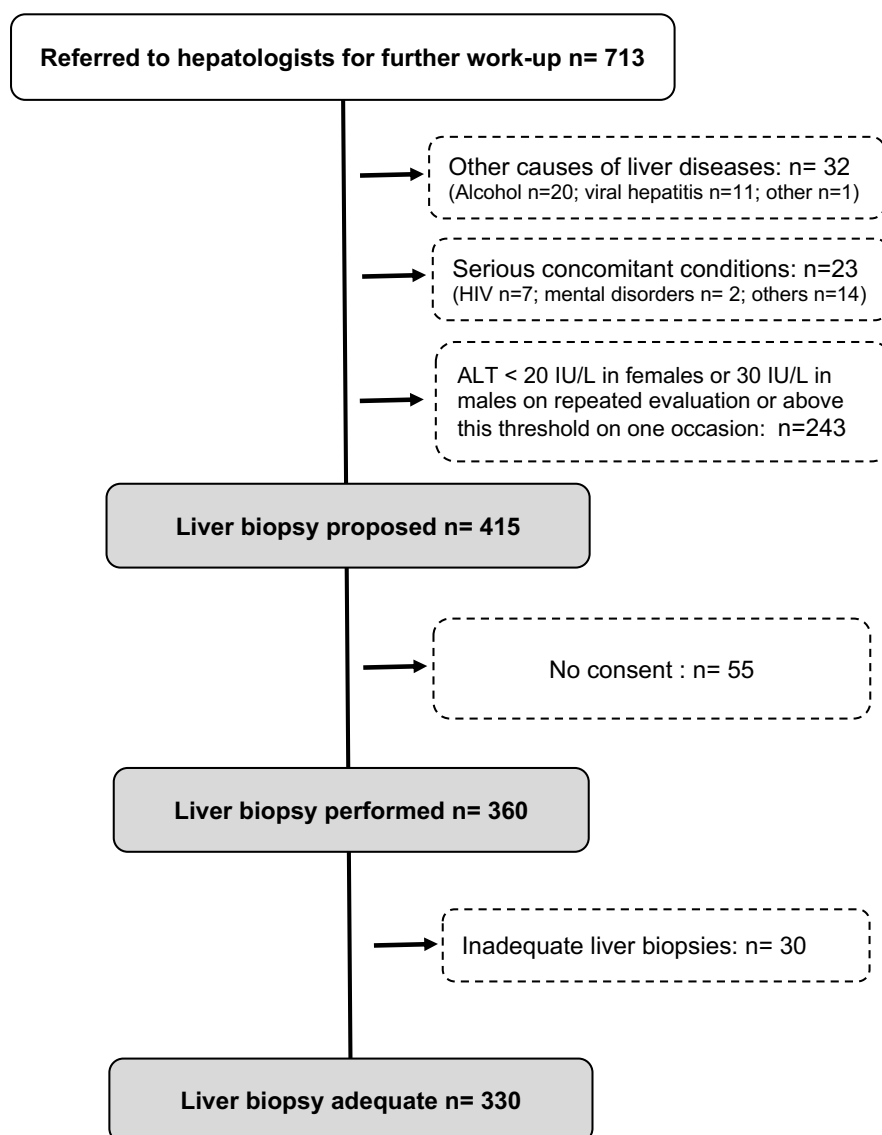
**Suppl. Table 5. Factors associated with NASH in univariate analysis**

Characteristic	Missing Data (n)	No NASH (N=138)	NASH (N=192)	Bivariate analysis	
				OR (95%CI)	p
Age - years		60 (52-67)	59 (52-65)	1.00 (0.97-1.02)	0.79
Female sex		45 (33%)	78 (41%)	1.41 (0.90-2.24)	0.14
BMI - kg/m <sup>2</sup>		31 (28-35)	32 (29-36)	1.05 (1.01-1.10)	0.02
WC >102 cm in M or >88 cm in F	7	99 (73%)	161 (86%)	2.17 (1.24-3.82)	0.01
Hypertension	1	87 (63%)	135 (71%)	1.41 (0.89-2.25)	0.15
HDL-C <1.03 mmol/L in M or <1.29 mmol/L in F		70 (51%)	125 (65%)	1.81 (1.16-2.84)	0.01
Triglycerides > 1.7mmol/L		56 (41%)	111 (58%)	2.01 (1.29-3.14)	0.002
Duration of diabetes - years	1	9 (5-15)	10 (5-15)	1.01 (0.98-1.03)	0.54
Retinopathy	4	26 (19%)	36 (19%)	0.97 (0.56-1.72)	0.93
Neuropathy	8	30 (22%)	38 (20%)	0.91 (0.53-1.56)	0.72
Nephropathy	1	55 (40%)	86 (45%)	1.21 (0.78-1.89)	0.40
Stroke		3 (2%)	9 (5%)	2.21 (0.65-10.1)	0.24
Peripheral arterial disease	1	5 (4%)	5 (3%)	0.72 (0.20-2.62)	0.60
Ischemic heart disease	1	25 (18%)	25 (13%)	0.68 (0.37-1.25)	0.21
Insulin without GLP1		11 (8%)	18 (9%)	1.19 [0.55;2.69]	0.66
GLP1 without insulin		25 (18%)	50 (26%)	1.59 [0.94;2.76]	0.09
GLP1 & Insulin		47 (34%)	66 (34%)	1.01 [0.64;1.61]	0.95
Cardiovascular drugs		96 (70%)	144 (75%)	1.31 (0.80-2.14)	0.27
Lipid lowering drugs		93 (67%)	120 (63%)	0.81 (0.51-1.28)	0.36
Platelet count - g/L		244 (200-292)	243 (197-292)	1.00 (1.00-1.00)	0.81
AST - IU/L	1	29 (24-36)	40 (32-53)	1.06 (1.04-1.09)	<0.0001
ALT - IU/L		40 (31-57)	55 (40-76)	1.03 (1.02-1.04)	<0.0001
GGT - IU/L		46 (27-66)	60 (40-109)	1.007 (1.003-1.011)	0.002
Total bilirubin - µmol/L		8.9 (6.3-12.1)	8.9 (6.0-12.5)	1.00 (0.96-1.04)	0.98
Serum albumin - g/L	8	44 (41-46)	43 (40-45)	0.96 (0.90-1.02)	0.19
Serum ferritin - µg/L	5	92 (44-180)	129 (67-235)	1.001 (1.0001-1.003)	0.04
Total cholesterol - g/L		1.52 (1.28-1.73)	1.58 (1.35-1.91)	1.78 (1.02-3.18)	0.046
LDL-C - mmol/L	15	2.07 (1.57-2.52)	2.09 (1.54-2.87)	1.01 (0.79-1.29)	0.93
HDL-C - mmol/L		1.09 (0.91-1.31)	1.02 (0.88-1.2)	0.88 (0.53-1.49)	0.64
Triglycerides - mmol/L		1.44 (0.98-2.08)	1.89 (1.45-2.69)	1.70 (1.35-2.20)	<0.0001
Fasting blood glucose - mmol/L	1	7.97 (6.65-9.79)	8.53 (7.2-11.05)	1.09 (1.003-1.18)	0.045
HbA1C - %	3	7.4 (6.6-8.1)	7.7 (6.9-8.6)	1.27 (1.06-1.53)	0.01
Serum creatinine - µmol/L	2	73 (63.6-84.1)	70 (58.4-83.5)	0.99 (0.981-0.998)	0.03
eGFR - ml/mn	2	93.9 (84.2-103.9)	95.2 (79.5-104.5)	1.01 (1.00-1.02)	0.16
UACR - mg/mmol	70	1.84 (0.87-5.50)	2.03 (1.06-6.15)	1.00 (1.00-1.01)	0.71
FIB-4	1	1.15 (0.8-1.51)	1.3 (0.96-1.85)	1.63 (1.18-2.34)	0.005
NAFLD fibrosis score	8	-0.8 (-1.6- -0.05)	-0.6 (-1.5-0.2)	1.11 (0.93-1.33)	0.25
CAP - dB/m	13	325 (283-359)	349 (321-378)	1.01 (1.01-1.02)	<0.0001
Liver stiffness - kPa	3	6.7 (5.4-9.5)	10.1 (7.3-13.7)	1.06 (1.02-1.11)	0.003

Footnote: Qualitative data were described with absolute and relative frequencies and quantitative data were described with median and interquartile interval. Abbreviations: OR corresponds to odd's ratio; aOR: adjusted odd's ratio; 95%CI : 95% confidence interval; p: the p-value of Wald test for bivariate analysis, respectively the adjusted p-value for multivariate analysis; ALT: alanine aminotransferase; AST: aspartate aminotransferase; BMI: body mass index; CAP: controlled attenuation parameter GGT: γ-glutamyl transferase; eGRF: estimated glomular filtration rate; HbA1C: Haemoglobin A1C; HDL;C: cholesterol high-density lipoprotein; LDL;C: cholesterol low-density lipoprotein; UACR: Urine albumin creatinine ratio; WC: waist circumference

Suppl. Figure 1: Study flow chart

### Flowchart



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