



# Diagnostic accuracy of non-invasive tests for advanced fibrosis in patients with NAFLD: an individual patient data meta-analysis

Ferenc Emil Mózes ,<sup>1</sup> Jenny A Lee,<sup>2</sup> Emmanuel Anandraj Selvaraj,<sup>1,3,4</sup> Arjun Narayan Ajmer Jayaswal,<sup>1</sup> Michael Trauner ,<sup>5</sup> Jerome Boursier ,<sup>6,7</sup> Céline Fournier,<sup>8</sup> Katharina Staufer,<sup>5,9,10</sup> Rudolf E Stauber,<sup>11</sup> Elisabetta Bugianesi ,<sup>12</sup> Ramy Younes ,<sup>13</sup> Silvia Gaia,<sup>12</sup> Monica Lupşor-Platon,<sup>14</sup> Salvatore Petta ,<sup>15</sup> Toshihide Shima,<sup>16</sup> Takeshi Okanoue,<sup>16</sup> Sanjiv Mahadeva ,<sup>17</sup> Wah-Kheong Chan,<sup>17</sup> Peter J Eddowes,<sup>18,19</sup> Gideon M Hirschfield,<sup>20</sup> Philip Noel Newsome ,<sup>18,21,22</sup> Vincent Wai-Sun Wong ,<sup>23</sup> Victor de Ledinghen,<sup>24,25</sup> Jiangao Fan,<sup>26</sup> Feng Shen,<sup>26</sup> Jeremy F Cobbald,<sup>3,4</sup> Yoshio Sumida,<sup>27</sup> Akira Okajima,<sup>28</sup> Jörn M Schattenberg,<sup>29</sup> Christian Labenz ,<sup>29</sup> Won Kim,<sup>30</sup> Myoung Seok Lee,<sup>31</sup> Johannes Wiegand ,<sup>32</sup> Thomas Karlas ,<sup>32</sup> Yusuf Yilmaz ,<sup>33,34</sup> Guruprasad Padur Aithal ,<sup>19,35</sup> Naaventhann Palaniyappan,<sup>19,35</sup> Christophe Cassinotto ,<sup>36</sup> Sandeep Aggarwal,<sup>37</sup> Harshit Garg,<sup>37</sup> Geraldine J Ooi ,<sup>38</sup> Atsushi Nakajima ,<sup>39</sup> Masato Yoneda,<sup>39</sup> Marianne Zioli,<sup>40</sup> Nathalie Barget,<sup>41</sup> Andreas Geier ,<sup>42</sup> Theresa Tuthill,<sup>43</sup> M. Julia Brosnan,<sup>43</sup> Quentin Mark Anstee,<sup>44</sup> Stefan Neubauer,<sup>1</sup> Stephen A. Harrison,<sup>1</sup> Patrick M Bossuyt,<sup>2</sup> Michael Pavlides ,<sup>1,3,4</sup> the LITMUS Investigators

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For numbered affiliations see end of article.

## Correspondence to

Dr Michael Pavlides, Cardiovascular Medicine, Radcliffe Department of Medicine, University of Oxford, Oxford, Oxfordshire, UK; [michael.pavlides@cardiov.ox.ac.uk](mailto:michael.pavlides@cardiov.ox.ac.uk)

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## ABSTRACT

**Objective** Liver biopsy is still needed for fibrosis staging in many patients with non-alcoholic fatty liver disease. The aims of this study were to evaluate the individual diagnostic performance of liver stiffness measurement by vibration controlled transient elastography (LSM-VCTE), Fibrosis-4 Index (FIB-4) and NAFLD (non-alcoholic fatty liver disease) Fibrosis Score (NFS) and to derive diagnostic strategies that could reduce the need for liver biopsies.

**Design** Individual patient data meta-analysis of studies evaluating LSM-VCTE against liver histology was conducted. FIB-4 and NFS were computed where possible. Sensitivity, specificity and area under the receiver operating curve (AUROC) were calculated. Biomarkers were assessed individually and in sequential combinations.

**Results** Data were included from 37 primary studies ( $n=5735$ ; 45% women; median age: 54 years; median body mass index: 30 kg/m<sup>2</sup>; 33% had type 2 diabetes; 30% had advanced fibrosis). AUROCs of individual LSM-VCTE, FIB-4 and NFS for advanced fibrosis were 0.85, 0.76 and 0.73. Sequential combination of FIB-4 cut-offs ( $<1.3$ ;  $\geq 2.67$ ) followed by LSM-VCTE cut-offs ( $<8.0$ ;  $\geq 10.0$  kPa) to rule-in or rule-out advanced fibrosis had sensitivity and specificity (95% CI) of 66% (63–68) and 86% (84–87) with 33% needing a biopsy to establish a final diagnosis. FIB-4 cut-offs ( $<1.3$ ;  $\geq 3.48$ ) followed by LSM cut-offs ( $<8.0$ ;  $\geq 20.0$  kPa) to rule out advanced fibrosis or rule in cirrhosis had a sensitivity of 38% (37–39) and specificity of 90% (89–91) with 19% needing biopsy.

## Significance of this study

### What is already known on this subject?

- Patients with non-alcoholic fatty liver disease (NAFLD) and advanced fibrosis (F3–4) are at risk of disease progression and adverse clinical outcomes.
- Non-invasive tests with predefined cut-offs are used as screening biomarkers to identify those at low risk of advanced fibrosis who can be safely managed in primary care.
- Liver biopsy is still needed in secondary care to further identify those with cirrhosis who would benefit from surveillance for hepatocellular cancer and screening for oesophageal varices.

**Conclusion** Sequential combinations of markers with a lower cut-off to rule-out advanced fibrosis and a higher cut-off to rule-in cirrhosis can reduce the need for liver biopsies.

## INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is the hepatic manifestation of the metabolic syndrome with high prevalence worldwide.<sup>1</sup> Most patients remain asymptomatic for long periods of time (years/decades) with slowly progressive disease, but

## Significance of this study

## What are the new findings?

- Existing non-invasive tests cut-offs are validated for their use as screening biomarkers to rule out advanced fibrosis in a study group of 5735 patients.
- The sequential combination of Fibrosis-4 Index (FIB-4) ( $<1.3$ ;  $\geq 2.67$ ) and liver stiffness measurement by vibration controlled transient elastography (LSM-VCTE) ( $<8.0$  kPa;  $\geq 10.0$  kPa) which is increasingly used in routine practice has a false negative rate of 9% for advanced fibrosis.
- The diagnostic performance of LSM-VCTE for advanced fibrosis is influenced by biopsy quality, body mass index and presence of type 2 diabetes.
- An algorithm combining FIB-4 and LSM-VCTE sequentially with lower cut-offs to rule out advanced fibrosis (FIB-4  $<1.3$ ; LSM-VCTE  $<8.0$  kPa) and with upper cut-offs to rule-in and positively diagnose cirrhosis without the need for liver biopsy with specificity of 95% (FIB-4  $\geq 3.48$ ; LSM-VCTE  $\geq 20.0$  kPa) or 98% (FIB-4  $\geq 4.63$ ; LSM-VCTE  $\geq 28.0$  kPa) can reduce the need for liver biopsies from 33% to 19% or 24%, respectively.

## How might it impact on clinical practice in the foreseeable future?

- The non-invasive test cut-offs for the diagnosis of cirrhosis can be incorporated into clinical practice as they have been validated in a large group of patients.
- Application of these cut-offs can lead to a decrease in the need for liver biopsies in secondary care.

a minority<sup>2</sup> progress to cirrhosis, liver failure and hepatocellular carcinoma (HCC).

NAFLD comprises several histological features ranging from simple steatosis to steatosis with lobular inflammation and ballooned hepatocytes (steatohepatitis), both of which can be accompanied by varying degrees of fibrosis. The currently accepted reference standard for diagnosing NAFLD is liver biopsy as its diagnostic features are based on histology.<sup>3</sup> Liver biopsy, however, is invasive and carries a risk of complications,<sup>4</sup> is limited by sampling variability<sup>5</sup> and high observer dependent variability in pathological reporting.<sup>6,7</sup>

NAFLD is often diagnosed after incidental findings of elevated liver transaminases on blood tests, or liver steatosis or cirrhosis on imaging. One challenge clinicians face is to identify which of these patients are at high risk of progression or clinical outcomes, as they would benefit from specialist follow-up. There is now substantial evidence showing that those with at least advanced fibrosis (F3–4) are at higher risk of liver-related events in later life.<sup>8–10</sup>

A large body of evidence also exists on how non-invasive tests (NITs) could be used to risk-stratify patients for the presence of advanced fibrosis. These approaches usually involve sequential application of two NITs, with the first tier of a simple, inexpensive, serum-based test performed in the community (eg, Fibrosis-4 Index (FIB-4) or NAFLD Fibrosis Score (NFS)), followed by a second tier of liver stiffness measurement (LSM) (eg, vibration controlled transient elastography: VCTE), or a proprietary serum-based test (eg, enhanced liver fibrosis test; ELF). A lower and an upper threshold are usually used in each tier of testing to rule out (those with a NIT result less than the lower threshold) or rule in (those with a NIT result more than the upper threshold)

patients at high risk of advanced fibrosis. Patients with indeterminate results in both tiers of testing would need a liver biopsy for risk stratification. The main value of these approaches lies in their high negative predictive value to rule out patients with low risk of advanced fibrosis who can be safely managed in primary care.

Despite the increasing evidence to support these approaches, some aspects of their application require further clarifications. First, there is no consensus on which NIT thresholds to use for this purpose. For example, FIB-4 upper cut-offs of 3.25<sup>11</sup> and 2.67<sup>12</sup> have been described, while other investigators omit the FIB-4 upper cut-off altogether.<sup>13</sup> There is also some uncertainty about the performance of NITs in specific patient subgroups, such as those with diabetes or obesity. Furthermore, for patients who are ruled in as being at high risk of advanced fibrosis (F3–4), liver biopsy is often needed to identify those with cirrhosis who would need surveillance for HCC.<sup>14</sup> Developing approaches that can minimise the need for liver biopsy in secondary care is therefore an area of unmet need.

To address these problems, we conducted an individual patient data meta-analysis (IPDMA) with three main aims: (1) to evaluate the performance of LSM-VCTE and compare it to the performance of FIB-4 and NFS as screening tests to rule out advanced fibrosis; (2) to evaluate NIT combination strategies to minimise the number of cases that would need a liver biopsy in secondary care; (3) to explore factors that influence diagnostic accuracy.

## METHODS

This IPDMA was reported in accordance with the recommendations of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses-IPD Statement<sup>15</sup> and was registered as PROSPERO CRD42019157661.

## Criteria for considering studies for the IPD meta-analysis Patients

Studies reporting data on adults ( $\geq 18$  years) with NAFLD and paired liver histology and LSM-VCTE were eligible. When studies reported study groups of participants with unselected aetiologies, only IPD of those with NAFLD were sought.

## Index tests

The index test of main interest was LSM-VCTE performed with FibroScan (Echosens, France). Results for serum-based biomarkers NSF,<sup>16</sup> FIB-4,<sup>17</sup> aspartate aminotransferase (AST) to alanine aminotransferase (ALT) ratio<sup>18</sup> and AST-to-platelet ratio index (APRI)<sup>19</sup> were also computed where data were available. Online supplemental table 1 summarises the definition of NITs considered in this IPDMA.

Universally accepted cut-offs for diagnosing different groups of fibrosis stages do not exist (several suggested cut-offs are presented in online supplemental table 2). For LSM-VCTE,  $<7.9$  kPa and  $\geq 9.6$  kPa are the most used for respectively ruling out and in, advanced fibrosis.<sup>20</sup>

## Reference standard

Only studies reporting histological classification of liver fibrosis based on the non-alcoholic steatohepatitis Clinical Research Network (NASH CRN) staging system were considered.<sup>21</sup>

## Target conditions

Advanced fibrosis (F3–4) and cirrhosis (F4) were the target conditions of interest. To fulfil the aims of the study, cut-offs

were selected to rule out or rule in advanced fibrosis, and to rule out advanced fibrosis or rule in cirrhosis.

### Study design

All study designs were considered if they were reporting on patients with NAFLD undergoing both liver biopsy and LSM-VCTE within 6 months. No language restrictions were applied.

### Establishing collaborations

Authors of eligible studies were contacted by email and reminders were sent if a response was not received within 2 weeks. Only data from studies that received ethical approval were used. Additional ethical approval was not sought for the meta-analysis as only anonymised data were provided.

### Data verification

Range checks of measurement values provided for individual patients were carried out and authors were asked to provide clarifications where necessary. Missing data were queried until received or confirmed as unavailable. Missing data were handled in the analysis by pairwise deletion.

LSM-VCTE with median stiffness  $\geq 7.1$  kPa and IQR-to-median LSM ratio  $>30\%$  were considered unreliable.<sup>22</sup> These were included in the main analysis and were later compared in a subgroup analysis to reliable measurements, to assess whether they can be reliably used to diagnose advanced fibrosis.

Authors were provided with a template table of required data (online supplemental table 3) and were asked to deduplicate data where possible. We also checked for duplicate entries and where identified these were removed.

### Data analysis

#### Quality and bias assessment

The quality of studies was assessed using the Quality Assessment of Diagnostic Accuracy Studies tool (QUADAS-2).<sup>23</sup>

#### IPD meta-analysis

The original data sets were merged, a study identification variable was added, and descriptive statistical analysis of the data sets was conducted. Dichotomous variables are displayed as percentages. Continuous variables are reported as means with SD, or medians with IQRs according to the distribution of the data.

Analyses were done per protocol, as we did not have information on failed LSM-VCTE. To express the diagnostic performance of NITs, non-parametric, empirical receiver operating characteristic (ROC) curves were constructed for the target conditions of interest. Diagnostic performance was expressed as the area under the ROC curve (AUROC) with 95% CI, based on De Long's method. AUROCs were compared using De Long's test statistic.

Thresholds to maximise the Youden index (ie, sensitivity + specificity – 1), for 90% sensitivity, and for 90% specificity were reported. The diagnostic performance of previously published cut-offs was also evaluated. Sequential combinations of serum biomarkers and LSM-VCTE were evaluated, by computing sensitivity, specificity and proportions of misclassified and indeterminate patients.

Positive and negative predictive values (PPV and NPV) were estimated for prevalences within the range of those reported in the original studies. The number of false positive and false negative results for 100 theoretical cases was also reported.

The main analysis was conducted to maximise data for each NIT. For a valid comparison of the performance of NITs, a separate analysis was conducted in the subgroup of patients where all three of VCTE, FIB-4 and NFS were available in each participant.

To fulfil the aim of developing testing strategies that reduce the number of patients in need of a liver biopsy, lower cut-offs for ruling out advanced fibrosis and upper cut-offs for ruling in cirrhosis were used. The rationale for this approach is illustrated in online supplemental figure 1. The upper cut-offs for identifying cirrhosis were chosen at 95% and 98% specificity in a derivation set and tested in a validation set. Derivation and validation sets were obtained by random sampling from the IPD study group in a 3:2 ratio. These upper cut-offs were combined with lower cut-offs from the literature for ruling out advanced fibrosis and the algorithm was tested in the whole IPD study group. For ease of reference, we also examined the cut-offs of 8 kPa and 10 kPa (corresponding to the most common VCTE cut-offs in the literature of 7.9 kPa and 9.6 kPa rounded to the nearest integer) and also rounded our cirrhosis cut-offs to the nearest integer to facilitate application in clinical practice.

Only test-positive and test-negative patients were included in the calculation of diagnostic performance indices, and patients in the indeterminate group were excluded from calculations.

Subgroup analysis was performed according to biopsy length ( $<20$  mm,  $\geq 20$  mm), number of portal tracts in biopsy samples ( $<11$ ,  $\geq 11$ ), biopsy quality (intermediate:  $10 \text{ mm} \leq \text{length} < 20 \text{ mm}$ ; high:  $\text{length} \geq 20 \text{ mm}$  and  $\geq 11$  tracts), age (four quartiles), sex, body mass index (BMI;  $\text{BMI} < 25 \text{ kg/m}^2$ ,  $25 \text{ kg/m}^2 \leq \text{BMI} < 30 \text{ kg/m}^2$ ,  $\text{BMI} \geq 30 \text{ kg/m}^2$ ), presence of type 2 diabetes mellitus (T2DM), continent of provenance (Europe, Asia), probes used (M, XL), reliability criteria for LSM-VCTE (reliable (median LSM  $< 7.1$  kPa or median LSM  $\geq 7.1$  kPa and IQR/median LSM  $< 0.30$ ) vs unreliable (median LSM  $\geq 7.1$  kPa and IQR/median LSM  $\geq 0.30$ );<sup>22</sup> reliable (IQR/median LSM  $< 0.30$ ) vs unreliable (IQR/median LSM  $\geq 0.30$ )), and aminotransferase levels (ALT or AST  $< 40$ ,  $40 \leq \text{ALT}$  or  $\text{AST} < 100$ , ALT or AST  $\geq 100$ ; ALT  $< 40$  and AST  $< 40$ , ALT  $\geq 40$  or AST  $\geq 40$ ).

All statistical analyses were performed using R (V.1.2.1335, R Foundation for Statistical Computing, Vienna, Austria) with the PROC package<sup>24,25</sup>; 95% CIs were calculated using 500 stratified bootstrap replicates using the boot package.<sup>26,27</sup>

#### VCTE probe types

The analysis to account for probe type is described in the online supplemental materials.

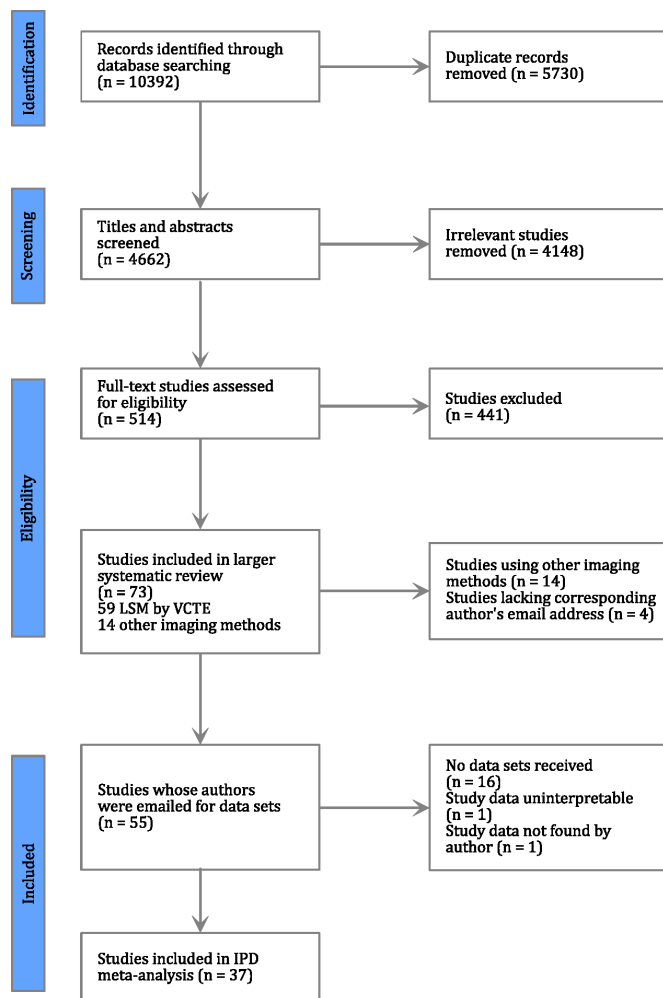
#### Patient and public involvement

Patients and the public were not involved in the conduct of this study as there was no direct patient participation in the study.

## RESULTS

### Search process and data collection

Ten thousand three hundred ninety-two articles were identified in a search performed for a larger systematic review evaluating the diagnostic performance of LSM-VCTE and other index tests for the staging of fibrosis and diagnosis of NASH in adult patients with NAFLD. After removing duplicates, and screening titles, abstracts, and full texts, 59 studies examining VCTE were identified. The authors of 37 studies shared useable data (figure 1). Authors of more than one study supplied data in a single dataset and, overall, we received 30 data sets including data from 6571 patients. After removing duplicates ( $n=628$ ) and patients with



**Figure 1** Preferred Reporting Items for Systematic Reviews and Meta-Analyses flow chart illustrating the identification and selection process for studies finally included in this individual patient data meta-analysis. IPD, individual patient data; LSM, liver stiffness measurement; VCTE, vibration controlled transient elastography.

missing biopsy ( $n=14$ ) or LSM-VCTE ( $n=194$ ) data, the final dataset consisted of 5735 unique patients.

### Study and population characteristics

The characteristics of the 30 data sets are summarised in [table 1](#). Studies were conducted in Europe (67%), Asia (40%) and Australia (3%). Data availability is shown in online supplemental table 3. FIB-4 and NFS were determined in 5393 (94%) and 3248 (57%) cases, respectively. Median age was 54 years, 2570 (45%) patients were women, 33% had diabetes and 43% had BMI  $\geq 30$  kg/m<sup>2</sup>. Overall, 30% had advanced fibrosis and 11% had cirrhosis. Details of the IPD study group are included in [table 2](#), and online supplemental tables 4 and 5.

### Study quality

The methodological quality of the studies assessed with the QUADAS-2 tool is summarised in online supplemental figures 2 and 3. Only one study had low risk of bias or low applicability concerns in all QUADAS-2 domains.<sup>28</sup> The flow and timing domain were judged to have high risk or unclear risk of bias in 65% of studies, as these either excluded technical failures from

their final diagnostic performance analysis or did not report them.

### Validating the diagnostic performance of LSM by VCTE and serum-based tests for detecting advanced fibrosis

LSM-VCTE, FIB-4, NFS, APRI and AST/ALT had corresponding AUROCs of 0.85, 0.76, 0.73, 0.70, 0.64 for identifying advanced fibrosis ([table 3](#)), and 0.90, 0.80, 0.78, 0.72, 0.69 for the identification of cirrhosis (online supplemental table 6). LSM-VCTE performed significantly better ( $p<10^{-15}$ ) in detecting both advanced fibrosis and cirrhosis than all serum-based tests. This relationship was preserved when performing a head-to-head comparison of LSM-VCTE, FIB-4 and NFS in the same group of patients (online supplemental tables 7 and 8).

When considering cut-offs from the literature, we evaluated lower and higher cut-offs separately. For any given test, as would be expected, low thresholds yielded higher sensitivity and high thresholds were associated with higher specificity (online supplemental table 9). Indicative PPV and NPV are also provided for the range of prevalences (5%–50%) reported in the primary studies (online supplemental tables 10–14).

APRI and AST/ALT ratio had only modest diagnostic performance for advanced fibrosis (AUROC  $\leq 0.70$ , [table 3](#)), and were therefore not considered further.

None of the thresholds regarded in isolation resulted in both a high sensitivity ( $\geq 80\%$ ) and high specificity ( $\geq 80\%$ ) ([figure 2](#), [table 3](#), online supplemental tables 9 and 15, and online supplemental figure 4). Therefore, we explored the use of a lower and an upper cut-off. LSM-VCTE literature cut-offs performed well in only two cases ( $<7.1$  kPa and  $\geq 14.1$  kPa: 83% sensitivity, 90% specificity; and  $<7.9$  kPa and  $\geq 9.6$  kPa: 84% sensitivity, 78% specificity), while for other LSM-VCTE, NFS and FIB-4 thresholds a high specificity was observed (FIB-4: 91% for  $<1.3$  and  $\geq 2.67$ , 95% for  $<1.3$ ,  $\geq 3.25$ ) but sensitivity was  $<60\%$  ([table 4](#)). In addition, the proportion of indeterminate cases was  $>30\%$  for serum-based NITs. Threshold pairs derived from the IPD study group did not reduce the proportion of misclassified and indeterminate patients seen with literature-based threshold pairs ([table 4](#)).

We further evaluated the performance of LSM-VCTE, FIB-4 and NFS to diagnose advanced fibrosis in sequential combinations of serum-based NITs and LSM-VCTE. When selecting threshold combinations for FIB-4 and NFS available in the literature ( $<1.3$  &  $\geq 2.67$ ,  $<1.3$  &  $\geq 3.25$  for FIB-4;  $<-1.455$  &  $\geq 0.676$  for NFS) and pairing them with the best threshold pair for LSM-VCTE ( $<7.9$  kPa &  $\geq 9.6$  kPa, identified as the one with highest sensitivity and lowest indeterminate proportion), the proportion of patients in the indeterminate group was 5%. While both the FIB-4+LSM VCTE and NFS+LSM VCTE sequential combinations had specificity  $>80\%$ , their sensitivity was  $\leq 80\%$  ([table 5](#)). A better sensitivity was reached by using thresholds derived from the IPD study group ( $<0.88$  &  $\geq 2.31$  for FIB-4;  $<-2.55$  &  $\geq 0.28$  for NFS), but the proportion of indeterminate cases was near 20% in those cases and the proportions of patients needing LSM-VCTE was also larger than when using literature cut-offs ([table 5](#)).

### Algorithms to minimise the need for liver biopsy

In the derivation set, the cut-offs for 95% and 98% specificity for the diagnosis of cirrhosis were respectively 20.4 kPa and 27.6 kPa for LSM-VCTE, 3.48 and 4.63 for FIB-4 and 1.01 and 1.57 for NFS. These cut-offs performed similarly in the validation set (online supplemental tables 16 and 17).

**Table 1** Details of individual patient data included in this meta-analysis

Data set ID	Country	Study design	Number of participants (n)	Age (year)	BMI (kg/m <sup>2</sup> )	WC (cm)	M/F	Recruitment interval	Hardware used	Probe used
Agrawal <i>et al</i> <sup>49</sup>	UK	MC, P, CS	25	47.8 (19–70)	27.7 (15.8–35.7)	95.4 (39–120)	18/7	2009–2012	–	M
Aykut <i>et al</i> <sup>50</sup>	Turkey	SC, P, CS	88	46.0 (24–62)	30.3 (18.3–41.8)	101.5 (70–143)	50/38	–	FibroScan 502 Touch	M
Boursier <i>et al</i> <sup>51–53</sup>	France	MC, P, CS	1063	56.1 (18–83)	31.6 (16.7–55.5)	108.3 (58–174)	613/450	–	–	M or XL
Cassinotto <i>et al</i> <sup>54</sup>	France	SC, P, CS	61	55.9 (22–81)	30.1 (16.7–46.6)	103.6 (72–125)	40/21	2010–2012	–	M and XL
Cassinotto <i>et al</i> <sup>55</sup>	France	MC, P, CS	286	56.6 (18–80)	32.2 (20.3–57.4)	109.8 (68–168)	171/115	2011–2015	–	M and XL
Chan <i>et al</i> <sup>56</sup>	Malaysia	SC, P, CS	146	50.4 (18–73)	29.4 (6.9–41.2)	98.3 (79–127)	80/66	2012–2013	FibroScan 502 Touch	M
Chan <i>et al</i> <sup>57</sup>	Malaysia, Hong Kong	MC, P, CC	153	54.0 (24–76)	29.9 (20.1–44.8)	98.4 (69–141)	68/85	–	FibroScan 502 Touch	M and XL
Eddowes <i>et al</i> <sup>31 58</sup>	UK	MC, P, CS	358	53.3 (19–77)	34.2 (19.5–53.2)	117.2 (65–158)	206/152	–	–	M or XL
Eddowes <i>et al</i> <sup>59</sup>	UK	MC, P, CS	50	50.2 (18–73)	33.6 (23.6–47.8)	109.4 (89–132)	28/22	2014–2015	–	M or XL
Gaia <i>et al</i> <sup>60</sup>	Italy	SC, P, CS	68	46.8 (28–65)	28.0 (21.2–40.2)	–	48/20	2007–2009	–	M
Garg <i>et al</i> <sup>61</sup>	India	SC, P, CS	76	38.2 (20–65)	45.2 (32.3–73.8)	–	16/60	2014–2016	FibroScan 502 Touch	XL
Karlas <i>et al</i> <sup>62</sup>	Germany	SC, P, CS	41	45.7 (28–64)	47.7 (33.7–60.1)	–	13/28	–	FibroScan 502	XL
Labenz <i>et al</i> <sup>63</sup>	Germany	SC, P, CS	126	47.4 (20–73)	31.6 (23.2–50.4)	–	72/54	–	FibroScan 402	M or XL
Lee <i>et al</i> <sup>64</sup>	Korea	SC, P, CS	94	55.5 (19–82)	27.2 (19.1–36.3)	–	41/53	2014–2015	–	M or XL
Lupsor <i>et al</i> <sup>65</sup>	Romania	SC, P, CS	72	42.4 (20–69)	29.7 (21.0–41.5)	102.4 (60–124)	51/21	2007–2009	–	M
Mahadeva <i>et al</i> <sup>66</sup>	Malaysia	SC, P, CS	131	49.9 (23–73)	28.7 (18.6–43.1)	93.5 (43–128)	66/65	2009–2010	–	M
Okajima <i>et al</i> <sup>67</sup>	Japan	SC, P, CS	173	56.3 (18–81)	27.2 (16.5–40.3)	–	84/89	2013–2015	–	M
Ooi <i>et al</i> <sup>68</sup>	Australia	MC, P, CS	82	44.5 (18–67)	46.2 (29.1–74.0)	136.5 (101–192)	23/59	2015–2016	–	M or XL
Pavlidis <i>et al</i> <sup>69</sup>	UK	SC, P, CS	70	53.5 (25–77)	34.5 (23.0–57.3)	112.5 (80–149)	42/28	2011–2015	–	M or XL
Petta <i>et al</i> <sup>70 71</sup>	Italy	MC, P&R, CS	234	45.5 (15–78)	28.2 (15.7–40.7)	99.4 (69–126)	169/65	2008–2013	–	M
Petta <i>et al</i> <sup>28</sup>	France, Hong Kong, Italy	MC, P, CS	260	54.6 (15–87)	29.4 (16.5–46.6)	100.9 (74–148)	122/138	–	–	M
Petta <i>et al</i> <sup>72</sup>	Italy	MC, P, CS	474	45.5 (19–77)	29.2 (15.2–49.5)	99.6 (47–164)	275/199	–	–	M
Seki <i>et al</i> <sup>73</sup>	Japan	SC, P, CS	181	57.7 (16–82)	27.1 (16.9–38.1)	95.1 (71–117)	91/90	2013–2015	–	M
Shen <i>et al</i> <sup>74</sup>	China	MC, P, CS	101	59.0 (16–67)	27.0 (20.1–37.3)	92.9 (75–120)	74/27	2012–2014	FibroScan 502	M
Stauffer <sup>75</sup>	Austria	MC, P, CS	186	49.6 (19–83)	32.5 (19.0–56.9)	–	106/80	2011–2016	FibroScan 502 Touch	M or XL
Wong <i>et al</i> <sup>76–79</sup>	Hong Kong, France	MC, P, CS	464	53.8 (20–83)	30.5 (17.3–48.0)	102.0 (71–148)	201/263	2009–2017	–	M and XL
Wong <i>et al</i> <sup>20</sup>	Hong Kong, France	MC, P, CS	273	51.6 (21–77)	28.8 (16.5–54.0)	96.2 (65–144)	147/126	2003–2009	–	M
Yoneda <i>et al</i> <sup>80</sup>	Japan	MC, P, CS	97	52.1 (19–76)	26.5 (17.9–38.5)	–	41/56	<2008	–	M
Younes <i>et al</i> <sup>81</sup>	Italy	MC, P, CS	289	44.8 (15–78)	28.8 (17.5–41.7)	98.9 (47–128)	199/90	–	–	M
Ziol <i>et al</i> <sup>82</sup>	France	SC, P, CS	13	49.3 (39–60)	29.4 (23.8–34.6)	–	10/3	2003–2005	–	–

–, Data not available; BMI, body mass index; CC, case-control; CS, cross-sectional; F, females; M, males; MC, multicentre; P, prospective; R, retrospective; SC, single-centre; WC, waist circumference.

Algorithms combining FIB-4 (lower cut-off of 1.3 as described in the literature and upper cut-offs of 3.48 and 4.63 as described above) and LSM by VCTE (lower cut-off rounded to 8.0 kPa and upper cut-offs rounded to 20.0 kPa

and 28.0 kPa, as described above) were then compared with the traditional way of applying these tests, also with rounded cut-offs for LSM by VCTE (8 kPa and 10 kPa) (figure 3). This approach increased the number of patients requiring a LSM

**Table 2** Demographic details of the entire cohort, and patients without (F0–2) and with (F3–4) advanced fibrosis

	Entire cohort (N=5735)	F0–2 (N=4013)	F3–4 (N=1722)
Females (%)	45	43	48
BMI $\geq 30$ kg/m <sup>2</sup> (%)	43	45	53
Waist circumference (cm)	103 (15)	102 (15)	106 (14)
Diabetes (%)	33	30	58
Age (years)*	54 (19)	50 (19)	59 (14)
BMI (kg/m <sup>2</sup> )*	30 (7)	29 (8)	30 (7)
<b>Biopsy data</b>			
Steatosis			
S0/S1/S2/S3 (%)	3/35/36/26	3/36/36/25	2/32/38/28
Ballooning			
B0/B1/B2 (%)	24/47/29	30/49/21	10/45/45
Inflammation			
I0/I1/I2/I3 (%)	13/60/24/3	17/62/20/1	5/55/34/6
NAS score†	4 (2)	4 (2)	5 (1)
NASH (%)	50	43	67
<b>Liver function tests</b>			
ALT (IU/L)*	55 (48)	53 (48)	60 (48)
AST (IU/L)*	40 (30)	36 (25)	50 (34)
Platelets ( $\times 10^9/L$ )†	230 (72)	241 (67)	205 (75)
Albumin (g/L)†	43 (9)	43 (7)	43 (13)
GGT (IU/L)*	69 (87)	62 (78)	87 (102)
<b>NITs</b>			
LSM (kPa)*	10.7 (6.1)	6.7 (3.5)	13.3 (12.0)
FIB-4*	1.7 (1.2)	1.1 (0.9)	1.9 (1.7)
NFS†	–1.5 (1.7)	–1.9 (1.6)	–0.6 (1.8)
APRI*	0.6 (0.4)	0.4 (0.3)	0.6 (0.6)
AST/ALT*	0.8 (0.4)	0.7 (0.4)	0.8 (0.5)

\*Data are reported as median (IQR).

†Data are reported as mean (SD).

ALT, alanine aminotransferase; APRI, AST-to-platelet ratio index; AST, aspartate aminotransferase; BMI, body mass index; FIB-4, Fibrosis-4 Index; GGT, gamma-glutamyltransferase; LSM, liver stiffness measurement; NAS, NAFLD activity score; NASH, non-alcoholic steatohepatitis; NFS, NAFLD (non-alcoholic fatty liver disease) Fibrosis Score; NIT, non-invasive test.

(from 34% to 40% and 44%) but decreased the number of patients needing liver biopsy (from 33% to 19% and 24% when using the 95% and 98% specificity cut-offs, respectively) (online supplemental table 18 and figure 3).

### Subgroup and sensitivity analyses

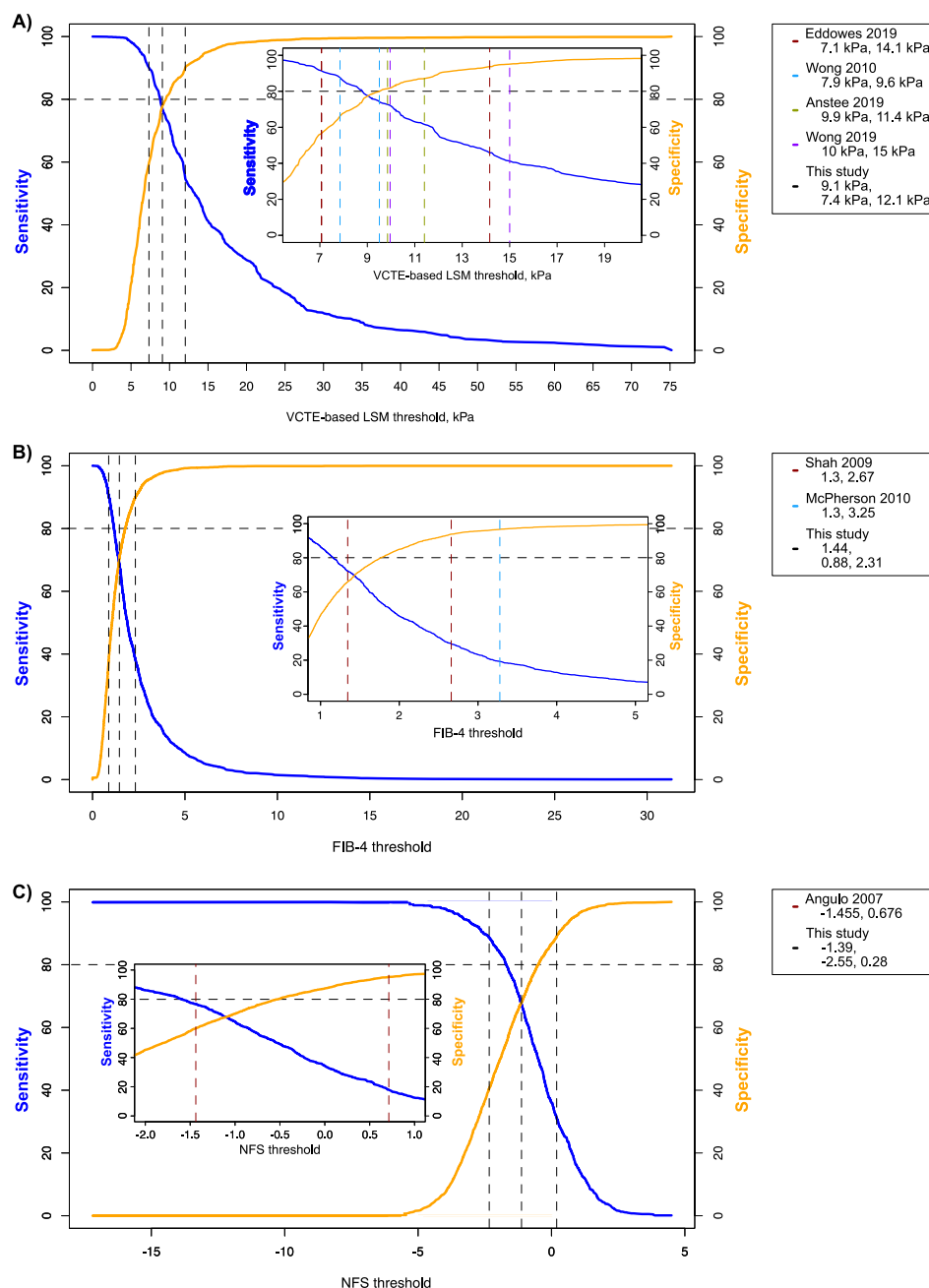
In subgroup analysis for the diagnosis of advanced fibrosis (online supplemental table 19), NITs performed better in patients with lower BMI (AUROCs LSM-VCTE: 0.91,  $p < 0.005$ ; FIB-4: 0.81,  $p < 0.001$ ; NFS: 0.76,  $p < 0.025$ ), without T2DM (LSM-VCTE: 0.87,  $p < 10^{-6}$ ; FIB-4: 0.77,  $p < 0.01$ ), and with biopsies shorter than 20 mm (LSM-VCTE: 0.87,  $p < 0.005$ ; FIB-4: 0.80,  $p < 0.001$ ; NFS: 0.79,  $p < 0.05$ ), or with fewer than 11 portal tracts (LSM-VCTE: 0.86,  $p = 0.01$ ; FIB-4: 0.79,  $p = 0.04$ ; NFS: 0.78,  $p < 0.005$ ). Diagnostic performance was also lower in patients in the youngest age quartile ( $< 43$  years, AUROC: 0.58,  $p < 0.001$ ) and in women (AUROC: 0.71,  $p = 0.03$ ) for NFS, while continent of provenance did not have a significant effect for any NITs. In patients with normal levels of ALT (ALT  $< 40$ ) FIB-4 performed worse (AUROC: 0.73) than in patients with ALT  $\geq 40$  and ALT  $< 100$  (AUROC: 0.77,  $p < 0.01$ ). NFS

**Table 3** Diagnostic performance of non-invasive tests for advanced fibrosis (F3–F4)

	LSM by VCTE (n=5489)			FIB-4 (n=5393)			NFS (n=3248)			APRI (n=5477)			AST/ALT (n=5434)		
Advanced fibrosis, %	30	30	30	30	30	30	29	30	30	30	30	30	30	30	30
AUROC	0.85 (0.84–0.86)	0.76 (0.74–0.77)	0.73 (0.71–0.75)	0.76 (0.74–0.77)	0.73 (0.71–0.75)	0.70 (0.69–0.72)	0.73 (0.71–0.75)	0.70 (0.69–0.72)	0.70 (0.69–0.72)	0.70 (0.69–0.72)	0.70 (0.69–0.72)	0.70 (0.69–0.72)	0.64 (0.62–0.65)	0.64 (0.62–0.65)	0.64 (0.62–0.65)
Threshold	9.1	12.1	14.4	12.1	14.4	16.7	9.1	12.1	14.4	16.7	19.0	21.3	9.1	12.1	14.4
Sensitivity, %	77 (75–79)	55 (52–57)	69 (67–72)	55 (52–57)	69 (67–72)	88 (88–91)	75 (73–78)	67 (64–69)	67 (64–69)	67 (64–69)	67 (64–69)	67 (64–69)	75 (73–77)	75 (73–77)	75 (73–77)
Specificity, %	78 (76–79)	90 (89–91)	90 (89–91)	90 (89–91)	90 (89–91)	90 (89–91)	63 (61–65)	63 (61–65)	63 (61–65)	63 (61–65)	63 (61–65)	63 (61–65)	47 (45–48)	47 (45–48)	47 (45–48)
Misclassified, %	22 (22–23)	21 (20–21)	30 (30–31)	21 (20–21)	30 (30–31)	26 (25–26)	34 (34–36)	36 (36–37)	36 (36–37)	36 (36–37)	36 (36–37)	36 (36–37)	45 (45–46)	45 (45–46)	45 (45–46)

For each non-invasive test thresholds were selected according to Youden's index (YI), and fixed at 90% sensitivity (90% Se) and 90% specificity (90% Sp). 95% CIs were estimated with 500 bootstrap replicates.

ALT, alanine aminotransferase; APRI, AST-to-platelet ratio index; AST, aspartate aminotransferase; FIB-4, Fibrosis-4 Index; LSM, liver stiffness measurement; NFS, NAFLD (non-alcoholic fatty liver disease) Fibrosis Score; VCTE, vibration transient elastography.



**Figure 2** Distribution of sensitivities and specificities over the possible threshold ranges for liver stiffness measurement (LSM) by vibration controlled transient elastography (VCTE) (A), Fibrosis-4 Index (FIB-4) (B) and NAFLD (non-alcoholic fatty liver disease) Fibrosis Score (NFS) (C) when considering the diagnosis of advanced fibrosis. Insets show the distribution of cut-offs identified from the literature. Horizontal dashed lines are representing the minimum acceptable criteria for considering a test as having high sensitivity ( $\geq 80\%$ ) and high specificity ( $\geq 80\%$ ).

performed better in patients with  $AST < 40$  (AUROC: 0.76), than in patients with  $AST \geq 100$  (AUROC: 0.65,  $p < 0.01$ ). FIB-4 performed better in patients with at least one abnormal aminotransferase measurement (AUROC: 0.72,  $p = 0.014$ ). For cirrhosis, the trends were similar, except that for the diagnosis of cirrhosis, LSM by VCTE performed better in the youngest age group (AUROC: 0.97,  $p < 10^{-4}$ ) and NIT diagnostic performance was independent of aminotransferase levels (online supplemental table 20).

The diagnostic performance of LSM-VCTE was significantly lower in patients with unreliable LSMs ( $p < 10^{-8}$ ; both for advanced fibrosis and cirrhosis) when applying the Boursier-criteria,<sup>22</sup> but not when only considering IQR/median LSM

$< 0.30$ . The proportion of unreliable results was 12% both in the advanced fibrosis and cirrhosis groups (online supplemental table 21).

There was no difference in the diagnostic performance of LSM-VCTE between the M and XL probes in the subgroup of patients who had undergone LSM by both probes (online supplemental table 22).

In a sensitivity analysis of patients with LSM matched to BMI (only M probe measurements if  $BMI < 30 \text{ kg/m}^2$  and only XL probe measurements if  $BMI \geq 30 \text{ kg/m}^2$ ), there was no significant difference between the diagnostic performance of LSM-VCTE when comparing to the entire IPD study group (online supplemental table 23).

**Table 4** Diagnostic accuracy of pairs of cut-offs from the literature for liver stiffness measurement (LSM) by vibration controlled transient elastography (VCTE), Fibrosis-4 Index (FIB-4) and NAFLD (non-alcoholic fatty liver disease) Fibrosis Score (NFS) for diagnosing advanced fibrosis

	LSM by VCTE (n=5489)		FIB-4 (n=5393)		NFS (n=3248)	
Advanced fibrosis, %	30		30		29	
AUROC	0.85 (0.84–0.86)		0.76 (0.74–0.77)		0.73 (0.71–0.75)	
Source	Anstee <i>et al.</i> <sup>29</sup>	Eddowes <i>et al.</i> <sup>31</sup>	Wong <i>et al.</i> <sup>20</sup>	This study	McPherson <i>et al.</i> <sup>64</sup>	This study
Thresholds	<9.9, ≥11.4	<7.1, ≥14.1	<7.9, ≥9.6	<7.4, ≥12.1*	<1.3, ≥3.25	<0.88, ≥2.31*
Sensitivity, %	69 (67–71)	83 (80–86)	84 (82–87)	84 (81–87)	44 (42–46)	80 (76–83)
Specificity, %	86 (85–88)	90 (88–92)	78 (76–80)	87 (85–88)	95 (93–96)	79 (77–81)
Misclassified, %	17 (16–19)	7 (6–8)	17 (16–19)	10 (9–11)	10 (9–11)	10 (9–11)
Indeterminate, %	7 (6–8)	39 (37–40)	13 (12–14)	31 (30–33)	39 (37–40)	52 (50–53)

\*Cut-offs determined from the individual patient data study group. Lower cut-offs correspond to a lower limit of 90% sensitivity, upper cut-offs correspond to a lower limit of 90% specificity. 95% CIs were determined with 500 bootstrap replicates.

AUROC, area under the receiver operating characteristic.

## DISCUSSION

Through an extensive collaboration network with authors of primary studies we were able to collect the largest dataset of its kind ever to be reported on. This includes a diverse set of study groups from Europe, Asia, and Australia, 30% of whom had advanced fibrosis. We believe that our findings are therefore relevant for patients typical of secondary care in these territories and may be applied in the development of new strategies or in the consolidation of existing practices in evaluating patients for referral to secondary care.

A few studies evaluated the diagnostic performance of LSM-VCTE and other NITs, but most report on fewer than 500 patients. One similarly large study reported on patients screened for inclusion in clinical trials, where the prevalence of advanced fibrosis was 71%,<sup>29</sup> making it difficult to make generalisations about its applicability in routine practice or compare its results to ours. A smaller study with 1073 patients with NAFLD of whom 29% had advanced fibrosis<sup>30</sup> examined the diagnostic performance of LSM by VCTE. The authors of that study reported AUC and specificity values similar to our findings, however they reported increased sensitivity. Other smaller studies reported similar prevalence of advanced fibrosis and similar AUROCs for LSM-VCTE.<sup>31–34</sup>

Overall, the diagnostic performance of LSM-VCTE for advanced fibrosis was good (AUROC=0.85), while that of FIB-4 and NFS in the same group was moderate (AUROC=0.76 for FIB-4, AUROC=0.73 for NFS). None of the studied NITs had both sufficiently high sensitivity and specificity (≥80%) when used with single cut-offs. Diagnostic performance was higher for detecting cirrhosis, as reported in previous studies.<sup>31 35 36</sup> LSM-VCTE had the highest sensitivity and specificity, both in the case of a single cut-off (9.1 kPa obtained by maximising the Youden index; 77% and 78%) and for two cut-offs (<7.4 kPa & ≥12.1 kPa; 84% and 87%). Of the LSM-VCTE cut-off pairs tested, <7.1 kPa and ≥14.1 kPa, first published by Eddowes *et al.*,<sup>31</sup> performed well for advanced fibrosis, with sensitivity of 83% and specificity of 90%, but with a proportion of 39% of patients ending up with an indeterminate result, similar to 41% indeterminate patients reported in the original paper.<sup>31</sup>

LSM-VCTE thresholds identified in our study group (<9.1 kPa; <7.4 kPa & ≥12.1 kPa) were similar to thresholds reported in the literature (<9.9 kPa; <7.1 kPa & ≥14.1 kPa, <7.9 kPa & ≥9.6 kPa). However, thresholds for FIB-4 (<1.44; <0.88 & ≥2.31) and NFS (<−1.39; <−2.55 & ≥0.28) defined in our IPD study group spanned a wider range than those reported in the literature (<1.3 & ≥2.67 or <1.3 & ≥3.25 for FIB-4; <−1.455 & ≥0.676 for NFS).

Our findings are in line with the existing literature suggesting that sequential combinations of NITs increase sensitivity and specificity.<sup>29</sup> Additionally, we have found NFS+LSM VCTE and FIB-4+LSM VCTE combinations to have similar sensitivity and specificity as recently reported by Boursier *et al.*<sup>37</sup> Such combined testing strategies can reduce the number of indeterminate cases and reduce the costs associated with liver biopsies.

Furthermore, we propose an approach that could minimise the need for liver biopsies further, by using upper cut-offs with 95% and 98% specificity for the identification of cirrhosis. The rationale for this approach is explained in the online supplemental discussion. When using the 95% specificity cut-off, the proportion of patients needing liver biopsy decreases from 33% to 19% (figure 3). However, in this approach, 345 of 656 patients 'ruled-in' as having cirrhosis do not have histologically diagnosed cirrhosis. While this may seem like a high proportion

**Table 5** Diagnostic performance of combinations of NAFLD (non-alcoholic fatty liver disease) Fibrosis Score (NFS) and liver stiffness measurement (LSM) by vibration controlled transient elastography (VCTE), and Fibrosis-4 Index (FIB-4) and LSM by VCTE tests to diagnose patients with advanced fibrosis

	FIB-4 & LSM by VCTE (n=5159)	NFS & LSM by VCTE (n=3094)	FIB-4 & LSM by VCTE (n=5159)	NFS & LSM by VCTE (n=3094)	FIB-4 & LSM by VCTE (n=5159)	NFS & LSM by VCTE (n=3094)
Advanced fibrosis, %	30	28	30	28	30	28
Thresholds for blood-based NIT	<0.88, $\geq 2.31^*$	<-2.55, $\geq 0.28^*$	<1.3, $\geq 2.67^\dagger$	<-1.455, $\geq 0.676^\dagger$	<1.3, $\geq 2.67^\dagger$	<-1.455, $\geq 0.676^\dagger$
Thresholds for LSM by VCTE, kPa	<7.4, $\geq 12.1^*$	<7.4, $\geq 12.1^*$	<7.9, $\geq 9.6^\dagger$	<7.9, $\geq 9.6^\dagger$	<8.0, $\geq 10.0^\dagger$	<8.0, $\geq 10.0^\dagger$
Sensitivity, %	80 (77–83)	77 (74–81)	67 (64–69)	65 (62–68)	66 (63–68)	64 (62–67)
Specificity, %	81 (79–83)	83 (81–85)	85 (84–87)	86 (84–88)	86 (84–87)	86 (84–88)
PPV, %	62 (60–65)	61 (58–64)	66 (64–68)	63 (61–67)	66 (64–68)	64 (61–67)
NPV, %	91 (90–92)	91 (89–93)	86 (85–87)	87 (85–88)	86 (85–87)	86 (85–88)
Indeterminate, %	18 (17–19)	20 (18–21)	5 (4–5)	5 (5–6)	5 (4–6)	5 (5–6)
Misclassification, %	16 (14–17)	15 (13–17)	19 (18–21)	19 (17–21)	19 (18–20)	19 (17–21)
Patients undergoing LSM by VCTE, %	51 (50–53)	56 (54–59)	34 (32–35)	38 (36–40)	34 (33–35)	38 (37–40)

95% CIs were estimated with 500 bootstrap replicates.

\*Thresholds were determined from the individual patient data study group as corresponding to 90% sensitivity (lower value) and 90% specificity (upper value)

$^\dagger$ Threshold were determined from the literature. For LSM by VCTE, a threshold pair yielding the highest sensitivity and specificity while having the smallest proportion of indeterminate cases in diagnosing advanced fibrosis was chosen.

NIT, non-invasive test; NPV, negative predictive value; PPV, positive predictive value.

of patients with false positive results, this must be interpreted in the light of two factors. First, the limitations of liver biopsy could mean that these patients are falsely classified as not having cirrhosis histologically. Furthermore, patients without cirrhosis on histology and with high NIT values could have equivalent risks as patients with cirrhosis on histology. For example, it is known from the hepatitis C literature<sup>38</sup> that patients without cirrhosis on liver biopsy but with a high FIB-4 ( $>3.25$ ) still had a significant risk of developing HCC after hepatitis C treatment, demonstrating that NITs can have added benefit beyond the histological diagnosis of cirrhosis alone. The rate of false positive results for cirrhosis can be decreased by choosing cut-offs with higher specificity, but this will come at the expense of doing more biopsies. Despite this encouraging result, this is an area where more information is needed, particularly longitudinal data comparing the prognostic value of LSM-VCTE and other NITs against histology, and ultimately, the cost effectiveness of the various cut-offs would need to be evaluated.

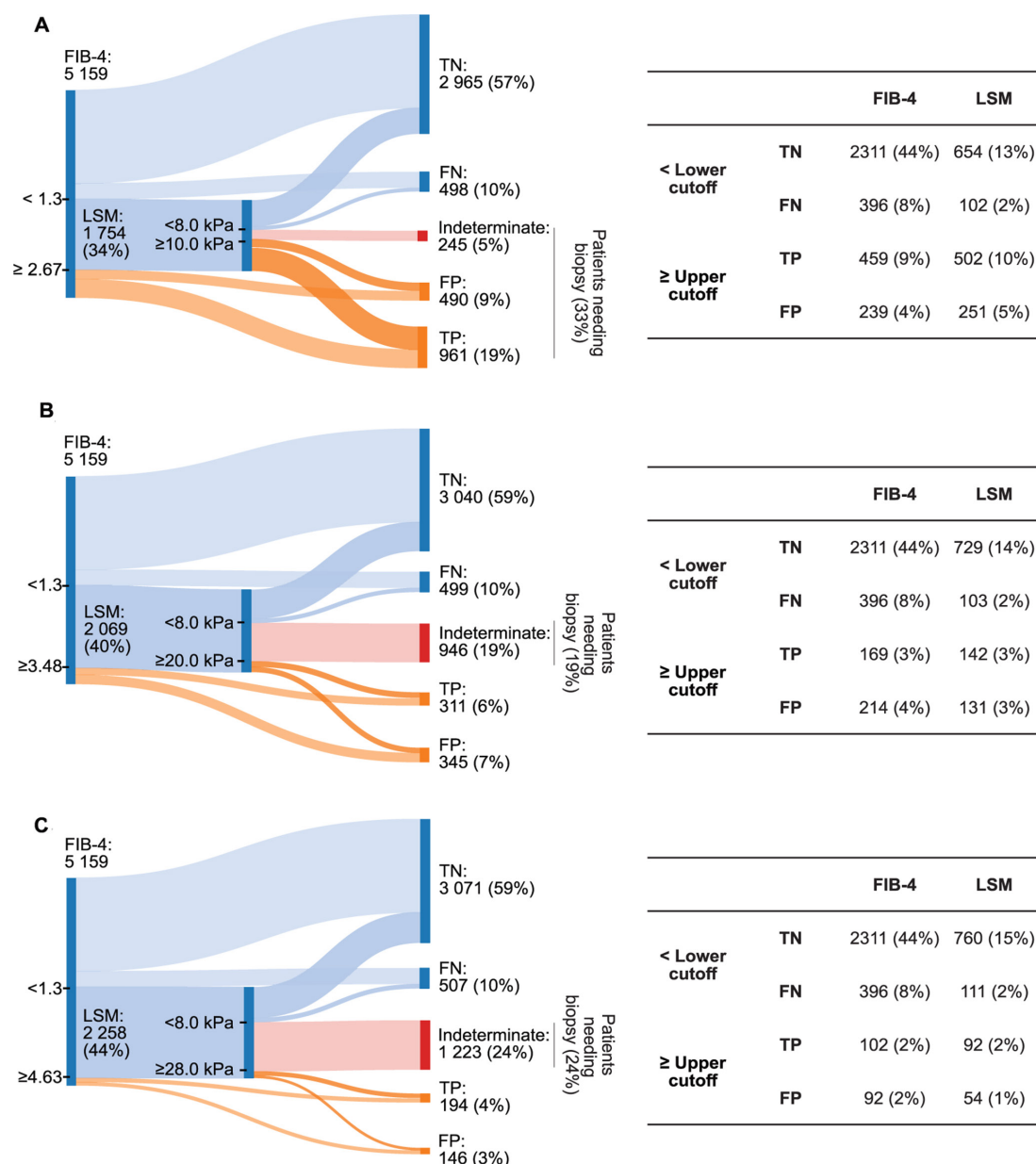
Surprisingly, subgroup analyses showed that the diagnostic accuracy of NITs was better in cases with poor biopsy quality. This finding is difficult to explain but a similar observation was reported previously in a large group of patients screened for clinical trials.<sup>29</sup> The use of local biopsy reports as reference standard and the well-known observer-dependent variability of biopsy interpretation, even among expert pathologists,<sup>7</sup> are factors that may have contributed to our finding. Spectrum bias was excluded as a source of this finding due to a near-identical proportion of patients in both the advanced fibrosis and cirrhosis group having short biopsies (online supplemental table 5).

Subgroup analysis showed better diagnostic performance of NITs in patients with lower BMI,<sup>39,40</sup> and patients without diabetes, in keeping with other studies.<sup>41,42</sup> This effect is likely to be primarily driven by BMI as there is thought to be a causal association between BMI and T2DM. NIT performance was impacted by age, with all NITs performing worse in the younger quartile of our study group for advanced fibrosis, but the trend was reversed for cirrhosis where NITs performed better in those younger than 43 years of age. The age dependence of FIB-4 and NFS is expected, as age is one of the parameters included in the algorithms, and has indeed been previously described.<sup>13,43</sup> It is, however, difficult to explain why performance of NITs is better in the younger age group for the diagnosis of cirrhosis.

Our study has several strengths, including the large size of the IPD study group and composition with prevalence of advanced fibrosis of 30%, which makes it relevant to routine practice. Furthermore, the proportion of unreliable VCTE measurements in our study was 12%, in keeping with the literature.<sup>22</sup> However, we acknowledge some limitations. We did not have any data from the USA and very few studies from Australia, so the results could not be globally applicable, due to differences in BMI across study populations. In addition, due to the nature of our study, we had to use the locally provided histology results possibly introducing bias. Furthermore, we covered a large chronological period, during which LSM-VCTE application underwent significant changes, initially with the introduction of the XL probe, followed by the advice to measure skin-to-capsule distance (SCD) and the introduction of the Automatic Probe Selection tool. There was therefore some heterogeneity in the performance of LSM-VCTE, with early studies using only the M probe to assess all patients, while only a subset of studies assessed SCD to guide probe selection. Furthermore, one third of the included studies was carried out in France, as the technology used for LSM by VCTE originates from there. Lastly, our data confirm that LSM-VCTE had superior accuracy to serum-based tests, and this is independent of probe type, sex, ALT, AST, and participants' continent of origin. There was, however, some dependence on the presence of T2DM, BMI and for the detection of cirrhosis, and we did not check for subgroup-specific cut-offs, but these should be explored in future studies.

Our study examined some of the most widely available NITs. While it cannot be considered exhaustive, it can be regarded as the benchmark against which newer NITs can be tested. This is particularly important as new tests are continuously being developed (FibroTest-FibroSURE, ActiTest,<sup>44</sup> ELF<sup>45</sup>). Furthermore, newer tests are also needed for patients with 'at risk' NASH (NASH+F2–3) who would be candidates for clinical trials or treatments, once approved therapies become available (FAST score,<sup>46</sup> NIS4,<sup>47</sup> cTAG<sup>48</sup>).

In conclusion, our study provides further validation of the use of sequential combination of FIB-4 and LSM-VCTE to rule out patients with NAFLD and advanced fibrosis who can be managed in primary care. We have shown how the use of upper cut-offs to rule in cirrhosis in combination with lower cut-offs to



**Figure 3** Sankey diagrams showing the distribution of patients in true positive, true negative, false positive, false negative and indeterminate groups for a sequential combination of Fibrosis-4 Index (FIB-4) and liver stiffness measurement (LSM) by vibration controlled transient elastography (VCTE) when using different thresholds for each testing tier. A lower threshold was used to rule out patients without advanced fibrosis and an upper threshold ruled in patients with advanced fibrosis when applying both tests (A). In an alternative model, a lower threshold was used to rule out patients without advanced fibrosis, but the upper threshold ruled in only patients with cirrhosis (B, C). Two different pairs of thresholds were chosen for this hybrid strategy: the lower cut-off for both FIB-4 and LSM by VCTE were determined from the literature; upper cut-offs were both determined as corresponding to 95% specificity in detecting cirrhosis (B) or both corresponding to 98% specificity in detecting cirrhosis (C). In the application of the algorithm described in (A) 33% of patients would need to have a liver biopsy for the diagnosis of cirrhosis (those in the indeterminate group to rule out advanced fibrosis and those in the rule in group to identify cirrhosis). With the application of an upper cut-off to rule in cirrhosis without the need of biopsy, only patients in the indeterminate group need to have a biopsy. The latter strategy results in fewer patients undergoing biopsy (18% and 24% depending on the threshold used). Tables next to each panel contain the number and proportion of patients in each of the true positive (TP), true negative (TN), false positive (FP) and false negative (FN) groups for FIB-4 and LSM by VCTE.

rule out advanced fibrosis can lead to a reduction in the number of patients who would need to undergo liver biopsy.

#### Author affiliations

<sup>1</sup>Cardiovascular Medicine, Radcliffe Department of Medicine, University of Oxford, Oxford, UK

<sup>2</sup>Department of Epidemiology and Data Science, Amsterdam UMC, University of Amsterdam, Amsterdam, The Netherlands

<sup>3</sup>Translational Gastroenterology Unit, University of Oxford, Oxford, UK

<sup>4</sup>NIHR Oxford Biomedical Research Centre, Oxford University Hospitals NHS Foundation Trust and the University of Oxford, Oxford, UK

<sup>5</sup>Division of Gastroenterology and Hepatology, Department of Medicine III, Medical University of Vienna, Vienna, Austria

<sup>6</sup>Laboratoire HIFIH, UPRES EA 3859, SFR ICAT 4208, Université d'Angers, Angers, Pays de la Loire, France

<sup>7</sup>Service d'Hépatogastroentérologie et Oncologie Digestive, Centre Hospitalier Universitaire d'Angers, Angers, Pays de la Loire, France

<sup>8</sup>Echosens SA, Paris, Île-de-France, France

<sup>9</sup>Department of Visceral Surgery and Medicine, Inselspital University Hospital Bern, Bern, Switzerland

<sup>10</sup>Department of Surgery, Division of Transplantation, Medical University of Vienna, Vienna, Austria

<sup>11</sup>Department of Internal Medicine, Medical University of Graz, Graz, Austria

<sup>12</sup>Medical Sciences, University of Turin, Torino, Italy

<sup>13</sup>Boehringer Ingelheim International GmbH, Ingelheim, Rheinland-Pfalz, Germany

<sup>14</sup>Department of Ultrasonography, University of Medicine and Pharmacy, Regional Institute of Gastroenterology and Hepatology "Prof. Dr. Octavian Fodor", Cluj Napoca, Romania

<sup>15</sup>Section of Gastroenterology and Hepatology, PROMISE, Palermo, Italy

<sup>16</sup>Hepatology Center, Saiseikai Suita Hospital, Suita, Osaka, Japan

<sup>17</sup>Faculty of Medicine, Department of Medicine, University of Malaya, Kuala Lumpur, Wilayah Persekutuan, Malaysia

<sup>18</sup>NIHR Biomedical Research Centre, University of Birmingham, Birmingham, UK

<sup>19</sup>NIHR Nottingham Biomedical Research Centre, University of Nottingham, Nottingham, UK

<sup>20</sup>Toronto Centre for Liver Disease, University Health Network, Toronto, Ontario, Canada

<sup>21</sup>Centre for Liver and Gastrointestinal Research, Institute of Immunology and Immunotherapy, University of Birmingham, Birmingham, UK

<sup>22</sup>Liver Unit, University Hospitals Birmingham NHS Foundation Trust, Birmingham, UK

<sup>23</sup>Department of Medicine and Therapeutics, Chinese University of Hong Kong, Hong Kong, Hong Kong

<sup>24</sup>Centre d'Investigation de la Fibrose Hépatique, Hôpital Haut-Leveque, Pessac, France

<sup>25</sup>INSERM1053, Université de Bordeaux, Talence, Aquitaine, France

<sup>26</sup>Department of Gastroenterology, Xinhua Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China

<sup>27</sup>Department of Internal Medicine, Aichi Medical University, Nagakute, Aichi, Japan

<sup>28</sup>Department of Gastroenterology, Koseikai Takeda Hospital, Kyoto, Japan

<sup>29</sup>Department of Internal Medicine I, University Medical Centre of the Johannes Gutenberg-University Mainz, Mainz, Rheinland-Palatinate, Germany

<sup>30</sup>Department of Gastroenterology and Hepatology, Department of Internal Medicine, Seoul National University College of Medicine, Seoul Metropolitan Government Boramae Medical Center, Seoul, South Korea

<sup>31</sup>Department of Radiology, Seoul National University Seoul Metropolitan Government Boramae Medical Center, Dongjak-gu, Seoul, The Republic of Korea

<sup>32</sup>Department of Medicine II, Leipzig University Medical Center, Leipzig, Sachsen, Germany

<sup>33</sup>Department of Gastroenterology, Marmara University School of Medicine, Istanbul, Turkey

<sup>34</sup>Institute of Gastroenterology, Marmara University, Istanbul, Turkey

<sup>35</sup>Nottingham Digestive Diseases Centre, School of Medicine, University of Nottingham, Nottingham, UK

<sup>36</sup>Diagnostic and Interventional Radiology, University Hospital Centre Montpellier, Montpellier, Languedoc-Roussillon, France

<sup>37</sup>Department of Surgical Disciplines, AIIMS, New Delhi, Delhi, India

<sup>38</sup>Department of Surgery, Monash University, Prahran, Victoria, Australia

<sup>39</sup>Department of Gastroenterology and Hepatology, Yokohama City University, Yokohama, Kanagawa, Japan

<sup>40</sup>Service d'Anatomie Pathologique et Centre de Ressources Biologiques, Hôpital Jean Verdier, Paris, France

<sup>41</sup>Centre de Ressources Biologiques, Hôpitaux Universitaires Paris-Seine-Saint-Denis, Bondy, Île-de-France, France

<sup>42</sup>Division of Hepatology, University Hospital Würzburg, Würzburg, Bayern, Germany

<sup>43</sup>Internal Medicine Research Unit, Pfizer Inc, Cambridge, Massachusetts, USA

<sup>44</sup>Translational and Clinical Research Institute, Faculty of Medicine, Newcastle University, Newcastle upon Tyne, UK

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**Collaborators** The LITMUS Investigators: Quentin Anstee, Ann Daly, Katherine Johnson, Olivier Govaere, Simon Cockell, Dina Tiniakos, Pierre Bedossa, Fiona Oakley, Heather Cordell, Chris Day, Kristy Wonders (Newcastle University); Patrick Bossuyt, Hadi Zafarmand, Yasaman Vali, Jenny Lee (AMC Amsterdam); Vlad Ratiuz, Karine Clement, Raluca Pais (Hôpital Pitié Salpêtrière, Assistance Publique -Hôpitaux de Paris, and Institute of Cardiometabolism and Nutrition, Paris, France); Detlef Schuppan, Jörn Schattenberg (University Medical Center Mainz); Detlef Schuppan, Jörn Schattenberg (University Medical Center Mainz); Toni Vidal-Puig, Michele Vacca, Sergio Rodriguez-Cuenca, Mike Allison, Ioannis Kamzolas, Evangelia Petsalaki (University of Cambridge); Matej Oresic, Tuulia Hyötyläinen, Aiden McGlinchey (Örebro University); Jose M Mato, Oscar Millet (Center for Cooperative Research in Biosciences); Jean-François Dufour, Annalisa Berzigotti (University of Bern); Michael Pavlides, Stephen Harrison, Stefan Neubauer, Jeremy Cobbold, Ferenc Mozes, Salma Akhtar (University of Oxford); Rajarshi Banerjee, Matt Kelly, Elizabeth Shumbayawonda, Andrea Dennis, Charlotte Epcum, Micheala Graham (Perspectum);

Manuel Romero-Gómez, Emilio Gómez-González, Javier Ampuero, Javier Castell, Rocío Gallego-Durán, Isabel Fernández, Rocío Montero-Vallejo (Servicio Andaluz de Salud, Seville); Morten Karsdal, Elisabeth Erhardt, Daniel Rasmussen, Diana Julie Leeming, Mette Juul Fisker, Antonia Sinisi, Kishwar Musa (Nordic Bioscience); Fay Betso, Estelle Sandt, Manuela Tonini (Integrated Biobank of Luxembourg); Elisabetta Bugianesi, Chiara Rosso, Angelo Armandi, Fabio Marra (UNIFI), Amalia Gastaldelli (CNR), Gianluca Sveglia (UNIPM) (University of Torino); Jérôme Boursier (University Hospital of Angers); Sven Francque; Luisa Vonghia (Antwerp University Hospital); Mattias Ekstedt, Stergios Kechagias (Linköping University); Hannele Yki-Jarvinen, Kimmu Porthan (University of Helsinki); Saskia van Mil (UMC Utrecht); George Papatheodoridis (National & Kapodistrian University of Athens); Helena Cortez-Pinto (Faculdade de Medicina de Lisboa); Luca Valenti (Università degli Studi di Milano); Salvatore Petta (Università degli Studi di Palermo); Luca Miele (Università Cattolica del Sacro Cuore); Andreas Geier (University Hospital Würzburg); Christian Trautwein (RWTH Aachen University Hospital); Guru Aithal (University of Nottingham); Paul Hockings (Antaros Medical); Philip Newsome (University Hospitals Birmingham NHS Foundation Trust); David Wenn (iXscent); Cecilia Maria Pereira Rodrigues (University of Lisbon); Pierre Chaumat, Rémy Hanf (Genfit); Aldo Trylesinski (Intercept Pharma); Pablo Ortiz (OWL); Kevin Duffin (Ely-Lilly); Julia Brosnan, Theresa Tuthill, Euan McLeod (Pfizer); Judith Ertle, Ramy Younes (Boehringer-Ingelheim); Rachel Ostroff, Leigh Alexander (Somalogic); Mette Skalskøi Kjaer (Novo Nordisk); Lars Friis Mikkelsen (Ellegaard Göttingen Minipigs); Maria-Magdalena Balp, Clifford Brass, Lori Jennings, Miljen Martić, Juergen Loeffler (Novartis Pharma AG); Guido Hanauer (Takeda Development Centre Europe Ltd); Sudha Shankar (AstraZeneca); Céline Fournier (Echosens); Kay Pepin, Richard Ehman (Resoundant); Joel Myers (Bristol-Myers Squibb); Gideon Ho (HistoIndex); Richard Torstenson (Allergan); Rob Myers (Gilead); Lynda Doward (RTI-HS).

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#### ORCID iDs

Ferenc Emil Mózes <http://orcid.org/0000-0002-1361-4349>  
 Michael Trauner <http://orcid.org/0000-0002-1275-6425>  
 Jerome Boursier <http://orcid.org/0000-0002-7282-1436>  
 Elisabetta Bugianesi <http://orcid.org/0000-0002-0502-4381>  
 Ramy Younes <http://orcid.org/0000-0003-2302-5318>  
 Salvatore Petta <http://orcid.org/0000-0002-0822-9673>  
 Sanjiv Mahadeva <http://orcid.org/0000-0001-5824-0590>  
 Philip Noel Newsome <http://orcid.org/0000-0001-6085-3652>  
 Vincent Wai-Sun Wong <http://orcid.org/0000-0003-2215-9410>

Christian Labenz <http://orcid.org/0000-0001-8390-9663>  
 Johannes Wiegand <http://orcid.org/0000-0001-9233-4064>  
 Thomas Karlas <http://orcid.org/0000-0002-8109-8526>  
 Yusuf Yilmaz <http://orcid.org/0000-0003-4518-5283>  
 Guruprasad Padur Aithal <http://orcid.org/0000-0003-3924-4830>  
 Christophe Cassinotto <http://orcid.org/0000-0001-5136-4742>  
 Geraldine J Ooi <http://orcid.org/0000-0002-4540-408X>  
 Atsushi Nakajima <http://orcid.org/0000-0002-6263-1436>  
 Andreas Geier <http://orcid.org/0000-0002-9626-5083>  
 Michael Pavlides <http://orcid.org/0000-0001-9882-8874>

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## Supporting information for:

### Diagnostic accuracy of non-invasive tests for diagnosing advanced fibrosis in patients with NAFLD – An individual patient data meta-analysis

Ferenc E. Mózes<sup>1</sup>, Jenny Lee<sup>2</sup>, Emmanuel A. Selvaraj<sup>1,3,4</sup>, Arjun N. A. Jayaswal<sup>1</sup>, Michael Trauner<sup>5</sup>, Jérôme Boursier<sup>6,7</sup>, Céline Fournier<sup>8</sup>, Katharina Stauffer<sup>5,9,10</sup>, Rudolf Stauber<sup>11</sup>, Elisabetta Bugianesi<sup>12</sup>, Ramy Younes<sup>13</sup>, Silvia Gaia<sup>12</sup>, Monica Lupşor-Platon<sup>14</sup>, Salvatore Petta<sup>15</sup>, Toshihide Shima<sup>16</sup>, Takeshi Okanoue<sup>16</sup>, Sanjiv Mahadeva<sup>17</sup>, Wah-Kheong Chan<sup>17</sup>, Peter J. Eddowes<sup>18</sup>, Philip N. Newsome<sup>19,20,21</sup>, Vincent Wai-Sun Wong<sup>22</sup>, Victor de Lédinghen<sup>23</sup>, Jian-Gao Fan<sup>24</sup>, Feng Shen<sup>24</sup>, Jeremy F. L. Cobbold<sup>25</sup>, Yoshio Sumida<sup>26</sup>, Akira Okajima<sup>27</sup>, Jörn M. Schattenberg<sup>28</sup>, Christian Labenz<sup>29</sup>, Won Kim<sup>30</sup>, Myoung Seok Lee<sup>31</sup>, Johannes Wiegand<sup>32</sup>, Thomas Karlas<sup>33</sup>, Yusuf Yilmaz<sup>34,35</sup>, Guruprasad Padur Aithal<sup>36,37</sup>, Naaventhana Palaniyappan<sup>36,37</sup>, Christophe Cassinotto<sup>38</sup>, Sandeep Aggarwal<sup>39</sup>, Harshit Garg<sup>39</sup>, Geraldine Ooi<sup>40</sup>, Atsushi Nakajima<sup>41</sup>, Masato Yoneda<sup>41</sup>, Marianne Ziolo<sup>42</sup>, Nathalie Barget<sup>43</sup>, Andreas Geier<sup>44</sup>, Theresa Tuthill<sup>45</sup>, Julia M. Brosnan<sup>45</sup>, Quentin M. Anstee<sup>46</sup>, Stefan Neubauer<sup>1</sup>, Stephen A. Harrison<sup>1</sup>, Patrick M. Bossuyt<sup>2</sup>, Michael Pavlides<sup>1,3,4</sup>, on behalf of the LITMUS Investigators

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## The LITMUS Investigators

Newcastle University	Quentin Anstee Ann Daly Katherine Johnson Olivier Govaere Simon Cockell Dina Tiniakos Pierre Bedossa Fiona Oakley Heather Cordell Chris Day Kristy Wonders
AMC Amsterdam	Patrick Bossuyt Hadi Zafarmand Yasaman Vali Jenny Lee
Hôpital Pitié Salpêtrière, Assistance Publique -Hôpitaux de Paris, and Institute of Cardiometabolism and Nutrition, Paris, France	Vlad Ratziu Karine Clement Raluca Pais
University Medical Center Mainz	Detlef Schuppan Jörn Schattenberg
University of Cambridge	Toni Vidal-Puig Michele Vacca Sergio Rodrigues-Cuenca Mike Allison Ioannis Kamzolas Evangelia Petsalaki
Örebro University	Matej Oresic Tuulia Hyötyläinen Aiden McGlinchey
Center for Cooperative Research in Biosciences	Jose M Mato Oscar Millet
University of Bern	Jean-François Dufour Annalisa Berzigotti
University of Oxford	Michael Pavlides Stephen Harrison Stefan Neubauer Jeremy Cobbold Ferenc Mozes Salma Akhtar
Perspectum	Rajarshi Banerjee Matt Kelly Elizabeth Shumbayawonda Andrea Dennis Charlotte Erpicum Micheala Graham
Servicio Andaluz de Salud, Seville	Manuel Romero-Gómez Emilio Gómez-González Javier Ampuero Javier Castell

	Rocío Gallego-Durán Isabel Fernández Rocío Montero-Vallejo
Nordic Bioscience	Morten Karsdal Elisabeth Erhardtsen Daniel Rasmussen Diana Julie Leeming Mette Juul Fisker Antonia Sinisi Kishwar Musa
Integrated Biobank of Luxembourg	Fay Betsou Estelle Sandt Manuela Tonini
University of Torino	Elisabetta Bugianesi Chiara Rosso Angelo Armandi Fabio Marra (UNIFI) Amalia Gastaldelli (CNR) Gianluca Svegliati (UNIPM)
University Hospital of Angers	Jérôme Boursier
Antwerp University Hospital	Sven Francque Luisa Vonghia
Linköping University	Mattias Ekstedt Stergios Kechagias
University of Helsinki	Hannele Yki-Jarvinen Kimmu Porthan
UMC Utrecht	Saskia van Mil
National & Kapodistrian University of Athens	George Papatheodoridis
Faculdade de Medicina de Lisboa	Helena Cortez-Pinto
Università degli Studi di Milano	Luca Valenti
Università degli Studi di Palermo	Salvatore Petta
Università Cattolica del Sacro Cuore	Luca Miele
University Hospital Würzburg	Andreas Geier
RWTH Aachen University Hospital	Christian Trautwein
University of Nottingham	Guru Aithal
Antaros Medical	Paul Hockings
University Hospitals Birmingham NHS Foundation Trust	Philip Newsome
iXscient	David Wenn
University of Lisbon	Cecília Maria Pereira Rodrigues
Genfit	Pierre Chaumat Rémy Hanf
Intercept Pharma	Aldo Trylesinski
OWL	Pablo Ortiz
Ely-Lilly	Kevin Duffin
Pfizer	Julia Brosnan Theresa Tuthill Euan McLeod
Boehringer-Ingelheim	Judith Ertle Ramy Younes
Somallogic	Rachel Ostroff

	Leigh Alexander
Novo Nordisk	Mette Skalskøi Kjær
Ellegaard Göttingen Minipigs	Lars Friis Mikkelsen
Novartis Pharma AG	Maria-Magdalena Balp Clifford Brass Lori Jennings Miljen Martić Juergen Loeffler
Takeda Development Centre Europe Ltd	Guido Hanauer
AstraZeneca	Sudha Shankar
Echosens	Céline Fournier
Resoundant	Kay Pepin Richard Ehman
Bristol-Myers Squibb	Joel Myers
HistoIndex	Gideon Ho
Allergan	Richard Torstenson
Gilead	Rob Myers
RTI-HS	Lynda Doward

## Supporting Methods

According to the manufacturer, probe selection should be driven by skin-to-liver capsule distance (SCD): M probe for  $SCD < 25$  mm and XL probe for  $25 \text{ mm} \leq SCD < 35$  mm. In the latest version of the FibroScan equipment this is done by the Automatic Probe Selection tool. Some investigators have suggested that BMI may be used as a surrogate of SCD, using the M probe if  $BMI < 30 \text{ kg/m}^2$  and XL probe if  $BMI \geq 30 \text{ kg/m}^2$  (1).

For this meta-analysis, if only one VCTE-based liver stiffness measurement was available then this was included in the main analysis irrespective of probe type and BMI. Where two VCTE-based LSM were available (one with each probe), the main analysis included the M-probe measurement for  $BMI < 30 \text{ kg/m}^2$  and the XL probe measurement for  $BMI \geq 30 \text{ kg/m}^2$ . Therefore, all LSM cut-offs were determined independent of probe type.

We further conducted sensitivity analysis to investigate the influence of probe selection by excluding patients with  $BMI \geq 30 \text{ kg/m}^2$  who had a measurement with the M probe and patients with  $BMI < 30 \text{ kg/m}^2$  who had measurement with the XL probe.

## Supporting Discussion

### Rationale for proposing new NIT combinations with higher cut-offs for diagnosis of cirrhosis

Up until now, the literature has focused on the application of non-invasive tests in screening strategies for advanced fibrosis (F3-4). These strategies are useful when applied at the interface of primary and secondary care. Patients assessed using these strategies are classified as low risk, high risk or indeterminate risk of having advanced fibrosis, based on which clinical decisions are made: those with low risk continue to be managed in primary care, those with high risk are referred to secondary care and those with indeterminate risk undergo liver biopsy to determine their risk category.

What is lacking from the literature and what we have tried to answer with our analysis is what happens to patients with high risk of advanced fibrosis that are referred to secondary care. Our view is that they remain an indeterminate group as they can have either F3 or F4 fibrosis stage. Therefore, to distinguish between F3 and cirrhosis (F4) they still need to undergo liver biopsy, as those with liver cirrhosis would be managed differently (ultrasound surveillance for HCC and screening for oesophageal varices is generally indicated in patients with cirrhosis, but not those with F3 fibrosis stage). The identification of patients with cirrhosis would also be important as potential treatments for NASH may be licenced exclusively for patients with or without cirrhosis. We therefore argue that in practice, both the indeterminate and high-risk groups need to have a liver biopsy to establish their disease stage. In the case of those in the indeterminate category, the biopsy is needed to decide whether they merit referral to secondary care, and in the case of those with high risk of advanced fibrosis a biopsy is needed in secondary care to identify those with cirrhosis. We illustrate this point in **Supporting Figure 1a** and in **Figure 3a**, we also show how the FIB4-VCTE combination performs in our cohort.

Our answer to the problem above is a hybrid algorithm, where the lower NIT cut-offs are used to rule out advanced fibrosis, and the upper cut-offs are used to rule in cirrhosis. We provide cut-offs

with 95% and 98% specificity for the diagnosis of cirrhosis. This approach still stratifies patients into 3 risk groups – those with low risk of advanced fibrosis remaining in primary care, those in the indeterminate group needing a biopsy and those with high risk for cirrhosis. We argue that the group with high risk for cirrhosis can be positively diagnosed with cirrhosis without needing to have a biopsy. The net effect is that even though the indeterminate group is larger, fewer patients need to have a biopsy overall. This new approach is illustrated in **Supporting Figure 1b**, with results from our cohort given in **Figures 3b** and **3c**.

## Supporting Tables

**Supporting Table 1** Definitions of NITs evaluated in the current meta-analysis.

NIT	Definition
LSM by VCTE	An ultrasound probe that can also generate shear waves is placed over the right liver lobe. A low frequency shear wave is then generated by the external vibrator located in the probe, and ultrasound is used to measure the velocity of this shear wave through the liver. This velocity is directly related to liver stiffness.
FIB-4	$\text{Age [years]} \times \text{AST [IU/L]} / (\text{platelets} [\times 10^9/\text{L}] \times (\text{ALT [U/L]})^{1/2})$
NFS	$-1.675 + 0.037 \times \text{age [years]} + 0.094 \times \text{BMI [kg/m}^2] + 1.13 \times \text{IFG/diabetes [yes = 1, no = 0]} + 0.99 \times \text{AST/ALT ratio} - 0.013 \times \text{platelet} [\times 10^9/\text{L}] - 0.66 \times \text{albumin [g/dL]}$
AST/ALT	$\text{AST [IU/L]} / \text{ALT [IU/L]}$
APRI	$\text{AST [IU/L]} / \text{AST ULN [IU/L]} / \text{platelet} [\times 10^9/\text{L}]$

Abbreviations: LSM – liver stiffness measurement; VCTE – vibration-controlled transient elastography; FIB-4 – Fibrosis-4 score; NFS – NAFLD fibrosis score; AST/ALT – AST to ALT ratio; APRI – AST to platelet ratio index; ULN – upper limit of normal; IU – international unit; IFG – impaired fasting glucose

**Supporting Table 2** Non-invasive test cut-offs to rule-in and rule-out advanced fibrosis in patients with NAFLD

Study ID	Rule out cut-off	Rule-in cut-off
<b>Vibration controlled transient elastography</b>		
Studies testing pre-defined cut-offs (kPa)		
Anstee 2019 (2)	< 9.0	> 11.4
Wong 2019 (3), Papatheodoridi 2021 (4)	< 10.0	> 15.0 <sup>^</sup>
Petta 2019 (5), Boursier 2019 (6), Petta 2017 (7)	< 7.9	> 9.6 <sup>*</sup>
Cut-offs identified from other primary studies (kPa)		
Tapper 2016 (8)	< 7.9	> 9.8
Eddowes 2019 (9)	< 7.1	> 14.1
Hsu 2019 (10)	< 5.9	> 13.4
Cassinotto 2016 (11)	< 8.2	> 12.5
Papatheodoridi 2021 (4)	< 8.0	< 12.0
<b>FIB-4</b>		
Studies testing pre-defined cut-offs		
Anstee 2019 (2), Xun 2012 (12), Petta 2019 (5)	< 1.30	> 2.67 <sup>#</sup>
Vilar-Gomez 2018 (13), Sun 2016 (14), McPherson 2010 (15), Srivastava 2019 (16)	< 1.30	> 3.25
Demir 2013 (17)	< 1.45	> 3.25
Cut-offs from other primary studies		
Siddiqui 2019 (18)	< 1.02	> 1.95
<b>NAFLD Fibrosis score</b>		
Studies testing pre-defined cut-offs		
Antsee 2019 (2), Tapper 2016 (8), Vilar-Gomez 2018 (13), Sun 2016 (14), McPherson 2010 (15), Xun 2012 (12), Demir 2013 (17), Petta 2014 (19), Dowman 2011 (20), Petta 2019 (5), Fowell 2020 (21)	< -1.455	> 0.676 <sup>%</sup>

<sup>^</sup>based on BavenoVI (22), <sup>\*</sup>based on Wong (23), <sup>#</sup>from Shah 2009 (24), <sup>%</sup>from Angulo 2007 (25)

**Supporting Table 3** Data fields requested from the authors of primary studies of LSM by VCTE

Category	Field	Units or possible values	Proportion of patients in whom reported, %
Study details	Name of first author	-	100.0
	Year of publication	-	100.0
	Country	-	100.0
	Centre	-	
Demographic and anthropometric details	Gender	M/F	100.0
	Age	years	99.9
	Ethnicity	-	38.6
	Height	m	92.4
	Weight	kg	94.9
	Waist circumference	cm	72.3
	Hip circumference	cm	21.8
	Smoking	Current/Ex/Never	10.0
	Presence of type 2 diabetes mellitus	Yes/No	86.4
	Presence of hypertension	Yes/No	48.8
	Presence of hyperlipidaemia	Yes/No	26.0
Laboratory data	Platelet count	$\times 10^9/l$	98.2
	INR	-	35.4
	Bilirubin	$\mu\text{mol/l}$	55.5
	ALT	IU/L	97.2
	AST	IU/L	96.2
	ALP	IU/L	48.3
	GGT	IU/L	82.2
	Albumin	g/l	67.2
	Sodium	mmol/l	6.7
	Urea	mmol/l	13.7
	Creatine	$\mu\text{mol/l}$	22.2
	Total cholesterol	mmol/l	62.8
	LDL cholesterol	mmol/l	32.8
	HDL cholesterol	mmol/l	77.6
	Triglycerides	mmol/l	79.3
	CRP	mg/l	7.9
	Fasting glucose	mmol/l	73.0
	Fasting insulin	mU/L	18.0
	HOMA-IR	-	16.8
Biopsy data	Date of biopsy	-	67.0
	Length of biopsy sample	mm	70.6
	Number of portal tracts	-	32.4
	Fibrosis stage	0-4	100.0
	Ballooning	0-2	63.7
	Lobular inflammation	0-3	64.2
	Steatosis	0-3	71.5
	NAS score	0-8	82.9
	Date of scan	-	68.9

Transient elastography details	Time between biopsy and scan	days	79.3
	Probe type	M/XL	91.9
	Number of valid shots	-	59.4
	Median stiffness	kPa	95.7
	IQR	kPa	83.4
	IQR/median	-	83.0
	Success rate	%	77.8

**Supporting Table 4** Demographic, biopsy, liver function test and NIT details of the entire cohort and broken down by fibrosis stage

	Entire cohort (n = 5735)	F0 (n = 1138)	F1 (n = 1613)	F2 (n = 1262)	F3 (n = 1101)	F4 (n = 621)
Females (%)	45	43	44	43	47	50
BMI > 30 kg/m <sup>2</sup> (%)	47	33	45	56	55	51
Waist circumference (cm)	103 (15)	99 (16)	101 (15)	106 (14)	106 (14)	106 (15)
Diabetes (%)	38	28	33	45	62	65
Age (years)*	54 (19)	48 (17)	50 (20)	53 (19)	59 (15)	60 (12)
BMI (kg/m <sup>2</sup> )*	30 (7)	28 (7)	29 (7)	31 (7)	31 (7)	30 (7)
<b>Biopsy data</b>						
Steatosis						
S0/S1/S2/S3 (%)	3/35/36/26	8/45/30/17	2/35/37/26	1/28/39/32	1/28/39/32	3/38/37/22
Ballooning						
B0/B1/B2 (%)	24/47/29	53/37/10	26/55/19	11/53/36	10/43/47	10/46/44
Inflammation						
I0/I1/I2/I3 (%)	13/60/24/3	3/60/9/4	13/65/21/1	6/60/31/3	5/53/36/6	8/57/29/6
NAS score <sup>+</sup>	4 (2)	3 (2)	4 (2)	4 (1)	5 (1)	4 (2)
NASH (%)	50	19	46	64	71	61
<b>Liver function tests</b>						
ALT (IU/L) *	55 (48)	46 (39)	54 (50)	59 (52)	63 (50)	55 (43)
AST (IU/L) *	40 (30)	31 (19)	36 (27)	41 (28)	49 (32)	53 (39)
Platelets (×10 <sup>9</sup> /l) <sup>+</sup>	230 (72)	247 (64)	243 (69)	232 (66)	217 (69)	184 (81)
Albumin (g/l) <sup>+</sup>	43 (9)	43 (8)	43 (7)	43 (5)	43 (6)	43 (20)
GGT (IU/L) *	69 (87)	59 (85)	61 (75)	63 (74)	82 (88)	104 (169)
Total cholesterol (mmol/l) <sup>+</sup>	5.1 (1.3)	5.2 (1.3)	5.1 (1.2)	5.2 (1.4)	4.9 (1.2)	4.6 (1.3)
HDL cholesterol (mmol/l) <sup>+</sup>	1.2 (0.5)	1.3 (0.5)	1.3 (0.5)	1.2 (0.5)	1.2 (0.4)	1.2 (0.5)
Triglycerides (mmol/l) *	1.6 (1.1)	1.4 (1.0)	1.6 (1.1)	1.6 (1.0)	1.6 (1.1)	1.5 (1.0)

Fasting glucose (mmol/l) *	5.6 (2.0)	5.3 (1.2)	5.4 (1.8)	5.6 (1.7)	6.3 (2.9)	6.4 (2.8)
Non-invasive tests						
LSM (kPa) *	10.7 (6.1)	5.7 (2.5)	6.7 (3.4)	7.9 (4.3)	11.3 (6.9)	20.9 (16.8)
AST/ALT*	0.8 (0.4)	0.7 (0.4)	0.7 (0.4)	0.7 (0.4)	0.8 (0.4)	0.9 (0.6)
FIB-4*	1.7 (1.2)	1.1 (0.7)	1.3 (1.2)	1.5 (1.1)	2.1 (1.6)	3.3 (2.9)
NFS*	-1.5 (1.7)	-2.3 (2.0)	-2.0 (2.2)	-1.4 (2.2)	-0.8 (1.8)	0.0 (1.8)
APRI*	0.6 (0.4)	0.3 (0.3)	0.4 (0.3)	0.5 (0.4)	0.6 (0.5)	0.8 (0.8)

\*Data are reported as median (IQR); \*Data are reported as mean (SD).

**Supporting Table 5** Details of biopsy and biopsy quality in the entire IPD cohort.

Biopsy details	Entire cohort (n = 5735)	Advanced fibrosis (n = 1722)	Cirrhosis (n = 621)
<b>Time between liver biopsy and LSM by VCTE</b>			
Patients with reported exact time period, %	79 (4549/5735)	80 (1371/1722)	76 (474/621)
Median (IQR) (days)	0 (14)	0 (9)	1 (26)
<b>Length of biopsy sample</b>			
Patients with reported length of biopsy, %	71 (4047/5735)	80 (1369/1722)	80 (495/621)
< 10 mm, %	3 (123/4047)	3 (42/1369)	5 (25/495)
≥ 10 mm and < 20 mm, %	35 (1432/4047)	33 (450/1369)	35 (172/495)
≥ 20 mm, %	62 (2492/4047)	64 (877/1369)	60 (298/495)
<b>Number of portal tracts in biopsy sample</b>			
Patients with reported portal tracts %	32 (1857/5735)	32 (544/1722)	26 (159/621)
< 11, %	54 (1006/1857)	42 (228/544)	47 (74/159)
≥ 11, %	46 (851/1857)	58 (316/544)	54 (85/159)
<b>Patients with both portal tracts and biopsy length reported, %</b>	32 (1854/5735)	32 (543/1722)	26 (159/621)
<b>Biopsy quality</b>			
Intermediate quality (length ≥ 10 mm and < 20 mm), %	46 (849/1854)	41 (220/543)	39 (62/159)
High quality (length ≥ 20 mm and ≥ 11 portal tracts), %	36 (670/1854)	45 (246/543)	39 (62/159)

Data are reported as percentage (number of patient satisfying conditions/total number of patients in subgroup)

**Supporting Table 6** Diagnostic performance of non-invasive tests for cirrhosis (F4)

	LSM by VCTE (n = 5489)			FIB-4 (n = 5393)			NFS (n = 3248)			APRI (n =5477)			AST/ALT ratio (n = 5434)		
Cirrhosis, %	11			11			11			11			11		
AUC	0.90 (.89-0.91)			0.80 (0.78-0.82)			0.77 (0.75-0.80)			0.72 (0.70-0.74)			0.69 (0.67-0.71)		
Threshold	10.4	<10.2	≥14.9	1.55	<1.13	≥2.66	-1.11	<-1.72	≥0.48	0.58	<0.30	≥1.04	0.82	<0.58	≥1.35
Sensitivity, %	89	90	67	77	90	44	82	90	36	66	90	35	64	90	24
	(86-91)	(8-92)	(64-70)	(72-80)	(87-92)	(40-48)	(76-85)	(86-93)	(31-40)	(61-69)	(87-92)	(31-39)	(59-67)	(87-92)	(20-28)
Specificity, %	75	74	90	67	48	90	63	49	90	68	28	90	66	33	90
	(74-76)	(72-75)	(89-90)	(65-68)	(46-49)	(89-90)	(61-64)	(46-50)	(88-91)	(66-69)	(26-28)	(89-90)	(64-67)	(31-33)	(89-90)
Misclassified, %	23	24	12	32	48	15	35	47	16	32	66	16	34	61	17
	(23-24)	(24-25)	(12-13)	(32-33)	(47-49)	(14-15)	(35-36)	(46-48)	(15-16)	(32-33)	(65-66)	(15-16)	(34-35)	(61-62)	(16-17)

For each non-invasive test thresholds were calculated according to Youden’s index (YI), and fixed at 90% sensitivity (90% Se) and 90% specificity (90% Sp). 95% confidence intervals were estimated with 500 bootstrap iterations.

**Supporting Table 7** Diagnostic performance of non-invasive tests for advanced fibrosis (F3-4) in a head-to-head comparison of NITs

	LSM by VCTE (n = 3248)			FIB-4 (n = 3248)			NFS (n = 3248)		
Advanced fibrosis, %	29			29			29		
AUC	0.86 (0.85-0.88)			0.75 (0.73-0.77)			0.73 (0.71-0.75)		
	YI	90% Se	90% Sp	YI	90% Se	90% Sp	YI	90% Se	90% Sp
Threshold	9.1	7.2	11.8	1.45	0.87	2.39	-1.39	-2.55	0.28
Sensitivity, %	77 (74-80)	90 (89-92)	59 (57-63)	69 (66-72)	90 (88-92)	36 (33-39)	75 (72-78)	90 (88-92)	29 (26-32)
Specificity, %	81 (79-82)	61 (59-63)	90 (89-92)	69 (67-71)	38 (36-39)	90 (89-91)	63 (61-65)	36 (33-37)	90 (89-91)
Misclassified, %	21 (19-22)	31 (29-32)	18 (17-20)	31 (29-32)	47 (46-49)	25 (24-27)	34 (34-36)	48 (49-50)	28 (28-29)

**Supporting Table 8** Diagnostic performance of non-invasive tests for cirrhosis (F4) in a head-to-head comparison of NITs

	LSM by VCTE (n = 3094)			FIB-4 (n = 3094)			NFS (n = 3094)		
Cirrhosis, %	11			11			11		
AUC	0.91 (0.89-0.92)			0.78 (0.76-0.81)			0.77 (0.75-0.80)		
	YI	90% Se	90% Sp	YI	90% Se	90% Sp	YI	90% Se	90% Sp
Threshold	10.3	9.7	14.4	1.35	1.08	2.76	-1.11	-1.93	0.46
Sensitivity, %	89 (86-92)	90 (87-93)	68 (63-72)	83 (79-87)	90 (87-93)	42 (37-47)	81 (77-86)	90 (87-93)	35 (29-40)
Specificity, %	78 (76-79)	74 (73-76)	91 (90-92)	59 (57-61)	45 (43-47)	90 (89-91)	64 (62-66)	45 (43-47)	90 (89-91)
Misclassified, %	21 (20-22)	24 (22-25)	12 (10-13)	39 (37-40)	50 (48-52)	15 (14-16)	34 (33-36)	50 (49-52)	16 (15-17)

**Supporting Table 9** Diagnostic performance of cut-offs from the literature for LSM by VCTE, FIB-4 and NFS for diagnosing advanced fibrosis.

	LSM by VCTE (n = 5489)								FIB-4 (n = 5393)		NFS (n = 3248)			
Source	Anstee 2019 (2)		Eddowes 2019 (9)		Wong 2019 (3)		Wong 2010 (23)		Shah 2009 (24)		McPherson 2010 (15)		Angulo 2007 (25)	
Thresholds	<9.9	≥11.4	<7.1	≥14.1	<10	≥15	<7.9	≥9.6	<1.3	≥2.67	<1.3	≥3.25	<-1.455	≥0.676
Sensitivity, %	72 (71-75)	61 (60-64)	91 (90-93)	46 (44-49)	71 (70-74)	41 (39-44)	86 (86-89)	73 (71-76)	74 (72-76)	30 (28-32)	74 (72-76)	20 (18-22)	76 (73-78)	22 (19-24)
Specificity, %	82 (80-83)	87 (86-88)	58 (55-58)	94 (93-94)	82 (81-83)	95 (94-96)	68 (65-68)	81 (79-81)	64 (63-66)	94 (93-94)	64 (63-66)	96 (96-97)	61 (60-64)	94 (93-95)
Misclassified, %	21 (21-22)	21 (20-22)	32 (32-34)	20 (20-21)	21 (21-22)	21 (21-22)	27 (27-29)	21 (21-23)	33 (33-34)	25 (25-26)	33 (33-34)	27 (26-27)	35 (34-36)	28 (27-28)

95% confidence intervals were estimated with 500 bootstrap iterations

**Supporting Table 10** Indicative PPV, NPV, false positive and false negative proportions for prevalences found in primary studies for the diagnostic performance of VCTE in identifying advanced fibrosis

Cut-off	Se, %	95% CI, %	Sp, %	95% CI, %	Prevalence, %	PPV, %	NPV, %	FP*	FN*
7.4 kPa	90	89-91	60	59-61	5	11	99	38	1
					10	20	98	36	1
					20	36	96	32	2
					<b>30</b>	<b>49</b>	<b>93</b>	<b>28</b>	<b>3</b>
					40	60	90	24	4
					50	69	86	20	5
9.1 kPa	77	75-79	78	76-79	5	16	98	21	1
					10	28	97	20	2
					20	47	93	18	5
					<b>30</b>	<b>60</b>	<b>89</b>	<b>15</b>	<b>7</b>
					40	70	84	13	9
					50	78	77	11	12
12.1 kPa	55	52-57	90	89-91	5	22	97	10	2
					10	38	95	9	5
					20	58	89	8	9
					<b>30</b>	<b>70</b>	<b>82</b>	<b>7</b>	<b>14</b>
					40	79	75	6	18
					50	85	67	5	23
<7.4 kPa, ≥12.1 kPa	84	81-87	87	85-88	5	25	99	12	1
					10	42	98	12	2
					20	62	96	10	3
					<b>30</b>	<b>73</b>	<b>93</b>	<b>9</b>	<b>5</b>
					40	81	89	8	6
					50	87	84	7	8
<9.9 kPa, ≥11.4 kPa (Anstee 2019)	69	67-71	86	85-88	5	21	98	13	2
					10	35	96	13	3
					20	55	92	11	6
					<b>30</b>	<b>68</b>	<b>87</b>	<b>10</b>	<b>9</b>
					40	77	81	8	12
					50	83	74	7	16
<7.1, ≥14.1 (Eddowes 2019)	83	80-86	90	88-92	5	30	99	10	1
					10	48	98	9	2
					20	67	95	8	3
					<b>30</b>	<b>78</b>	<b>93</b>	<b>7</b>	<b>5</b>
					40	85	89	6	7
					50	89	84	5	9
<10, ≥15 (Wong 2019)	59	57-61	94	93-96	5	34	98	6	2
					10	52	95	5	4
					20	71	90	5	8
					<b>30</b>	<b>81</b>	<b>84</b>	<b>4</b>	<b>12</b>
					40	87	77	4	16
					50	91	70	3	21
<7.9, ≥9.6 (Wong 2010)	84	82-87	78	76-80	5	17	99	21	1
					10	30	98	20	2
					20	49	95	18	3
					<b>30</b>	<b>62</b>	<b>92</b>	<b>15</b>	<b>5</b>
					40	72	88	13	6

	50	79	83	11	8
*Number of false positives and false negatives for 100 hypothetical cases; Values in bold face correspond to the proportion of patients with advanced fibrosis in the IPDMA cohort					

**Supporting Table 11** Indicative PPV, NPV, false positive and false negative proportions for prevalences found in primary studies for the diagnostic performance of FIB-4 in identifying advanced fibrosis

Cut-off	Se, %	95% CI, %	Sp, %	95% CI, %	Prevalence, %	PPV, %	NPV, %	FP*	FN*
0.88	90	88-91	39	37-40	5	7	99	58	1
					10	14	97	55	1
					20	27	94	49	2
					<b>30</b>	<b>39</b>	<b>90</b>	<b>43</b>	<b>3</b>
					40	50	85	37	4
					50	60	80	31	5
1.44	69	67-72	70	69-72	5	11	98	29	2
					10	20	95	27	3
					20	37	90	24	6
					<b>30</b>	<b>50</b>	<b>84</b>	<b>21</b>	<b>9</b>
					40	61	77	18	12
					50	70	69	15	16
2.31	38	36-41	90	89-91	5	17	97	10	3
					10	30	93	9	6
					20	49	85	8	12
					<b>30</b>	<b>62</b>	<b>77</b>	<b>7</b>	<b>19</b>
					40	72	69	6	25
					50	79	59	5	31
<1.3, ≥2.67 (Shah 2009)	54	52-56	91	89-92	5	24	97	9	2
					10	40	95	8	5
					20	60	89	7	9
					<b>30</b>	<b>72</b>	<b>82</b>	<b>6</b>	<b>14</b>
					40	80	75	5	18
					50	86	66	5	23
<1.3, ≥3.25 (McPherson 2010)	44	42-46	95	93-96	5	32	97	5	3
					10	49	94	5	6
					20	69	87	4	11
					<b>30</b>	<b>79</b>	<b>80</b>	<b>4</b>	<b>17</b>
					40	85	72	3	22
					50	90	63	3	28

\*Number of false positives and false negatives for 100 hypothetical cases; Values in bold face correspond to the proportion of patients with advanced fibrosis in the IPDMA cohort

**Supporting Table 12** Indicative PPV, NPV, false positive and false negative proportions for prevalences found in primary studies for the diagnostic performance of NFS in identifying advanced fibrosis

Cut-off	Se, %	95% CI, %	Sp, %	95% CI, %	Prevalence, %	PPV, %	NPV, %	FP*	FN*
-2.55	90	88-92	36	33-37	5	7	99	61	1
					10	14	97	58	1
					20	26	94	51	2
					<b>30</b>	<b>38</b>	<b>89</b>	<b>45</b>	<b>3</b>
					40	48	84	38	4
					50	58	78	32	5
-1.39	75	72-78	63	61-65	5	10	98	35	1
					10	18	96	33	3
					20	34	91	30	5
					<b>30</b>	<b>46</b>	<b>85</b>	<b>26</b>	<b>8</b>
					40	57	79	22	10
					50	67	72	19	13
0.28	29	26-32	90	89-91	5	13	96	10	4
					10	24	92	9	7
					20	42	84	8	14
					<b>30</b>	<b>55</b>	<b>75</b>	<b>7</b>	<b>21</b>
					40	66	66	6	28
					50	74	56	5	36
<-1.455, ≥0.676 (Angulo 2007)	47	44-50	91	89-93	5	22	97	9	3
					10	37	94	8	5
					20	57	87	7	11
					<b>30</b>	<b>69</b>	<b>80</b>	<b>6</b>	<b>16</b>
					40	78	72	5	21
					50	84	63	5	27

\*Number of false positives and false negatives for 100 hypothetical cases; Values in bold face correspond to the proportion of patients with advanced fibrosis in the IPDMA cohort

**Supporting Table 13** Indicative PPV, NPV, false positive and false negative proportions for prevalences found in primary studies for the diagnostic performance of APRI in identifying advanced fibrosis

Cut-off	Se, %	95% CI, %	Sp, %	95% CI, %	Prevalence, %	PPV, %	NPV, %	FP*	FN*
0.29	90	89-92	29	28-30	5	6	98	67	1
					10	12	96	64	1
					20	24	92	57	2
					<b>30</b>	<b>35</b>	<b>87</b>	<b>50</b>	<b>3</b>
					40	46	81	43	4
					50	56	74	36	5
0.49	67	64-69	63	62-65	5	9	97	35	2
					10	17	95	33	3
					20	31	88	30	7
					<b>30</b>	<b>44</b>	<b>82</b>	<b>26</b>	<b>10</b>
					40	55	74	22	13
					50	64	66	19	17
0.91	32	30-34	90	89-91	5	14	96	10	3
					10	26	92	9	7
					20	44	84	8	14
					<b>30</b>	<b>58</b>	<b>76</b>	<b>7</b>	<b>20</b>
					40	68	67	6	27
					50	76	57	5	34

\*Number of false positives and false negatives for 100 hypothetical cases; Values in bold face correspond to the proportion of patients with advanced fibrosis in the IPDMA cohort

**Supporting Table 14** Indicative PPV, NPV, false positive and false negative proportions for prevalences found in primary studies for the diagnostic performance of AST/ALT in identifying advanced fibrosis

Cut-off	Se, %	95% CI, %	Sp, %	95% CI, %	Prevalence, %	PPV, %	NPV, %	FP*	FN*
0.51	90	87-91	25	23-26	5	6	98	71	1
					10	12	96	68	1
					20	23	91	60	2
					<b>30</b>	<b>34</b>	<b>85</b>	<b>53</b>	<b>3</b>
					40	44	79	45	4
					50	55	71	38	5
0.64	75	73-77	47	45-48	5	7	97	50	1
					10	14	94	48	3
					20	26	88	42	5
					<b>30</b>	<b>38</b>	<b>81</b>	<b>37</b>	<b>8</b>
					40	49	74	32	10
					50	59	65	27	13
1.34	16	14-18	90	89-91	5	8	95	10	4
					10	15	91	9	8
					20	29	81	8	17
					<b>30</b>	<b>41</b>	<b>71</b>	<b>7</b>	<b>25</b>
					40	52	62	6	34
					50	62	52	5	42

\*Number of false positives and false negatives for 100 hypothetical cases; Values in bold face correspond to the proportion of patients with advanced fibrosis in the IPDMA cohort

**Supporting Table 15** Diagnostic accuracy of pairs of cut-offs from the literature for NITs for diagnosing advanced fibrosis. Patient proportions used to calculate performance statistics are displayed as ratios.

LSM by VCTE (n = 5489)						FIB-4 (n = 5393)			NFS (n = 3248)	
Prevalence, %						30			29	
AUROC						0.85 (0.84-0.86)			0.73 (0.71-0.75)	
Source of thresholds	Anstee 2019 (2)	Eddowes 2019 (9)	Wong 2019 (3)	Wong 2010 (23)	This study	Shah 2009 (24)	McPherson 2010 (15)	This study	Angulo 2007 (25)	This study
Thresholds	<9.9, ≥11.4	<7.1, ≥14.1	<10, ≥15	<7.9, ≥9.6	<7.4, ≥12.1	<1.3, ≥2.67	<1.3, ≥3.25	<0.88, ≥2.31	<-1.455, ≥0.676	<-2.55, ≥0.28
Sensitivity, %	69 (1009/1456)	83 (754/905)	59 (674/1145)	84 (1205/1431)	84 (889/1060)	54 (485/901)	44 (328/744)	80 (621/780)	47 (202/429)	74 (270/363)
Specificity, %	86 (3147/3639)	90 (2216/2457)	94 (3165/3351)	78 (2599/3330)	87 (2338/2702)	91 (2423/2668)	95 (2423/2563)	79 (1448/1831)	91 (1423/1562)	78 (821/1050)
Misclassified, %	17 (948/5489)	7 (392/5489)	12 (657/5489)	17 (957/5489)	10 (535/5489)	12 (661/5393)	10 (556/5393)	10 (542/5393)	11 (366/3248)	10 (322/3248)
Indeterminate, %	7 (385/5489)	39 (2127/5489)	18 (993/5489)	13 (728/5489)	31 (1727/5489)	34 (1824/5393)	39 (2086/5393)	52 (2782/5393)	39 (1257/3248)	56 (1835/3248)

95% confidence intervals were estimated with 500 bootstrap replicates.

**Supporting Table 16** Derivation of new cut-offs corresponding to 95% specificity for cirrhosis. The cut-offs were calculated in the training cohort and were validated in the validation cohort. Training and validation cohorts were obtained by random sampling from the IPD cohort in a 3:2 ratio.

	LSM by VCTE (n = 5489)		FIB-4 (n = 5393)		NFS (n = 3248)	
	Training (n = 3290)	Validation (n = 2199)	Training (n = 3254)	Validation (n = 2139)	Training (n = 1963)	Validation (n = 1285)
Proportion of patients with cirrhosis, %	11	10	11	11	10	11
AUC	0.90 (0.89-0.92)	0.90 (0.88-0.92)	0.81 (0.78-0.83)	0.79 (0.77-0.82)	0.78 (0.75-0.81)	0.77 (0.72-0.81)
Threshold	20.4		3.48		1.01	
Sensitivity, %	52 (47-57)	49 (43-56)	33 (28-37)	30 (24-36)	21 (16-27)	28 (21-36)
Specificity, %	95 (95-96)	95 (95-97)	95 (94-96)	96 (95-97)	95 (94-96)	95 (94-96)
Misclassified, %	10 (10-11)	9 (9-10)	12 (12-13)	11 (11-12)	13 (13-14)	13 (13-14)

95% confidence intervals were estimated with 500 bootstrap replicates.

**Supporting Table 17** Derivation of new cut-offs corresponding to 98% specificity for cirrhosis. The cut-offs were calculated in the training cohort and were validated in the validation cohort. Training and validation cohorts were obtained by random sampling from the IPD cohort in a 3:2 ratio.

	LSM by VCTE (n = 5489)		FIB-4 (n = 5393)		NFS (n = 3248)	
	Training (n = 3290)	Validation (n = 2199)	Training (n = 3254)	Validation (n = 2139)	Training (n = 1963)	Validation (n = 1285)
Proportion of patients with cirrhosis, %	11	10	11	11	10	11
AUC	0.90 (0.89-0.92)	0.90 (0.88-0.92)	0.81 (0.78-0.83)	0.79 (0.77-0.82)	0.78 (0.75-0.81)	0.77 (0.72-0.81)
Threshold	27.6		4.63		1.57	
Sensitivity, %	27 (23-32)	29 (22-34)	19 (15-23)	20 (15-26)	12 (8-17)	18 (13-27)
Specificity, %	98 (98-99)	98 (98-99)	98 (97-98)	98 (97-99)	98 (97-99)	98 (97-99)
Misclassified, %	10 (10-11)	9 (9-10)	10 (10-11)	10 (10-11)	11 (11-12)	11 (11-12)

95% confidence intervals were estimated with 500 bootstrap replicates.

**Supporting Table 18** Diagnostic performance of combinations of NFS and LSM by VCTE, and FIB-4 and LSM by VCTE tests to reduce need for liver biopsies

	FIB-4 & LSM by VCTE (n = 5159)	NFS & LSM by VCTE (n = 3094)	FIB-4 & LSM by VCTE (n = 5159)	NFS & LSM by VCTE (n = 3094)	FIB-4 & LSM by VCTE (n = 5159)	NFS & LSM by VCTE (n = 3094)	FIB-4 & LSM by VCTE (n = 5159)	NFS & LSM by VCTE (n = 3094)	FIB-4 & LSM by VCTE (n = 5159)	NFS & LSM by VCTE (n = 3094)
Prevalence, %	30	28	30	28	30	28	30	28	30	28
Threshold for blood-based NIT*	< 1.3, ≥ 3.48	< -1.455, ≥ 1.010	< 1.3, ≥ 3.48	< -1.455, ≥ 1.010	< 1.3, ≥ 3.48	< -1.455, ≥ 1.010	< 1.3, ≥ 4.63	< -1.455, ≥ 1.570	< 1.3, ≥ 4.63	< -1.455, ≥ 1.570
Threshold for VCTE, kPa*	< 7.9, ≥ 16.1	< 7.9, ≥ 16.1	< 7.9, ≥ 20.4	< 7.9, ≥ 20.4	< 8.0, ≥ 20.0	< 8.0, ≥ 20.0	< 7.9, ≥ 27.6	< 7.9, ≥ 27.6	< 8.0, ≥ 28.0	< 8.0, ≥ 28.0
Sensitivity, %	41 (40-43)	41 (39-42)	38 (37-40)	37 (35-38)	38 (37-39)	36 (34-38)	28 (27-29)	25 (24-26)	27 (26-28)	24 (23-25)
Specificity, %	88 (86-89)	88 (87-90)	90 (89-91)	90 (89-92)	90 (89-91)	90 (89-92)	95 (94-97)	96 (95-98)	96 (94-97)	96 (95-98)
PPV, %	45 (43-47)	45 (41-47)	48 (45-50)	46 (43-49)	47 (45-50)	45 (43-49)	57 (54-61)	57 (52-63)	57 (54-61)	57 (52-61)
NPV, %	86 (85-87)	87 (85-88)	86 (85-87)	87 (85-88)	86 (85-87)	86 (85-88)	86 (85-87)	86 (85-88)	86 (85-87)	86 (85-88)
Indeterminate, %	16 (15-17)	17 (16-19)	19 (18-20)	20 (18-21)	18 (17-19)	17 (18-21)	24 (23-25)	25 (23-27)	24 (23-25)	21 (23-26)
Misclassification, %	18 (17-19)	17 (15-19)	16 (15-17)	15 (14-17)	17 (15-18)	14 (14-17)	13 (12-14)	12 (10-13)	13 (12-14)	11 (10-13)
Patients undergoing VCTE, %	40 (39-42)	42 (40-44)	40 (39-42)	42 (40-44)	40 (39-42)	42 (40-44)	44 (42-45)	45 (43-47)	44 (42-45)	45 (43-47)

95% confidence intervals were estimated with 500 bootstrap replicates

\*A lower cut-off was used to rule out patients with advanced fibrosis and an upper cut-off was used to rule in patients with cirrhosis. Lower cut-offs were the same as used in **Table 6** of the main manuscript. Upper cut-offs for were calculated to obtain a 95% and 98% specificity in diagnosing cirrhosis in the IPD cohort.

**Supporting Table 19** Diagnostic performance of non-invasive tests in subgroup for discriminating advanced fibrosis (F3-F4).

	LSM by VCTE	FIB-4	NFS
Biopsy length < 20 mm (n = 1555)	<b>0.87 (0.86-0.89)</b>	<b>0.80 (0.78-0.83)</b>	<b>0.79 (0.75-0.82)</b>
Biopsy length ≥ 20 mm (n = 2492)	<b>0.83 (0.82-0.85)</b>	<b>0.75 (0.72-0.77)</b>	<b>0.72 (0.69-0.75)</b>
Number of portal tracts < 11 (n = 1006)	<b>0.86 (0.83-0.88)</b>	<b>0.79 (0.75-0.82)</b>	<b>0.78 (0.74-0.81)</b>
Number of portal tracts ≥ 11 (n = 851)	<b>0.80 (0.77-0.83)</b>	<b>0.73 (0.70-0.77)</b>	<b>0.68 (0.63-0.72)</b>
Intermediate quality biopsy (n = 1432)	<b>0.87 (0.85-0.89)</b>	<b>0.79 (0.77-0.82)</b>	<b>0.78 (0.74-0.81)</b>
High quality biopsy (n = 670)	<b>0.79 (0.75-0.83)</b>	<b>0.72 (0.68-0.76)</b>	<b>0.67 (0.62-0.73)</b>
BMI < 25 kg/m <sup>2</sup> (n = 868)	<b>0.91 (0.89-0.94)</b>	<b>0.81 (0.78-0.84)</b>	<b>0.76 (0.71-0.81)<sup>#</sup></b>
25 kg/m <sup>2</sup> ≤ BMI < 30 kg/m <sup>2</sup> (n = 2127)	<b>0.87 (0.85-0.89)</b>	0.77 (0.75-0.80)	<b>0.74 (0.71-0.77)<sup>*</sup></b>
BMI ≥ 30 kg/m <sup>2</sup> (n = 2710)	<b>0.81 (0.79-0.83)</b>	<b>0.74 (0.72-0.76)</b>	<b>0.69 (0.66-0.72)<sup>*,#</sup></b>
Continent – Europe (n = 3560)	0.85 (0.84-0.87)	0.75 (0.73-0.77)	0.72 (0.69-0.75)
Continent - Asia (n = 1278)	0.85 (0.82-0.88)	0.77 (0.73-0.80)	0.76 (0.73-0.80)
Sex – Male (n = 3165)	0.85 (0.83-0.86)	0.76 (0.74-0.78)	<b>0.75 (0.72-0.77)</b>
Sex – Female (n = 2570)	0.86 (0.84-0.87)	0.76 (0.73-0.78)	<b>0.71 (0.68-0.74)</b>
Presence of T2DM (n = 2191)	<b>0.81 (0.79-0.83)</b>	<b>0.73 (0.71-0.75)</b>	0.68 (0.65-0.70)
Lack of T2DM (n = 2763)	<b>0.87 (0.86-0.89)</b>	<b>0.77 (0.75-0.79)</b>	0.71 (0.68-0.74)
ALT < 40 U/L (n = 1656)	0.85 (0.83-0.88)	<b>0.73 (0.70-0.76)</b>	0.74 (0.70-0.78)
40 U/L ≤ ALT < 100 U/L (n = 2933)	0.86 (0.85-0.87)	<b>0.77 (0.76-0.79)</b>	0.75 (0.73-0.78)
ALT ≥ 100 U/L (n = 984)	0.83 (0.80-0.86)	0.76 (0.73-0.79)	0.77 (0.73-0.81)
AST < 40 U/L (n = 2759)	0.84 (0.82-0.86)	0.73 (0.70-0.75)	<b>0.76 (0.73-0.78)</b>
40 U/L ≤ AST < 100 U/L (n = 2385)	0.85 (0.83-0.86)	0.74 (0.72-0.76)	0.72 (0.69-0.75)
AST ≥ 100 U/L (n = 373)	0.86 (0.82-0.90)	0.71 (0.66-0.76)	<b>0.65 (0.58-0.72)</b>
ALT < 40 U/L AND AST < 40 U/L (n = 1323)	0.84 (0.81-0.87)	<b>0.72 (0.68-0.75)</b>	0.73 (0.69-0.77)
ALT ≥ 40 U/L OR AST ≥ 40 U/L (n = 4235)	0.86 (0.84-0.87)	<b>0.76 (0.75-0.78)</b>	0.75 (0.73-0.77)
Age < 43 yrs (n = 1401)	0.81 (0.77-0.84)	<b>0.65 (0.61-0.70)</b>	<b>0.58 (0.52-0.64)<sup>*,#</sup></b>
43 yrs ≤ Age < 54 yrs (n = 1478)	0.84 (0.82-0.86)	0.69 (0.66-0.72)	<b>0.70 (0.66-0.74)<sup>*</sup></b>
54 yrs ≤ Age < 62 yrs (n = 1423)	0.85 (0.83-0.87)	<b>0.72 (0.69-0.75)</b>	<b>0.70 (0.67-0.74)<sup>#</sup></b>
62 yrs ≤ Age (n = 1430)	0.84 (0.81-0.86)	0.70 (0.67-0.72)	0.66 (0.62-0.70)

Bold AUCs within a column and subgroup category are significantly different. Bonferroni's correction was applied when performing multiple comparisons. AUCs marked with \* or # are pairwise significantly different.

**Supporting Table 20** Diagnostic performance of non-invasive tests in subgroup for discriminating cirrhosis (F4).

	LSM by VCTE	FIB-4	NFS
Biopsy length < 20 mm (n = 1555)	0.91 (0.88-0.93)	0.84 (0.81-0.86)	<b>0.83 (0.79-0.87)</b>
Biopsy length ≥ 20 mm (n = 2492)	0.89 (0.88-0.91)	0.78 (0.76-0.81)	<b>0.75 (0.71-0.78)</b>
Number of portal tracts < 11 (n = 1006)	<b>0.90 (0.87-0.94)</b>	0.81 (0.76-0.87)	0.76 (0.70-0.83)
Number of portal tracts ≥ 11 (n = 851)	<b>0.84 (0.81-0.88)</b>	0.77 (0.72-0.81)	0.71 (0.65-0.77)
Intermediate quality biopsy (n = 1432)	0.91 (0.88-0.93)	0.83 (0.80-0.86)	<b>0.83 (0.78-0.87)</b>
High quality biopsy (n = 670)	0.87 (0.83-0.90)	0.87 (0.83-0.90)	<b>0.69 (0.62-0.76)</b>
BMI < 25 kg/m <sup>2</sup> (n = 868)	<b>0.93 (0.91-0.95)<sup>#</sup></b>	<b>0.84 (0.80-0.88)</b>	0.77 (0.69-0.84)
25 kg/m <sup>2</sup> ≤ BMI < 30 kg/m <sup>2</sup> (n = 2127)	<b>0.92 (0.91-0.94)<sup>*</sup></b>	0.82 (0.78-0.85)	<b>0.83 (0.80-0.86)</b>
BMI ≥ 30 kg/m <sup>2</sup> (n = 2710)	<b>0.87 (0.85-0.89)<sup>*,#</sup></b>	<b>0.77 (0.75-0.80)</b>	<b>0.73 (0.69-0.76)</b>
Continent – Europe (n = 3560)	0.90 (0.89-0.92)	0.80 (0.78-0.82)	0.77 (0.74-0.81)
Continent - Asia (n = 1278)	0.92 (0.89-0.94)	0.81 (0.77-0.85)	0.80 (0.75-0.85)
Sex – Male (n = 3165)	0.91 (0.89-0.92)	0.81 (0.78-0.83)	<b>0.80 (0.77-0.83)</b>
Sex – Female (n = 2570)	0.89 (0.88-0.91)	0.78 (0.76-0.81)	<b>0.74 (0.71-0.78)</b>
Presence of T2DM (n = 2191)	<b>0.85 (0.83-0.87)</b>	<b>0.74 (0.72-0.77)</b>	<b>0.70 (0.67-0.70)</b>
Lack of T2DM (n = 2763)	<b>0.94 (0.92-0.95)</b>	<b>0.85 (0.83-0.88)</b>	<b>0.80 (0.76-0.84)</b>
ALT < 40 U/L (n = 1656)	0.91 (0.89-0.93)	0.79 (0.75-0.83)	0.77 (0.73-0.82)
40 U/L ≤ ALT < 100 U/L (n = 2933)	0.90 (0.88-0.92)	0.80 (0.78-0.83)	0.77 (0.74-0.80)
ALT ≥ 100 U/L (n = 984)	0.90 (0.87-0.93)	0.79 (0.75-0.84)	0.82 (0.76-0.88)
AST < 40 U/L (n = 2759)	0.90 (0.88-0.92)	0.78 (0.75-0.81)	0.80 (0.77-0.84)
40 U/L ≤ AST < 100 U/L (n = 2385)	0.89 (0.87-0.91)	0.78 (0.76-0.81)	0.75 (0.72-0.79)
AST ≥ 100 U/L (n = 373)	0.90 (0.86-0.94)	0.77 (0.71-0.84)	0.75 (0.66-0.84)
ALT < 40 U/L AND AST < 40 U/L (n = 1323)	0.91 (0.89-0.93)	0.76 (0.72-0.81)	0.75 (0.69-0.80)
ALT ≥ 40 U/L OR AST ≥ 40 U/L (n = 4235)	0.90 (0.89-0.91)	0.80 (0.78-0.82)	0.79 (0.76-0.81)
Age < 43 yrs (n = 1401)	<b>0.97 (0.95-0.99)<sup>*,#,%</sup></b>	0.82 (0.75-0.88)	0.72 (0.55-0.89)
43 yrs ≤ Age < 54 yrs (n = 1478)	<b>0.90 (0.87-0.93)<sup>*</sup></b>	0.77 (0.72-0.82)	0.74 (0.67-0.81)
54 yrs ≤ Age < 62 yrs (n = 1423)	<b>0.87 (0.85-0.90)<sup>#</sup></b>	0.75 (0.71-0.78)	0.74 (0.69-0.78)
62 yrs ≤ Age (n = 1430)	<b>0.86 (0.84-0.89)<sup>%</sup></b>	0.72 (0.69-0.76)	0.66 (0.62-0.71)

Bold AUCs within a column and subgroup category are significantly different. Bonferroni's correction was applied when performing multiple comparisons. AUCs marked with <sup>\*</sup>, <sup>#</sup> or <sup>%</sup> are pairwise significantly different.

**Supporting Table 21** Subgroup analysis on the impact of reliability of liver stiffness measurements (LSM) on diagnostic performance in detecting advanced fibrosis.

	AUROC (95% CI) for diagnosing advanced fibrosis	AUROC (95% CI) for diagnosing cirrhosis
Reliable LSM by VCTE (median LSM < 7.1 kPa OR (median LSM ≥ 7.1 kPa AND IQR/median LSM < 0.30)	<b>0.86 (0.85-0.87)</b>	<b>0.91 (0.90-0.92)</b>
Unreliable LSM by VCTE (median LSM ≥ 7.1 kPa AND IQR/median LSM > 0.30)	<b>0.75 (0.70-0.80)</b>	<b>0.81 (0.76-0.86)</b>
Reliable LSM by VCTE (IQR/median LSM < 0.30)	0.86 (0.84-0.87)	0.90 (0.89-0.92)
Unreliable LSM by VCTE (IQR/median LSM ≥ 0.30)	0.84 (0.82-0.86)	0.88 (0.86-0.91)

VCTE – vibration-controlled transient elastography; 95% confidence intervals were estimated using 500 bootstrap iterations. Bold AUCs within a column and subgroup category are significantly different (p < 0.05).

**Supporting Table 22** Subgroup analysis based on choice of probe type (in patients with data available from both probes) compared to the diagnostic accuracy of LSM by VCTE calculated in the entire IPD cohort.

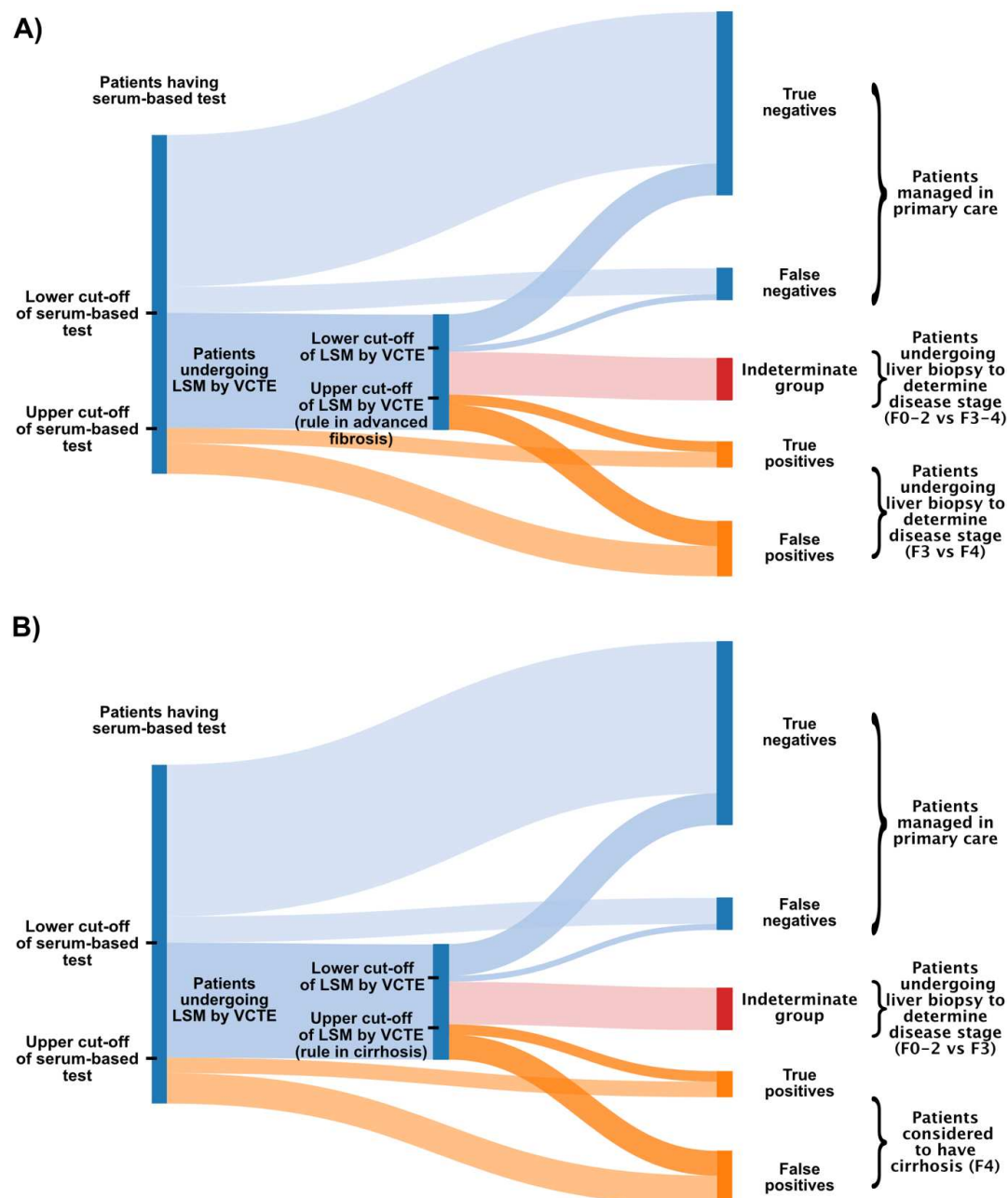
	AUROC (95% CI) for diagnosing advanced fibrosis	AUROC (95% CI) for diagnosing cirrhosis
Entire cohort (n = 5489)	0.85 (0.84-0.86)	0.90 (0.89-0.91)
M probe only (where measurements performed with both probes were performed) (n = 799)	0.84 (0.82-0.87)	0.86 (0.83-0.90)
XL probe only (where measurements performed with both probes were performed) (n = 799)	0.83 (0.80-0.86)	0.87 (0.84-0.90)

**Supporting Table 23** Sensitivity analysis on the impact of probe selection on diagnostic performance in detecting advanced fibrosis. Thresholds were calculated from the entire IPD cohort.

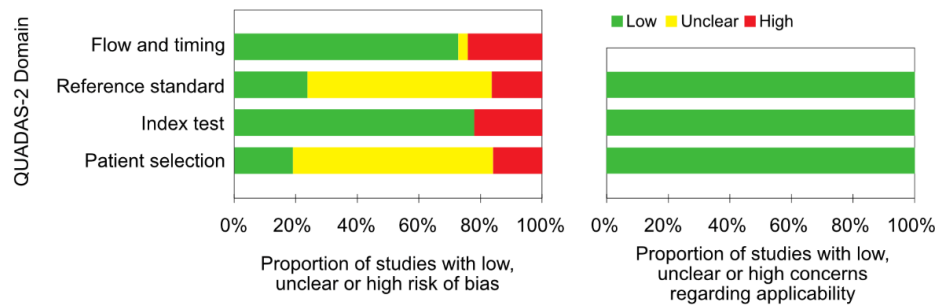
	All patients with LSM (n = 5489)			Patients with BMI < 30 kg/m <sup>2</sup> and M probe OR BMI ≥ 30 kg/m <sup>2</sup> and XL probe (n = 4464)		
AUC (95% CI)	0.85 (0.84-0.86)			0.86 (0.85-0.87)		
Thresholds, kPa	9.1	< 7.4	≥ 12.1	9.1	< 7.4	≥ 12.1
Sensitivity, %	77 (75-79)	90 (89-91)	55 (52-57)	75 (72-78)	89 (87-91)	53 (50-56)
Specificity, %	78 (76-79)	60 (59-61)	90 (89-91)	81 (79-82)	65 (63-67)	92 (91-93)
Misclassified, %	22 (22-23)	31 (31-32)	21 (20-21)	21 (20-22)	28 (27-29)	20 (18-21)

95% confidence intervals were estimated with 500 bootstrap replicates.

## Supporting Figures



**Supporting Figure 1** “Traditional” (A) and newly proposed two-tier algorithms (B) for using non-invasive tests in clinical care. (A) In the traditional application of NITs, patients with NIT values below the lower cut-offs are “ruled out” and are managed in primary care. Those with indeterminate NIT values and those “ruled in” with values above the upper cut-offs still need to undergo liver biopsy in order to stage their disease. Patients with indeterminate NITs need a liver biopsy to rule out advanced fibrosis, while patients ruled in for advanced fibrosis still need a biopsy to diagnose cirrhosis, as those with cirrhosis are managed differently (they need surveillance for hepatocellular cancer and screening for oesophageal varices). (B) In the proposed algorithms we use upper cut-off values to rule in cirrhosis, where those who are ruled in are thereby managed as having cirrhosis without the need for liver biopsy. Patients in the indeterminate group still require biopsy to correctly stage their disease.

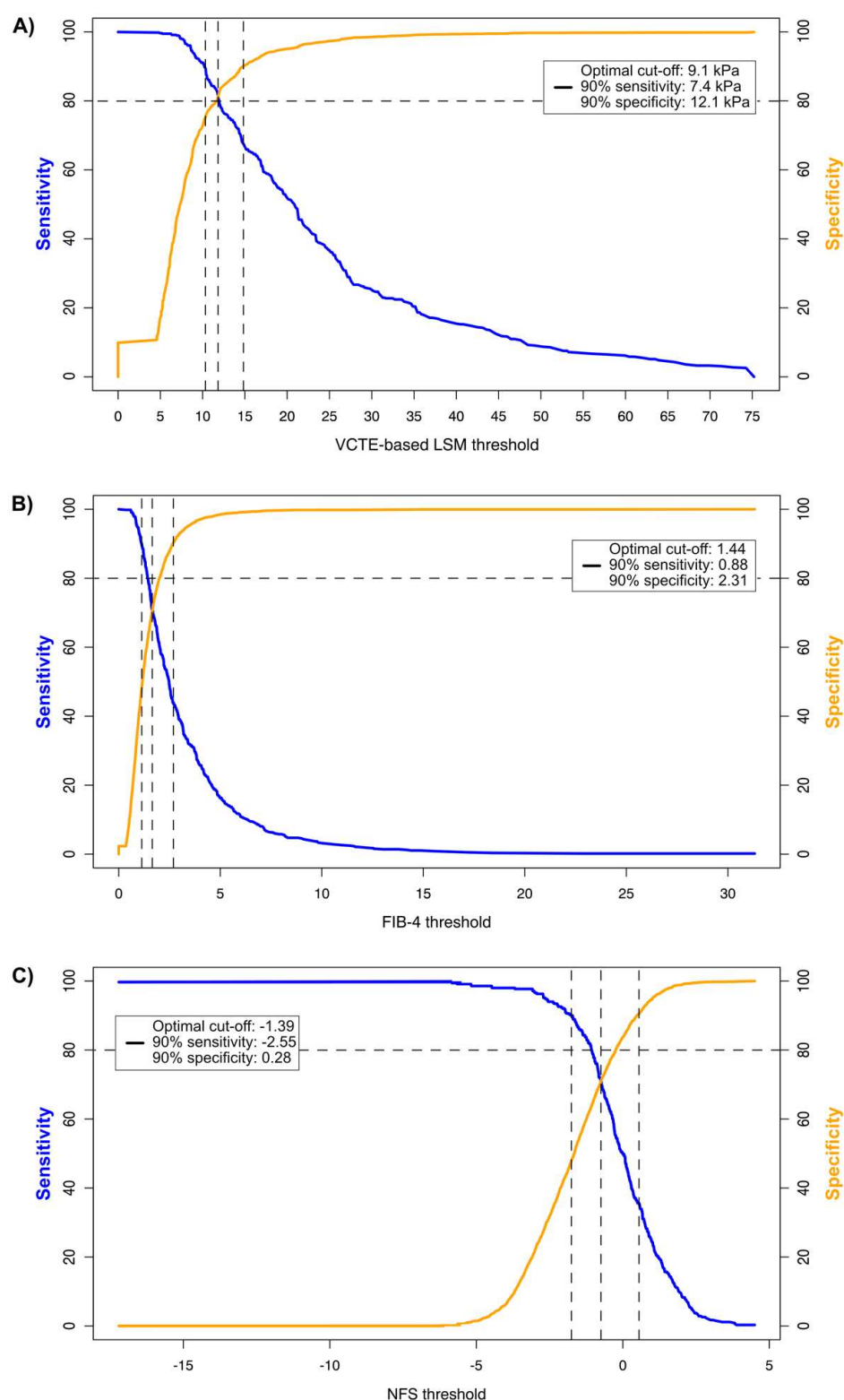


Supporting Figure 2 Risk of bias and applicability concerns

Study ID	Risk of bias				Applicability concerns		
	Patient selection	Index test	Reference test	Flow and timing	Patient selection	Index test	Reference test
Agrawal 2017	+	-	+	-	+	+	+
Aykut 2014	?	?	?	?	+	+	+
Boursier 2016	+	-	+	-	+	+	+
Boursier 2017	?	?	?	+	+	+	+
Boursier 2018	?	?	?	?	+	+	+
Cassinotto 2013	+	-	+	-	+	+	+
Cassinotto 2016	+	-	+	-	+	+	+
Chan 2015	+	+	+	-	+	+	+
Chan 2017	-	-	+	-	+	+	+
Clet 2018	?	-	+	?	+	+	+
Eddowes 2016	?	?	+	+	+	+	+
Eddowes 2019	+	-	+	-	+	+	+
Gaia 2011	+	-	+	?	+	+	+
Garg 2018	+	-	+	-	+	+	+
Karlas 2015	?	?	+	-	+	+	+
Kwok 2016	+	+	+	-	+	+	+
Labenz 2018	+	+	?	-	+	+	+
Lee 2017	+	-	+	-	+	+	+
Loong 2017	+	+	+	-	+	+	+
Lupsor 2010	?	-	+	-	+	+	+
Mahadeva 2013	+	-	+	?	+	+	+
Okajima 2017	+	-	?	-	+	+	+
Ooi 2018	+	?	+	+	+	+	+
Pavlidis 2017	+	-	+	-	+	+	+
Petta 2015 Liv Int	+	+	+	-	+	+	+
Petta 2015 Hepatol	+	-	+	-	+	+	+
Petta 2017 APT	+	-	+	?	+	+	+
Petta 2017 Hepatol	+	+	+	+	+	+	+
Seki 2017	+	-	?	+	+	+	+
Shen 2015	+	-	+	-	+	+	+
Staufer 2019	+	+	+	-	+	+	+
Wong 2010	+	-	+	-	+	+	+
Wong 2012	+	-	+	-	+	+	+
Wong 2019	+	+	+	-	+	+	+
Yoneda 2008	?	-	?	-	+	+	+
Younes 2018	?	+	+	+	+	+	+
Ziol 2009	+	-	?	+	+	+	+

 Low
  High
  Unclear

Supporting Figure 3 Methodological quality summary



**Supporting Figure 4** Distribution of sensitivities and specificities over the possible threshold ranges for LSM by VCTE (A), FIB-4 (B) and NFS (C) when considering the diagnosis of cirrhosis. Horizontal dashed lines are representing the minimum acceptable criteria for considering a test as having high sensitivity ( $\geq 80\%$ ) and high specificity ( $\geq 80\%$ ).

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## Supporting information for:

### Diagnostic accuracy of non-invasive tests for diagnosing advanced fibrosis in patients with NAFLD – An individual patient data meta-analysis

Ferenc E. Mózes<sup>1</sup>, Jenny Lee<sup>2</sup>, Emmanuel A. Selvaraj<sup>1,3,4</sup>, Arjun N. A. Jayaswal<sup>1</sup>, Michael Trauner<sup>5</sup>, Jérôme Boursier<sup>6,7</sup>, Céline Fournier<sup>8</sup>, Katharina Stauffer<sup>5,9,10</sup>, Rudolf Stauber<sup>11</sup>, Elisabetta Bugianesi<sup>12</sup>, Ramy Younes<sup>13</sup>, Silvia Gaia<sup>12</sup>, Monica Lupşor-Platon<sup>14</sup>, Salvatore Petta<sup>15</sup>, Toshihide Shima<sup>16</sup>, Takeshi Okanoue<sup>16</sup>, Sanjiv Mahadeva<sup>17</sup>, Wah-Kheong Chan<sup>17</sup>, Peter J. Eddowes<sup>18</sup>, Philip N. Newsome<sup>19,20,21</sup>, Vincent Wai-Sun Wong<sup>22</sup>, Victor de Lédinghen<sup>23</sup>, Jian-Gao Fan<sup>24</sup>, Feng Shen<sup>24</sup>, Jeremy F. L. Cobbold<sup>25</sup>, Yoshio Sumida<sup>26</sup>, Akira Okajima<sup>27</sup>, Jörn M. Schattenberg<sup>28</sup>, Christian Labenz<sup>29</sup>, Won Kim<sup>30</sup>, Myoung Seok Lee<sup>31</sup>, Johannes Wiegand<sup>32</sup>, Thomas Karlas<sup>33</sup>, Yusuf Yilmaz<sup>34,35</sup>, Guruprasad Padur Aithal<sup>36,37</sup>, Naaventhana Palaniyappan<sup>36,37</sup>, Christophe Cassinotto<sup>38</sup>, Sandeep Aggarwal<sup>39</sup>, Harshit Garg<sup>39</sup>, Geraldine Ooi<sup>40</sup>, Atsushi Nakajima<sup>41</sup>, Masato Yoneda<sup>41</sup>, Marianne Ziolo<sup>42</sup>, Nathalie Barget<sup>43</sup>, Andreas Geier<sup>44</sup>, Theresa Tuthill<sup>45</sup>, Julia M. Brosnan<sup>45</sup>, Quentin M. Anstee<sup>46</sup>, Stefan Neubauer<sup>1</sup>, Stephen A. Harrison<sup>1</sup>, Patrick M. Bossuyt<sup>2</sup>, Michael Pavlides<sup>1,3,4</sup>, on behalf of the LITMUS Investigators

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## The LITMUS Investigators

Newcastle University	Quentin Anstee Ann Daly Katherine Johnson Olivier Govaere Simon Cockell Dina Tiniakos Pierre Bedossa Fiona Oakley Heather Cordell Chris Day Kristy Wonders
AMC Amsterdam	Patrick Bossuyt Hadi Zafarmand Yasaman Vali Jenny Lee
Hôpital Pitié Salpêtrière, Assistance Publique -Hôpitaux de Paris, and Institute of Cardiometabolism and Nutrition, Paris, France	Vlad Ratziu Karine Clement Raluca Pais
University Medical Center Mainz	Detlef Schuppan Jörn Schattenberg
University of Cambridge	Toni Vidal-Puig Michele Vacca Sergio Rodrigues-Cuenca Mike Allison Ioannis Kamzolas Evangelia Petsalaki
Örebro University	Matej Oresic Tuulia Hyötyläinen Aiden McGlinchey
Center for Cooperative Research in Biosciences	Jose M Mato Oscar Millet
University of Bern	Jean-François Dufour Annalisa Berzigotti
University of Oxford	Michael Pavlides Stephen Harrison Stefan Neubauer Jeremy Cobbold Ferenc Mozes Salma Akhtar
Perspectum	Rajarshi Banerjee Matt Kelly Elizabeth Shumbayawonda Andrea Dennis Charlotte Erpicum Micheala Graham
Servicio Andaluz de Salud, Seville	Manuel Romero-Gómez Emilio Gómez-González Javier Ampuero Javier Castell

	Rocío Gallego-Durán Isabel Fernández Rocío Montero-Vallejo
Nordic Bioscience	Morten Karsdal Elisabeth Erhardtsen Daniel Rasmussen Diana Julie Leeming Mette Juul Fisker Antonia Sinisi Kishwar Musa
Integrated Biobank of Luxembourg	Fay Betsou Estelle Sandt Manuela Tonini
University of Torino	Elisabetta Bugianesi Chiara Rosso Angelo Armandi Fabio Marra (UNIFI) Amalia Gastaldelli (CNR) Gianluca Svegliati (UNIPM)
University Hospital of Angers	Jérôme Boursier
Antwerp University Hospital	Sven Francque Luisa Vonghia
Linköping University	Mattias Ekstedt Stergios Kechagias
University of Helsinki	Hannele Yki-Jarvinen Kimmu Porthan
UMC Utrecht	Saskia van Mil
National & Kapodistrian University of Athens	George Papatheodoridis
Faculdade de Medicina de Lisboa	Helena Cortez-Pinto
Università degli Studi di Milano	Luca Valenti
Università degli Studi di Palermo	Salvatore Petta
Università Cattolica del Sacro Cuore	Luca Miele
University Hospital Würzburg	Andreas Geier
RWTH Aachen University Hospital	Christian Trautwein
University of Nottingham	Guru Aithal
Antaros Medical	Paul Hockings
University Hospitals Birmingham NHS Foundation Trust	Philip Newsome
iXscient	David Wenn
University of Lisbon	Cecília Maria Pereira Rodrigues
Genfit	Pierre Chaumat Rémy Hanf
Intercept Pharma	Aldo Trylesinski
OWL	Pablo Ortiz
Ely-Lilly	Kevin Duffin
Pfizer	Julia Brosnan Theresa Tuthill Euan McLeod
Boehringer-Ingelheim	Judith Ertle Ramy Younes
Somallogic	Rachel Ostroff

	Leigh Alexander
Novo Nordisk	Mette Skalhøj Kjær
Ellegaard Göttingen Minipigs	Lars Friis Mikkelsen
Novartis Pharma AG	Maria-Magdalena Balp Clifford Brass Lori Jennings Miljen Martić Juergen Loeffler
Takeda Development Centre Europe Ltd	Guido Hanauer
AstraZeneca	Sudha Shankar
Echosens	Céline Fournier
Resoundant	Kay Pepin Richard Ehman
Bristol-Myers Squibb	Joel Myers
HistoIndex	Gideon Ho
Allergan	Richard Torstenson
Gilead	Rob Myers
RTI-HS	Lynda Doward

## Supporting Methods

According to the manufacturer, probe selection should be driven by skin-to-liver capsule distance (SCD): M probe for  $SCD < 25$  mm and XL probe for  $25 \text{ mm} \leq SCD < 35$  mm. In the latest version of the FibroScan equipment this is done by the Automatic Probe Selection tool. Some investigators have suggested that BMI may be used as a surrogate of SCD, using the M probe if  $BMI < 30 \text{ kg/m}^2$  and XL probe if  $BMI \geq 30 \text{ kg/m}^2$  (1).

For this meta-analysis, if only one VCTE-based liver stiffness measurement was available then this was included in the main analysis irrespective of probe type and BMI. Where two VCTE-based LSM were available (one with each probe), the main analysis included the M-probe measurement for  $BMI < 30 \text{ kg/m}^2$  and the XL probe measurement for  $BMI \geq 30 \text{ kg/m}^2$ . Therefore, all LSM cut-offs were determined independent of probe type.

We further conducted sensitivity analysis to investigate the influence of probe selection by excluding patients with  $BMI \geq 30 \text{ kg/m}^2$  who had a measurement with the M probe and patients with  $BMI < 30 \text{ kg/m}^2$  who had measurement with the XL probe.

## Supporting Discussion

### Rationale for proposing new NIT combinations with higher cut-offs for diagnosis of cirrhosis

Up until now, the literature has focused on the application of non-invasive tests in screening strategies for advanced fibrosis (F3-4). These strategies are useful when applied at the interface of primary and secondary care. Patients assessed using these strategies are classified as low risk, high risk or indeterminate risk of having advanced fibrosis, based on which clinical decisions are made: those with low risk continue to be managed in primary care, those with high risk are referred to secondary care and those with indeterminate risk undergo liver biopsy to determine their risk category.

What is lacking from the literature and what we have tried to answer with our analysis is what happens to patients with high risk of advanced fibrosis that are referred to secondary care. Our view is that they remain an indeterminate group as they can have either F3 or F4 fibrosis stage. Therefore, to distinguish between F3 and cirrhosis (F4) they still need to undergo liver biopsy, as those with liver cirrhosis would be managed differently (ultrasound surveillance for HCC and screening for oesophageal varices is generally indicated in patients with cirrhosis, but not those with F3 fibrosis stage). The identification of patients with cirrhosis would also be important as potential treatments for NASH may be licenced exclusively for patients with or without cirrhosis. We therefore argue that in practice, both the indeterminate and high-risk groups need to have a liver biopsy to establish their disease stage. In the case of those in the indeterminate category, the biopsy is needed to decide whether they merit referral to secondary care, and in the case of those with high risk of advanced fibrosis a biopsy is needed in secondary care to identify those with cirrhosis. We illustrate this point in **Supporting Figure 1a** and in **Figure 3a**, we also show how the FIB4-VCTE combination performs in our cohort.

Our answer to the problem above is a hybrid algorithm, where the lower NIT cut-offs are used to rule out advanced fibrosis, and the upper cut-offs are used to rule in cirrhosis. We provide cut-offs

with 95% and 98% specificity for the diagnosis of cirrhosis. This approach still stratifies patients into 3 risk groups – those with low risk of advanced fibrosis remaining in primary care, those in the indeterminate group needing a biopsy and those with high risk for cirrhosis. We argue that the group with high risk for cirrhosis can be positively diagnosed with cirrhosis without needing to have a biopsy. The net effect is that even though the indeterminate group is larger, fewer patients need to have a biopsy overall. This new approach is illustrated in **Supporting Figure 1b**, with results from our cohort given in **Figures 3b** and **3c**.

## Supporting Tables

**Supporting Table 1** Definitions of NITs evaluated in the current meta-analysis.

NIT	Definition
LSM by VCTE	An ultrasound probe that can also generate shear waves is placed over the right liver lobe. A low frequency shear wave is then generated by the external vibrator located in the probe, and ultrasound is used to measure the velocity of this shear wave through the liver. This velocity is directly related to liver stiffness.
FIB-4	$\text{Age [years]} \times \text{AST [IU/L]} / (\text{platelets} [\times 10^9/\text{L}] \times (\text{ALT [U/L]})^{1/2})$
NFS	$-1.675 + 0.037 \times \text{age [years]} + 0.094 \times \text{BMI [kg/m}^2] + 1.13 \times \text{IFG/diabetes [yes = 1, no = 0]} + 0.99 \times \text{AST/ALT ratio} - 0.013 \times \text{platelet} [\times 10^9/\text{L}] - 0.66 \times \text{albumin [g/dL]}$
AST/ALT	$\text{AST [IU/L]} / \text{ALT [IU/L]}$
APRI	$\text{AST [IU/L]} / \text{AST ULN [IU/L]} / \text{platelet} [\times 10^9/\text{L}]$

Abbreviations: LSM – liver stiffness measurement; VCTE – vibration-controlled transient elastography; FIB-4 – Fibrosis-4 score; NFS – NAFLD fibrosis score; AST/ALT – AST to ALT ratio; APRI – AST to platelet ratio index; ULN – upper limit of normal; IU – international unit; IFG – impaired fasting glucose

**Supporting Table 2** Non-invasive test cut-offs to rule-in and rule-out advanced fibrosis in patients with NAFLD

Study ID	Rule out cut-off	Rule-in cut-off
<b>Vibration controlled transient elastography</b>		
Studies testing pre-defined cut-offs (kPa)		
Anstee 2019 (2)	< 9.0	> 11.4
Wong 2019 (3), Papatheodoridi 2021 (4)	< 10.0	> 15.0 <sup>^</sup>
Petta 2019 (5), Boursier 2019 (6), Petta 2017 (7)	< 7.9	> 9.6 <sup>*</sup>
Cut-offs identified from other primary studies (kPa)		
Tapper 2016 (8)	< 7.9	> 9.8
Eddowes 2019 (9)	< 7.1	> 14.1
Hsu 2019 (10)	< 5.9	> 13.4
Cassinotto 2016 (11)	< 8.2	> 12.5
Papatheodoridi 2021 (4)	< 8.0	< 12.0
<b>FIB-4</b>		
Studies testing pre-defined cut-offs		
Anstee 2019 (2), Xun 2012 (12), Petta 2019 (5)	< 1.30	> 2.67 <sup>#</sup>
Vilar-Gomez 2018 (13), Sun 2016 (14), McPherson 2010 (15), Srivastava 2019 (16)	< 1.30	> 3.25
Demir 2013 (17)	< 1.45	> 3.25
Cut-offs from other primary studies		
Siddiqui 2019 (18)	< 1.02	> 1.95
<b>NAFLD Fibrosis score</b>		
Studies testing pre-defined cut-offs		
Antsee 2019 (2), Tapper 2016 (8), Vilar-Gomez 2018 (13), Sun 2016 (14), McPherson 2010 (15), Xun 2012 (12), Demir 2013 (17), Petta 2014 (19), Dowman 2011 (20), Petta 2019 (5), Fowell 2020 (21)	< -1.455	> 0.676 <sup>%</sup>

<sup>^</sup>based on BavenoVI (22), <sup>\*</sup>based on Wong (23), <sup>#</sup>from Shah 2009 (24), <sup>%</sup>from Angulo 2007 (25)

**Supporting Table 3** Data fields requested from the authors of primary studies of LSM by VCTE

Category	Field	Units or possible values	Proportion of patients in whom reported, %
Study details	Name of first author	-	100.0
	Year of publication	-	100.0
	Country	-	100.0
	Centre	-	
Demographic and anthropometric details	Gender	M/F	100.0
	Age	years	99.9
	Ethnicity	-	38.6
	Height	m	92.4
	Weight	kg	94.9
	Waist circumference	cm	72.3
	Hip circumference	cm	21.8
	Smoking	Current/Ex/Never	10.0
	Presence of type 2 diabetes mellitus	Yes/No	86.4
	Presence of hypertension	Yes/No	48.8
	Presence of hyperlipidaemia	Yes/No	26.0
Laboratory data	Platelet count	$\times 10^9/l$	98.2
	INR	-	35.4
	Bilirubin	$\mu\text{mol/l}$	55.5
	ALT	IU/L	97.2
	AST	IU/L	96.2
	ALP	IU/L	48.3
	GGT	IU/L	82.2
	Albumin	g/l	67.2
	Sodium	mmol/l	6.7
	Urea	mmol/l	13.7
	Creatine	$\mu\text{mol/l}$	22.2
	Total cholesterol	mmol/l	62.8
	LDL cholesterol	mmol/l	32.8
	HDL cholesterol	mmol/l	77.6
	Triglycerides	mmol/l	79.3
	CRP	mg/l	7.9
	Fasting glucose	mmol/l	73.0
	Fasting insulin	mU/L	18.0
	HOMA-IR	-	16.8
Biopsy data	Date of biopsy	-	67.0
	Length of biopsy sample	mm	70.6
	Number of portal tracts	-	32.4
	Fibrosis stage	0-4	100.0
	Ballooning	0-2	63.7
	Lobular inflammation	0-3	64.2
	Steatosis	0-3	71.5
	NAS score	0-8	82.9
	Date of scan	-	68.9

Transient elastography details	Time between biopsy and scan	days	79.3
	Probe type	M/XL	91.9
	Number of valid shots	-	59.4
	Median stiffness	kPa	95.7
	IQR	kPa	83.4
	IQR/median	-	83.0
	Success rate	%	77.8

**Supporting Table 4** Demographic, biopsy, liver function test and NIT details of the entire cohort and broken down by fibrosis stage

	Entire cohort (n = 5735)	F0 (n = 1138)	F1 (n = 1613)	F2 (n = 1262)	F3 (n = 1101)	F4 (n = 621)
Females (%)	45	43	44	43	47	50
BMI > 30 kg/m <sup>2</sup> (%)	47	33	45	56	55	51
Waist circumference (cm)	103 (15)	99 (16)	101 (15)	106 (14)	106 (14)	106 (15)
Diabetes (%)	38	28	33	45	62	65
Age (years)*	54 (19)	48 (17)	50 (20)	53 (19)	59 (15)	60 (12)
BMI (kg/m <sup>2</sup> )*	30 (7)	28 (7)	29 (7)	31 (7)	31 (7)	30 (7)
Biopsy data						
Steatosis						
S0/S1/S2/S3 (%)	3/35/36/26	8/45/30/17	2/35/37/26	1/28/39/32	1/28/39/32	3/38/37/22
Ballooning						
B0/B1/B2 (%)	24/47/29	53/37/10	26/55/19	11/53/36	10/43/47	10/46/44
Inflammation						
I0/I1/I2/I3 (%)	13/60/24/3	3/60/9/4	13/65/21/1	6/60/31/3	5/53/36/6	8/57/29/6
NAS score <sup>+</sup>	4 (2)	3 (2)	4 (2)	4 (1)	5 (1)	4 (2)
NASH (%)	50	19	46	64	71	61
Liver function tests						
ALT (IU/L) *	55 (48)	46 (39)	54 (50)	59 (52)	63 (50)	55 (43)
AST (IU/L) *	40 (30)	31 (19)	36 (27)	41 (28)	49 (32)	53 (39)
Platelets (×10 <sup>9</sup> /l) <sup>+</sup>	230 (72)	247 (64)	243 (69)	232 (66)	217 (69)	184 (81)
Albumin (g/l) <sup>+</sup>	43 (9)	43 (8)	43 (7)	43 (5)	43 (6)	43 (20)
GGT (IU/L) *	69 (87)	59 (85)	61 (75)	63 (74)	82 (88)	104 (169)
Total cholesterol (mmol/l) <sup>+</sup>	5.1 (1.3)	5.2 (1.3)	5.1 (1.2)	5.2 (1.4)	4.9 (1.2)	4.6 (1.3)
HDL cholesterol (mmol/l) <sup>+</sup>	1.2 (0.5)	1.3 (0.5)	1.3 (0.5)	1.2 (0.5)	1.2 (0.4)	1.2 (0.5)
Triglycerides (mmol/l) *	1.6 (1.1)	1.4 (1.0)	1.6 (1.1)	1.6 (1.0)	1.6 (1.1)	1.5 (1.0)

Fasting glucose (mmol/l) *	5.6 (2.0)	5.3 (1.2)	5.4 (1.8)	5.6 (1.7)	6.3 (2.9)	6.4 (2.8)
Non-invasive tests						
LSM (kPa) *	10.7 (6.1)	5.7 (2.5)	6.7 (3.4)	7.9 (4.3)	11.3 (6.9)	20.9 (16.8)
AST/ALT*	0.8 (0.4)	0.7 (0.4)	0.7 (0.4)	0.7 (0.4)	0.8 (0.4)	0.9 (0.6)
FIB-4*	1.7 (1.2)	1.1 (0.7)	1.3 (1.2)	1.5 (1.1)	2.1 (1.6)	3.3 (2.9)
NFS*	-1.5 (1.7)	-2.3 (2.0)	-2.0 (2.2)	-1.4 (2.2)	-0.8 (1.8)	0.0 (1.8)
APRI*	0.6 (0.4)	0.3 (0.3)	0.4 (0.3)	0.5 (0.4)	0.6 (0.5)	0.8 (0.8)

\*Data are reported as median (IQR); \*Data are reported as mean (SD).

**Supporting Table 5** Details of biopsy and biopsy quality in the entire IPD cohort.

Biopsy details	Entire cohort (n = 5735)	Advanced fibrosis (n = 1722)	Cirrhosis (n = 621)
<b>Time between liver biopsy and LSM by VCTE</b>			
Patients with reported exact time period, %	79 (4549/5735)	80 (1371/1722)	76 (474/621)
Median (IQR) (days)	0 (14)	0 (9)	1 (26)
<b>Length of biopsy sample</b>			
Patients with reported length of biopsy, %	71 (4047/5735)	80 (1369/1722)	80 (495/621)
< 10 mm, %	3 (123/4047)	3 (42/1369)	5 (25/495)
≥ 10 mm and < 20 mm, %	35 (1432/4047)	33 (450/1369)	35 (172/495)
≥ 20 mm, %	62 (2492/4047)	64 (877/1369)	60 (298/495)
<b>Number of portal tracts in biopsy sample</b>			
Patients with reported portal tracts %	32 (1857/5735)	32 (544/1722)	26 (159/621)
< 11, %	54 (1006/1857)	42 (228/544)	47 (74/159)
≥ 11, %	46 (851/1857)	58 (316/544)	54 (85/159)
<b>Patients with both portal tracts and biopsy length reported, %</b>	32 (1854/5735)	32 (543/1722)	26 (159/621)
<b>Biopsy quality</b>			
Intermediate quality (length ≥ 10 mm and < 20 mm), %	46 (849/1854)	41 (220/543)	39 (62/159)
High quality (length ≥ 20 mm and ≥ 11 portal tracts), %	36 (670/1854)	45 (246/543)	39 (62/159)

Data are reported as percentage (number of patient satisfying conditions/total number of patients in subgroup)

**Supporting Table 6** Diagnostic performance of non-invasive tests for cirrhosis (F4)

	LSM by VCTE (n = 5489)			FIB-4 (n = 5393)			NFS (n = 3248)			APRI (n =5477)			AST/ALT ratio (n = 5434)		
Cirrhosis, %	11			11			11			11			11		
AUC	0.90 (.89-0.91)			0.80 (0.78-0.82)			0.77 (0.75-0.80)			0.72 (0.70-0.74)			0.69 (0.67-0.71)		
Threshold	10.4	<10.2	≥14.9	1.55	<1.13	≥2.66	-1.11	<-1.72	≥0.48	0.58	<0.30	≥1.04	0.82	<0.58	≥1.35
Sensitivity, %	89	90	67	77	90	44	82	90	36	66	90	35	64	90	24
	(86-91)	(8-92)	(64-70)	(72-80)	(87-92)	(40-48)	(76-85)	(86-93)	(31-40)	(61-69)	(87-92)	(31-39)	(59-67)	(87-92)	(20-28)
Specificity, %	75	74	90	67	48	90	63	49	90	68	28	90	66	33	90
	(74-76)	(72-75)	(89-90)	(65-68)	(46-49)	(89-90)	(61-64)	(46-50)	(88-91)	(66-69)	(26-28)	(89-90)	(64-67)	(31-33)	(89-90)
Misclassified, %	23	24	12	32	48	15	35	47	16	32	66	16	34	61	17
	(23-24)	(24-25)	(12-13)	(32-33)	(47-49)	(14-15)	(35-36)	(46-48)	(15-16)	(32-33)	(65-66)	(15-16)	(34-35)	(61-62)	(16-17)

For each non-invasive test thresholds were calculated according to Youden’s index (YI), and fixed at 90% sensitivity (90% Se) and 90% specificity (90% Sp). 95% confidence intervals were estimated with 500 bootstrap iterations.

**Supporting Table 7** Diagnostic performance of non-invasive tests for advanced fibrosis (F3-4) in a head-to-head comparison of NITs

	LSM by VCTE (n = 3248)			FIB-4 (n = 3248)			NFS (n = 3248)		
Advanced fibrosis, %	29			29			29		
AUC	0.86 (0.85-0.88)			0.75 (0.73-0.77)			0.73 (0.71-0.75)		
	YI	90% Se	90% Sp	YI	90% Se	90% Sp	YI	90% Se	90% Sp
Threshold	9.1	7.2	11.8	1.45	0.87	2.39	-1.39	-2.55	0.28
Sensitivity, %	77 (74-80)	90 (89-92)	59 (57-63)	69 (66-72)	90 (88-92)	36 (33-39)	75 (72-78)	90 (88-92)	29 (26-32)
Specificity, %	81 (79-82)	61 (59-63)	90 (89-92)	69 (67-71)	38 (36-39)	90 (89-91)	63 (61-65)	36 (33-37)	90 (89-91)
Misclassified, %	21 (19-22)	31 (29-32)	18 (17-20)	31 (29-32)	47 (46-49)	25 (24-27)	34 (34-36)	48 (49-50)	28 (28-29)

**Supporting Table 8** Diagnostic performance of non-invasive tests for cirrhosis (F4) in a head-to-head comparison of NITs

	LSM by VCTE (n = 3094)			FIB-4 (n = 3094)			NFS (n = 3094)		
Cirrhosis, %	11			11			11		
AUC	0.91 (0.89-0.92)			0.78 (0.76-0.81)			0.77 (0.75-0.80)		
	YI	90% Se	90% Sp	YI	90% Se	90% Sp	YI	90% Se	90% Sp
Threshold	10.3	9.7	14.4	1.35	1.08	2.76	-1.11	-1.93	0.46
Sensitivity, %	89 (86-92)	90 (87-93)	68 (63-72)	83 (79-87)	90 (87-93)	42 (37-47)	81 (77-86)	90 (87-93)	35 (29-40)
Specificity, %	78 (76-79)	74 (73-76)	91 (90-92)	59 (57-61)	45 (43-47)	90 (89-91)	64 (62-66)	45 (43-47)	90 (89-91)
Misclassified, %	21 (20-22)	24 (22-25)	12 (10-13)	39 (37-40)	50 (48-52)	15 (14-16)	34 (33-36)	50 (49-52)	16 (15-17)

**Supporting Table 9** Diagnostic performance of cut-offs from the literature for LSM by VCTE, FIB-4 and NFS for diagnosing advanced fibrosis.

	LSM by VCTE (n = 5489)						FIB-4 (n = 5393)				NFS (n = 3248)			
Source	Anstee 2019 (2)		Eddowes 2019 (9)		Wong 2019 (3)		Wong 2010 (23)		Shah 2009 (24)		McPherson 2010 (15)		Angulo 2007 (25)	
Thresholds	<9.9	≥11.4	<7.1	≥14.1	<10	≥15	<7.9	≥9.6	<1.3	≥2.67	<1.3	≥3.25	<-1.455	≥0.676
Sensitivity, %	72 (71-75)	61 (60-64)	91 (90-93)	46 (44-49)	71 (70-74)	41 (39-44)	86 (86-89)	73 (71-76)	74 (72-76)	30 (28-32)	74 (72-76)	20 (18-22)	76 (73-78)	22 (19-24)
Specificity, %	82 (80-83)	87 (86-88)	58 (55-58)	94 (93-94)	82 (81-83)	95 (94-96)	68 (65-68)	81 (79-81)	64 (63-66)	94 (93-94)	64 (63-66)	96 (96-97)	61 (60-64)	94 (93-95)
Misclassified, %	21 (21-22)	21 (20-22)	32 (32-34)	20 (20-21)	21 (21-22)	21 (21-22)	27 (27-29)	21 (21-23)	33 (33-34)	25 (25-26)	33 (33-34)	27 (26-27)	35 (34-36)	28 (27-28)

95% confidence intervals were estimated with 500 bootstrap iterations

**Supporting Table 10** Indicative PPV, NPV, false positive and false negative proportions for prevalences found in primary studies for the diagnostic performance of VCTE in identifying advanced fibrosis

Cut-off	Se, %	95% CI, %	Sp, %	95% CI, %	Prevalence, %	PPV, %	NPV, %	FP*	FN*
7.4 kPa	90	89-91	60	59-61	5	11	99	38	1
					10	20	98	36	1
					20	36	96	32	2
					<b>30</b>	<b>49</b>	<b>93</b>	<b>28</b>	<b>3</b>
					40	60	90	24	4
					50	69	86	20	5
9.1 kPa	77	75-79	78	76-79	5	16	98	21	1
					10	28	97	20	2
					20	47	93	18	5
					<b>30</b>	<b>60</b>	<b>89</b>	<b>15</b>	<b>7</b>
					40	70	84	13	9
					50	78	77	11	12
12.1 kPa	55	52-57	90	89-91	5	22	97	10	2
					10	38	95	9	5
					20	58	89	8	9
					<b>30</b>	<b>70</b>	<b>82</b>	<b>7</b>	<b>14</b>
					40	79	75	6	18
					50	85	67	5	23
<7.4 kPa, ≥12.1 kPa	84	81-87	87	85-88	5	25	99	12	1
					10	42	98	12	2
					20	62	96	10	3
					<b>30</b>	<b>73</b>	<b>93</b>	<b>9</b>	<b>5</b>
					40	81	89	8	6
					50	87	84	7	8
<9.9 kPa, ≥11.4 kPa (Anstee 2019)	69	67-71	86	85-88	5	21	98	13	2
					10	35	96	13	3
					20	55	92	11	6
					<b>30</b>	<b>68</b>	<b>87</b>	<b>10</b>	<b>9</b>
					40	77	81	8	12
					50	83	74	7	16
<7.1, ≥14.1 (Eddowes 2019)	83	80-86	90	88-92	5	30	99	10	1
					10	48	98	9	2
					20	67	95	8	3
					<b>30</b>	<b>78</b>	<b>93</b>	<b>7</b>	<b>5</b>
					40	85	89	6	7
					50	89	84	5	9
<10, ≥15 (Wong 2019)	59	57-61	94	93-96	5	34	98	6	2
					10	52	95	5	4
					20	71	90	5	8
					<b>30</b>	<b>81</b>	<b>84</b>	<b>4</b>	<b>12</b>
					40	87	77	4	16
					50	91	70	3	21
<7.9, ≥9.6 (Wong 2010)	84	82-87	78	76-80	5	17	99	21	1
					10	30	98	20	2
					20	49	95	18	3
					<b>30</b>	<b>62</b>	<b>92</b>	<b>15</b>	<b>5</b>
					40	72	88	13	6

	50	79	83	11	8
*Number of false positives and false negatives for 100 hypothetical cases; Values in bold face correspond to the proportion of patients with advanced fibrosis in the IPDMA cohort					

**Supporting Table 11** Indicative PPV, NPV, false positive and false negative proportions for prevalences found in primary studies for the diagnostic performance of FIB-4 in identifying advanced fibrosis

Cut-off	Se, %	95% CI, %	Sp, %	95% CI, %	Prevalence, %	PPV, %	NPV, %	FP*	FN*
0.88	90	88-91	39	37-40	5	7	99	58	1
					10	14	97	55	1
					20	27	94	49	2
					<b>30</b>	<b>39</b>	<b>90</b>	<b>43</b>	<b>3</b>
					40	50	85	37	4
					50	60	80	31	5
1.44	69	67-72	70	69-72	5	11	98	29	2
					10	20	95	27	3
					20	37	90	24	6
					<b>30</b>	<b>50</b>	<b>84</b>	<b>21</b>	<b>9</b>
					40	61	77	18	12
					50	70	69	15	16
2.31	38	36-41	90	89-91	5	17	97	10	3
					10	30	93	9	6
					20	49	85	8	12
					<b>30</b>	<b>62</b>	<b>77</b>	<b>7</b>	<b>19</b>
					40	72	69	6	25
					50	79	59	5	31
<1.3, ≥2.67 (Shah 2009)	54	52-56	91	89-92	5	24	97	9	2
					10	40	95	8	5
					20	60	89	7	9
					<b>30</b>	<b>72</b>	<b>82</b>	<b>6</b>	<b>14</b>
					40	80	75	5	18
					50	86	66	5	23
<1.3, ≥3.25 (McPherson 2010)	44	42-46	95	93-96	5	32	97	5	3
					10	49	94	5	6
					20	69	87	4	11
					<b>30</b>	<b>79</b>	<b>80</b>	<b>4</b>	<b>17</b>
					40	85	72	3	22
					50	90	63	3	28

\*Number of false positives and false negatives for 100 hypothetical cases; Values in bold face correspond to the proportion of patients with advanced fibrosis in the IPDMA cohort

**Supporting Table 12** Indicative PPV, NPV, false positive and false negative proportions for prevalences found in primary studies for the diagnostic performance of NFS in identifying advanced fibrosis

Cut-off	Se, %	95% CI, %	Sp, %	95% CI, %	Prevalence, %	PPV, %	NPV, %	FP*	FN*
-2.55	90	88-92	36	33-37	5	7	99	61	1
					10	14	97	58	1
					20	26	94	51	2
					<b>30</b>	<b>38</b>	<b>89</b>	<b>45</b>	<b>3</b>
					40	48	84	38	4
					50	58	78	32	5
-1.39	75	72-78	63	61-65	5	10	98	35	1
					10	18	96	33	3
					20	34	91	30	5
					<b>30</b>	<b>46</b>	<b>85</b>	<b>26</b>	<b>8</b>
					40	57	79	22	10
					50	67	72	19	13
0.28	29	26-32	90	89-91	5	13	96	10	4
					10	24	92	9	7
					20	42	84	8	14
					<b>30</b>	<b>55</b>	<b>75</b>	<b>7</b>	<b>21</b>
					40	66	66	6	28
					50	74	56	5	36
<-1.455, ≥0.676 (Angulo 2007)	47	44-50	91	89-93	5	22	97	9	3
					10	37	94	8	5
					20	57	87	7	11
					<b>30</b>	<b>69</b>	<b>80</b>	<b>6</b>	<b>16</b>
					40	78	72	5	21
					50	84	63	5	27

\*Number of false positives and false negatives for 100 hypothetical cases; Values in bold face correspond to the proportion of patients with advanced fibrosis in the IPDMA cohort

**Supporting Table 13** Indicative PPV, NPV, false positive and false negative proportions for prevalences found in primary studies for the diagnostic performance of APRI in identifying advanced fibrosis

Cut-off	Se, %	95% CI, %	Sp, %	95% CI, %	Prevalence, %	PPV, %	NPV, %	FP*	FN*
0.29	90	89-92	29	28-30	5	6	98	67	1
					10	12	96	64	1
					20	24	92	57	2
					<b>30</b>	<b>35</b>	<b>87</b>	<b>50</b>	<b>3</b>
					40	46	81	43	4
					50	56	74	36	5
0.49	67	64-69	63	62-65	5	9	97	35	2
					10	17	95	33	3
					20	31	88	30	7
					<b>30</b>	<b>44</b>	<b>82</b>	<b>26</b>	<b>10</b>
					40	55	74	22	13
					50	64	66	19	17
0.91	32	30-34	90	89-91	5	14	96	10	3
					10	26	92	9	7
					20	44	84	8	14
					<b>30</b>	<b>58</b>	<b>76</b>	<b>7</b>	<b>20</b>
					40	68	67	6	27
					50	76	57	5	34

\*Number of false positives and false negatives for 100 hypothetical cases; Values in bold face correspond to the proportion of patients with advanced fibrosis in the IPDMA cohort

**Supporting Table 14** Indicative PPV, NPV, false positive and false negative proportions for prevalences found in primary studies for the diagnostic performance of AST/ALT in identifying advanced fibrosis

Cut-off	Se, %	95% CI, %	Sp, %	95% CI, %	Prevalence, %	PPV, %	NPV, %	FP*	FN*
0.51	90	87-91	25	23-26	5	6	98	71	1
					10	12	96	68	1
					20	23	91	60	2
					<b>30</b>	<b>34</b>	<b>85</b>	<b>53</b>	<b>3</b>
					40	44	79	45	4
					50	55	71	38	5
0.64	75	73-77	47	45-48	5	7	97	50	1
					10	14	94	48	3
					20	26	88	42	5
					<b>30</b>	<b>38</b>	<b>81</b>	<b>37</b>	<b>8</b>
					40	49	74	32	10
					50	59	65	27	13
1.34	16	14-18	90	89-91	5	8	95	10	4
					10	15	91	9	8
					20	29	81	8	17
					<b>30</b>	<b>41</b>	<b>71</b>	<b>7</b>	<b>25</b>
					40	52	62	6	34
					50	62	52	5	42

\*Number of false positives and false negatives for 100 hypothetical cases; Values in bold face correspond to the proportion of patients with advanced fibrosis in the IPDMA cohort

**Supporting Table 15** Diagnostic accuracy of pairs of cut-offs from the literature for NITs for diagnosing advanced fibrosis. Patient proportions used to calculate performance statistics are displayed as ratios.

LSM by VCTE (n = 5489)						FIB-4 (n = 5393)			NFS (n = 3248)	
Prevalence, %						30			29	
AUROC						0.85 (0.84-0.86)			0.73 (0.71-0.75)	
Source of thresholds	Anstee 2019 (2)	Eddowes 2019 (9)	Wong 2019 (3)	Wong 2010 (23)	This study	Shah 2009 (24)	McPherson 2010 (15)	This study	Angulo 2007 (25)	This study
Thresholds	<9.9, ≥11.4	<7.1, ≥14.1	<10, ≥15	<7.9, ≥9.6	<7.4, ≥12.1	<1.3, ≥2.67	<1.3, ≥3.25	<0.88, ≥2.31	<1.455, ≥0.676	<-2.55, ≥0.28
Sensitivity, %	69 (1009/1456)	83 (754/905)	59 (674/1145)	84 (1205/1431)	84 (889/1060)	54 (485/901)	44 (328/744)	80 (621/780)	47 (202/429)	74 (270/363)
Specificity, %	86 (3147/3639)	90 (2216/2457)	94 (3165/3351)	78 (2599/3330)	87 (2338/2702)	91 (2423/2668)	95 (2423/2563)	79 (1448/1831)	91 (1423/1562)	78 (821/1050)
Misclassified, %	17 (948/5489)	7 (392/5489)	12 (657/5489)	17 (957/5489)	10 (535/5489)	12 (661/5393)	10 (556/5393)	10 (542/5393)	11 (366/3248)	10 (322/3248)
Indeterminate, %	7 (385/5489)	39 (2127/5489)	18 (993/5489)	13 (728/5489)	31 (1727/5489)	34 (1824/5393)	39 (2086/5393)	52 (2782/5393)	39 (1257/3248)	56 (1835/3248)

95% confidence intervals were estimated with 500 bootstrap replicates.

**Supporting Table 16** Derivation of new cut-offs corresponding to 95% specificity for cirrhosis. The cut-offs were calculated in the training cohort and were validated in the validation cohort. Training and validation cohorts were obtained by random sampling from the IPD cohort in a 3:2 ratio.

	LSM by VCTE (n = 5489)		FIB-4 (n = 5393)		NFS (n = 3248)	
	Training (n = 3290)	Validation (n = 2199)	Training (n = 3254)	Validation (n = 2139)	Training (n = 1963)	Validation (n = 1285)
Proportion of patients with cirrhosis, %	11	10	11	11	10	11
AUC	0.90 (0.89-0.92)	0.90 (0.88-0.92)	0.81 (0.78-0.83)	0.79 (0.77-0.82)	0.78 (0.75-0.81)	0.77 (0.72-0.81)
Threshold	20.4		3.48		1.01	
Sensitivity, %	52 (47-57)	49 (43-56)	33 (28-37)	30 (24-36)	21 (16-27)	28 (21-36)
Specificity, %	95 (95-96)	95 (95-97)	95 (94-96)	96 (95-97)	95 (94-96)	95 (94-96)
Misclassified, %	10 (10-11)	9 (9-10)	12 (12-13)	11 (11-12)	13 (13-14)	13 (13-14)

95% confidence intervals were estimated with 500 bootstrap replicates.

**Supporting Table 17** Derivation of new cut-offs corresponding to 98% specificity for cirrhosis. The cut-offs were calculated in the training cohort and were validated in the validation cohort. Training and validation cohorts were obtained by random sampling from the IPD cohort in a 3:2 ratio.

	LSM by VCTE (n = 5489)		FIB-4 (n = 5393)		NFS (n = 3248)	
	Training (n = 3290)	Validation (n = 2199)	Training (n = 3254)	Validation (n = 2139)	Training (n = 1963)	Validation (n = 1285)
Proportion of patients with cirrhosis, %	11	10	11	11	10	11
AUC	0.90 (0.89-0.92)	0.90 (0.88-0.92)	0.81 (0.78-0.83)	0.79 (0.77-0.82)	0.78 (0.75-0.81)	0.77 (0.72-0.81)
Threshold	27.6		4.63		1.57	
Sensitivity, %	27 (23-32)	29 (22-34)	19 (15-23)	20 (15-26)	12 (8-17)	18 (13-27)
Specificity, %	98 (98-99)	98 (98-99)	98 (97-98)	98 (97-99)	98 (97-99)	98 (97-99)
Misclassified, %	10 (10-11)	9 (9-10)	10 (10-11)	10 (10-11)	11 (11-12)	11 (11-12)

95% confidence intervals were estimated with 500 bootstrap replicates.

**Supporting Table 18** Diagnostic performance of combinations of NFS and LSM by VCTE, and FIB-4 and LSM by VCTE tests to reduce need for liver biopsies

	FIB-4 & LSM by VCTE (n = 5159)	NFS & LSM by VCTE (n = 3094)	FIB-4 & LSM by VCTE (n = 5159)	NFS & LSM by VCTE (n = 3094)	FIB-4 & LSM by VCTE (n = 5159)	NFS & LSM by VCTE (n = 3094)	FIB-4 & LSM by VCTE (n = 5159)	NFS & LSM by VCTE (n = 3094)	FIB-4 & LSM by VCTE (n = 5159)	NFS & LSM by VCTE (n = 3094)
Prevalence, %	30	28	30	28	30	28	30	28	30	28
Threshold for blood-based NIT*	< 1.3, ≥ 3.48	< -1.455, ≥ 1.010	< 1.3, ≥ 3.48	< -1.455, ≥ 1.010	< 1.3, ≥ 3.48	< -1.455, ≥ 1.010	< 1.3, ≥ 4.63	< -1.455, ≥ 1.570	< 1.3, ≥ 4.63	< -1.455, ≥ 1.570
Threshold for VCTE, kPa*	< 7.9, ≥ 16.1	< 7.9, ≥ 16.1	< 7.9, ≥ 20.4	< 7.9, ≥ 20.4	< 8.0, ≥ 20.0	< 8.0, ≥ 20.0	< 7.9, ≥ 27.6	< 7.9, ≥ 27.6	< 8.0, ≥ 28.0	< 8.0, ≥ 28.0
Sensitivity, %	41 (40-43)	41 (39-42)	38 (37-40)	37 (35-38)	38 (37-39)	36 (34-38)	28 (27-29)	25 (24-26)	27 (26-28)	24 (23-25)
Specificity, %	88 (86-89)	88 (87-90)	90 (89-91)	90 (89-92)	90 (89-91)	90 (89-92)	95 (94-97)	96 (95-98)	96 (94-97)	96 (95-98)
PPV, %	45 (43-47)	45 (41-47)	48 (45-50)	46 (43-49)	47 (45-50)	45 (43-49)	57 (54-61)	57 (52-63)	57 (54-61)	57 (52-61)
NPV, %	86 (85-87)	87 (85-88)	86 (85-87)	87 (85-88)	86 (85-87)	86 (85-88)	86 (85-87)	86 (85-88)	86 (85-87)	86 (85-88)
Indeterminate, %	16 (15-17)	17 (16-19)	19 (18-20)	20 (18-21)	18 (17-19)	17 (18-21)	24 (23-25)	25 (23-27)	24 (23-25)	21 (23-26)
Misclassification, %	18 (17-19)	17 (15-19)	16 (15-17)	15 (14-17)	17 (15-18)	14 (14-17)	13 (12-14)	12 (10-13)	13 (12-14)	11 (10-13)
Patients undergoing VCTE, %	40 (39-42)	42 (40-44)	40 (39-42)	42 (40-44)	40 (39-42)	42 (40-44)	44 (42-45)	45 (43-47)	44 (42-45)	45 (43-47)

95% confidence intervals were estimated with 500 bootstrap replicates

\*A lower cut-off was used to rule out patients with advanced fibrosis and an upper cut-off was used to rule in patients with cirrhosis. Lower cut-offs were the same as used in **Table 6** of the main manuscript. Upper cut-offs for were calculated to obtain a 95% and 98% specificity in diagnosing cirrhosis in the IPD cohort.

**Supporting Table 19** Diagnostic performance of non-invasive tests in subgroup for discriminating advanced fibrosis (F3-F4).

	LSM by VCTE	FIB-4	NFS
Biopsy length < 20 mm (n = 1555)	<b>0.87 (0.86-0.89)</b>	<b>0.80 (0.78-0.83)</b>	<b>0.79 (0.75-0.82)</b>
Biopsy length ≥ 20 mm (n = 2492)	<b>0.83 (0.82-0.85)</b>	<b>0.75 (0.72-0.77)</b>	<b>0.72 (0.69-0.75)</b>
Number of portal tracts < 11 (n = 1006)	<b>0.86 (0.83-0.88)</b>	<b>0.79 (0.75-0.82)</b>	<b>0.78 (0.74-0.81)</b>
Number of portal tracts ≥ 11 (n = 851)	<b>0.80 (0.77-0.83)</b>	<b>0.73 (0.70-0.77)</b>	<b>0.68 (0.63-0.72)</b>
Intermediate quality biopsy (n = 1432)	<b>0.87 (0.85-0.89)</b>	<b>0.79 (0.77-0.82)</b>	<b>0.78 (0.74-0.81)</b>
High quality biopsy (n = 670)	<b>0.79 (0.75-0.83)</b>	<b>0.72 (0.68-0.76)</b>	<b>0.67 (0.62-0.73)</b>
BMI < 25 kg/m <sup>2</sup> (n = 868)	<b>0.91 (0.89-0.94)</b>	<b>0.81 (0.78-0.84)</b>	<b>0.76 (0.71-0.81)<sup>#</sup></b>
25 kg/m <sup>2</sup> ≤ BMI < 30 kg/m <sup>2</sup> (n = 2127)	<b>0.87 (0.85-0.89)</b>	0.77 (0.75-0.80)	<b>0.74 (0.71-0.77)<sup>*</sup></b>
BMI ≥ 30 kg/m <sup>2</sup> (n = 2710)	<b>0.81 (0.79-0.83)</b>	<b>0.74 (0.72-0.76)</b>	<b>0.69 (0.66-0.72)<sup>*,#</sup></b>
Continent – Europe (n = 3560)	0.85 (0.84-0.87)	0.75 (0.73-0.77)	0.72 (0.69-0.75)
Continent - Asia (n = 1278)	0.85 (0.82-0.88)	0.77 (0.73-0.80)	0.76 (0.73-0.80)
Sex – Male (n = 3165)	0.85 (0.83-0.86)	0.76 (0.74-0.78)	<b>0.75 (0.72-0.77)</b>
Sex – Female (n = 2570)	0.86 (0.84-0.87)	0.76 (0.73-0.78)	<b>0.71 (0.68-0.74)</b>
Presence of T2DM (n = 2191)	<b>0.81 (0.79-0.83)</b>	<b>0.73 (0.71-0.75)</b>	0.68 (0.65-0.70)
Lack of T2DM (n = 2763)	<b>0.87 (0.86-0.89)</b>	<b>0.77 (0.75-0.79)</b>	0.71 (0.68-0.74)
ALT < 40 U/L (n = 1656)	0.85 (0.83-0.88)	<b>0.73 (0.70-0.76)</b>	0.74 (0.70-0.78)
40 U/L ≤ ALT < 100 U/L (n = 2933)	0.86 (0.85-0.87)	<b>0.77 (0.76-0.79)</b>	0.75 (0.73-0.78)
ALT ≥ 100 U/L (n = 984)	0.83 (0.80-0.86)	0.76 (0.73-0.79)	0.77 (0.73-0.81)
AST < 40 U/L (n = 2759)	0.84 (0.82-0.86)	0.73 (0.70-0.75)	<b>0.76 (0.73-0.78)</b>
40 U/L ≤ AST < 100 U/L (n = 2385)	0.85 (0.83-0.86)	0.74 (0.72-0.76)	0.72 (0.69-0.75)
AST ≥ 100 U/L (n = 373)	0.86 (0.82-0.90)	0.71 (0.66-0.76)	<b>0.65 (0.58-0.72)</b>
ALT < 40 U/L AND AST < 40 U/L (n = 1323)	0.84 (0.81-0.87)	<b>0.72 (0.68-0.75)</b>	0.73 (0.69-0.77)
ALT ≥ 40 U/L OR AST ≥ 40 U/L (n = 4235)	0.86 (0.84-0.87)	<b>0.76 (0.75-0.78)</b>	0.75 (0.73-0.77)
Age < 43 yrs (n = 1401)	0.81 (0.77-0.84)	<b>0.65 (0.61-0.70)</b>	<b>0.58 (0.52-0.64)<sup>*,#</sup></b>
43 yrs ≤ Age < 54 yrs (n = 1478)	0.84 (0.82-0.86)	0.69 (0.66-0.72)	<b>0.70 (0.66-0.74)<sup>*</sup></b>
54 yrs ≤ Age < 62 yrs (n = 1423)	0.85 (0.83-0.87)	<b>0.72 (0.69-0.75)</b>	<b>0.70 (0.67-0.74)<sup>#</sup></b>
62 yrs ≤ Age (n = 1430)	0.84 (0.81-0.86)	0.70 (0.67-0.72)	0.66 (0.62-0.70)

Bold AUCs within a column and subgroup category are significantly different. Bonferroni's correction was applied when performing multiple comparisons. AUCs marked with \* or # are pairwise significantly different.

**Supporting Table 20** Diagnostic performance of non-invasive tests in subgroup for discriminating cirrhosis (F4).

	LSM by VCTE	FIB-4	NFS
Biopsy length < 20 mm (n = 1555)	0.91 (0.88-0.93)	0.84 (0.81-0.86)	<b>0.83 (0.79-0.87)</b>
Biopsy length ≥ 20 mm (n = 2492)	0.89 (0.88-0.91)	0.78 (0.76-0.81)	<b>0.75 (0.71-0.78)</b>
Number of portal tracts < 11 (n = 1006)	<b>0.90 (0.87-0.94)</b>	0.81 (0.76-0.87)	0.76 (0.70-0.83)
Number of portal tracts ≥ 11 (n = 851)	<b>0.84 (0.81-0.88)</b>	0.77 (0.72-0.81)	0.71 (0.65-0.77)
Intermediate quality biopsy (n = 1432)	0.91 (0.88-0.93)	0.83 (0.80-0.86)	<b>0.83 (0.78-0.87)</b>
High quality biopsy (n = 670)	0.87 (0.83-0.90)	0.87 (0.83-0.90)	<b>0.69 (0.62-0.76)</b>
BMI < 25 kg/m <sup>2</sup> (n = 868)	<b>0.93 (0.91-0.95)<sup>#</sup></b>	<b>0.84 (0.80-0.88)</b>	0.77 (0.69-0.84)
25 kg/m <sup>2</sup> ≤ BMI < 30 kg/m <sup>2</sup> (n = 2127)	<b>0.92 (0.91-0.94)<sup>*</sup></b>	0.82 (0.78-0.85)	<b>0.83 (0.80-0.86)</b>
BMI ≥ 30 kg/m <sup>2</sup> (n = 2710)	<b>0.87 (0.85-0.89)<sup>*,#</sup></b>	<b>0.77 (0.75-0.80)</b>	<b>0.73 (0.69-0.76)</b>
Continent – Europe (n = 3560)	0.90 (0.89-0.92)	0.80 (0.78-0.82)	0.77 (0.74-0.81)
Continent - Asia (n = 1278)	0.92 (0.89-0.94)	0.81 (0.77-0.85)	0.80 (0.75-0.85)
Sex – Male (n = 3165)	0.91 (0.89-0.92)	0.81 (0.78-0.83)	<b>0.80 (0.77-0.83)</b>
Sex – Female (n = 2570)	0.89 (0.88-0.91)	0.78 (0.76-0.81)	<b>0.74 (0.71-0.78)</b>
Presence of T2DM (n = 2191)	<b>0.85 (0.83-0.87)</b>	<b>0.74 (0.72-0.77)</b>	<b>0.70 (0.67-0.70)</b>
Lack of T2DM (n = 2763)	<b>0.94 (0.92-0.95)</b>	<b>0.85 (0.83-0.88)</b>	<b>0.80 (0.76-0.84)</b>
ALT < 40 U/L (n = 1656)	0.91 (0.89-0.93)	0.79 (0.75-0.83)	0.77 (0.73-0.82)
40 U/L ≤ ALT < 100 U/L (n = 2933)	0.90 (0.88-0.92)	0.80 (0.78-0.83)	0.77 (0.74-0.80)
ALT ≥ 100 U/L (n = 984)	0.90 (0.87-0.93)	0.79 (0.75-0.84)	0.82 (0.76-0.88)
AST < 40 U/L (n = 2759)	0.90 (0.88-0.92)	0.78 (0.75-0.81)	0.80 (0.77-0.84)
40 U/L ≤ AST < 100 U/L (n = 2385)	0.89 (0.87-0.91)	0.78 (0.76-0.81)	0.75 (0.72-0.79)
AST ≥ 100 U/L (n = 373)	0.90 (0.86-0.94)	0.77 (0.71-0.84)	0.75 (0.66-0.84)
ALT < 40 U/L AND AST < 40 U/L (n = 1323)	0.91 (0.89-0.93)	0.76 (0.72-0.81)	0.75 (0.69-0.80)
ALT ≥ 40 U/L OR AST ≥ 40 U/L (n = 4235)	0.90 (0.89-0.91)	0.80 (0.78-0.82)	0.79 (0.76-0.81)
Age < 43 yrs (n = 1401)	<b>0.97 (0.95-0.99)<sup>*,#,%</sup></b>	0.82 (0.75-0.88)	0.72 (0.55-0.89)
43 yrs ≤ Age < 54 yrs (n = 1478)	<b>0.90 (0.87-0.93)<sup>*</sup></b>	0.77 (0.72-0.82)	0.74 (0.67-0.81)
54 yrs ≤ Age < 62 yrs (n = 1423)	<b>0.87 (0.85-0.90)<sup>#</sup></b>	0.75 (0.71-0.78)	0.74 (0.69-0.78)
62 yrs ≤ Age (n = 1430)	<b>0.86 (0.84-0.89)<sup>%</sup></b>	0.72 (0.69-0.76)	0.66 (0.62-0.71)

Bold AUCs within a column and subgroup category are significantly different. Bonferroni's correction was applied when performing multiple comparisons. AUCs marked with <sup>\*</sup>, <sup>#</sup> or <sup>%</sup> are pairwise significantly different.

**Supporting Table 21** Subgroup analysis on the impact of reliability of liver stiffness measurements (LSM) on diagnostic performance in detecting advanced fibrosis.

	AUROC (95% CI) for diagnosing advanced fibrosis	AUROC (95% CI) for diagnosing cirrhosis
Reliable LSM by VCTE (median LSM < 7.1 kPa OR (median LSM ≥ 7.1 kPa AND IQR/median LSM < 0.30)	<b>0.86 (0.85-0.87)</b>	<b>0.91 (0.90-0.92)</b>
Unreliable LSM by VCTE (median LSM ≥ 7.1 kPa AND IQR/median LSM > 0.30)	<b>0.75 (0.70-0.80)</b>	<b>0.81 (0.76-0.86)</b>
Reliable LSM by VCTE (IQR/median LSM < 0.30)	0.86 (0.84-0.87)	0.90 (0.89-0.92)
Unreliable LSM by VCTE (IQR/median LSM ≥ 0.30)	0.84 (0.82-0.86)	0.88 (0.86-0.91)

VCTE – vibration-controlled transient elastography; 95% confidence intervals were estimated using 500 bootstrap iterations. Bold AUCs within a column and subgroup category are significantly different (p < 0.05).

**Supporting Table 22** Subgroup analysis based on choice of probe type (in patients with data available from both probes) compared to the diagnostic accuracy of LSM by VCTE calculated in the entire IPD cohort.

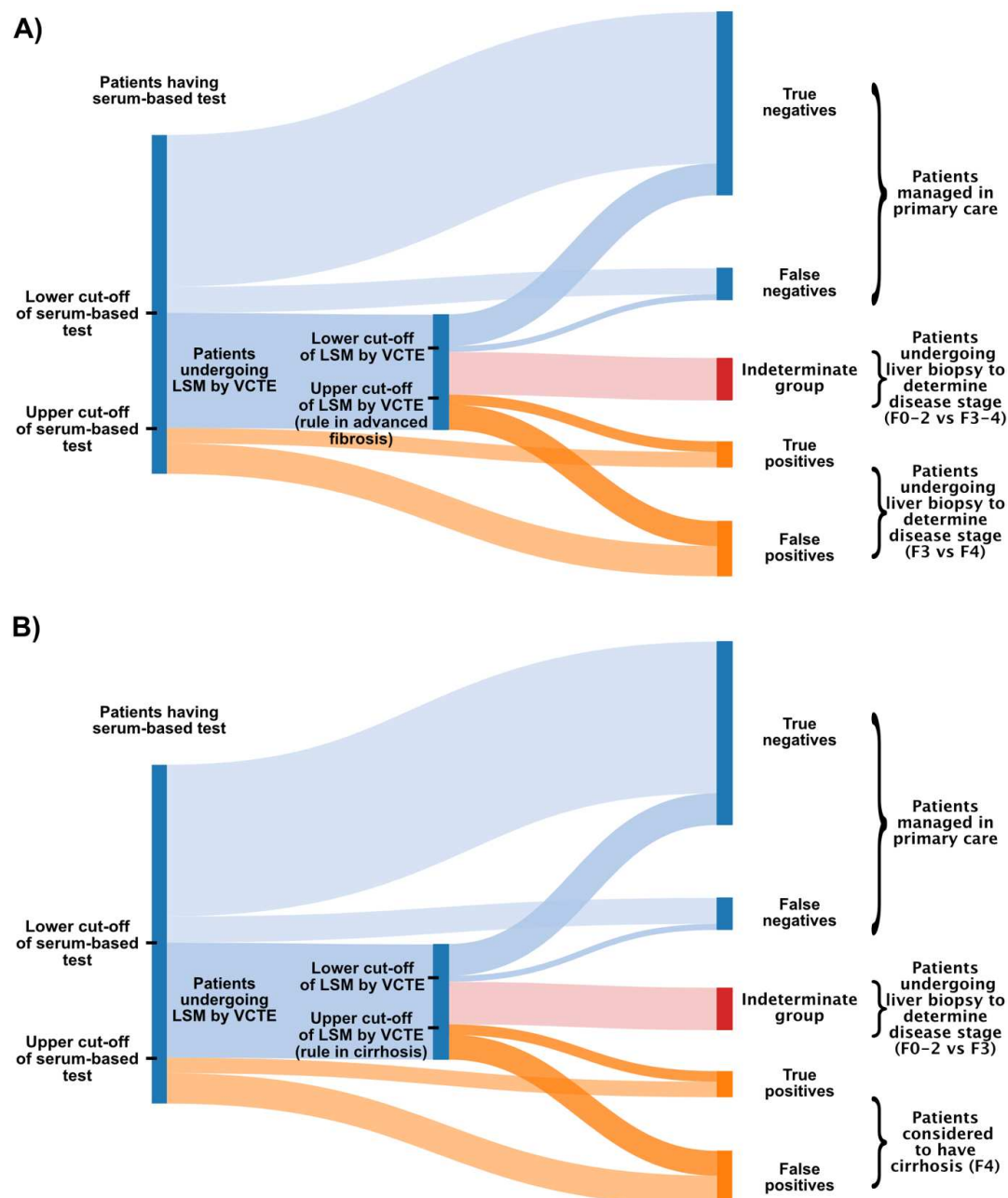
	AUROC (95% CI) for diagnosing advanced fibrosis	AUROC (95% CI) for diagnosing cirrhosis
Entire cohort (n = 5489)	0.85 (0.84-0.86)	0.90 (0.89-0.91)
M probe only (where measurements performed with both probes were performed) (n = 799)	0.84 (0.82-0.87)	0.86 (0.83-0.90)
XL probe only (where measurements performed with both probes were performed) (n = 799)	0.83 (0.80-0.86)	0.87 (0.84-0.90)

**Supporting Table 23** Sensitivity analysis on the impact of probe selection on diagnostic performance in detecting advanced fibrosis. Thresholds were calculated from the entire IPD cohort.

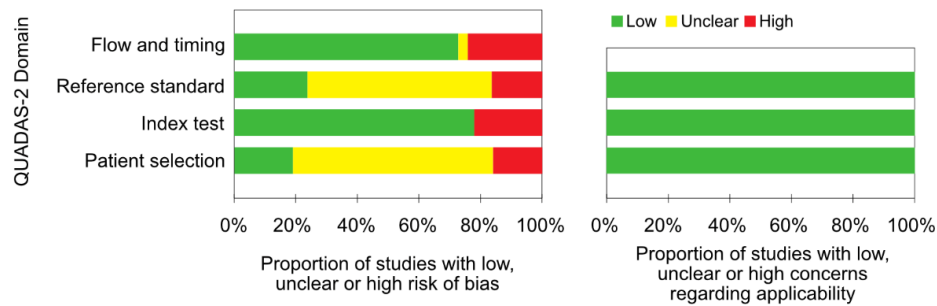
	All patients with LSM (n = 5489)			Patients with BMI < 30 kg/m <sup>2</sup> and M probe OR BMI ≥ 30 kg/m <sup>2</sup> and XL probe (n = 4464)		
AUC (95% CI)	0.85 (0.84-0.86)			0.86 (0.85-0.87)		
Thresholds, kPa	9.1	< 7.4	≥ 12.1	9.1	< 7.4	≥ 12.1
Sensitivity, %	77 (75-79)	90 (89-91)	55 (52-57)	75 (72-78)	89 (87-91)	53 (50-56)
Specificity, %	78 (76-79)	60 (59-61)	90 (89-91)	81 (79-82)	65 (63-67)	92 (91-93)
Misclassified, %	22 (22-23)	31 (31-32)	21 (20-21)	21 (20-22)	28 (27-29)	20 (18-21)

95% confidence intervals were estimated with 500 bootstrap replicates.

## Supporting Figures



**Supporting Figure 1** “Traditional” (A) and newly proposed two-tier algorithms (B) for using non-invasive tests in clinical care. (A) In the traditional application of NITs, patients with NIT values below the lower cut-offs are “ruled out” and are managed in primary care. Those with indeterminate NIT values and those “ruled in” with values above the upper cut-offs still need to undergo liver biopsy in order to stage their disease. Patients with indeterminate NITs need a liver biopsy to rule out advanced fibrosis, while patients ruled in for advanced fibrosis still need a biopsy to diagnose cirrhosis, as those with cirrhosis are managed differently (they need surveillance for hepatocellular cancer and screening for oesophageal varices). (B) In the proposed algorithms we use upper cut-off values to rule in cirrhosis, where those who are ruled in are thereby managed as having cirrhosis without the need for liver biopsy. Patients in the indeterminate group still require biopsy to correctly stage their disease.

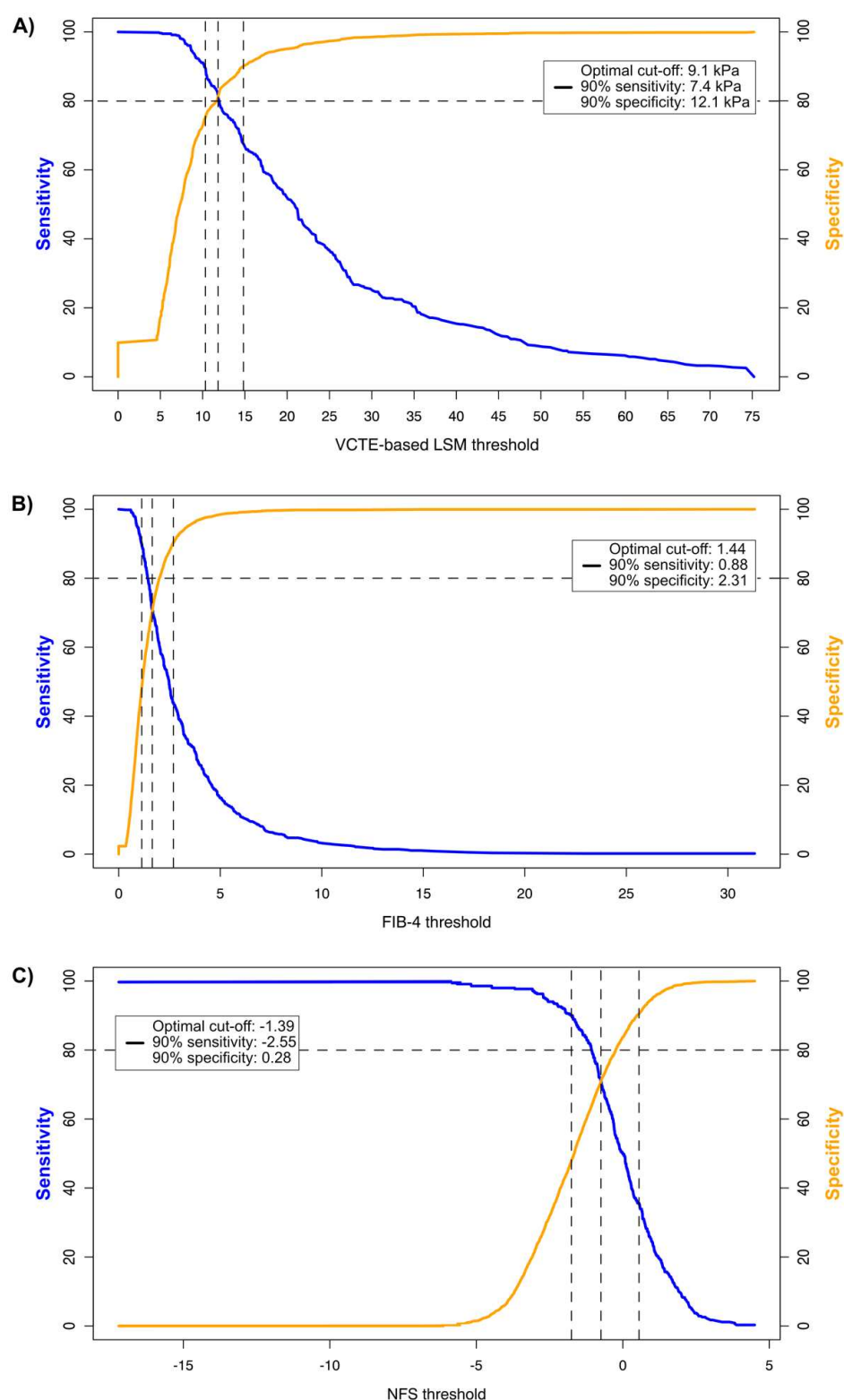


Supporting Figure 2 Risk of bias and applicability concerns

Study ID	Risk of bias				Applicability concerns		
	Patient selection	Index test	Reference test	Flow and timing	Patient selection	Index test	Reference test
Agrawal 2017	+	-	+	-	+	+	+
Aykut 2014	?	?	?	?	+	+	+
Boursier 2016	+	-	+	-	+	+	+
Boursier 2017	?	?	?	+	+	+	+
Boursier 2018	?	?	?	?	+	+	+
Cassinotto 2013	+	-	+	-	+	+	+
Cassinotto 2016	+	-	+	-	+	+	+
Chan 2015	+	+	+	-	+	+	+
Chan 2017	-	-	+	-	+	+	+
Clet 2018	?	-	+	?	+	+	+
Eddowes 2016	?	?	+	+	+	+	+
Eddowes 2019	+	-	+	-	+	+	+
Gaia 2011	+	-	+	?	+	+	+
Garg 2018	+	-	+	-	+	+	+
Karlas 2015	?	?	+	-	+	+	+
Kwok 2016	+	+	+	-	+	+	+
Labenz 2018	+	+	?	-	+	+	+
Lee 2017	+	-	+	-	+	+	+
Loong 2017	+	+	+	-	+	+	+
Lupsor 2010	?	-	+	-	+	+	+
Mahadeva 2013	+	-	+	?	+	+	+
Okajima 2017	+	-	?	-	+	+	+
Ooi 2018	+	?	+	+	+	+	+
Pavlidis 2017	+	-	+	-	+	+	+
Petta 2015 Liv Int	+	+	+	-	+	+	+
Petta 2015 Hepatol	+	-	+	-	+	+	+
Petta 2017 APT	+	-	+	?	+	+	+
Petta 2017 Hepatol	+	+	+	+	+	+	+
Seki 2017	+	-	?	+	+	+	+
Shen 2015	+	-	+	-	+	+	+
Staufer 2019	+	+	+	-	+	+	+
Wong 2010	+	-	+	-	+	+	+
Wong 2012	+	-	+	-	+	+	+
Wong 2019	+	+	+	-	+	+	+
Yoneda 2008	?	-	?	-	+	+	+
Younes 2018	?	+	+	+	+	+	+
Ziol 2009	+	-	?	+	+	+	+

Low 
 High 
 Unclear

Supporting Figure 3 Methodological quality summary



**Supporting Figure 4** Distribution of sensitivities and specificities over the possible threshold ranges for LSM by VCTE (A), FIB-4 (B) and NFS (C) when considering the diagnosis of cirrhosis. Horizontal dashed lines are representing the minimum acceptable criteria for considering a test as having high sensitivity ( $\geq 80\%$ ) and high specificity ( $\geq 80\%$ ).

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## Supporting information for:

### Diagnostic accuracy of non-invasive tests for diagnosing advanced fibrosis in patients with NAFLD – An individual patient data meta-analysis

Ferenc E. Mózes<sup>1</sup>, Jenny Lee<sup>2</sup>, Emmanuel A. Selvaraj<sup>1,3,4</sup>, Arjun N. A. Jayaswal<sup>1</sup>, Michael Trauner<sup>5</sup>, Jérôme Boursier<sup>6,7</sup>, Céline Fournier<sup>8</sup>, Katharina Stauffer<sup>5,9,10</sup>, Rudolf Stauber<sup>11</sup>, Elisabetta Bugianesi<sup>12</sup>, Ramy Younes<sup>13</sup>, Silvia Gaia<sup>12</sup>, Monica Lupşor-Platon<sup>14</sup>, Salvatore Petta<sup>15</sup>, Toshihide Shima<sup>16</sup>, Takeshi Okanoue<sup>16</sup>, Sanjiv Mahadeva<sup>17</sup>, Wah-Kheong Chan<sup>17</sup>, Peter J. Eddowes<sup>18</sup>, Philip N. Newsome<sup>19,20,21</sup>, Vincent Wai-Sun Wong<sup>22</sup>, Victor de Lédinghen<sup>23</sup>, Jian-Gao Fan<sup>24</sup>, Feng Shen<sup>24</sup>, Jeremy F. L. Cobbold<sup>25</sup>, Yoshio Sumida<sup>26</sup>, Akira Okajima<sup>27</sup>, Jörn M. Schattenberg<sup>28</sup>, Christian Labenz<sup>29</sup>, Won Kim<sup>30</sup>, Myoung Seok Lee<sup>31</sup>, Johannes Wiegand<sup>32</sup>, Thomas Karlas<sup>33</sup>, Yusuf Yilmaz<sup>34,35</sup>, Guruprasad Padur Aithal<sup>36,37</sup>, Naaventhana Palaniyappan<sup>36,37</sup>, Christophe Cassinotto<sup>38</sup>, Sandeep Aggarwal<sup>39</sup>, Harshit Garg<sup>39</sup>, Geraldine Ooi<sup>40</sup>, Atsushi Nakajima<sup>41</sup>, Masato Yoneda<sup>41</sup>, Marianne Ziolo<sup>42</sup>, Nathalie Barget<sup>43</sup>, Andreas Geier<sup>44</sup>, Theresa Tuthill<sup>45</sup>, Julia M. Brosnan<sup>45</sup>, Quentin M. Anstee<sup>46</sup>, Stefan Neubauer<sup>1</sup>, Stephen A. Harrison<sup>1</sup>, Patrick M. Bossuyt<sup>2</sup>, Michael Pavlides<sup>1,3,4</sup>, on behalf of the LITMUS Investigators

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## The LITMUS Investigators

Newcastle University	Quentin Anstee Ann Daly Katherine Johnson Olivier Govaere Simon Cockell Dina Tiniakos Pierre Bedossa Fiona Oakley Heather Cordell Chris Day Kristy Wonders
AMC Amsterdam	Patrick Bossuyt Hadi Zafarmand Yasaman Vali Jenny Lee
Hôpital Pitié Salpêtrière, Assistance Publique -Hôpitaux de Paris, and Institute of Cardiometabolism and Nutrition, Paris, France	Vlad Ratziu Karine Clement Raluca Pais
University Medical Center Mainz	Detlef Schuppan Jörn Schattenberg
University of Cambridge	Toni Vidal-Puig Michele Vacca Sergio Rodrigues-Cuenca Mike Allison Ioannis Kamzolas Evangelia Petsalaki
Örebro University	Matej Oresic Tuulia Hyötyläinen Aiden McGlinchey
Center for Cooperative Research in Biosciences	Jose M Mato Oscar Millet
University of Bern	Jean-François Dufour Annalisa Berzigotti
University of Oxford	Michael Pavlides Stephen Harrison Stefan Neubauer Jeremy Cobbold Ferenc Mozes Salma Akhtar
Perspectum	Rajarshi Banerjee Matt Kelly Elizabeth Shumbayawonda Andrea Dennis Charlotte Erpicum Micheala Graham
Servicio Andaluz de Salud, Seville	Manuel Romero-Gómez Emilio Gómez-González Javier Ampuero Javier Castell

	Rocío Gallego-Durán Isabel Fernández Rocío Montero-Vallejo
Nordic Bioscience	Morten Karsdal Elisabeth Erhardtsen Daniel Rasmussen Diana Julie Leeming Mette Juul Fisker Antonia Sinisi Kishwar Musa
Integrated Biobank of Luxembourg	Fay Betsou Estelle Sandt Manuela Tonini
University of Torino	Elisabetta Bugianesi Chiara Rosso Angelo Armandi Fabio Marra (UNIFI) Amalia Gastaldelli (CNR) Gianluca Svegliati (UNIPM)
University Hospital of Angers	Jérôme Boursier
Antwerp University Hospital	Sven Francque Luisa Vonghia
Linköping University	Mattias Ekstedt Stergios Kechagias
University of Helsinki	Hannele Yki-Jarvinen Kimmu Porthan
UMC Utrecht	Saskia van Mil
National & Kapodistrian University of Athens	George Papatheodoridis
Faculdade de Medicina de Lisboa	Helena Cortez-Pinto
Università degli Studi di Milano	Luca Valenti
Università degli Studi di Palermo	Salvatore Petta
Università Cattolica del Sacro Cuore	Luca Miele
University Hospital Würzburg	Andreas Geier
RWTH Aachen University Hospital	Christian Trautwein
University of Nottingham	Guru Aithal
Antaros Medical	Paul Hockings
University Hospitals Birmingham NHS Foundation Trust	Philip Newsome
iXscient	David Wenn
University of Lisbon	Cecília Maria Pereira Rodrigues
Genfit	Pierre Chaumat Rémy Hanf
Intercept Pharma	Aldo Trylesinski
OWL	Pablo Ortiz
Ely-Lilly	Kevin Duffin
Pfizer	Julia Brosnan Theresa Tuthill Euan McLeod
Boehringer-Ingelheim	Judith Ertle Ramy Younes
Somallogic	Rachel Ostroff

	Leigh Alexander
Novo Nordisk	Mette Skalhøj Kjær
Ellegaard Göttingen Minipigs	Lars Friis Mikkelsen
Novartis Pharma AG	Maria-Magdalena Balp Clifford Brass Lori Jennings Miljen Martić Juergen Loeffler
Takeda Development Centre Europe Ltd	Guido Hanauer
AstraZeneca	Sudha Shankar
Echosens	Céline Fournier
Resoundant	Kay Pepin Richard Ehman
Bristol-Myers Squibb	Joel Myers
HistoIndex	Gideon Ho
Allergan	Richard Torstenson
Gilead	Rob Myers
RTI-HS	Lynda Doward

## Supporting Methods

According to the manufacturer, probe selection should be driven by skin-to-liver capsule distance (SCD): M probe for  $SCD < 25$  mm and XL probe for  $25 \text{ mm} \leq SCD < 35$  mm. In the latest version of the FibroScan equipment this is done by the Automatic Probe Selection tool. Some investigators have suggested that BMI may be used as a surrogate of SCD, using the M probe if  $BMI < 30 \text{ kg/m}^2$  and XL probe if  $BMI \geq 30 \text{ kg/m}^2$  (28).

For this meta-analysis, if only one VCTE-based liver stiffness measurement was available then this was included in the main analysis irrespective of probe type and BMI. Where two VCTE-based LSM were available (one with each probe), the main analysis included the M-probe measurement for  $BMI < 30 \text{ kg/m}^2$  and the XL probe measurement for  $BMI \geq 30 \text{ kg/m}^2$ . Therefore, all LSM cut-offs were determined independent of probe type.

We further conducted sensitivity analysis to investigate the influence of probe selection by excluding patients with  $BMI \geq 30 \text{ kg/m}^2$  who had a measurement with the M probe and patients with  $BMI < 30 \text{ kg/m}^2$  who had measurement with the XL probe.

## Supporting Discussion

### Rationale for proposing new NIT combinations with higher cut-offs for diagnosis of cirrhosis

Up until now, the literature has focused on the application of non-invasive tests in screening strategies for advanced fibrosis (F3-4). These strategies are useful when applied at the interface of primary and secondary care. Patients assessed using these strategies are classified as low risk, high risk or indeterminate risk of having advanced fibrosis, based on which clinical decisions are made: those with low risk continue to be managed in primary care, those with high risk are referred to secondary care and those with indeterminate risk undergo liver biopsy to determine their risk category.

What is lacking from the literature and what we have tried to answer with our analysis is what happens to patients with high risk of advanced fibrosis that are referred to secondary care. Our view is that they remain an indeterminate group as they can have either F3 or F4 fibrosis stage. Therefore, to distinguish between F3 and cirrhosis (F4) they still need to undergo liver biopsy, as those with liver cirrhosis would be managed differently (ultrasound surveillance for HCC and screening for oesophageal varices is generally indicated in patients with cirrhosis, but not those with F3 fibrosis stage). The identification of patients with cirrhosis would also be important as potential treatments for NASH may be licenced exclusively for patients with or without cirrhosis. We therefore argue that in practice, both the indeterminate and high-risk groups need to have a liver biopsy to establish their disease stage. In the case of those in the indeterminate category, the biopsy is needed to decide whether they merit referral to secondary care, and in the case of those with high risk of advanced fibrosis a biopsy is needed in secondary care to identify those with cirrhosis. We illustrate this point in **Supporting Figure 1a** and in **Figure 3a**, we also show how the FIB4-VCTE combination performs in our cohort.

Our answer to the problem above is a hybrid algorithm, where the lower NIT cut-offs are used to rule out advanced fibrosis, and the upper cut-offs are used to rule in cirrhosis. We provide cut-offs

with 95% and 98% specificity for the diagnosis of cirrhosis. This approach still stratifies patients into 3 risk groups – those with low risk of advanced fibrosis remaining in primary care, those in the indeterminate group needing a biopsy and those with high risk for cirrhosis. We argue that the group with high risk for cirrhosis can be positively diagnosed with cirrhosis without needing to have a biopsy. The net effect is that even though the indeterminate group is larger, fewer patients need to have a biopsy overall. This new approach is illustrated in **Supporting Figure 1b**, with results from our cohort given in **Figures 3b** and **3c**.

## Supporting Tables

**Supporting Table 1** Definitions of NITs evaluated in the current meta-analysis.

NIT	Definition
LSM by VCTE	An ultrasound probe that can also generate shear waves is placed over the right liver lobe. A low frequency shear wave is then generated by the external vibrator located in the probe, and ultrasound is used to measure the velocity of this shear wave through the liver. This velocity is directly related to liver stiffness.
FIB-4	$\text{Age [years]} \times \text{AST [IU/L]} / (\text{platelets} [\times 10^9/\text{L}] \times (\text{ALT [U/L]})^{1/2})$
NFS	$-1.675 + 0.037 \times \text{age [years]} + 0.094 \times \text{BMI [kg/m}^2] + 1.13 \times \text{IFG/diabetes [yes = 1, no = 0]} + 0.99 \times \text{AST/ALT ratio} - 0.013 \times \text{platelet} [\times 10^9/\text{L}] - 0.66 \times \text{albumin [g/dL]}$
AST/ALT	$\text{AST [IU/L]} / \text{ALT [IU/L]}$
APRI	$\text{AST [IU/L]} / \text{AST ULN [IU/L]} / \text{platelet} [\times 10^9/\text{L}]$

Abbreviations: LSM – liver stiffness measurement; VCTE – vibration-controlled transient elastography; FIB-4 – Fibrosis-4 score; NFS – NAFLD fibrosis score; AST/ALT – AST to ALT ratio; APRI – AST to platelet ratio index; ULN – upper limit of normal; IU – international unit; IFG – impaired fasting glucose

**Supporting Table 2** Non-invasive test cut-offs to rule-in and rule-out advanced fibrosis in patients with NAFLD

Study ID	Rule out cut-off	Rule-in cut-off
<b>Vibration controlled transient elastography</b>		
Studies testing pre-defined cut-offs (kPa)		
Anstee 2019 (1)	< 9.0	> 11.4
Wong 2019 (2), Papatheodoridi 2021 (ref)	< 10.0	> 15.0 <sup>^</sup>
Petta 2019 (3), Boursier 2019 (4), Petta 2017 (5)	< 7.9	> 9.6 <sup>*</sup>
Cut-offs identified from other primary studies (kPa)		
Tapper 2016 (6)	< 7.9	> 9.8
Eddowes 2019 (7)	< 7.1	> 14.1
Hsu 2019 (8)	< 5.9	> 13.4
Cassinotto 2016 (9)	< 8.2	> 12.5
Papatheodoridi 2021 (ref)		
<b>FIB-4</b>		
Studies testing pre-defined cut-offs		
Anstee 2019 (1), Xun 2012 (10), Petta 2019 (3)	< 1.30	> 2.67 <sup>#</sup>
Vilar-Gomez 2018 (11), Sun 2016 (12), McPherson 2010 (13), Srivastava 2019 (14)	< 1.30	> 3.25
Demir 2013 (15)	< 1.45	> 3.25
Cut-offs from other primary studies		
Siddiqui 2019 (16)	< 1.02	> 1.95
<b>NAFLD Fibrosis score</b>		
Studies testing pre-defined cut-offs		
Antsee 2019 (1), Tapper 2016 (6), Vilar-Gomez 2018 (11), Sun 2016 (12), McPherson 2010 (13), Xun 2012 (10), Demir 2013 (15), Petta 2014 (17), Dowman 2011 (18), Petta 2019 (3), Fowell 2020 (19)	< -1.455	> 0.676 <sup>%</sup>
<sup>^</sup> based on BavenoVI (20), <sup>*</sup> based on Wong (21), <sup>#</sup> from Shah 2009 (22), <sup>%</sup> from Angulo 2007 (23)		

**Supporting Table 3** Data fields requested from the authors of primary studies of LSM by VCTE

Category	Field	Units or possible values	Proportion of patients in whom reported, %
Study details	Name of first author	-	100.0
	Year of publication	-	100.0
	Country	-	100.0
	Centre	-	
Demographic and anthropometric details	Gender	M/F	100.0
	Age	years	99.9
	Ethnicity	-	38.6
	Height	m	92.4
	Weight	kg	94.9
	Waist circumference	cm	72.3
	Hip circumference	cm	21.8
	Smoking	Current/Ex/Never	10.0
	Presence of type 2 diabetes mellitus	Yes/No	86.4
	Presence of hypertension	Yes/No	48.8
	Presence of hyperlipidaemia	Yes/No	26.0
Laboratory data	Platelet count	$\times 10^9/l$	98.2
	INR	-	35.4
	Bilirubin	$\mu\text{mol/l}$	55.5
	ALT	IU/L	97.2
	AST	IU/L	96.2
	ALP	IU/L	48.3
	GGT	IU/L	82.2
	Albumin	g/l	67.2
	Sodium	mmol/l	6.7
	Urea	mmol/l	13.7
	Creatine	$\mu\text{mol/l}$	22.2
	Total cholesterol	mmol/l	62.8
	LDL cholesterol	mmol/l	32.8
	HDL cholesterol	mmol/l	77.6
	Triglycerides	mmol/l	79.3
	CRP	mg/l	7.9
	Fasting glucose	mmol/l	73.0
	Fasting insulin	mU/L	18.0
	HOMA-IR	-	16.8
Biopsy data	Date of biopsy	-	67.0
	Length of biopsy sample	mm	70.6
	Number of portal tracts	-	32.4
	Fibrosis stage	0-4	100.0
	Ballooning	0-2	63.7
	Lobular inflammation	0-3	64.2
	Steatosis	0-3	71.5
	NAS score	0-8	82.9
	Date of scan	-	68.9

Transient elastography details	Time between biopsy and scan	days	79.3
	Probe type	M/XL	91.9
	Number of valid shots	-	59.4
	Median stiffness	kPa	95.7
	IQR	kPa	83.4
	IQR/median	-	83.0
	Success rate	%	77.8

**Supporting Table 4** Demographic, biopsy, liver function test and NIT details of the entire cohort and broken down by fibrosis stage

	Entire cohort (n = 5735)	F0 (n = 1138)	F1 (n = 1613)	F2 (n = 1262)	F3 (n = 1101)	F4 (n = 621)
Females (%)	45	43	44	43	47	50
BMI > 30 kg/m <sup>2</sup> (%)	47	33	45	56	55	51
Waist circumference (cm)	103 (15)	99 (16)	101 (15)	106 (14)	106 (14)	106 (15)
Diabetes (%)	38	28	33	45	62	65
Age (years)*	54 (19)	48 (17)	50 (20)	53 (19)	59 (15)	60 (12)
BMI (kg/m <sup>2</sup> )*	30 (7)	28 (7)	29 (7)	31 (7)	31 (7)	30 (7)
Biopsy data						
Steatosis						
S0/S1/S2/S3 (%)	3/35/36/26	8/45/30/17	2/35/37/26	1/28/39/32	1/28/39/32	3/38/37/22
Ballooning						
B0/B1/B2 (%)	24/47/29	53/37/10	26/55/19	11/53/36	10/43/47	10/46/44
Inflammation						
I0/I1/I2/I3 (%)	13/60/24/3	3/60/9/4	13/65/21/1	6/60/31/3	5/53/36/6	8/57/29/6
NAS score <sup>+</sup>	4 (2)	3 (2)	4 (2)	4 (1)	5 (1)	4 (2)
NASH (%)	50	19	46	64	71	61
Liver function tests						
ALT (IU/L) *	55 (48)	46 (39)	54 (50)	59 (52)	63 (50)	55 (43)
AST (IU/L) *	40 (30)	31 (19)	36 (27)	41 (28)	49 (32)	53 (39)
Platelets (×10 <sup>9</sup> /l) <sup>+</sup>	230 (72)	247 (64)	243 (69)	232 (66)	217 (69)	184 (81)
Albumin (g/l) <sup>+</sup>	43 (9)	43 (8)	43 (7)	43 (5)	43 (6)	43 (20)
GGT (IU/L) *	69 (87)	59 (85)	61 (75)	63 (74)	82 (88)	104 (169)
Total cholesterol (mmol/l) <sup>+</sup>	5.1 (1.3)	5.2 (1.3)	5.1 (1.2)	5.2 (1.4)	4.9 (1.2)	4.6 (1.3)
HDL cholesterol (mmol/l) <sup>+</sup>	1.2 (0.5)	1.3 (0.5)	1.3 (0.5)	1.2 (0.5)	1.2 (0.4)	1.2 (0.5)
Triglycerides (mmol/l) *	1.6 (1.1)	1.4 (1.0)	1.6 (1.1)	1.6 (1.0)	1.6 (1.1)	1.5 (1.0)

Fasting glucose (mmol/l) *	5.6 (2.0)	5.3 (1.2)	5.4 (1.8)	5.6 (1.7)	6.3 (2.9)	6.4 (2.8)
Non-invasive tests						
LSM (kPa) *	10.7 (6.1)	5.7 (2.5)	6.7 (3.4)	7.9 (4.3)	11.3 (6.9)	20.9 (16.8)
AST/ALT*	0.8 (0.4)	0.7 (0.4)	0.7 (0.4)	0.7 (0.4)	0.8 (0.4)	0.9 (0.6)
FIB-4*	1.7 (1.2)	1.1 (0.7)	1.3 (1.2)	1.5 (1.1)	2.1 (1.6)	3.3 (2.9)
NFS*	-1.5 (1.7)	-2.3 (2.0)	-2.0 (2.2)	-1.4 (2.2)	-0.8 (1.8)	0.0 (1.8)
APRI*	0.6 (0.4)	0.3 (0.3)	0.4 (0.3)	0.5 (0.4)	0.6 (0.5)	0.8 (0.8)

\*Data are reported as median (IQR); \*Data are reported as mean (SD).

**Supporting Table 5** Details of biopsy and biopsy quality in the entire IPD cohort.

Biopsy details	Entire cohort (n = 5735)	Advanced fibrosis (n = 1722)	Cirrhosis (n = 621)
<b>Time between liver biopsy and LSM by VCTE</b>			
Patients with reported exact time period, %	79 (4549/5735)	80 (1371/1722)	76 (474/621)
Median (IQR) (days)	0 (14)	0 (9)	1 (26)
<b>Length of biopsy sample</b>			
Patients with reported length of biopsy, %	71 (4047/5735)	80 (1369/1722)	80 (495/621)
< 10 mm, %	3 (123/4047)	3 (42/1369)	5 (25/495)
≥ 10 mm and < 20 mm, %	35 (1432/4047)	33 (450/1369)	35 (172/495)
≥ 20 mm, %	62 (2492/4047)	64 (877/1369)	60 (298/495)
<b>Number of portal tracts in biopsy sample</b>			
Patients with reported portal tracts %	32 (1857/5735)	32 (544/1722)	26 (159/621)
< 11, %	54 (1006/1857)	42 (228/544)	47 (74/159)
≥ 11, %	46 (851/1857)	58 (316/544)	54 (85/159)
<b>Patients with both portal tracts and biopsy length reported, %</b>	32 (1854/5735)	32 (543/1722)	26 (159/621)
<b>Biopsy quality</b>			
Intermediate quality (length ≥ 10 mm and < 20 mm), %	46 (849/1854)	41 (220/543)	39 (62/159)
High quality (length ≥ 20 mm and ≥ 11 portal tracts), %	36 (670/1854)	45 (246/543)	39 (62/159)

Data are reported as percentage (number of patient satisfying conditions/total number of patients in subgroup)

**Supporting Table 6** Diagnostic performance of non-invasive tests for cirrhosis (F4)

	LSM by VCTE (n = 5489)			FIB-4 (n = 5393)			NFS (n = 3248)			APRI (n =5477)			AST/ALT ratio (n = 5434)		
Cirrhosis, %	11			11			11			11			11		
AUC	0.90 (.89-0.91)			0.80 (0.78-0.82)			0.77 (0.75-0.80)			0.72 (0.70-0.74)			0.69 (0.67-0.71)		
Threshold	10.4	<10.2	≥14.9	1.55	<1.13	≥2.66	-1.11	<-1.72	≥0.48	0.58	<0.30	≥1.04	0.82	<0.58	≥1.35
Sensitivity, %	89	90	67	77	90	44	82	90	36	66	90	35	64	90	24
	(86-91)	(8-92)	(64-70)	(72-80)	(87-92)	(40-48)	(76-85)	(86-93)	(31-40)	(61-69)	(87-92)	(31-39)	(59-67)	(87-92)	(20-28)
Specificity, %	75	74	90	67	48	90	63	49	90	68	28	90	66	33	90
	(74-76)	(72-75)	(89-90)	(65-68)	(46-49)	(89-90)	(61-64)	(46-50)	(88-91)	(66-69)	(26-28)	(89-90)	(64-67)	(31-33)	(89-90)
Misclassified, %	23	24	12	32	48	15	35	47	16	32	66	16	34	61	17
	(23-24)	(24-25)	(12-13)	(32-33)	(47-49)	(14-15)	(35-36)	(46-48)	(15-16)	(32-33)	(65-66)	(15-16)	(34-35)	(61-62)	(16-17)

For each non-invasive test thresholds were calculated according to Youden’s index (YI), and fixed at 90% sensitivity (90% Se) and 90% specificity (90% Sp). 95% confidence intervals were estimated with 500 bootstrap iterations.

**Supporting Table 7** Diagnostic performance of non-invasive tests for advanced fibrosis (F3-4) in a head-to-head comparison of NITs

	LSM by VCTE (n = 3248)			FIB-4 (n = 3248)			NFS (n = 3248)		
Advanced fibrosis, %	29			29			29		
AUC	0.86 (0.85-0.88)			0.75 (0.73-0.77)			0.73 (0.71-0.75)		
	YI	90% Se	90% Sp	YI	90% Se	90% Sp	YI	90% Se	90% Sp
Threshold	9.1	7.2	11.8	1.45	0.87	2.39	-1.39	-2.55	0.28
Sensitivity, %	77 (74-80)	90 (89-92)	59 (57-63)	69 (66-72)	90 (88-92)	36 (33-39)	75 (72-78)	90 (88-92)	29 (26-32)
Specificity, %	81 (79-82)	61 (59-63)	90 (89-92)	69 (67-71)	38 (36-39)	90 (89-91)	63 (61-65)	36 (33-37)	90 (89-91)
Misclassified, %	21 (19-22)	31 (29-32)	18 (17-20)	31 (29-32)	47 (46-49)	25 (24-27)	34 (34-36)	48 (49-50)	28 (28-29)

**Supporting Table 8** Diagnostic performance of non-invasive tests for cirrhosis (F4) in a head-to-head comparison of NITs

	LSM by VCTE (n = 3094)			FIB-4 (n = 3094)			NFS (n = 3094)		
Cirrhosis, %	11			11			11		
AUC	0.91 (0.89-0.92)			0.78 (0.76-0.81)			0.77 (0.75-0.80)		
	YI	90% Se	90% Sp	YI	90% Se	90% Sp	YI	90% Se	90% Sp
Threshold	10.3	9.7	14.4	1.35	1.08	2.76	-1.11	-1.93	0.46
Sensitivity, %	89 (86-92)	90 (87-93)	68 (63-72)	83 (79-87)	90 (87-93)	42 (37-47)	81 (77-86)	90 (87-93)	35 (29-40)
Specificity, %	78 (76-79)	74 (73-76)	91 (90-92)	59 (57-61)	45 (43-47)	90 (89-91)	64 (62-66)	45 (43-47)	90 (89-91)
Misclassified, %	21 (20-22)	24 (22-25)	12 (10-13)	39 (37-40)	50 (48-52)	15 (14-16)	34 (33-36)	50 (49-52)	16 (15-17)

**Supporting Table 9** Diagnostic performance of cut-offs from the literature for LSM by VCTE, FIB-4 and NFS for diagnosing advanced fibrosis.

	LSM by VCTE (n = 5489)								FIB-4 (n = 5393)		NFS (n = 3248)			
Source	Anstee 2019 (30)		Eddowes 2019 (31)		Wong 2019 (71)		Wong 2010 (21)		Shah 2009 (78)		McPherson 2010 (79)		Angulo 2007 (17)	
Thresholds	<9.9	≥11.4	<7.1	≥14.1	<10	≥15	<7.9	≥9.6	<1.3	≥2.67	<1.3	≥3.25	<-1.455	≥0.676
Sensitivity, %	72 (71-75)	61 (60-64)	91 (90-93)	46 (44-49)	71 (70-74)	41 (39-44)	86 (86-89)	73 (71-76)	74 (72-76)	30 (28-32)	74 (72-76)	20 (18-22)	76 (73-78)	22 (19-24)
Specificity, %	82 (80-83)	87 (86-88)	58 (55-58)	94 (93-94)	82 (81-83)	95 (94-96)	68 (65-68)	81 (79-81)	64 (63-66)	94 (93-94)	64 (63-66)	96 (96-97)	61 (60-64)	94 (93-95)
Misclassified, %	21 (21-22)	21 (20-22)	32 (32-34)	20 (20-21)	21 (21-22)	21 (21-22)	27 (27-29)	21 (21-23)	33 (33-34)	25 (25-26)	33 (33-34)	27 (26-27)	35 (34-36)	28 (27-28)

95% confidence intervals were estimated with 500 bootstrap iterations

**Supporting Table 10** Indicative PPV, NPV, false positive and false negative proportions for prevalences found in primary studies for the diagnostic performance of VCTE in identifying advanced fibrosis

Cut-off	Se, %	95% CI, %	Sp, %	95% CI, %	Prevalence, %	PPV, %	NPV, %	FP*	FN*
7.4 kPa	90	89-91	60	59-61	5	11	99	38	1
					10	20	98	36	1
					20	36	96	32	2
					<b>30</b>	<b>49</b>	<b>93</b>	<b>28</b>	<b>3</b>
					40	60	90	24	4
					50	69	86	20	5
9.1 kPa	77	75-79	78	76-79	5	16	98	21	1
					10	28	97	20	2
					20	47	93	18	5
					<b>30</b>	<b>60</b>	<b>89</b>	<b>15</b>	<b>7</b>
					40	70	84	13	9
					50	78	77	11	12
12.1 kPa	55	52-57	90	89-91	5	22	97	10	2
					10	38	95	9	5
					20	58	89	8	9
					<b>30</b>	<b>70</b>	<b>82</b>	<b>7</b>	<b>14</b>
					40	79	75	6	18
					50	85	67	5	23
<7.4 kPa, ≥12.1 kPa	84	81-87	87	85-88	5	25	99	12	1
					10	42	98	12	2
					20	62	96	10	3
					<b>30</b>	<b>73</b>	<b>93</b>	<b>9</b>	<b>5</b>
					40	81	89	8	6
					50	87	84	7	8
<9.9 kPa, ≥11.4 kPa (Anstee 2019)	69	67-71	86	85-88	5	21	98	13	2
					10	35	96	13	3
					20	55	92	11	6
					<b>30</b>	<b>68</b>	<b>87</b>	<b>10</b>	<b>9</b>
					40	77	81	8	12
					50	83	74	7	16
<7.1, ≥14.1 (Eddowes 2019)	83	80-86	90	88-92	5	30	99	10	1
					10	48	98	9	2
					20	67	95	8	3
					<b>30</b>	<b>78</b>	<b>93</b>	<b>7</b>	<b>5</b>
					40	85	89	6	7
					50	89	84	5	9
<10, ≥15 (Wong 2019)	59	57-61	94	93-96	5	34	98	6	2
					10	52	95	5	4
					20	71	90	5	8
					<b>30</b>	<b>81</b>	<b>84</b>	<b>4</b>	<b>12</b>
					40	87	77	4	16
					50	91	70	3	21
<7.9, ≥9.6 (Wong 2010)	84	82-87	78	76-80	5	17	99	21	1
					10	30	98	20	2
					20	49	95	18	3
					<b>30</b>	<b>62</b>	<b>92</b>	<b>15</b>	<b>5</b>
					40	72	88	13	6

	50	79	83	11	8
*Number of false positives and false negatives for 100 hypothetical cases; Values in bold face correspond to the proportion of patients with advanced fibrosis in the IPDMA cohort					

**Supporting Table 11** Indicative PPV, NPV, false positive and false negative proportions for prevalences found in primary studies for the diagnostic performance of FIB-4 in identifying advanced fibrosis

Cut-off	Se, %	95% CI, %	Sp, %	95% CI, %	Prevalence, %	PPV, %	NPV, %	FP*	FN*
0.88	90	88-91	39	37-40	5	7	99	58	1
					10	14	97	55	1
					20	27	94	49	2
					<b>30</b>	<b>39</b>	<b>90</b>	<b>43</b>	<b>3</b>
					40	50	85	37	4
					50	60	80	31	5
1.44	69	67-72	70	69-72	5	11	98	29	2
					10	20	95	27	3
					20	37	90	24	6
					<b>30</b>	<b>50</b>	<b>84</b>	<b>21</b>	<b>9</b>
					40	61	77	18	12
					50	70	69	15	16
2.31	38	36-41	90	89-91	5	17	97	10	3
					10	30	93	9	6
					20	49	85	8	12
					<b>30</b>	<b>62</b>	<b>77</b>	<b>7</b>	<b>19</b>
					40	72	69	6	25
					50	79	59	5	31
<1.3, ≥2.67 (Shah 2009)	54	52-56	91	89-92	5	24	97	9	2
					10	40	95	8	5
					20	60	89	7	9
					<b>30</b>	<b>72</b>	<b>82</b>	<b>6</b>	<b>14</b>
					40	80	75	5	18
					50	86	66	5	23
<1.3, ≥3.25 (McPherson 2010)	44	42-46	95	93-96	5	32	97	5	3
					10	49	94	5	6
					20	69	87	4	11
					<b>30</b>	<b>79</b>	<b>80</b>	<b>4</b>	<b>17</b>
					40	85	72	3	22
					50	90	63	3	28

\*Number of false positives and false negatives for 100 hypothetical cases; Values in bold face correspond to the proportion of patients with advanced fibrosis in the IPDMA cohort

**Supporting Table 12** Indicative PPV, NPV, false positive and false negative proportions for prevalences found in primary studies for the diagnostic performance of NFS in identifying advanced fibrosis

Cut-off	Se, %	95% CI, %	Sp, %	95% CI, %	Prevalence, %	PPV, %	NPV, %	FP*	FN*
-2.55	90	88-92	36	33-37	5	7	99	61	1
					10	14	97	58	1
					20	26	94	51	2
					<b>30</b>	<b>38</b>	<b>89</b>	<b>45</b>	<b>3</b>
					40	48	84	38	4
					50	58	78	32	5
-1.39	75	72-78	63	61-65	5	10	98	35	1
					10	18	96	33	3
					20	34	91	30	5
					<b>30</b>	<b>46</b>	<b>85</b>	<b>26</b>	<b>8</b>
					40	57	79	22	10
					50	67	72	19	13
0.28	29	26-32	90	89-91	5	13	96	10	4
					10	24	92	9	7
					20	42	84	8	14
					<b>30</b>	<b>55</b>	<b>75</b>	<b>7</b>	<b>21</b>
					40	66	66	6	28
					50	74	56	5	36
<-1.455, ≥0.676 (Angulo 2007)	47	44-50	91	89-93	5	22	97	9	3
					10	37	94	8	5
					20	57	87	7	11
					<b>30</b>	<b>69</b>	<b>80</b>	<b>6</b>	<b>16</b>
					40	78	72	5	21
					50	84	63	5	27

\*Number of false positives and false negatives for 100 hypothetical cases; Values in bold face correspond to the proportion of patients with advanced fibrosis in the IPDMA cohort

**Supporting Table 13** Indicative PPV, NPV, false positive and false negative proportions for prevalences found in primary studies for the diagnostic performance of APRI in identifying advanced fibrosis

Cut-off	Se, %	95% CI, %	Sp, %	95% CI, %	Prevalence, %	PPV, %	NPV, %	FP*	FN*
0.29	90	89-92	29	28-30	5	6	98	67	1
					10	12	96	64	1
					20	24	92	57	2
					<b>30</b>	<b>35</b>	<b>87</b>	<b>50</b>	<b>3</b>
					40	46	81	43	4
					50	56	74	36	5
0.49	67	64-69	63	62-65	5	9	97	35	2
					10	17	95	33	3
					20	31	88	30	7
					<b>30</b>	<b>44</b>	<b>82</b>	<b>26</b>	<b>10</b>
					40	55	74	22	13
					50	64	66	19	17
0.91	32	30-34	90	89-91	5	14	96	10	3
					10	26	92	9	7
					20	44	84	8	14
					<b>30</b>	<b>58</b>	<b>76</b>	<b>7</b>	<b>20</b>
					40	68	67	6	27
					50	76	57	5	34

\*Number of false positives and false negatives for 100 hypothetical cases; Values in bold face correspond to the proportion of patients with advanced fibrosis in the IPDMA cohort

**Supporting Table 14** Indicative PPV, NPV, false positive and false negative proportions for prevalences found in primary studies for the diagnostic performance of AST/ALT in identifying advanced fibrosis

Cut-off	Se, %	95% CI, %	Sp, %	95% CI, %	Prevalence, %	PPV, %	NPV, %	FP*	FN*
0.51	90	87-91	25	23-26	5	6	98	71	1
					10	12	96	68	1
					20	23	91	60	2
					<b>30</b>	<b>34</b>	<b>85</b>	<b>53</b>	<b>3</b>
					40	44	79	45	4
					50	55	71	38	5
0.64	75	73-77	47	45-48	5	7	97	50	1
					10	14	94	48	3
					20	26	88	42	5
					<b>30</b>	<b>38</b>	<b>81</b>	<b>37</b>	<b>8</b>
					40	49	74	32	10
					50	59	65	27	13
1.34	16	14-18	90	89-91	5	8	95	10	4
					10	15	91	9	8
					20	29	81	8	17
					<b>30</b>	<b>41</b>	<b>71</b>	<b>7</b>	<b>25</b>
					40	52	62	6	34
					50	62	52	5	42

\*Number of false positives and false negatives for 100 hypothetical cases; Values in bold face correspond to the proportion of patients with advanced fibrosis in the IPDMA cohort

**Supporting Table 15** Diagnostic accuracy of pairs of cut-offs from the literature for NITs for diagnosing advanced fibrosis. Patient proportions used to calculate performance statistics are displayed as ratios.

LSM by VCTE (n = 5489)						FIB-4 (n = 5393)			NFS (n = 3248)	
Prevalence, %		30				30			29	
AUROC		0.85 (0.84-0.86)				0.76 (0.74-0.77)			0.73 (0.71-0.75)	
Source of thresholds	Anstee 2019 (1)	Eddowes 2019 (7)	Wong 2019 (2)	Wong 2010 (21)	This study	Shah 2009 (22)	McPherson 2010 (13)	This study	Angulo 2007 (23)	This study
Thresholds	<9.9, ≥11.4	<7.1, ≥14.1	<10, ≥15	<7.9, ≥9.6	<7.4, ≥12.1	<1.3, ≥2.67	<1.3, ≥3.25	<0.88, ≥2.31	<-1.455, ≥0.676	<-2.55, ≥0.28
Sensitivity, %	69 (1009/1456)	83 (754/905)	59 (674/1145)	84 (1205/1431)	84 (889/1060)	54 (485/901)	44 (328/744)	80 (621/780)	47 (202/429)	74 (270/363)
Specificity, %	86 (3147/3639)	90 (2216/2457)	94 (3165/3351)	78 (2599/3330)	87 (2338/2702)	91 (2423/2668)	95 (2423/2563)	79 (1448/1831)	91 (1423/1562)	78 (821/1050)
Misclassified, %	17 (948/5489)	7 (392/5489)	12 (657/5489)	17 (957/5489)	10 (535/5489)	12 (661/5393)	10 (556/5393)	10 (542/5393)	11 (366/3248)	10 (322/3248)
Indeterminate, %	7 (385/5489)	39 (2127/5489)	18 (993/5489)	13 (728/5489)	31 (1727/5489)	34 (1824/5393)	39 (2086/5393)	52 (2782/5393)	39 (1257/3248)	56 (1835/3248)

95% confidence intervals were estimated with 500 bootstrap replicates.

**Supporting Table 16** Derivation of new cut-offs corresponding to 95% specificity for cirrhosis. The cut-offs were calculated in the training cohort and were validated in the validation cohort. Training and validation cohorts were obtained by random sampling from the IPD cohort in a 3:2 ratio.

	LSM by VCTE (n = 5489)		FIB-4 (n = 5393)		NFS (n = 3248)	
	Training (n = 3290)	Validation (n = 2199)	Training (n = 3254)	Validation (n = 2139)	Training (n = 1963)	Validation (n = 1285)
Proportion of patients with cirrhosis, %	11	10	11	11	10	11
AUC	0.90 (0.89-0.92)	0.90 (0.88-0.92)	0.81 (0.78-0.83)	0.79 (0.77-0.82)	0.78 (0.75-0.81)	0.77 (0.72-0.81)
Threshold	20.4		3.48		1.01	
Sensitivity, %	52 (47-57)	49 (43-56)	33 (28-37)	30 (24-36)	21 (16-27)	28 (21-36)
Specificity, %	95 (95-96)	95 (95-97)	95 (94-96)	96 (95-97)	95 (94-96)	95 (94-96)
Misclassified, %	10 (10-11)	9 (9-10)	12 (12-13)	11 (11-12)	13 (13-14)	13 (13-14)

95% confidence intervals were estimated with 500 bootstrap replicates.

**Supporting Table 17** Derivation of new cut-offs corresponding to 98% specificity for cirrhosis. The cut-offs were calculated in the training cohort and were validated in the validation cohort. Training and validation cohorts were obtained by random sampling from the IPD cohort in a 3:2 ratio.

	LSM by VCTE (n = 5489)		FIB-4 (n = 5393)		NFS (n = 3248)	
	Training (n = 3290)	Validation (n = 2199)	Training (n = 3254)	Validation (n = 2139)	Training (n = 1963)	Validation (n = 1285)
Proportion of patients with cirrhosis, %	11	10	11	11	10	11
AUC	0.90 (0.89-0.92)	0.90 (0.88-0.92)	0.81 (0.78-0.83)	0.79 (0.77-0.82)	0.78 (0.75-0.81)	0.77 (0.72-0.81)
Threshold	27.6		4.63		1.57	
Sensitivity, %	27 (23-32)	29 (22-34)	19 (15-23)	20 (15-26)	12 (8-17)	18 (13-27)
Specificity, %	98 (98-99)	98 (98-99)	98 (97-98)	98 (97-99)	98 (97-99)	98 (97-99)
Misclassified, %	10 (10-11)	9 (9-10)	10 (10-11)	10 (10-11)	11 (11-12)	11 (11-12)

95% confidence intervals were estimated with 500 bootstrap replicates.

**Supporting Table 18** Diagnostic performance of combinations of NFS and LSM by VCTE, and FIB-4 and LSM by VCTE tests to reduce need for liver biopsies

	FIB-4 & LSM by VCTE (n = 5159)	NFS & LSM by VCTE (n = 3094)	FIB-4 & LSM by VCTE (n = 5159)	NFS & LSM by VCTE (n = 3094)	FIB-4 & LSM by VCTE (n = 5159)	NFS & LSM by VCTE (n = 3094)	FIB-4 & LSM by VCTE (n = 5159)	NFS & LSM by VCTE (n = 3094)	FIB-4 & LSM by VCTE (n = 5159)	NFS & LSM by VCTE (n = 3094)
Prevalence, %	30	28	30	28	30	28	30	28	30	28
Threshold for blood-based NIT*	< 1.3, ≥ 3.48	< -1.455, ≥ 1.010	< 1.3, ≥ 3.48	< -1.455, ≥ 1.010	< 1.3, ≥ 3.48	< -1.455, ≥ 1.010	< 1.3, ≥ 4.63	< -1.455, ≥ 1.570	< 1.3, ≥ 4.63	< -1.455, ≥ 1.570
Threshold for VCTE, kPa*	< 7.9, ≥ 16.1	< 7.9, ≥ 16.1	< 7.9, ≥ 20.4	< 7.9, ≥ 20.4	< 8.0, ≥ 20.0	< 8.0, ≥ 20.0	< 7.9, ≥ 27.6	< 7.9, ≥ 27.6	< 8.0, ≥ 28.0	< 8.0, ≥ 28.0
Sensitivity, %	41 (40-43)	41 (39-42)	38 (37-40)	37 (35-38)	38 (37-39)	36 (34-38)	28 (27-29)	25 (24-26)	27 (26-28)	24 (23-25)
Specificity, %	88 (86-89)	88 (87-90)	90 (89-91)	90 (89-92)	90 (89-91)	90 (89-92)	95 (94-97)	96 (95-98)	96 (94-97)	96 (95-98)
PPV, %	45 (43-47)	45 (41-47)	48 (45-50)	46 (43-49)	47 (45-50)	45 (43-49)	57 (54-61)	57 (52-63)	57 (54-61)	57 (52-61)
NPV, %	86 (85-87)	87 (85-88)	86 (85-87)	87 (85-88)	86 (85-87)	86 (85-88)	86 (85-87)	86 (85-88)	86 (85-87)	86 (85-88)
Indeterminate, %	16 (15-17)	17 (16-19)	19 (18-20)	20 (18-21)	18 (17-19)	17 (18-21)	24 (23-25)	25 (23-27)	24 (23-25)	21 (23-26)
Misclassification, %	18 (17-19)	17 (15-19)	16 (15-17)	15 (14-17)	17 (15-18)	14 (14-17)	13 (12-14)	12 (10-13)	13 (12-14)	11 (10-13)
Patients undergoing VCTE, %	40 (39-42)	42 (40-44)	40 (39-42)	42 (40-44)	40 (39-42)	42 (40-44)	44 (42-45)	45 (43-47)	44 (42-45)	45 (43-47)

95% confidence intervals were estimated with 500 bootstrap replicates

\*A lower cut-off was used to rule out patients with advanced fibrosis and an upper cut-off was used to rule in patients with cirrhosis. Lower cut-offs were the same as used in **Table 6** of the main manuscript. Upper cut-offs for were calculated to obtain a 95% and 98% specificity in diagnosing cirrhosis in the IPD cohort.

**Supporting Table 19** Diagnostic performance of non-invasive tests in subgroup for discriminating advanced fibrosis (F3-F4).

	LSM by VCTE	FIB-4	NFS
Biopsy length < 20 mm (n = 1555)	<b>0.87 (0.86-0.89)</b>	<b>0.80 (0.78-0.83)</b>	<b>0.79 (0.75-0.82)</b>
Biopsy length ≥ 20 mm (n = 2492)	<b>0.83 (0.82-0.85)</b>	<b>0.75 (0.72-0.77)</b>	<b>0.72 (0.69-0.75)</b>
Number of portal tracts < 11 (n = 1006)	<b>0.86 (0.83-0.88)</b>	<b>0.79 (0.75-0.82)</b>	<b>0.78 (0.74-0.81)</b>
Number of portal tracts ≥ 11 (n = 851)	<b>0.80 (0.77-0.83)</b>	<b>0.73 (0.70-0.77)</b>	<b>0.68 (0.63-0.72)</b>
Intermediate quality biopsy (n = 1432)	<b>0.87 (0.85-0.89)</b>	<b>0.79 (0.77-0.82)</b>	<b>0.78 (0.74-0.81)</b>
High quality biopsy (n = 670)	<b>0.79 (0.75-0.83)</b>	<b>0.72 (0.68-0.76)</b>	<b>0.67 (0.62-0.73)</b>
BMI < 25 kg/m <sup>2</sup> (n = 868)	<b>0.91 (0.89-0.94)</b>	<b>0.81 (0.78-0.84)</b>	<b>0.76 (0.71-0.81)<sup>#</sup></b>
25 kg/m <sup>2</sup> ≤ BMI < 30 kg/m <sup>2</sup> (n = 2127)	<b>0.87 (0.85-0.89)</b>	0.77 (0.75-0.80)	<b>0.74 (0.71-0.77)<sup>*</sup></b>
BMI ≥ 30 kg/m <sup>2</sup> (n = 2710)	<b>0.81 (0.79-0.83)</b>	<b>0.74 (0.72-0.76)</b>	<b>0.69 (0.66-0.72)<sup>*,#</sup></b>
Continent – Europe (n = 3560)	0.85 (0.84-0.87)	0.75 (0.73-0.77)	0.72 (0.69-0.75)
Continent - Asia (n = 1278)	0.85 (0.82-0.88)	0.77 (0.73-0.80)	0.76 (0.73-0.80)
Sex – Male (n = 3165)	0.85 (0.83-0.86)	0.76 (0.74-0.78)	<b>0.75 (0.72-0.77)</b>
Sex – Female (n = 2570)	0.86 (0.84-0.87)	0.76 (0.73-0.78)	<b>0.71 (0.68-0.74)</b>
Presence of T2DM (n = 2191)	<b>0.81 (0.79-0.83)</b>	<b>0.73 (0.71-0.75)</b>	0.68 (0.65-0.70)
Lack of T2DM (n = 2763)	<b>0.87 (0.86-0.89)</b>	<b>0.77 (0.75-0.79)</b>	0.71 (0.68-0.74)
ALT < 40 U/L (n = 1656)	0.85 (0.83-0.88)	<b>0.73 (0.70-0.76)</b>	0.74 (0.70-0.78)
40 U/L ≤ ALT < 100 U/L (n = 2933)	0.86 (0.85-0.87)	<b>0.77 (0.76-0.79)</b>	0.75 (0.73-0.78)
ALT ≥ 100 U/L (n = 984)	0.83 (0.80-0.86)	0.76 (0.73-0.79)	0.77 (0.73-0.81)
AST < 40 U/L (n = 2759)	0.84 (0.82-0.86)	0.73 (0.70-0.75)	<b>0.76 (0.73-0.78)</b>
40 U/L ≤ AST < 100 U/L (n = 2385)	0.85 (0.83-0.86)	0.74 (0.72-0.76)	0.72 (0.69-0.75)
AST ≥ 100 U/L (n = 373)	0.86 (0.82-0.90)	0.71 (0.66-0.76)	<b>0.65 (0.58-0.72)</b>
ALT < 40 U/L AND AST < 40 U/L (n = 1323)	0.84 (0.81-0.87)	<b>0.72 (0.68-0.75)</b>	0.73 (0.69-0.77)
ALT ≥ 40 U/L OR AST ≥ 40 U/L (n = 4235)	0.86 (0.84-0.87)	<b>0.76 (0.75-0.78)</b>	0.75 (0.73-0.77)
Age < 43 yrs (n = 1401)	0.81 (0.77-0.84)	<b>0.65 (0.61-0.70)</b>	<b>0.58 (0.52-0.64)<sup>*,#</sup></b>
43 yrs ≤ Age < 54 yrs (n = 1478)	0.84 (0.82-0.86)	0.69 (0.66-0.72)	<b>0.70 (0.66-0.74)<sup>*</sup></b>
54 yrs ≤ Age < 62 yrs (n = 1423)	0.85 (0.83-0.87)	<b>0.72 (0.69-0.75)</b>	<b>0.70 (0.67-0.74)<sup>#</sup></b>
62 yrs ≤ Age (n = 1430)	0.84 (0.81-0.86)	0.70 (0.67-0.72)	0.66 (0.62-0.70)

Bold AUCs within a column and subgroup category are significantly different. Bonferroni's correction was applied when performing multiple comparisons. AUCs marked with \* or # are pairwise significantly different.

**Supporting Table 20** Diagnostic performance of non-invasive tests in subgroup for discriminating cirrhosis (F4).

	LSM by VCTE	FIB-4	NFS
Biopsy length < 20 mm (n = 1555)	0.91 (0.88-0.93)	0.84 (0.81-0.86)	<b>0.83 (0.79-0.87)</b>
Biopsy length ≥ 20 mm (n = 2492)	0.89 (0.88-0.91)	0.78 (0.76-0.81)	<b>0.75 (0.71-0.78)</b>
Number of portal tracts < 11 (n = 1006)	<b>0.90 (0.87-0.94)</b>	0.81 (0.76-0.87)	0.76 (0.70-0.83)
Number of portal tracts ≥ 11 (n = 851)	<b>0.84 (0.81-0.88)</b>	0.77 (0.72-0.81)	0.71 (0.65-0.77)
Intermediate quality biopsy (n = 1432)	0.91 (0.88-0.93)	0.83 (0.80-0.86)	<b>0.83 (0.78-0.87)</b>
High quality biopsy (n = 670)	0.87 (0.83-0.90)	0.87 (0.83-0.90)	<b>0.69 (0.62-0.76)</b>
BMI < 25 kg/m <sup>2</sup> (n = 868)	<b>0.93 (0.91-0.95)<sup>#</sup></b>	<b>0.84 (0.80-0.88)</b>	0.77 (0.69-0.84)
25 kg/m <sup>2</sup> ≤ BMI < 30 kg/m <sup>2</sup> (n = 2127)	<b>0.92 (0.91-0.94)<sup>*</sup></b>	0.82 (0.78-0.85)	<b>0.83 (0.80-0.86)</b>
BMI ≥ 30 kg/m <sup>2</sup> (n = 2710)	<b>0.87 (0.85-0.89)<sup>*,#</sup></b>	<b>0.77 (0.75-0.80)</b>	<b>0.73 (0.69-0.76)</b>
Continent – Europe (n = 3560)	0.90 (0.89-0.92)	0.80 (0.78-0.82)	0.77 (0.74-0.81)
Continent - Asia (n = 1278)	0.92 (0.89-0.94)	0.81 (0.77-0.85)	0.80 (0.75-0.85)
Sex – Male (n = 3165)	0.91 (0.89-0.92)	0.81 (0.78-0.83)	<b>0.80 (0.77-0.83)</b>
Sex – Female (n = 2570)	0.89 (0.88-0.91)	0.78 (0.76-0.81)	<b>0.74 (0.71-0.78)</b>
Presence of T2DM (n = 2191)	<b>0.85 (0.83-0.87)</b>	<b>0.74 (0.72-0.77)</b>	<b>0.70 (0.67-0.70)</b>
Lack of T2DM (n = 2763)	<b>0.94 (0.92-0.95)</b>	<b>0.85 (0.83-0.88)</b>	<b>0.80 (0.76-0.84)</b>
ALT < 40 U/L (n = 1656)	0.91 (0.89-0.93)	0.79 (0.75-0.83)	0.77 (0.73-0.82)
40 U/L ≤ ALT < 100 U/L (n = 2933)	0.90 (0.88-0.92)	0.80 (0.78-0.83)	0.77 (0.74-0.80)
ALT ≥ 100 U/L (n = 984)	0.90 (0.87-0.93)	0.79 (0.75-0.84)	0.82 (0.76-0.88)
AST < 40 U/L (n = 2759)	0.90 (0.88-0.92)	0.78 (0.75-0.81)	0.80 (0.77-0.84)
40 U/L ≤ AST < 100 U/L (n = 2385)	0.89 (0.87-0.91)	0.78 (0.76-0.81)	0.75 (0.72-0.79)
AST ≥ 100 U/L (n = 373)	0.90 (0.86-0.94)	0.77 (0.71-0.84)	0.75 (0.66-0.84)
ALT < 40 U/L AND AST < 40 U/L (n = 1323)	0.91 (0.89-0.93)	0.76 (0.72-0.81)	0.75 (0.69-0.80)
ALT ≥ 40 U/L OR AST ≥ 40 U/L (n = 4235)	0.90 (0.89-0.91)	0.80 (0.78-0.82)	0.79 (0.76-0.81)
Age < 43 yrs (n = 1401)	<b>0.97 (0.95-0.99)<sup>*,#,%</sup></b>	0.82 (0.75-0.88)	0.72 (0.55-0.89)
43 yrs ≤ Age < 54 yrs (n = 1478)	<b>0.90 (0.87-0.93)<sup>*</sup></b>	0.77 (0.72-0.82)	0.74 (0.67-0.81)
54 yrs ≤ Age < 62 yrs (n = 1423)	<b>0.87 (0.85-0.90)<sup>#</sup></b>	0.75 (0.71-0.78)	0.74 (0.69-0.78)
62 yrs ≤ Age (n = 1430)	<b>0.86 (0.84-0.89)<sup>%</sup></b>	0.72 (0.69-0.76)	0.66 (0.62-0.71)

Bold AUCs within a column and subgroup category are significantly different. Bonferroni's correction was applied when performing multiple comparisons. AUCs marked with <sup>\*</sup>, <sup>#</sup> or <sup>%</sup> are pairwise significantly different.

**Supporting Table 21** Subgroup analysis on the impact of reliability of liver stiffness measurements (LSM) on diagnostic performance in detecting advanced fibrosis.

	AUROC (95% CI) for diagnosing advanced fibrosis	AUROC (95% CI) for diagnosing cirrhosis
Reliable LSM by VCTE (median LSM < 7.1 kPa OR (median LSM ≥ 7.1 kPa AND IQR/median LSM < 0.30)	<b>0.86 (0.85-0.87)</b>	<b>0.91 (0.90-0.92)</b>
Unreliable LSM by VCTE (median LSM ≥ 7.1 kPa AND IQR/median LSM > 0.30)	<b>0.75 (0.70-0.80)</b>	<b>0.81 (0.76-0.86)</b>
Reliable LSM by VCTE (IQR/median LSM < 0.30)	0.86 (0.84-0.87)	0.90 (0.89-0.92)
Unreliable LSM by VCTE (IQR/median LSM ≥ 0.30)	0.84 (0.82-0.86)	0.88 (0.86-0.91)

VCTE – vibration-controlled transient elastography; 95% confidence intervals were estimated using 500 bootstrap iterations. Bold AUCs within a column and subgroup category are significantly different (p < 0.05).

**Supporting Table 22** Subgroup analysis based on choice of probe type (in patients with data available from both probes) compared to the diagnostic accuracy of LSM by VCTE calculated in the entire IPD cohort.

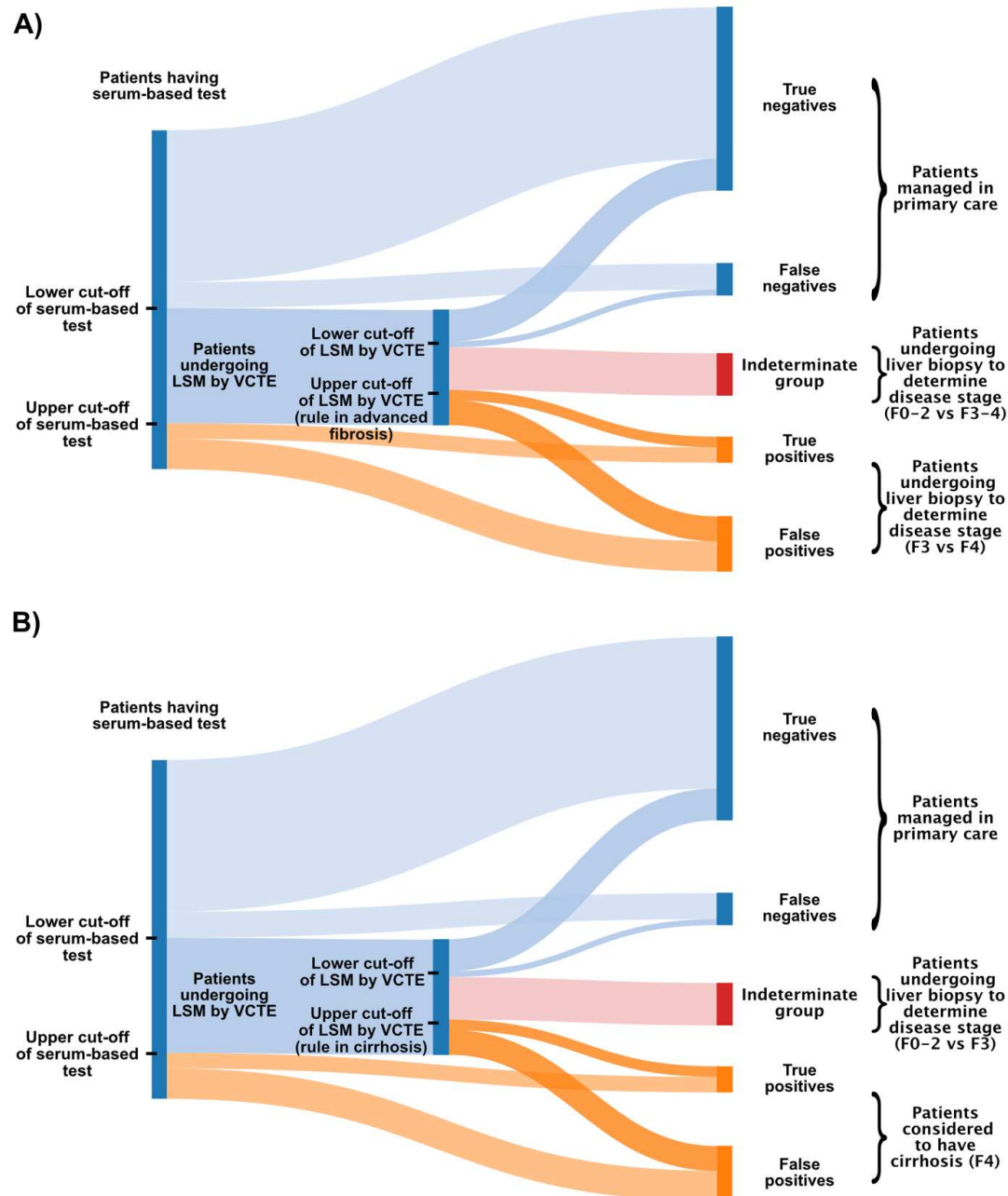
	AUROC (95% CI) for diagnosing advanced fibrosis	AUROC (95% CI) for diagnosing cirrhosis
Entire cohort (n = 5489)	0.85 (0.84-0.86)	0.90 (0.89-0.91)
M probe only (where measurements performed with both probes were performed) (n = 799)	0.84 (0.82-0.87)	0.86 (0.83-0.90)
XL probe only (where measurements performed with both probes were performed) (n = 799)	0.83 (0.80-0.86)	0.87 (0.84-0.90)

**Supporting Table 23** Sensitivity analysis on the impact of probe selection on diagnostic performance in detecting advanced fibrosis. Thresholds were calculated from the entire IPD cohort.

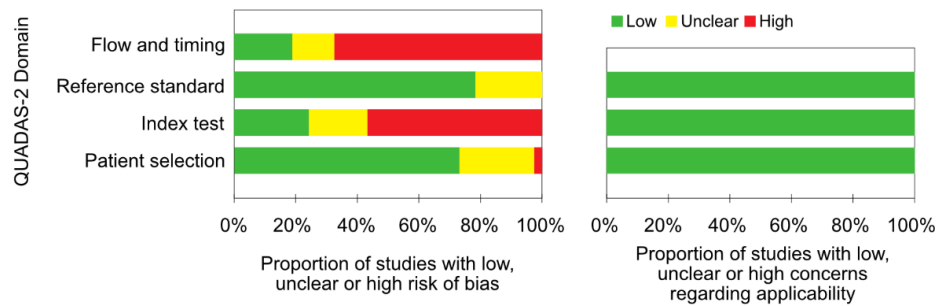
	All patients with LSM (n = 5489)			Patients with BMI < 30 kg/m <sup>2</sup> and M probe OR BMI ≥ 30 kg/m <sup>2</sup> and XL probe (n = 4464)		
AUC (95% CI)	0.85 (0.84-0.86)			0.86 (0.85-0.87)		
Thresholds, kPa	9.1	< 7.4	≥ 12.1	9.1	< 7.4	≥ 12.1
Sensitivity, %	77 (75-79)	90 (89-91)	55 (52-57)	75 (72-78)	89 (87-91)	53 (50-56)
Specificity, %	78 (76-79)	60 (59-61)	90 (89-91)	81 (79-82)	65 (63-67)	92 (91-93)
Misclassified, %	22 (22-23)	31 (31-32)	21 (20-21)	21 (20-22)	28 (27-29)	20 (18-21)

95% confidence intervals were estimated with 500 bootstrap replicates.

## Supporting Figures



**Supporting Figure 1** “Traditional” (A) and newly proposed two-tier algorithms (B) for using non-invasive tests in clinical care. (A) In the traditional application of NITs, patients with NIT values below the lower cut-offs are “ruled out” and are managed in primary care. Those with indeterminate NIT values and those “ruled in” with values above the upper cut-offs still need to undergo liver biopsy in order to stage their disease. Patients with indeterminate NITs need a liver biopsy to rule out advanced fibrosis, while patients ruled in for advanced fibrosis still need a biopsy to diagnose cirrhosis, as those with cirrhosis are managed differently (they need surveillance for hepatocellular cancer and screening for oesophageal varices). (B) In the proposed algorithms we use upper cut-off values to rule in cirrhosis, where those who are ruled in are thereby managed as having cirrhosis without the need for liver biopsy. Patients in the indeterminate group still require biopsy to correctly stage their disease.

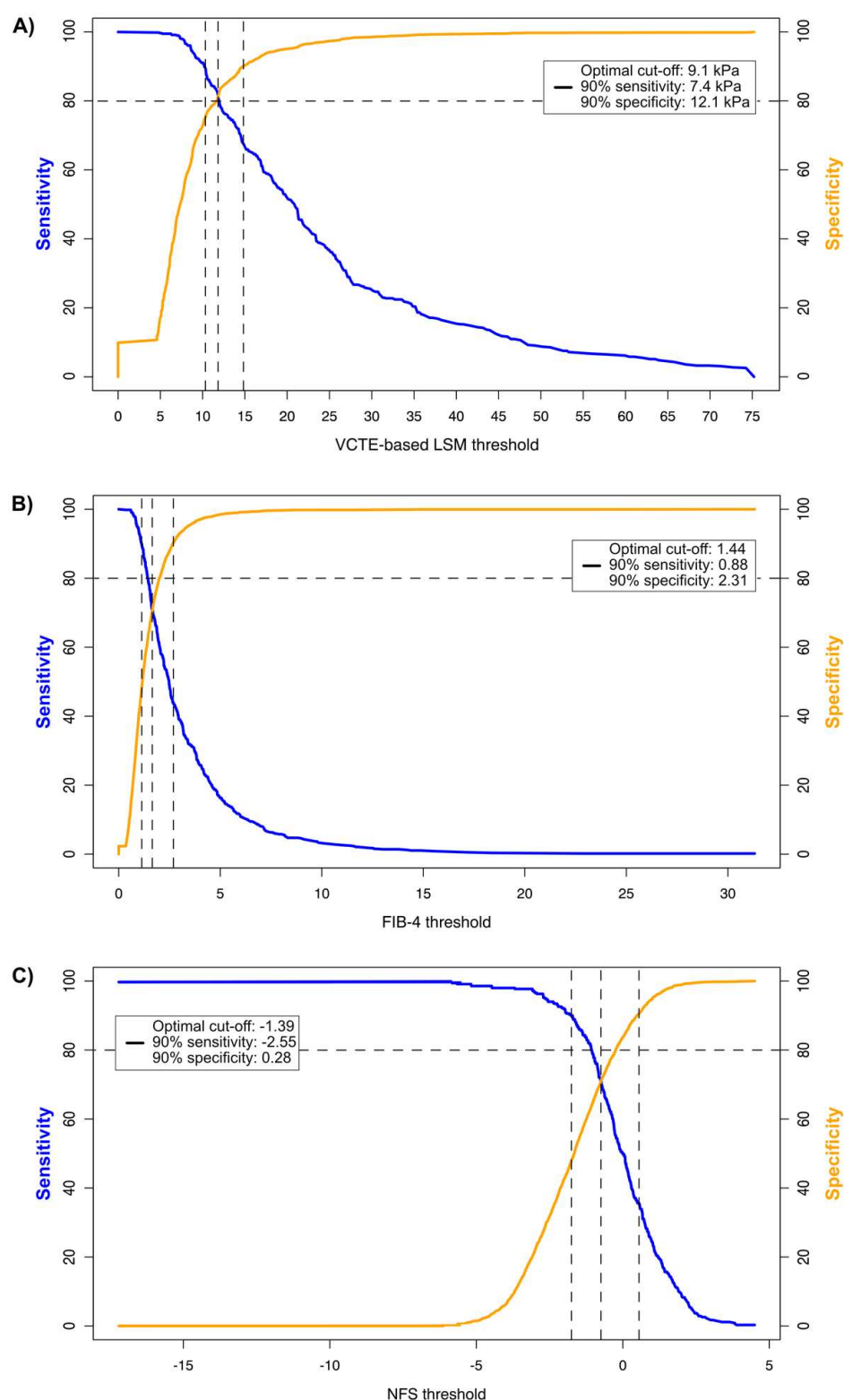


Supporting Figure 2 Risk of bias and applicability concerns

Study ID	Risk of bias				Applicability concerns		
	Patient selection	Index test	Reference test	Flow and timing	Patient selection	Index test	Reference test
Agrawal 2017	+	-	+	-	+	+	+
Aykut 2014	?	?	?	?	+	+	+
Boursier 2016	+	-	+	-	+	+	+
Boursier 2017	?	?	?	+	+	+	+
Boursier 2018	?	?	?	?	+	+	+
Cassinotto 2013	+	-	+	-	+	+	+
Cassinotto 2016	+	-	+	-	+	+	+
Chan 2015	+	+	+	-	+	+	+
Chan 2017	-	-	+	-	+	+	+
Eddowes 2016	?	?	+	+	+	+	+
Eddowes 2018	?	?	+	-	+	+	+
Eddowes 2019	+	-	+	-	+	+	+
Gaia 2011	+	-	+	?	+	+	+
Garg 2018	+	-	+	-	+	+	+
Karlas 2015	?	?	+	-	+	+	+
Kwok 2016	+	+	+	-	+	+	+
Labenz 2018	+	+	?	-	+	+	+
Lee 2017	+	-	+	-	+	+	+
Loong 2017	+	+	+	-	+	+	+
Lupsor 2010	?	-	+	-	+	+	+
Mahadeva 2013	+	-	+	?	+	+	+
Okajima 2017	+	-	?	-	+	+	+
Ooi 2018	+	?	+	+	+	+	+
Pavlidis 2017	+	-	+	-	+	+	+
Petta 2015 Liv Int	+	+	+	-	+	+	+
Petta 2015 Hepatol	+	-	+	-	+	+	+
Petta 2017 APT	+	-	+	?	+	+	+
Petta 2017 Hepatol	+	+	+	+	+	+	+
Seki 2017	+	-	?	+	+	+	+
Shen 2015	+	-	+	-	+	+	+
Staufer 2019	+	+	+	-	+	+	+
Wong 2010	+	-	+	-	+	+	+
Wong 2012	+	-	+	-	+	+	+
Wong 2019	+	+	+	-	+	+	+
Yoneda 2008	?	-	?	-	+	+	+
Younes 2018	?	+	+	+	+	+	+
Ziol 2009	+	-	?	+	+	+	+

 Low 
  High 
  Unclear

Supporting Figure 3 Methodological quality summary



**Supporting Figure 4** Distribution of sensitivities and specificities over the possible threshold ranges for LSM by VCTE (A), FIB-4 (B) and NFS (C) when considering the diagnosis of cirrhosis. Horizontal dashed lines are representing the minimum acceptable criteria for considering a test as having high sensitivity ( $\geq 80\%$ ) and high specificity ( $\geq 80\%$ ).

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## Supporting information for:

### Diagnostic accuracy of non-invasive tests for diagnosing advanced fibrosis in patients with NAFLD – An individual patient data meta-analysis

Ferenc E. Mózes<sup>1</sup>, Jenny Lee<sup>2</sup>, Emmanuel A. Selvaraj<sup>1,3,4</sup>, Arjun N. A. Jayaswal<sup>1</sup>, Michael Trauner<sup>5</sup>, Jérôme Boursier<sup>6,7</sup>, Céline Fournier<sup>8</sup>, Katharina Stauffer<sup>5,9,10</sup>, Rudolf Stauber<sup>11</sup>, Elisabetta Bugianesi<sup>12</sup>, Ramy Younes<sup>13</sup>, Silvia Gaia<sup>12</sup>, Monica Lupşor-Platon<sup>14</sup>, Salvatore Petta<sup>15</sup>, Toshihide Shima<sup>16</sup>, Takeshi Okanoue<sup>16</sup>, Sanjiv Mahadeva<sup>17</sup>, Wah-Kheong Chan<sup>17</sup>, Peter J. Eddowes<sup>18</sup>, Philip N. Newsome<sup>19,20,21</sup>, Vincent Wai-Sun Wong<sup>22</sup>, Victor de Lédinghen<sup>23</sup>, Jian-Gao Fan<sup>24</sup>, Feng Shen<sup>24</sup>, Jeremy F. L. Cobbold<sup>25</sup>, Yoshio Sumida<sup>26</sup>, Akira Okajima<sup>27</sup>, Jörn M. Schattenberg<sup>28</sup>, Christian Labenz<sup>29</sup>, Won Kim<sup>30</sup>, Myoung Seok Lee<sup>31</sup>, Johannes Wiegand<sup>32</sup>, Thomas Karlas<sup>33</sup>, Yusuf Yilmaz<sup>34,35</sup>, Guruprasad Padur Aithal<sup>36,37</sup>, Naaventhana Palaniyappan<sup>36,37</sup>, Christophe Cassinotto<sup>38</sup>, Sandeep Aggarwal<sup>39</sup>, Harshit Garg<sup>39</sup>, Geraldine Ooi<sup>40</sup>, Atsushi Nakajima<sup>41</sup>, Masato Yoneda<sup>41</sup>, Marianne Ziolo<sup>42</sup>, Nathalie Barget<sup>43</sup>, Andreas Geier<sup>44</sup>, Theresa Tuthill<sup>45</sup>, Julia M. Brosnan<sup>45</sup>, Quentin M. Anstee<sup>46</sup>, Stefan Neubauer<sup>1</sup>, Stephen A. Harrison<sup>1</sup>, Patrick M. Bossuyt<sup>2</sup>, Michael Pavlides<sup>1,3,4</sup>, on behalf of the LITMUS Investigators

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## The LITMUS Investigators

Newcastle University	Quentin Anstee Ann Daly Katherine Johnson Olivier Govaere Simon Cockell Dina Tiniakos Pierre Bedossa Fiona Oakley Heather Cordell Chris Day Kristy Wonders
AMC Amsterdam	Patrick Bossuyt Hadi Zafarmand Yasaman Vali Jenny Lee
Hôpital Pitié Salpêtrière, Assistance Publique -Hôpitaux de Paris, and Institute of Cardiometabolism and Nutrition, Paris, France	Vlad Ratziu Karine Clement Raluca Pais
University Medical Center Mainz	Detlef Schuppan Jörn Schattenberg
University of Cambridge	Toni Vidal-Puig Michele Vacca Sergio Rodrigues-Cuenca Mike Allison Ioannis Kamzolas Evangelia Petsalaki
Örebro University	Matej Oresic Tuulia Hyötyläinen Aiden McGlinchey
Center for Cooperative Research in Biosciences	Jose M Mato Oscar Millet
University of Bern	Jean-François Dufour Annalisa Berzigotti
University of Oxford	Michael Pavlides Stephen Harrison Stefan Neubauer Jeremy Cobbold Ferenc Mozes Salma Akhtar
Perspectum	Rajarshi Banerjee Matt Kelly Elizabeth Shumbayawonda Andrea Dennis Charlotte Erpicum Micheala Graham
Servicio Andaluz de Salud, Seville	Manuel Romero-Gómez Emilio Gómez-González Javier Ampuero Javier Castell

	Rocío Gallego-Durán Isabel Fernández Rocío Montero-Vallejo
Nordic Bioscience	Morten Karsdal Elisabeth Erhardtsen Daniel Rasmussen Diana Julie Leeming Mette Juul Fisker Antonia Sinisi Kishwar Musa
Integrated Biobank of Luxembourg	Fay Betsou Estelle Sandt Manuela Tonini
University of Torino	Elisabetta Bugianesi Chiara Rosso Angelo Armandi Fabio Marra (UNIFI) Amalia Gastaldelli (CNR) Gianluca Svegliati (UNIPM)
University Hospital of Angers	Jérôme Boursier
Antwerp University Hospital	Sven Francque Luisa Vonghia
Linköping University	Mattias Ekstedt Stergios Kechagias
University of Helsinki	Hannele Yki-Jarvinen Kimmu Porthan
UMC Utrecht	Saskia van Mil
National & Kapodistrian University of Athens	George Papatheodoridis
Faculdade de Medicina de Lisboa	Helena Cortez-Pinto
Università degli Studi di Milano	Luca Valenti
Università degli Studi di Palermo	Salvatore Petta
Università Cattolica del Sacro Cuore	Luca Miele
University Hospital Würzburg	Andreas Geier
RWTH Aachen University Hospital	Christian Trautwein
University of Nottingham	Guru Aithal
Antaros Medical	Paul Hockings
University Hospitals Birmingham NHS Foundation Trust	Philip Newsome
iXscient	David Wenn
University of Lisbon	Cecília Maria Pereira Rodrigues
Genfit	Pierre Chaumat Rémy Hanf
Intercept Pharma	Aldo Trylesinski
OWL	Pablo Ortiz
Ely-Lilly	Kevin Duffin
Pfizer	Julia Brosnan Theresa Tuthill Euan McLeod
Boehringer-Ingelheim	Judith Ertle Ramy Younes
Somallogic	Rachel Ostroff

	Leigh Alexander
Novo Nordisk	Mette Skalhøj Kjær
Ellegaard Göttingen Minipigs	Lars Friis Mikkelsen
Novartis Pharma AG	Maria-Magdalena Balp Clifford Brass Lori Jennings Miljen Martić Juergen Loeffler
Takeda Development Centre Europe Ltd	Guido Hanauer
AstraZeneca	Sudha Shankar
Echosens	Céline Fournier
Resoundant	Kay Pepin Richard Ehman
Bristol-Myers Squibb	Joel Myers
HistoIndex	Gideon Ho
Allergan	Richard Torstenson
Gilead	Rob Myers
RTI-HS	Lynda Doward

## Supporting Methods

According to the manufacturer, probe selection should be driven by skin-to-liver capsule distance (SCD): M probe for  $SCD < 25$  mm and XL probe for  $25 \text{ mm} \leq SCD < 35$  mm. In the latest version of the FibroScan equipment this is done by the Automatic Probe Selection tool. Some investigators have suggested that BMI may be used as a surrogate of SCD, using the M probe if  $BMI < 30 \text{ kg/m}^2$  and XL probe if  $BMI \geq 30 \text{ kg/m}^2$  (28).

For this meta-analysis, if only one VCTE-based liver stiffness measurement was available then this was included in the main analysis irrespective of probe type and BMI. Where two VCTE-based LSM were available (one with each probe), the main analysis included the M-probe measurement for  $BMI < 30 \text{ kg/m}^2$  and the XL probe measurement for  $BMI \geq 30 \text{ kg/m}^2$ . Therefore, all LSM cut-offs were determined independent of probe type.

We further conducted sensitivity analysis to investigate the influence of probe selection by excluding patients with  $BMI \geq 30 \text{ kg/m}^2$  who had a measurement with the M probe and patients with  $BMI < 30 \text{ kg/m}^2$  who had measurement with the XL probe.

## Supporting Discussion

### Rationale for proposing new NIT combinations with higher cut-offs for diagnosis of cirrhosis

Up until now, the literature has focused on the application of non-invasive tests in screening strategies for advanced fibrosis (F3-4). These strategies are useful when applied at the interface of primary and secondary care. Patients assessed using these strategies are classified as low risk, high risk or indeterminate risk of having advanced fibrosis, based on which clinical decisions are made: those with low risk continue to be managed in primary care, those with high risk are referred to secondary care and those with indeterminate risk undergo liver biopsy to determine their risk category.

What is lacking from the literature and what we have tried to answer with our analysis is what happens to patients with high risk of advanced fibrosis that are referred to secondary care. Our view is that they remain an indeterminate group as they can have either F3 or F4 fibrosis stage. Therefore, to distinguish between F3 and cirrhosis (F4) they still need to undergo liver biopsy, as those with liver cirrhosis would be managed differently (ultrasound surveillance for HCC and screening for oesophageal varices is generally indicated in patients with cirrhosis, but not those with F3 fibrosis stage). The identification of patients with cirrhosis would also be important as potential treatments for NASH may be licenced exclusively for patients with or without cirrhosis. We therefore argue that in practice, both the indeterminate and high-risk groups need to have a liver biopsy to establish their disease stage. In the case of those in the indeterminate category, the biopsy is needed to decide whether they merit referral to secondary care, and in the case of those with high risk of advanced fibrosis a biopsy is needed in secondary care to identify those with cirrhosis. We illustrate this point in **Supporting Figure 1a** and in **Figure 3a**, we also show how the FIB4-VCTE combination performs in our cohort.

Our answer to the problem above is a hybrid algorithm, where the lower NIT cut-offs are used to rule out advanced fibrosis, and the upper cut-offs are used to rule in cirrhosis. We provide cut-offs

with 95% and 98% specificity for the diagnosis of cirrhosis. This approach still stratifies patients into 3 risk groups – those with low risk of advanced fibrosis remaining in primary care, those in the indeterminate group needing a biopsy and those with high risk for cirrhosis. We argue that the group with high risk for cirrhosis can be positively diagnosed with cirrhosis without needing to have a biopsy. The net effect is that even though the indeterminate group is larger, fewer patients need to have a biopsy overall. This new approach is illustrated in **Supporting Figure 1b**, with results from our cohort given in **Figures 3b** and **3c**.

## Supporting Tables

**Supporting Table 1** Definitions of NITs evaluated in the current meta-analysis.

NIT	Definition
LSM by VCTE	An ultrasound probe that can also generate shear waves is placed over the right liver lobe. A low frequency shear wave is then generated by the external vibrator located in the probe, and ultrasound is used to measure the velocity of this shear wave through the liver. This velocity is directly related to liver stiffness.
FIB-4	$\text{Age [years]} \times \text{AST [IU/L]} / (\text{platelets} [\times 10^9/\text{L}] \times (\text{ALT [U/L]})^{1/2})$
NFS	$-1.675 + 0.037 \times \text{age [years]} + 0.094 \times \text{BMI [kg/m}^2] + 1.13 \times \text{IFG/diabetes [yes = 1, no = 0]} + 0.99 \times \text{AST/ALT ratio} - 0.013 \times \text{platelet} [\times 10^9/\text{L}] - 0.66 \times \text{albumin [g/dL]}$
AST/ALT	$\text{AST [IU/L]} / \text{ALT [IU/L]}$
APRI	$\text{AST [IU/L]} / \text{AST ULN [IU/L]} / \text{platelet} [\times 10^9/\text{L}]$

Abbreviations: LSM – liver stiffness measurement; VCTE – vibration-controlled transient elastography; FIB-4 – Fibrosis-4 score; NFS – NAFLD fibrosis score; AST/ALT – AST to ALT ratio; APRI – AST to platelet ratio index; ULN – upper limit of normal; IU – international unit; IFG – impaired fasting glucose

**Supporting Table 2** Non-invasive test cut-offs to rule-in and rule-out advanced fibrosis in patients with NAFLD

Study ID	Rule out cut-off	Rule-in cut-off
<b>Vibration controlled transient elastography</b>		
Studies testing pre-defined cut-offs (kPa)		
Anstee 2019 (1)	< 9.0	> 11.4
Wong 2019 (2), Papatheodoridi 2021 (ref)	< 10.0	> 15.0 <sup>^</sup>
Petta 2019 (3), Boursier 2019 (4), Petta 2017 (5)	< 7.9	> 9.6*
Cut-offs identified from other primary studies (kPa)		
Tapper 2016 (6)	< 7.9	> 9.8
Eddowes 2019 (7)	< 7.1	> 14.1
Hsu 2019 (8)	< 5.9	> 13.4
Cassinotto 2016 (9)	< 8.2	> 12.5
Papatheodoridi 2021 (ref)		
<b>FIB-4</b>		
Studies testing pre-defined cut-offs		
Anstee 2019 (1), Xun 2012 (10), Petta 2019 (3)	< 1.30	> 2.67 <sup>#</sup>
Vilar-Gomez 2018 (11), Sun 2016 (12), McPherson 2010 (13), Srivastava 2019 (14)	< 1.30	> 3.25
Demir 2013 (15)	< 1.45	> 3.25
Cut-offs from other primary studies		
Siddiqui 2019 (16)	< 1.02	> 1.95
<b>NAFLD Fibrosis score</b>		
Studies testing pre-defined cut-offs		
Antsee 2019 (1), Tapper 2016 (6), Vilar-Gomez 2018 (11), Sun 2016 (12), McPherson 2010 (13), Xun 2012 (10), Demir 2013 (15), Petta 2014 (17), Dowman 2011 (18), Petta 2019 (3), Fowell 2020 (19)	< -1.455	> 0.676 <sup>%</sup>
<sup>^</sup> based on BavenoVI (20), *based on Wong (21), <sup>#</sup> from Shah 2009 (22), <sup>%</sup> from Angulo 2007 (23)		

**Supporting Table 3** Data fields requested from the authors of primary studies of LSM by VCTE

Category	Field	Units or possible values	Proportion of patients in whom reported, %
Study details	Name of first author	-	100.0
	Year of publication	-	100.0
	Country	-	100.0
	Centre	-	
Demographic and anthropometric details	Gender	M/F	100.0
	Age	years	99.9
	Ethnicity	-	38.6
	Height	m	92.4
	Weight	kg	94.9
	Waist circumference	cm	72.3
	Hip circumference	cm	21.8
	Smoking	Current/Ex/Never	10.0
	Presence of type 2 diabetes mellitus	Yes/No	86.4
	Presence of hypertension	Yes/No	48.8
	Presence of hyperlipidaemia	Yes/No	26.0
Laboratory data	Platelet count	$\times 10^9/l$	98.2
	INR	-	35.4
	Bilirubin	$\mu\text{mol/l}$	55.5
	ALT	IU/L	97.2
	AST	IU/L	96.2
	ALP	IU/L	48.3
	GGT	IU/L	82.2
	Albumin	g/l	67.2
	Sodium	mmol/l	6.7
	Urea	mmol/l	13.7
	Creatine	$\mu\text{mol/l}$	22.2
	Total cholesterol	mmol/l	62.8
	LDL cholesterol	mmol/l	32.8
	HDL cholesterol	mmol/l	77.6
	Triglycerides	mmol/l	79.3
	CRP	mg/l	7.9
	Fasting glucose	mmol/l	73.0
	Fasting insulin	mU/L	18.0
	HOMA-IR	-	16.8
Biopsy data	Date of biopsy	-	67.0
	Length of biopsy sample	mm	70.6
	Number of portal tracts	-	32.4
	Fibrosis stage	0-4	100.0
	Ballooning	0-2	63.7
	Lobular inflammation	0-3	64.2
	Steatosis	0-3	71.5
	NAS score	0-8	82.9
	Date of scan	-	68.9

Transient elastography details	Time between biopsy and scan	days	79.3
	Probe type	M/XL	91.9
	Number of valid shots	-	59.4
	Median stiffness	kPa	95.7
	IQR	kPa	83.4
	IQR/median	-	83.0
	Success rate	%	77.8

**Supporting Table 4** Demographic, biopsy, liver function test and NIT details of the entire cohort and broken down by fibrosis stage

	Entire cohort (n = 5735)	F0 (n = 1138)	F1 (n = 1613)	F2 (n = 1262)	F3 (n = 1101)	F4 (n = 621)
Females (%)	45	43	44	43	47	50
BMI > 30 kg/m <sup>2</sup> (%)	47	33	45	56	55	51
Waist circumference (cm)	103 (15)	99 (16)	101 (15)	106 (14)	106 (14)	106 (15)
Diabetes (%)	38	28	33	45	62	65
Age (years)*	54 (19)	48 (17)	50 (20)	53 (19)	59 (15)	60 (12)
BMI (kg/m <sup>2</sup> )*	30 (7)	28 (7)	29 (7)	31 (7)	31 (7)	30 (7)
Biopsy data						
Steatosis						
S0/S1/S2/S3 (%)	3/35/36/26	8/45/30/17	2/35/37/26	1/28/39/32	1/28/39/32	3/38/37/22
Ballooning						
B0/B1/B2 (%)	24/47/29	53/37/10	26/55/19	11/53/36	10/43/47	10/46/44
Inflammation						
I0/I1/I2/I3 (%)	13/60/24/3	3/60/9/4	13/65/21/1	6/60/31/3	5/53/36/6	8/57/29/6
NAS score <sup>+</sup>	4 (2)	3 (2)	4 (2)	4 (1)	5 (1)	4 (2)
NASH (%)	50	19	46	64	71	61
Liver function tests						
ALT (IU/L) *	55 (48)	46 (39)	54 (50)	59 (52)	63 (50)	55 (43)
AST (IU/L) *	40 (30)	31 (19)	36 (27)	41 (28)	49 (32)	53 (39)
Platelets (×10 <sup>9</sup> /l) <sup>+</sup>	230 (72)	247 (64)	243 (69)	232 (66)	217 (69)	184 (81)
Albumin (g/l) <sup>+</sup>	43 (9)	43 (8)	43 (7)	43 (5)	43 (6)	43 (20)
GGT (IU/L) *	69 (87)	59 (85)	61 (75)	63 (74)	82 (88)	104 (169)
Total cholesterol (mmol/l) <sup>+</sup>	5.1 (1.3)	5.2 (1.3)	5.1 (1.2)	5.2 (1.4)	4.9 (1.2)	4.6 (1.3)
HDL cholesterol (mmol/l) <sup>+</sup>	1.2 (0.5)	1.3 (0.5)	1.3 (0.5)	1.2 (0.5)	1.2 (0.4)	1.2 (0.5)
Triglycerides (mmol/l) *	1.6 (1.1)	1.4 (1.0)	1.6 (1.1)	1.6 (1.0)	1.6 (1.1)	1.5 (1.0)

Fasting glucose (mmol/l) *	5.6 (2.0)	5.3 (1.2)	5.4 (1.8)	5.6 (1.7)	6.3 (2.9)	6.4 (2.8)
Non-invasive tests						
LSM (kPa) *	10.7 (6.1)	5.7 (2.5)	6.7 (3.4)	7.9 (4.3)	11.3 (6.9)	20.9 (16.8)
AST/ALT*	0.8 (0.4)	0.7 (0.4)	0.7 (0.4)	0.7 (0.4)	0.8 (0.4)	0.9 (0.6)
FIB-4*	1.7 (1.2)	1.1 (0.7)	1.3 (1.2)	1.5 (1.1)	2.1 (1.6)	3.3 (2.9)
NFS*	-1.5 (1.7)	-2.3 (2.0)	-2.0 (2.2)	-1.4 (2.2)	-0.8 (1.8)	0.0 (1.8)
APRI*	0.6 (0.4)	0.3 (0.3)	0.4 (0.3)	0.5 (0.4)	0.6 (0.5)	0.8 (0.8)

\*Data are reported as median (IQR); \*Data are reported as mean (SD).

**Supporting Table 5** Details of biopsy and biopsy quality in the entire IPD cohort.

Biopsy details	Entire cohort (n = 5735)	Advanced fibrosis (n = 1722)	Cirrhosis (n = 621)
<b>Time between liver biopsy and LSM by VCTE</b>			
Patients with reported exact time period, %	79 (4549/5735)	80 (1371/1722)	76 (474/621)
Median (IQR) (days)	0 (14)	0 (9)	1 (26)
<b>Length of biopsy sample</b>			
Patients with reported length of biopsy, %	71 (4047/5735)	80 (1369/1722)	80 (495/621)
< 10 mm, %	3 (123/4047)	3 (42/1369)	5 (25/495)
≥ 10 mm and < 20 mm, %	35 (1432/4047)	33 (450/1369)	35 (172/495)
≥ 20 mm, %	62 (2492/4047)	64 (877/1369)	60 (298/495)
<b>Number of portal tracts in biopsy sample</b>			
Patients with reported portal tracts %	32 (1857/5735)	32 (544/1722)	26 (159/621)
< 11, %	54 (1006/1857)	42 (228/544)	47 (74/159)
≥ 11, %	46 (851/1857)	58 (316/544)	54 (85/159)
<b>Patients with both portal tracts and biopsy length reported, %</b>	32 (1854/5735)	32 (543/1722)	26 (159/621)
<b>Biopsy quality</b>			
Intermediate quality (length ≥ 10 mm and < 20 mm), %	46 (849/1854)	41 (220/543)	39 (62/159)
High quality (length ≥ 20 mm and ≥ 11 portal tracts), %	36 (670/1854)	45 (246/543)	39 (62/159)

Data are reported as percentage (number of patient satisfying conditions/total number of patients in subgroup)

**Supporting Table 6** Diagnostic performance of non-invasive tests for cirrhosis (F4)

	LSM by VCTE (n = 5489)			FIB-4 (n = 5393)			NFS (n = 3248)			APRI (n =5477)			AST/ALT ratio (n = 5434)		
Cirrhosis, %	11			11			11			11			11		
AUC	0.90 (.89-0.91)			0.80 (0.78-0.82)			0.77 (0.75-0.80)			0.72 (0.70-0.74)			0.69 (0.67-0.71)		
Threshold	10.4	<10.2	≥14.9	1.55	<1.13	≥2.66	-1.11	<-1.72	≥0.48	0.58	<0.30	≥1.04	0.82	<0.58	≥1.35
Sensitivity, %	89	90	67	77	90	44	82	90	36	66	90	35	64	90	24
	(86-91)	(8-92)	(64-70)	(72-80)	(87-92)	(40-48)	(76-85)	(86-93)	(31-40)	(61-69)	(87-92)	(31-39)	(59-67)	(87-92)	(20-28)
Specificity, %	75	74	90	67	48	90	63	49	90	68	28	90	66	33	90
	(74-76)	(72-75)	(89-90)	(65-68)	(46-49)	(89-90)	(61-64)	(46-50)	(88-91)	(66-69)	(26-28)	(89-90)	(64-67)	(31-33)	(89-90)
Misclassified, %	23	24	12	32	48	15	35	47	16	32	66	16	34	61	17
	(23-24)	(24-25)	(12-13)	(32-33)	(47-49)	(14-15)	(35-36)	(46-48)	(15-16)	(32-33)	(65-66)	(15-16)	(34-35)	(61-62)	(16-17)

For each non-invasive test thresholds were calculated according to Youden’s index (YI), and fixed at 90% sensitivity (90% Se) and 90% specificity (90% Sp). 95% confidence intervals were estimated with 500 bootstrap iterations.

**Supporting Table 7** Diagnostic performance of non-invasive tests for advanced fibrosis (F3-4) in a head-to-head comparison of NITs

	LSM by VCTE (n = 3248)			FIB-4 (n = 3248)			NFS (n = 3248)		
Advanced fibrosis, %	29			29			29		
AUC	0.86 (0.85-0.88)			0.75 (0.73-0.77)			0.73 (0.71-0.75)		
	YI	90% Se	90% Sp	YI	90% Se	90% Sp	YI	90% Se	90% Sp
Threshold	9.1	7.2	11.8	1.45	0.87	2.39	-1.39	-2.55	0.28
Sensitivity, %	77 (74-80)	90 (89-92)	59 (57-63)	69 (66-72)	90 (88-92)	36 (33-39)	75 (72-78)	90 (88-92)	29 (26-32)
Specificity, %	81 (79-82)	61 (59-63)	90 (89-92)	69 (67-71)	38 (36-39)	90 (89-91)	63 (61-65)	36 (33-37)	90 (89-91)
Misclassified, %	21 (19-22)	31 (29-32)	18 (17-20)	31 (29-32)	47 (46-49)	25 (24-27)	34 (34-36)	48 (49-50)	28 (28-29)

**Supporting Table 8** Diagnostic performance of non-invasive tests for cirrhosis (F4) in a head-to-head comparison of NITs

	LSM by VCTE (n = 3094)			FIB-4 (n = 3094)			NFS (n = 3094)		
Cirrhosis, %	11			11			11		
AUC	0.91 (0.89-0.92)			0.78 (0.76-0.81)			0.77 (0.75-0.80)		
	YI	90% Se	90% Sp	YI	90% Se	90% Sp	YI	90% Se	90% Sp
Threshold	10.3	9.7	14.4	1.35	1.08	2.76	-1.11	-1.93	0.46
Sensitivity, %	89 (86-92)	90 (87-93)	68 (63-72)	83 (79-87)	90 (87-93)	42 (37-47)	81 (77-86)	90 (87-93)	35 (29-40)
Specificity, %	78 (76-79)	74 (73-76)	91 (90-92)	59 (57-61)	45 (43-47)	90 (89-91)	64 (62-66)	45 (43-47)	90 (89-91)
Misclassified, %	21 (20-22)	24 (22-25)	12 (10-13)	39 (37-40)	50 (48-52)	15 (14-16)	34 (33-36)	50 (49-52)	16 (15-17)

**Supporting Table 9** Diagnostic performance of cut-offs from the literature for LSM by VCTE, FIB-4 and NFS for diagnosing advanced fibrosis.

	LSM by VCTE (n = 5489)								FIB-4 (n = 5393)		NFS (n = 3248)			
Source	Anstee 2019 (30)		Eddowes 2019 (31)		Wong 2019 (71)		Wong 2010 (21)		Shah 2009 (78)		McPherson 2010 (79)		Angulo 2007 (17)	
Thresholds	<9.9	≥11.4	<7.1	≥14.1	<10	≥15	<7.9	≥9.6	<1.3	≥2.67	<1.3	≥3.25	<-1.455	≥0.676
Sensitivity, %	72 (71-75)	61 (60-64)	91 (90-93)	46 (44-49)	71 (70-74)	41 (39-44)	86 (86-89)	73 (71-76)	74 (72-76)	30 (28-32)	74 (72-76)	20 (18-22)	76 (73-78)	22 (19-24)
Specificity, %	82 (80-83)	87 (86-88)	58 (55-58)	94 (93-94)	82 (81-83)	95 (94-96)	68 (65-68)	81 (79-81)	64 (63-66)	94 (93-94)	64 (63-66)	96 (96-97)	61 (60-64)	94 (93-95)
Misclassified, %	21 (21-22)	21 (20-22)	32 (32-34)	20 (20-21)	21 (21-22)	21 (21-22)	27 (27-29)	21 (21-23)	33 (33-34)	25 (25-26)	33 (33-34)	27 (26-27)	35 (34-36)	28 (27-28)

95% confidence intervals were estimated with 500 bootstrap iterations

**Supporting Table 10** Indicative PPV, NPV, false positive and false negative proportions for prevalences found in primary studies for the diagnostic performance of VCTE in identifying advanced fibrosis

Cut-off	Se, %	95% CI, %	Sp, %	95% CI, %	Prevalence, %	PPV, %	NPV, %	FP*	FN*
7.4 kPa	90	89-91	60	59-61	5	11	99	38	1
					10	20	98	36	1
					20	36	96	32	2
					<b>30</b>	<b>49</b>	<b>93</b>	<b>28</b>	<b>3</b>
					40	60	90	24	4
					50	69	86	20	5
9.1 kPa	77	75-79	78	76-79	5	16	98	21	1
					10	28	97	20	2
					20	47	93	18	5
					<b>30</b>	<b>60</b>	<b>89</b>	<b>15</b>	<b>7</b>
					40	70	84	13	9
					50	78	77	11	12
12.1 kPa	55	52-57	90	89-91	5	22	97	10	2
					10	38	95	9	5
					20	58	89	8	9
					<b>30</b>	<b>70</b>	<b>82</b>	<b>7</b>	<b>14</b>
					40	79	75	6	18
					50	85	67	5	23
<7.4 kPa, ≥12.1 kPa	84	81-87	87	85-88	5	25	99	12	1
					10	42	98	12	2
					20	62	96	10	3
					<b>30</b>	<b>73</b>	<b>93</b>	<b>9</b>	<b>5</b>
					40	81	89	8	6
					50	87	84	7	8
<9.9 kPa, ≥11.4 kPa (Anstee 2019)	69	67-71	86	85-88	5	21	98	13	2
					10	35	96	13	3
					20	55	92	11	6
					<b>30</b>	<b>68</b>	<b>87</b>	<b>10</b>	<b>9</b>
					40	77	81	8	12
					50	83	74	7	16
<7.1, ≥14.1 (Eddowes 2019)	83	80-86	90	88-92	5	30	99	10	1
					10	48	98	9	2
					20	67	95	8	3
					<b>30</b>	<b>78</b>	<b>93</b>	<b>7</b>	<b>5</b>
					40	85	89	6	7
					50	89	84	5	9
<10, ≥15 (Wong 2019)	59	57-61	94	93-96	5	34	98	6	2
					10	52	95	5	4
					20	71	90	5	8
					<b>30</b>	<b>81</b>	<b>84</b>	<b>4</b>	<b>12</b>
					40	87	77	4	16
					50	91	70	3	21
<7.9, ≥9.6 (Wong 2010)	84	82-87	78	76-80	5	17	99	21	1
					10	30	98	20	2
					20	49	95	18	3
					<b>30</b>	<b>62</b>	<b>92</b>	<b>15</b>	<b>5</b>
					40	72	88	13	6

	50	79	83	11	8
*Number of false positives and false negatives for 100 hypothetical cases; Values in bold face correspond to the proportion of patients with advanced fibrosis in the IPDMA cohort					

**Supporting Table 11** Indicative PPV, NPV, false positive and false negative proportions for prevalences found in primary studies for the diagnostic performance of FIB-4 in identifying advanced fibrosis

Cut-off	Se, %	95% CI, %	Sp, %	95% CI, %	Prevalence, %	PPV, %	NPV, %	FP*	FN*
0.88	90	88-91	39	37-40	5	7	99	58	1
					10	14	97	55	1
					20	27	94	49	2
					<b>30</b>	<b>39</b>	<b>90</b>	<b>43</b>	<b>3</b>
					40	50	85	37	4
					50	60	80	31	5
1.44	69	67-72	70	69-72	5	11	98	29	2
					10	20	95	27	3
					20	37	90	24	6
					<b>30</b>	<b>50</b>	<b>84</b>	<b>21</b>	<b>9</b>
					40	61	77	18	12
					50	70	69	15	16
2.31	38	36-41	90	89-91	5	17	97	10	3
					10	30	93	9	6
					20	49	85	8	12
					<b>30</b>	<b>62</b>	<b>77</b>	<b>7</b>	<b>19</b>
					40	72	69	6	25
					50	79	59	5	31
<1.3, ≥2.67 (Shah 2009)	54	52-56	91	89-92	5	24	97	9	2
					10	40	95	8	5
					20	60	89	7	9
					<b>30</b>	<b>72</b>	<b>82</b>	<b>6</b>	<b>14</b>
					40	80	75	5	18
					50	86	66	5	23
<1.3, ≥3.25 (McPherson 2010)	44	42-46	95	93-96	5	32	97	5	3
					10	49	94	5	6
					20	69	87	4	11
					<b>30</b>	<b>79</b>	<b>80</b>	<b>4</b>	<b>17</b>
					40	85	72	3	22
					50	90	63	3	28

\*Number of false positives and false negatives for 100 hypothetical cases; Values in bold face correspond to the proportion of patients with advanced fibrosis in the IPDMA cohort

**Supporting Table 12** Indicative PPV, NPV, false positive and false negative proportions for prevalences found in primary studies for the diagnostic performance of NFS in identifying advanced fibrosis

Cut-off	Se, %	95% CI, %	Sp, %	95% CI, %	Prevalence, %	PPV, %	NPV, %	FP*	FN*
-2.55	90	88-92	36	33-37	5	7	99	61	1
					10	14	97	58	1
					20	26	94	51	2
					<b>30</b>	<b>38</b>	<b>89</b>	<b>45</b>	<b>3</b>
					40	48	84	38	4
					50	58	78	32	5
-1.39	75	72-78	63	61-65	5	10	98	35	1
					10	18	96	33	3
					20	34	91	30	5
					<b>30</b>	<b>46</b>	<b>85</b>	<b>26</b>	<b>8</b>
					40	57	79	22	10
					50	67	72	19	13
0.28	29	26-32	90	89-91	5	13	96	10	4
					10	24	92	9	7
					20	42	84	8	14
					<b>30</b>	<b>55</b>	<b>75</b>	<b>7</b>	<b>21</b>
					40	66	66	6	28
					50	74	56	5	36
<-1.455, ≥0.676 (Angulo 2007)	47	44-50	91	89-93	5	22	97	9	3
					10	37	94	8	5
					20	57	87	7	11
					<b>30</b>	<b>69</b>	<b>80</b>	<b>6</b>	<b>16</b>
					40	78	72	5	21
					50	84	63	5	27

\*Number of false positives and false negatives for 100 hypothetical cases; Values in bold face correspond to the proportion of patients with advanced fibrosis in the IPDMA cohort

**Supporting Table 13** Indicative PPV, NPV, false positive and false negative proportions for prevalences found in primary studies for the diagnostic performance of APRI in identifying advanced fibrosis

Cut-off	Se, %	95% CI, %	Sp, %	95% CI, %	Prevalence, %	PPV, %	NPV, %	FP*	FN*
0.29	90	89-92	29	28-30	5	6	98	67	1
					10	12	96	64	1
					20	24	92	57	2
					<b>30</b>	<b>35</b>	<b>87</b>	<b>50</b>	<b>3</b>
					40	46	81	43	4
					50	56	74	36	5
0.49	67	64-69	63	62-65	5	9	97	35	2
					10	17	95	33	3
					20	31	88	30	7
					<b>30</b>	<b>44</b>	<b>82</b>	<b>26</b>	<b>10</b>
					40	55	74	22	13
					50	64	66	19	17
0.91	32	30-34	90	89-91	5	14	96	10	3
					10	26	92	9	7
					20	44	84	8	14
					<b>30</b>	<b>58</b>	<b>76</b>	<b>7</b>	<b>20</b>
					40	68	67	6	27
					50	76	57	5	34

\*Number of false positives and false negatives for 100 hypothetical cases; Values in bold face correspond to the proportion of patients with advanced fibrosis in the IPDMA cohort

**Supporting Table 14** Indicative PPV, NPV, false positive and false negative proportions for prevalences found in primary studies for the diagnostic performance of AST/ALT in identifying advanced fibrosis

Cut-off	Se, %	95% CI, %	Sp, %	95% CI, %	Prevalence, %	PPV, %	NPV, %	FP*	FN*
0.51	90	87-91	25	23-26	5	6	98	71	1
					10	12	96	68	1
					20	23	91	60	2
					<b>30</b>	<b>34</b>	<b>85</b>	<b>53</b>	<b>3</b>
					40	44	79	45	4
					50	55	71	38	5
0.64	75	73-77	47	45-48	5	7	97	50	1
					10	14	94	48	3
					20	26	88	42	5
					<b>30</b>	<b>38</b>	<b>81</b>	<b>37</b>	<b>8</b>
					40	49	74	32	10
					50	59	65	27	13
1.34	16	14-18	90	89-91	5	8	95	10	4
					10	15	91	9	8
					20	29	81	8	17
					<b>30</b>	<b>41</b>	<b>71</b>	<b>7</b>	<b>25</b>
					40	52	62	6	34
					50	62	52	5	42

\*Number of false positives and false negatives for 100 hypothetical cases; Values in bold face correspond to the proportion of patients with advanced fibrosis in the IPDMA cohort

**Supporting Table 15** Diagnostic accuracy of pairs of cut-offs from the literature for NITs for diagnosing advanced fibrosis. Patient proportions used to calculate performance statistics are displayed as ratios.

LSM by VCTE (n = 5489)						FIB-4 (n = 5393)			NFS (n = 3248)	
Prevalence, %						30			29	
AUROC						0.85 (0.84-0.86)			0.73 (0.71-0.75)	
Source of thresholds	Anstee 2019 (1)	Eddowes 2019 (7)	Wong 2019 (2)	Wong 2010 (21)	This study	Shah 2009 (22)	McPherson 2010 (13)	This study	Angulo 2007 (23)	This study
Thresholds	<9.9, ≥11.4	<7.1, ≥14.1	<10, ≥15	<7.9, ≥9.6	<7.4, ≥12.1	<1.3, ≥2.67	<1.3, ≥3.25	<0.88, ≥2.31	<1.455, ≥0.676	<-2.55, ≥0.28
Sensitivity, %	69 (1009/1456)	83 (754/905)	59 (674/1145)	84 (1205/1431)	84 (889/1060)	54 (485/901)	44 (328/744)	80 (621/780)	47 (202/429)	74 (270/363)
Specificity, %	86 (3147/3639)	90 (2216/2457)	94 (3165/3351)	78 (2599/3330)	87 (2338/2702)	91 (2423/2668)	95 (2423/2563)	79 (1448/1831)	91 (1423/1562)	78 (821/1050)
Misclassified, %	17 (948/5489)	7 (392/5489)	12 (657/5489)	17 (957/5489)	10 (535/5489)	12 (661/5393)	10 (556/5393)	10 (542/5393)	11 (366/3248)	10 (322/3248)
Indeterminate, %	7 (385/5489)	39 (2127/5489)	18 (993/5489)	13 (728/5489)	31 (1727/5489)	34 (1824/5393)	39 (2086/5393)	52 (2782/5393)	39 (1257/3248)	56 (1835/3248)

95% confidence intervals were estimated with 500 bootstrap replicates.

**Supporting Table 16** Derivation of new cut-offs corresponding to 95% specificity for cirrhosis. The cut-offs were calculated in the training cohort and were validated in the validation cohort. Training and validation cohorts were obtained by random sampling from the IPD cohort in a 3:2 ratio.

	LSM by VCTE (n = 5489)		FIB-4 (n = 5393)		NFS (n = 3248)	
	Training (n = 3290)	Validation (n = 2199)	Training (n = 3254)	Validation (n = 2139)	Training (n = 1963)	Validation (n = 1285)
Proportion of patients with cirrhosis, %	11	10	11	11	10	11
AUC	0.90 (0.89-0.92)	0.90 (0.88-0.92)	0.81 (0.78-0.83)	0.79 (0.77-0.82)	0.78 (0.75-0.81)	0.77 (0.72-0.81)
Threshold	20.4		3.48		1.01	
Sensitivity, %	52 (47-57)	49 (43-56)	33 (28-37)	30 (24-36)	21 (16-27)	28 (21-36)
Specificity, %	95 (95-96)	95 (95-97)	95 (94-96)	96 (95-97)	95 (94-96)	95 (94-96)
Misclassified, %	10 (10-11)	9 (9-10)	12 (12-13)	11 (11-12)	13 (13-14)	13 (13-14)

95% confidence intervals were estimated with 500 bootstrap replicates.

**Supporting Table 17** Derivation of new cut-offs corresponding to 98% specificity for cirrhosis. The cut-offs were calculated in the training cohort and were validated in the validation cohort. Training and validation cohorts were obtained by random sampling from the IPD cohort in a 3:2 ratio.

	LSM by VCTE (n = 5489)		FIB-4 (n = 5393)		NFS (n = 3248)	
	Training (n = 3290)	Validation (n = 2199)	Training (n = 3254)	Validation (n = 2139)	Training (n = 1963)	Validation (n = 1285)
Proportion of patients with cirrhosis, %	11	10	11	11	10	11
AUC	0.90 (0.89-0.92)	0.90 (0.88-0.92)	0.81 (0.78-0.83)	0.79 (0.77-0.82)	0.78 (0.75-0.81)	0.77 (0.72-0.81)
Threshold	27.6		4.63		1.57	
Sensitivity, %	27 (23-32)	29 (22-34)	19 (15-23)	20 (15-26)	12 (8-17)	18 (13-27)
Specificity, %	98 (98-99)	98 (98-99)	98 (97-98)	98 (97-99)	98 (97-99)	98 (97-99)
Misclassified, %	10 (10-11)	9 (9-10)	10 (10-11)	10 (10-11)	11 (11-12)	11 (11-12)

95% confidence intervals were estimated with 500 bootstrap replicates.

**Supporting Table 18** Diagnostic performance of combinations of NFS and LSM by VCTE, and FIB-4 and LSM by VCTE tests to reduce need for liver biopsies

	FIB-4 & LSM by VCTE (n = 5159)	NFS & LSM by VCTE (n = 3094)	FIB-4 & LSM by VCTE (n = 5159)	NFS & LSM by VCTE (n = 3094)	FIB-4 & LSM by VCTE (n = 5159)	NFS & LSM by VCTE (n = 3094)	FIB-4 & LSM by VCTE (n = 5159)	NFS & LSM by VCTE (n = 3094)	FIB-4 & LSM by VCTE (n = 5159)	NFS & LSM by VCTE (n = 3094)
Prevalence, %	30	28	30	28	30	28	30	28	30	28
Threshold for blood-based NIT*	< 1.3, ≥ 3.48	< -1.455, ≥ 1.010	< 1.3, ≥ 3.48	< -1.455, ≥ 1.010	< 1.3, ≥ 3.48	< -1.455, ≥ 1.010	< 1.3, ≥ 4.63	< -1.455, ≥ 1.570	< 1.3, ≥ 4.63	< -1.455, ≥ 1.570
Threshold for VCTE, kPa*	< 7.9, ≥ 16.1	< 7.9, ≥ 16.1	< 7.9, ≥ 20.4	< 7.9, ≥ 20.4	< 8.0, ≥ 20.0	< 8.0, ≥ 20.0	< 7.9, ≥ 27.6	< 7.9, ≥ 27.6	< 8.0, ≥ 28.0	< 8.0, ≥ 28.0
Sensitivity, %	41 (40-43)	41 (39-42)	38 (37-40)	37 (35-38)	38 (37-39)	36 (34-38)	28 (27-29)	25 (24-26)	27 (26-28)	24 (23-25)
Specificity, %	88 (86-89)	88 (87-90)	90 (89-91)	90 (89-92)	90 (89-91)	90 (89-92)	95 (94-97)	96 (95-98)	96 (94-97)	96 (95-98)
PPV, %	45 (43-47)	45 (41-47)	48 (45-50)	46 (43-49)	47 (45-50)	45 (43-49)	57 (54-61)	57 (52-63)	57 (54-61)	57 (52-61)
NPV, %	86 (85-87)	87 (85-88)	86 (85-87)	87 (85-88)	86 (85-87)	86 (85-88)	86 (85-87)	86 (85-88)	86 (85-87)	86 (85-88)
Indeterminate, %	16 (15-17)	17 (16-19)	19 (18-20)	20 (18-21)	18 (17-19)	17 (18-21)	24 (23-25)	25 (23-27)	24 (23-25)	21 (23-26)
Misclassification, %	18 (17-19)	17 (15-19)	16 (15-17)	15 (14-17)	17 (15-18)	14 (14-17)	13 (12-14)	12 (10-13)	13 (12-14)	11 (10-13)
Patients undergoing VCTE, %	40 (39-42)	42 (40-44)	40 (39-42)	42 (40-44)	40 (39-42)	42 (40-44)	44 (42-45)	45 (43-47)	44 (42-45)	45 (43-47)

95% confidence intervals were estimated with 500 bootstrap replicates

\*A lower cut-off was used to rule out patients with advanced fibrosis and an upper cut-off was used to rule in patients with cirrhosis. Lower cut-offs were the same as used in **Table 6** of the main manuscript. Upper cut-offs for were calculated to obtain a 95% and 98% specificity in diagnosing cirrhosis in the IPD cohort.

**Supporting Table 19** Diagnostic performance of non-invasive tests in subgroup for discriminating advanced fibrosis (F3-F4).

	LSM by VCTE	FIB-4	NFS
Biopsy length < 20 mm (n = 1555)	<b>0.87 (0.86-0.89)</b>	<b>0.80 (0.78-0.83)</b>	<b>0.79 (0.75-0.82)</b>
Biopsy length ≥ 20 mm (n = 2492)	<b>0.83 (0.82-0.85)</b>	<b>0.75 (0.72-0.77)</b>	<b>0.72 (0.69-0.75)</b>
Number of portal tracts < 11 (n = 1006)	<b>0.86 (0.83-0.88)</b>	<b>0.79 (0.75-0.82)</b>	<b>0.78 (0.74-0.81)</b>
Number of portal tracts ≥ 11 (n = 851)	<b>0.80 (0.77-0.83)</b>	<b>0.73 (0.70-0.77)</b>	<b>0.68 (0.63-0.72)</b>
Intermediate quality biopsy (n = 1432)	<b>0.87 (0.85-0.89)</b>	<b>0.79 (0.77-0.82)</b>	<b>0.78 (0.74-0.81)</b>
High quality biopsy (n = 670)	<b>0.79 (0.75-0.83)</b>	<b>0.72 (0.68-0.76)</b>	<b>0.67 (0.62-0.73)</b>
BMI < 25 kg/m <sup>2</sup> (n = 868)	<b>0.91 (0.89-0.94)</b>	<b>0.81 (0.78-0.84)</b>	<b>0.76 (0.71-0.81)<sup>#</sup></b>
25 kg/m <sup>2</sup> ≤ BMI < 30 kg/m <sup>2</sup> (n = 2127)	<b>0.87 (0.85-0.89)</b>	0.77 (0.75-0.80)	<b>0.74 (0.71-0.77)<sup>*</sup></b>
BMI ≥ 30 kg/m <sup>2</sup> (n = 2710)	<b>0.81 (0.79-0.83)</b>	<b>0.74 (0.72-0.76)</b>	<b>0.69 (0.66-0.72)<sup>*,#</sup></b>
Continent – Europe (n = 3560)	0.85 (0.84-0.87)	0.75 (0.73-0.77)	0.72 (0.69-0.75)
Continent - Asia (n = 1278)	0.85 (0.82-0.88)	0.77 (0.73-0.80)	0.76 (0.73-0.80)
Sex – Male (n = 3165)	0.85 (0.83-0.86)	0.76 (0.74-0.78)	<b>0.75 (0.72-0.77)</b>
Sex – Female (n = 2570)	0.86 (0.84-0.87)	0.76 (0.73-0.78)	<b>0.71 (0.68-0.74)</b>
Presence of T2DM (n = 2191)	<b>0.81 (0.79-0.83)</b>	<b>0.73 (0.71-0.75)</b>	0.68 (0.65-0.70)
Lack of T2DM (n = 2763)	<b>0.87 (0.86-0.89)</b>	<b>0.77 (0.75-0.79)</b>	0.71 (0.68-0.74)
ALT < 40 U/L (n = 1656)	0.85 (0.83-0.88)	<b>0.73 (0.70-0.76)</b>	0.74 (0.70-0.78)
40 U/L ≤ ALT < 100 U/L (n = 2933)	0.86 (0.85-0.87)	<b>0.77 (0.76-0.79)</b>	0.75 (0.73-0.78)
ALT ≥ 100 U/L (n = 984)	0.83 (0.80-0.86)	0.76 (0.73-0.79)	0.77 (0.73-0.81)
AST < 40 U/L (n = 2759)	0.84 (0.82-0.86)	0.73 (0.70-0.75)	<b>0.76 (0.73-0.78)</b>
40 U/L ≤ AST < 100 U/L (n = 2385)	0.85 (0.83-0.86)	0.74 (0.72-0.76)	0.72 (0.69-0.75)
AST ≥ 100 U/L (n = 373)	0.86 (0.82-0.90)	0.71 (0.66-0.76)	<b>0.65 (0.58-0.72)</b>
ALT < 40 U/L AND AST < 40 U/L (n = 1323)	0.84 (0.81-0.87)	<b>0.72 (0.68-0.75)</b>	0.73 (0.69-0.77)
ALT ≥ 40 U/L OR AST ≥ 40 U/L (n = 4235)	0.86 (0.84-0.87)	<b>0.76 (0.75-0.78)</b>	0.75 (0.73-0.77)
Age < 43 yrs (n = 1401)	0.81 (0.77-0.84)	<b>0.65 (0.61-0.70)</b>	<b>0.58 (0.52-0.64)<sup>*,#</sup></b>
43 yrs ≤ Age < 54 yrs (n = 1478)	0.84 (0.82-0.86)	0.69 (0.66-0.72)	<b>0.70 (0.66-0.74)<sup>*</sup></b>
54 yrs ≤ Age < 62 yrs (n = 1423)	0.85 (0.83-0.87)	<b>0.72 (0.69-0.75)</b>	<b>0.70 (0.67-0.74)<sup>#</sup></b>
62 yrs ≤ Age (n = 1430)	0.84 (0.81-0.86)	0.70 (0.67-0.72)	0.66 (0.62-0.70)

Bold AUCs within a column and subgroup category are significantly different. Bonferroni's correction was applied when performing multiple comparisons. AUCs marked with \* or # are pairwise significantly different.

**Supporting Table 20** Diagnostic performance of non-invasive tests in subgroup for discriminating cirrhosis (F4).

	LSM by VCTE	FIB-4	NFS
Biopsy length < 20 mm (n = 1555)	0.91 (0.88-0.93)	0.84 (0.81-0.86)	<b>0.83 (0.79-0.87)</b>
Biopsy length ≥ 20 mm (n = 2492)	0.89 (0.88-0.91)	0.78 (0.76-0.81)	<b>0.75 (0.71-0.78)</b>
Number of portal tracts < 11 (n = 1006)	<b>0.90 (0.87-0.94)</b>	0.81 (0.76-0.87)	0.76 (0.70-0.83)
Number of portal tracts ≥ 11 (n = 851)	<b>0.84 (0.81-0.88)</b>	0.77 (0.72-0.81)	0.71 (0.65-0.77)
Intermediate quality biopsy (n = 1432)	0.91 (0.88-0.93)	0.83 (0.80-0.86)	<b>0.83 (0.78-0.87)</b>
High quality biopsy (n = 670)	0.87 (0.83-0.90)	0.87 (0.83-0.90)	<b>0.69 (0.62-0.76)</b>
BMI < 25 kg/m <sup>2</sup> (n = 868)	<b>0.93 (0.91-0.95)<sup>#</sup></b>	<b>0.84 (0.80-0.88)</b>	0.77 (0.69-0.84)
25 kg/m <sup>2</sup> ≤ BMI < 30 kg/m <sup>2</sup> (n = 2127)	<b>0.92 (0.91-0.94)<sup>*</sup></b>	0.82 (0.78-0.85)	<b>0.83 (0.80-0.86)</b>
BMI ≥ 30 kg/m <sup>2</sup> (n = 2710)	<b>0.87 (0.85-0.89)<sup>*,#</sup></b>	<b>0.77 (0.75-0.80)</b>	<b>0.73 (0.69-0.76)</b>
Continent – Europe (n = 3560)	0.90 (0.89-0.92)	0.80 (0.78-0.82)	0.77 (0.74-0.81)
Continent - Asia (n = 1278)	0.92 (0.89-0.94)	0.81 (0.77-0.85)	0.80 (0.75-0.85)
Sex – Male (n = 3165)	0.91 (0.89-0.92)	0.81 (0.78-0.83)	<b>0.80 (0.77-0.83)</b>
Sex – Female (n = 2570)	0.89 (0.88-0.91)	0.78 (0.76-0.81)	<b>0.74 (0.71-0.78)</b>
Presence of T2DM (n = 2191)	<b>0.85 (0.83-0.87)</b>	<b>0.74 (0.72-0.77)</b>	<b>0.70 (0.67-0.70)</b>
Lack of T2DM (n = 2763)	<b>0.94 (0.92-0.95)</b>	<b>0.85 (0.83-0.88)</b>	<b>0.80 (0.76-0.84)</b>
ALT < 40 U/L (n = 1656)	0.91 (0.89-0.93)	0.79 (0.75-0.83)	0.77 (0.73-0.82)
40 U/L ≤ ALT < 100 U/L (n = 2933)	0.90 (0.88-0.92)	0.80 (0.78-0.83)	0.77 (0.74-0.80)
ALT ≥ 100 U/L (n = 984)	0.90 (0.87-0.93)	0.79 (0.75-0.84)	0.82 (0.76-0.88)
AST < 40 U/L (n = 2759)	0.90 (0.88-0.92)	0.78 (0.75-0.81)	0.80 (0.77-0.84)
40 U/L ≤ AST < 100 U/L (n = 2385)	0.89 (0.87-0.91)	0.78 (0.76-0.81)	0.75 (0.72-0.79)
AST ≥ 100 U/L (n = 373)	0.90 (0.86-0.94)	0.77 (0.71-0.84)	0.75 (0.66-0.84)
ALT < 40 U/L AND AST < 40 U/L (n = 1323)	0.91 (0.89-0.93)	0.76 (0.72-0.81)	0.75 (0.69-0.80)
ALT ≥ 40 U/L OR AST ≥ 40 U/L (n = 4235)	0.90 (0.89-0.91)	0.80 (0.78-0.82)	0.79 (0.76-0.81)
Age < 43 yrs (n = 1401)	<b>0.97 (0.95-0.99)<sup>*,#,%</sup></b>	0.82 (0.75-0.88)	0.72 (0.55-0.89)
43 yrs ≤ Age < 54 yrs (n = 1478)	<b>0.90 (0.87-0.93)<sup>*</sup></b>	0.77 (0.72-0.82)	0.74 (0.67-0.81)
54 yrs ≤ Age < 62 yrs (n = 1423)	<b>0.87 (0.85-0.90)<sup>#</sup></b>	0.75 (0.71-0.78)	0.74 (0.69-0.78)
62 yrs ≤ Age (n = 1430)	<b>0.86 (0.84-0.89)<sup>%</sup></b>	0.72 (0.69-0.76)	0.66 (0.62-0.71)

Bold AUCs within a column and subgroup category are significantly different. Bonferroni's correction was applied when performing multiple comparisons. AUCs marked with <sup>\*</sup>, <sup>#</sup> or <sup>%</sup> are pairwise significantly different.

**Supporting Table 21** Subgroup analysis on the impact of reliability of liver stiffness measurements (LSM) on diagnostic performance in detecting advanced fibrosis.

	AUROC (95% CI) for diagnosing advanced fibrosis	AUROC (95% CI) for diagnosing cirrhosis
Reliable LSM by VCTE (median LSM < 7.1 kPa OR (median LSM ≥ 7.1 kPa AND IQR/median LSM < 0.30)	<b>0.86 (0.85-0.87)</b>	<b>0.91 (0.90-0.92)</b>
Unreliable LSM by VCTE (median LSM ≥ 7.1 kPa AND IQR/median LSM > 0.30)	<b>0.75 (0.70-0.80)</b>	<b>0.81 (0.76-0.86)</b>
Reliable LSM by VCTE (IQR/median LSM < 0.30)	0.86 (0.84-0.87)	0.90 (0.89-0.92)
Unreliable LSM by VCTE (IQR/median LSM ≥ 0.30)	0.84 (0.82-0.86)	0.88 (0.86-0.91)

VCTE – vibration-controlled transient elastography; 95% confidence intervals were estimated using 500 bootstrap iterations. Bold AUCs within a column and subgroup category are significantly different (p < 0.05).

**Supporting Table 22** Subgroup analysis based on choice of probe type (in patients with data available from both probes) compared to the diagnostic accuracy of LSM by VCTE calculated in the entire IPD cohort.

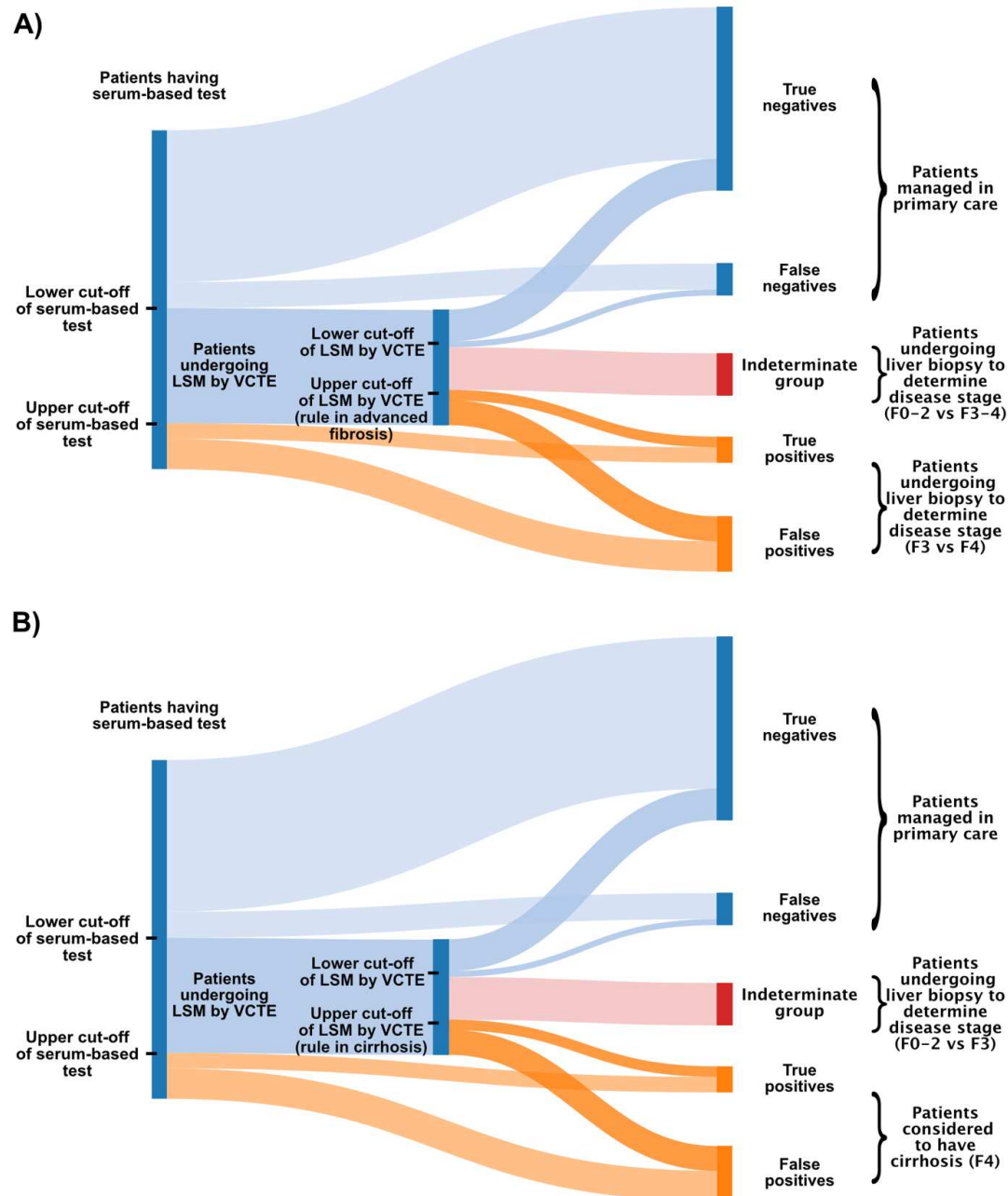
	AUROC (95% CI) for diagnosing advanced fibrosis	AUROC (95% CI) for diagnosing cirrhosis
Entire cohort (n = 5489)	0.85 (0.84-0.86)	0.90 (0.89-0.91)
M probe only (where measurements performed with both probes were performed) (n = 799)	0.84 (0.82-0.87)	0.86 (0.83-0.90)
XL probe only (where measurements performed with both probes were performed) (n = 799)	0.83 (0.80-0.86)	0.87 (0.84-0.90)

**Supporting Table 23** Sensitivity analysis on the impact of probe selection on diagnostic performance in detecting advanced fibrosis. Thresholds were calculated from the entire IPD cohort.

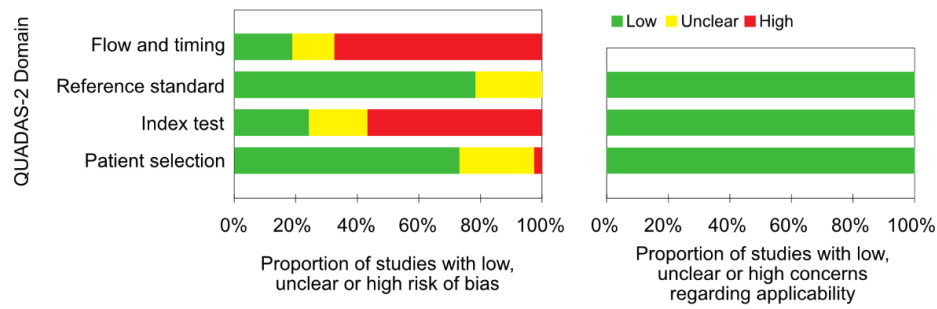
	All patients with LSM (n = 5489)			Patients with BMI < 30 kg/m <sup>2</sup> and M probe OR BMI ≥ 30 kg/m <sup>2</sup> and XL probe (n = 4464)		
AUC (95% CI)	0.85 (0.84-0.86)			0.86 (0.85-0.87)		
Thresholds, kPa	9.1	< 7.4	≥ 12.1	9.1	< 7.4	≥ 12.1
Sensitivity, %	77 (75-79)	90 (89-91)	55 (52-57)	75 (72-78)	89 (87-91)	53 (50-56)
Specificity, %	78 (76-79)	60 (59-61)	90 (89-91)	81 (79-82)	65 (63-67)	92 (91-93)
Misclassified, %	22 (22-23)	31 (31-32)	21 (20-21)	21 (20-22)	28 (27-29)	20 (18-21)

95% confidence intervals were estimated with 500 bootstrap replicates.

## Supporting Figures



**Supporting Figure 1** “Traditional” (A) and newly proposed two-tier algorithms (B) for using non-invasive tests in clinical care. (A) In the traditional application of NITs, patients with NIT values below the lower cut-offs are “ruled out” and are managed in primary care. Those with indeterminate NIT values and those “ruled in” with values above the upper cut-offs still need to undergo liver biopsy in order to stage their disease. Patients with indeterminate NITs need a liver biopsy to rule out advanced fibrosis, while patients ruled in for advanced fibrosis still need a biopsy to diagnose cirrhosis, as those with cirrhosis are managed differently (they need surveillance for hepatocellular cancer and screening for oesophageal varices). (B) In the proposed algorithms we use upper cut-off values to rule in cirrhosis, where those who are ruled in are thereby managed as having cirrhosis without the need for liver biopsy. Patients in the indeterminate group still require biopsy to correctly stage their disease.

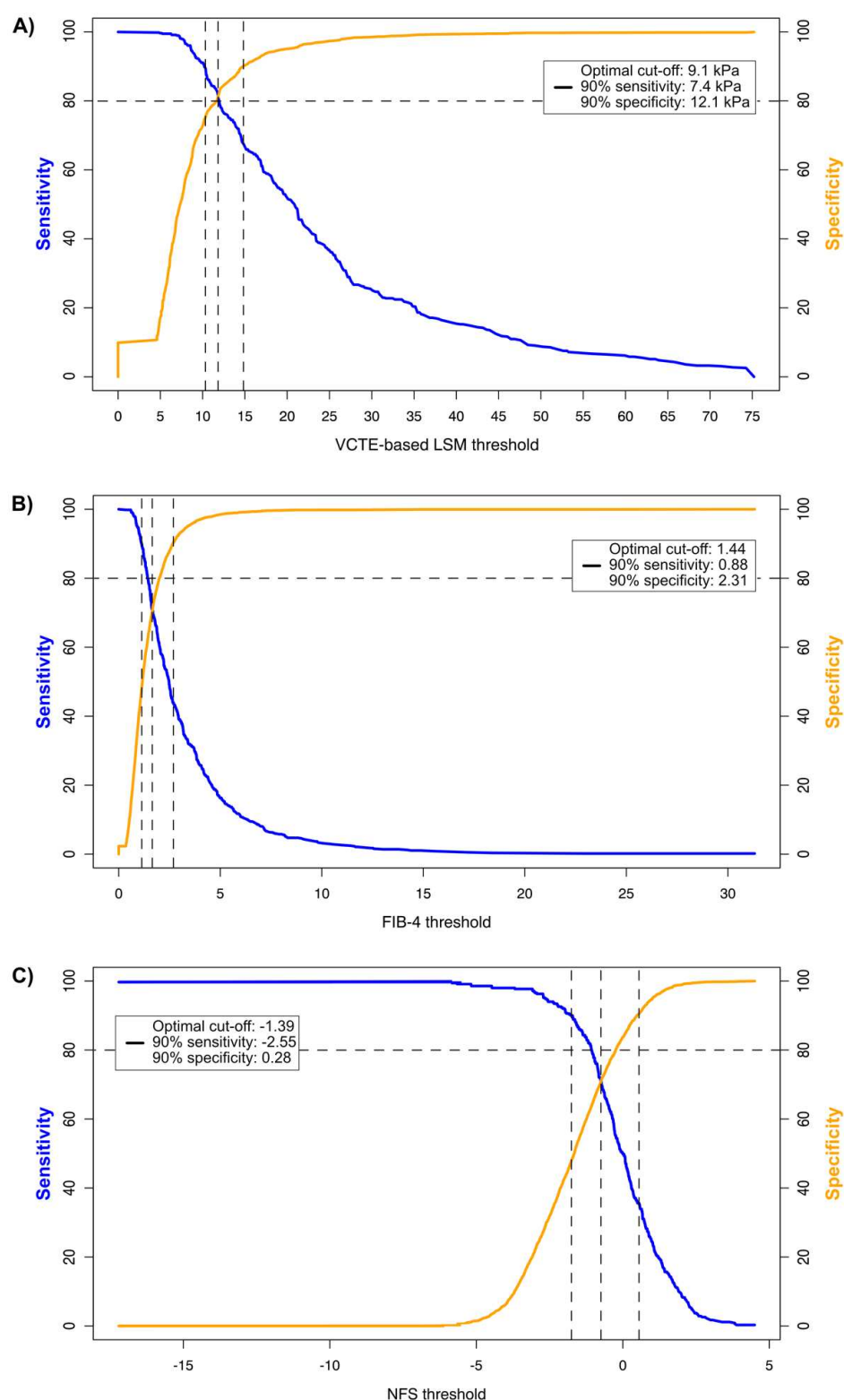


Supporting Figure 2 Risk of bias and applicability concerns

Study ID	Risk of bias				Applicability concerns		
	Patient selection	Index test	Reference test	Flow and timing	Patient selection	Index test	Reference test
Agrawal 2017	+	-	+	-	+	+	+
Aykut 2014	?	?	?	?	+	+	+
Boursier 2016	+	-	+	-	+	+	+
Boursier 2017	?	?	?	+	+	+	+
Boursier 2018	?	?	?	?	+	+	+
Cassinotto 2013	+	-	+	-	+	+	+
Cassinotto 2016	+	-	+	-	+	+	+
Chan 2015	+	+	+	-	+	+	+
Chan 2017	-	-	+	-	+	+	+
Eddowes 2016	?	?	+	+	+	+	+
Eddowes 2018	?	?	+	-	+	+	+
Eddowes 2019	+	-	+	-	+	+	+
Gaia 2011	+	-	+	?	+	+	+
Garg 2018	+	-	+	-	+	+	+
Karlas 2015	?	?	+	-	+	+	+
Kwok 2016	+	+	+	-	+	+	+
Labenz 2018	+	+	?	-	+	+	+
Lee 2017	+	-	+	-	+	+	+
Loong 2017	+	+	+	-	+	+	+
Lupsor 2010	?	-	+	-	+	+	+
Mahadeva 2013	+	-	+	?	+	+	+
Okajima 2017	+	-	?	-	+	+	+
Ooi 2018	+	?	+	+	+	+	+
Pavlidis 2017	+	-	+	-	+	+	+
Petta 2015 Liv Int	+	+	+	-	+	+	+
Petta 2015 Hepatol	+	-	+	-	+	+	+
Petta 2017 APT	+	-	+	?	+	+	+
Petta 2017 Hepatol	+	+	+	+	+	+	+
Seki 2017	+	-	?	+	+	+	+
Shen 2015	+	-	+	-	+	+	+
Staufer 2019	+	+	+	-	+	+	+
Wong 2010	+	-	+	-	+	+	+
Wong 2012	+	-	+	-	+	+	+
Wong 2019	+	+	+	-	+	+	+
Yoneda 2008	?	-	?	-	+	+	+
Younes 2018	?	+	+	+	+	+	+
Ziol 2009	+	-	?	+	+	+	+

Low 
 High 
 Unclear

Supporting Figure 3 Methodological quality summary



**Supporting Figure 4** Distribution of sensitivities and specificities over the possible threshold ranges for LSM by VCTE (A), FIB-4 (B) and NFS (C) when considering the diagnosis of cirrhosis. Horizontal dashed lines are representing the minimum acceptable criteria for considering a test as having high sensitivity ( $\geq 80\%$ ) and high specificity ( $\geq 80\%$ ).

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