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Key words: NAFLD, fibrosis, blood test, VCTE, non-invasive diagnosis, algorithm

Background & Aims: Advanced liver fibrosis is an important diagnostic target in non-alcoholic fatty liver disease (NAFLD) as it defines the subgroup of patients with impaired prognosis. The non-invasive diagnosis of advanced fibrosis is currently limited by the suboptimal positive predictive value and the grey zone (representing indeterminate diagnosis) of fibrosis tests. The combination of fibrosis tests significantly improves the diagnosis of liver fibrosis in chronic hepatitis C. Here, we aimed to determine the best combination of non-invasive tests for the diagnosis of advanced fibrosis in NAFLD.

Methods: A total of 938 patients with biopsy-proven NAFLD were randomized 2:1 into derivation and validation sets. All patients had liver stiffness measurement with vibration controlled transient elastography (VCTE), blood fibrosis tests (NAFLD fibrosis score, FIB-4, Fibrotest, Hepascore, FibroMeter), and calculation of FibroMeter^{VCTE}, which combines VCTE results and FibroMeter markers in a single test.

Results: For the diagnosis of advanced fibrosis, VCTE was significantly more accurate than the blood tests (area under the receiver operating characteristic curve [AUROC]: 0.840±0.013, p≤0.005) and among these latter, FibroMeter was the most accurate (AUROC: 0.793±0.015, p≤0.017). The combinatory test FibroMeter^{VCTE} outperformed VCTE and blood tests (AUROC: 0.866±0.012, p ≤0.005). The sequential combination of FIB-4 then FibroMeter^{VCTE} (FIB-4-FM^{VCTE} algorithm) or VCTE then FibroMeter^{VCTE} (VCTE-FM^{VCTE} algorithm) provided an excellent 90% diagnostic accuracy for advanced fibrosis with a very low 20% rate of required liver biopsy. The FIB-4-FM^{VCTE} and VCTE-FM^{VCTE} algorithms were significantly more accurate than the pragmatic algorithms currently proposed.

Conclusion: The sequential combination of fibrosis tests in the FIB-4-FM^{VCTE} and VCTE-FM^{VCTE} algorithms provides a highly accurate solution for the diagnosis of

advanced fibrosis in NAFLD. These algorithms should be now validated for case finding of advanced liver fibrosis in diabetology or primary care settings.

Lay summary

Liver fibrosis evaluation is mandatory in NAFLD as advanced fibrosis identifies the subgroup of patients with impaired prognosis. FibroMeter^{VCTE} is a new fibrosis test combining blood markers (aspartate aminotransferase, gamma-GT, prothrombin time, platelets, alpha2-macroglobulin) and VCTE result in a single diagnostic test. Our results show that FibroMeter^{VCTE} outperforms the others blood fibrosis tests and VCTE for the diagnosis of advanced fibrosis in a large multi-centric cohort of 938 biopsy-proven NAFLD patients. Sequential algorithms using a simple blood test (FIB-4) or VCTE as a first-line procedure, then FibroMeter^{VCTE} as a second-line test well classified 90% of patients for advanced fibrosis, with only 20% liver biopsy requirement.

Introduction [H1]

Non-alcoholic fatty liver disease (NAFLD), the liver manifestation of the metabolic syndrome linked to obesity and insulin resistance, affects 25% of the general population both in western and developing countries (1). As in the other causes of chronic liver disease, liver fibrosis is the main determinant of prognosis in NAFLD (2). The risk of liver-related mortality increases from fibrosis stage 2 and is exponentially higher when transitioning to stage F3 (bridging fibrosis) then F4 (cirrhosis) (2). Therefore, as recommended by international guidelines, patients with NAFLD should be assessed for the presence of advanced F3/4 fibrosis, because of its prognostic implications (3, 4).

Only a small number of patients with NAFLD develop advanced liver fibrosis and it is a challenge for physicians to identify them within the large NAFLD population (5). Non-invasive tests, mainly blood tests and elastography devices, are now available to facilitate the evaluation of liver fibrosis in chronic liver diseases. A recent meta-analysis showed that non-invasive fibrosis tests can accurately diagnose advanced fibrosis in NAFLD, with an area under the receiver operating characteristic curve (AUROC) around 0.80–0.85 (6). These tests have excellent negative predictive values to confidently exclude advanced fibrosis, but also have high rates of false positive results, limiting their ability to affirm the diagnosis (6). In addition, non-invasive fibrosis tests are usually used with 2 diagnostic thresholds framing a grey zone where the diagnosis remains undetermined. Several studies, mainly performed in chronic viral hepatitis, have shown that combining non-invasive fibrosis tests helps to reduce this grey zone and furthermore increases the positive predictive value of the diagnosis (7-9). For example, in the setting of chronic hepatitis C, we have developed the FibroMeter^{VCTE}, which is a combination of the result of transient elastography with the biomarkers of the blood test

FibroMeter (10). This concept of combining tests remains poorly evaluated in NAFLD. A stepwise algorithm (simple blood test first-line, specialized blood test or elastography second-line) has recently been proposed and is now presented in the slide deck of the guidelines of the European Association for the Study of the Liver (EASL) (11, 12). However, the development of this algorithm was based on a pragmatic approach and literature results, and its diagnostic accuracy has never been evaluated.

The aim of the present study was to determine the best combination of non-invasive tests for the diagnosis of advanced liver fibrosis in NAFLD, and to compare its accuracy to that of the recent EASL guidelines algorithm.

Patients and methods [H1]

Patients [H2]

Adults aged ≥18 years with biopsy-proven NAFLD were included in 4 French University Hospitals: Angers, Bordeaux, Grenoble and Toulouse. NAFLD was defined as ≥5% liver steatosis on liver biopsy after exclusion of concomitant steatosis-inducing drugs, excessive alcohol consumption (>210 g/week in men or >140 g/week in women), chronic hepatitis B or C infection, and histological evidence of other concomitant chronic liver disease. Patients were not included if they had liver complications (liver failure, encephalopathy, ascites, variceal bleeding, systemic infection or hepatocellular carcinoma). In each center, liver biopsy was performed mainly for suspected NAFLD with abnormal liver function test, hyperferritinemia, or abnormal fibrosis tests. All patients came from hepatology clinics and no biopsy was performed during bariatric surgery. The periods of inclusion were 2004-2017 for Angers, 2006-2017 for Bordeaux, 2014-2016 for Grenoble and 2015-2017 for Toulouse. The study protocol conformed to the ethical

guidelines of the current Declaration of Helsinki and was approved by the local Ethic Committees. All patients gave written informed consent before being included in the study.

Liver biopsy [H2]

Pathological examinations were performed in each center by the same senior expert specialized in hepatology and blinded to patient data. We and others have shown the excellent inter-observer reproducibility for liver fibrosis evaluation when performed by expert pathologists (13-15). Liver fibrosis was evaluated according to the non-alcoholic steatohepatitis (NASH) Clinical Research Network scoring system (13), *i.e.*, F0: no fibrosis; F1: perisinusoidal or portal/periportal fibrosis, F2: perisinusoidal and portal/periportal fibrosis, F3: bridging fibrosis and F4: cirrhosis. Advanced liver fibrosis was defined as F3/4 fibrosis stages and was the primary diagnostic target of the study.

Liver stiffness measurement [H2]

Liver stiffness measurements were performed using vibration controlled transient elastography (VCTE) technology (FibroScan® device; Echosens, Paris, France). The examinations were performed according to the manufacturer's recommendations (16), the day of or no more than 3 months before or after liver biopsy, with patients in fasting conditions. An experienced observer (>500 examinations), who was blinded to patient data, recorded 10 valid measurements. The VCTE results were expressed in kPa, as the median of these valid measurements.

Blood fibrosis tests [H2]

Fasting blood samples were taken the day of or within the week preceding liver biopsy. The following blood fibrosis tests were calculated according to published or patented formulas: NAFLD fibrosis score (NFS) (17), Fibrosis-4 (FIB-4) (18), Fibrotest (19), Hepascore (20), FibroMeter^{V2G} (FM) (21), and FibroMeter^{VCTE3G} (FM^{VCTE}) (10). The last of which is a new fibrosis test that combines, in a single formula, age, sex, the result of liver stiffness measured by VCTE, and the blood markers of FM (aspartate aminotransferase, gamma-GT, platelet count, prothrombin time, alpha-2-macroglobulin). All blood assays were performed in the laboratories of the investigating centers. We have previously demonstrated the excellent inter-laboratory reproducibility of blood fibrosis tests (22).

EASL guidelines algorithm [H2]

The EASL guidelines algorithm uses a simple blood test, either NFS or FIB-4, as the first-line procedure (Fig. 1): NFS <-1.455 or FIB-4 <1.30 rules out advanced fibrosis, whereas NFS >0.676 or FIB-4 >3.25 indicates a high risk of advanced fibrosis requiring confirmation by liver biopsy. Following previously published data (23), the algorithm recommends using age-specific cut-offs to rule out advanced fibrosis in patients aged >65 years (<0.12 for NFS, <2.0 for FIB-4). Should the first-line test give an intermediate result (in the grey zone), a second-line evaluation with a specialized blood test or elastography is performed.

Statistical analysis [H2]

Identification of the best-performing fibrosis tests – The diagnostic accuracy of the fibrosis tests was evaluated using the AUROC and the Obuchowski index. The

Obuchowski index is a multinomial version of the AUROC adapted to ordinal references such as pathological fibrosis staging (24). This index measures the probability that 2 randomly chosen patients from different fibrosis stages are correctly classified, with a penalty for incorrect classification (1 when the difference between stages is 1, 2 when the difference is 2, *etc.*).

New algorithm development – The study population was randomized 2:1 into derivation and validation sets. Two diagnostic cut-offs, corresponding to the 90% sensitivity and 90% specificity thresholds for advanced fibrosis, were calculated in the derivation set for the best-performing fibrosis tests. If a positive predictive value (PPV) ≥80% was not reached with the 90% specificity threshold, a 95% specificity threshold was calculated. Fibrosis tests were combined according to their ease of use: the simplest as a first-line test and the most complex as a second-line test. Finally, the diagnostic accuracy of the algorithm was evaluated in the validation set.

Statistical analyses were performed using SPSS version 18.0 software (IBM, Armonk, NY, USA). Results are reported in accordance with the recently published LiverFibroSTARD statements (25).

Results [H1]

Patients [H2]

The characteristics of the 938 patients included in the study are detailed in Table 1. A total of 396 patients were included in Angers, 441 in Bordeaux, 61 in Toulouse and 40 in Grenoble. Mean age was 56.5 ± 12.1 years, mean body mass index was 31.8 ± 5.8 kg/m^2, half of the patients were diabetic and 58.5% were male. Mean biopsy length was 27 ± 12 mm (median: 26 mm; 1st quartile: 19 mm; 3rd quartile: 33 mm) and

89.0% of the liver biopsies were ≥15 mm in length. The median VCTE result was 8.9 kPa (1st quartile: 6.3 kPa; 3rd quartile: 13.8 kPa). Bridging F3 fibrosis was present in 27.4% of patients and cirrhosis in 13.4%.

Comparison of fibrosis tests [H2]

We first evaluated the most validated fibrosis tests used with their published cut-offs (NFS: -1.455 and 0.676, FIB-4: 1.30 and 3.25, VCTE: 7.9 and 9.6 kPa). NFS had good sensitivity (85.4%) and negative predictive value (NPV) (81.9%), but insufficient PPV (70.6%) (Table S1). FIB-4 had good PPV (82.9%), but <80% sensitivity and NPV. VCTE had excellent sensitivity and NPV (>90%), included many less patients than blood tests in the grey zone between the 2 diagnostic thresholds (16.3% *vs.* 49.7% for NFS and 47.8% for FIB-4; both p <0.001), but had insufficient 68.5% PPV.

The comparison of AUROCs for the diagnosis of advanced fibrosis showed that FM was significantly more accurate than other blood fibrosis tests, and that VCTE was significantly more accurate than all blood tests (Table 2, see Table S2 for pairwise comparisons). The combinatorial test FM^{VCTE} was significantly more accurate than FM alone or VCTE alone. The same results were obtained when the AUROCs for the other diagnostic targets (F≥2 and cirrhosis) were compared, and when Obuchowski indexes were compared. Therefore, FM, VCTE, and their combination in FM^{VCTE} were selected to develop the new study algorithms, as well as NFS and FIB-4 which are the most validated blood fibrosis tests in the literature.

New diagnostic algorithms for advanced liver fibrosis in NAFLD [H2]

The characteristics of the patients in the derivation and validation sets did not differ significantly (Table 1). In the derivation set, the 90% sensitivity thresholds of NFS, FIB-4, FM, VCTE, and FM^{VCTE} were -1.669, 1.04, 0.26, 8.0 kPa and 0.32, respectively. Using these cut-offs, advanced fibrosis was ruled out with an NPV of 85–90% (Table S3). FM^{VCTE} attained the objective of a >80% PPV (81.5% PPV) using its 90% specificity threshold (0.69). However, the 4 other tests did not attain that objective (Table S3). Therefore, for these tests, we calculated the 95% thresholds (0.927 for NFS, 2.67 for FIB-4, 0.77 for FM and 15.7 kPa for VCTE). Using the 95% specificity threshold, FM and VCTE reached the >80% PPV objective (80.8% and 83.7%, respectively), whereas PPV was 78.3% for FIB-4 and only 74.4% for NFS.

We have previously shown that an interquartile range/median ratio (IQR/M) >0.30 in intermediate/high VCTE results indicates an unreliable VCTE examination with poor diagnostic accuracy (26). In the derivation set, the rates of advanced fibrosis in patients with VCTE results <8.0 kPa (false negatives) did not significantly differ between IQR/M ≤0.30 and IQR/M >0.30 (10.6% *vs.* 6.9%, $p = 0.749$; Table 3). In contrast, they significantly differed in patients with VCTE results ≥8.0 kPa, with respective rates of 67.2% *vs.* 40.0% ($p < 0.001$). That same trend was obtained for FM^{VCTE} (Table 3).

Based on the results above, we designed several stepwise algorithms for the diagnosis of advanced fibrosis in NAFLD (Fig. S1): blood tests as a first-line procedure then VCTE in second-line (NFS-VCTE, FIB-4-VCTE and FM-VCTE algorithms); blood tests then FM^{VCTE} (NFS-FM^{VCTE}, FIB-4-FM^{VCTE}, FM-FM^{VCTE} algorithms); VCTE then FM^{VCTE} (VCTE-FM^{VCTE} algorithm). The accuracy of these algorithms in the derivation set is detailed in Table S4 (see Table S5 for contingency tables).

Validation of the new algorithms [H2]

In the validation set, results showed that using VCTE as a second-line test decreased the need for liver biopsy by 2-fold compared to single tests, while maintaining high diagnostic accuracy (Table 4). Using FM^{VCTE} instead of VCTE as a second-line test reduced the need for liver biopsy even more: NFS-VCTE required 32.9% liver biopsy *vs.* 20.1% with NFS-FM^{VCTE} (p <0.001, 39% decrease), FIB-4-VCTE required 30.4% liver biopsy *vs.* 21.1% with FIB-4-FM^{VCTE} (p <0.001, 31% decrease), and FM-VCTE required 27.5% liver biopsy *vs.* 19.2% with FM-FM^{VCTE} (p = 0.001, 30% decrease). These results demonstrate the interest of FM^{VCTE} as a second-line specialized fibrosis test rather than VCTE alone.

Among the 4 algorithms using FM^{VCTE} as a second-line procedure, the VCTE-FM^{VCTE} provided the highest diagnostic accuracy (89.8%) and the lowest rate of second-line test requirement (46.3%, Table 4). Conversely, the NFS-FM^{VCTE} provided the lowest diagnostic accuracy (85.6%) and the highest rate of FM^{VCTE} requirement (63.6%). Despite FM having a significantly higher AUROC (Table 2) and a lower grey zone than FIB-4 for advanced fibrosis (Table 4), this did not translate into a significantly different diagnostic accuracy or rate of liver biopsy requirement between FM-FM^{VCTE} and FIB-4-FM^{VCTE} algorithms. Considering the advantages of FIB-4 and VCTE (no additional cost for the former, immediate result during the consultation for the latter), we selected the FIB-4-FM^{VCTE} and the VCTE-FM^{VCTE} algorithms (Fig. 2) for further analyses. FIB-4-FM^{VCTE} and VCTE-FM^{VCTE} had excellent diagnostic accuracy for advanced fibrosis in the validation population, correctly classifying 90% of patients, with 85% sensitivity, 90% specificity, 90% NPV, 85% PPV, and a requirement for liver biopsy in only 20% of patients (Table 4). The "FM^{VCTE} for all" strategy significantly increased sensitivity to 90%

but required both blood markers and VCTE for all patients and significantly increased the liver biopsy requirement to 28.4%.

The diagnostic accuracy of FIB-4-FM^{VCTE} and VCTE-FM^{VCTE} algorithms was not significantly different between the derivation and the validation sets. In multivariate analysis (adjusted on age, sex, body mass index, diabetes, derivation/validation set, F3/4, biopsy length, and AST), neither the period of liver biopsy (2004-2009 *vs.* 2010-2013 *vs.* 2014-2017) nor the investigating center were independently associated with diagnostic accuracy of the FIB-4-FM^{VCTE} or the VCTE-FM^{VCTE} algorithms (detailed data not shown).

Comparison to the EASL guidelines algorithm [H2]

Age-specific cut-offs were recently proposed for NFS (<0.12) and FIB-4 (<2.0) in patients aged ≥65 years (23). In the subgroup of patients aged ≥65 years and using these age-specific cut-offs, advanced fibrosis was ruled out for 50% of patients and specificity was increased from 15–25% to 50–60% (Table S6). However, there was an important concomitant decrease in sensitivity, from 90% to 60%. When considering the whole population, using the age-specific cut-offs led to a >10% decrease in sensitivity to only 72.6% for NFS and 66.8% for FIB-4.

As there was no significant difference in diagnostic accuracy for both the FIB-4-FM^{VCTE} and VCTE-FM^{VCTE} algorithms between the derivation and validation sets, we compared them with the EASL guidelines algorithm in the whole study population. According to the diagnostic tests used, the guidelines algorithm had 80–85% diagnostic accuracy, 50–70% sensitivity, 100% specificity, 75–80% NPV, 100% PPV, and 30–45% liver biopsy requirement (Table 5). Compared to the guidelines algorithm, both FIB-4-

FM^{VCTE} and VCTE-FM^{VCTE} showed greater accuracy and sensitivity for advanced fibrosis, and required fewer liver biopsies (Table 5). We also evaluated “modified” EASL algorithms where VCTE was used to confirm the diagnosis of advanced fibrosis suggested by the first-line blood test NFS or FIB-4, and liver biopsy was performed only when the diagnosis remained undetermined after VCTE evaluation (Fig. S2). Compared to these modified EASL algorithms, FIB-4-FM^{VCTE} and VCTE-FM^{VCTE} algorithms showed significantly higher diagnostic accuracy, sensitivity and NPV for advanced fibrosis (Table 5).

Discussion [H1]

Liver fibrosis must be accurately evaluated to assess the severity of NAFLD (3, 4), a pathology now affecting 25% of the general population (1). In such a large patient set, non-invasive tests of liver fibrosis are a very attractive option. These non-invasive tests include simple blood tests using common parameters available to all physicians, more specialized blood tests using costly but more accurate direct markers of liver fibrosis, and elastography devices (27). In the present study, we have extended the concept of combining tests previously developed in chronic viral hepatitis to NAFLD, demonstrating that the association of the blood test FM with VCTE in the FM^{VCTE} algorithm provides a powerful solution for the diagnosis of advanced fibrosis (10). We thus developed an algorithmic approach wherein VCTE or FIB-4 may be followed by the combinatory FM^{VCTE}. This approach correctly classified 90% of patients and reduced the requirement for liver biopsy to only 20%. The strengths of our study were the large sample of nearly a thousand patients with NAFLD and high quality liver biopsies, and the large panel of non-invasive tests including simple blood tests, specialized blood tests and VCTE,

through which we were able to identify the best combinations for advanced fibrosis diagnosis in NAFLD.

FM and VCTE were the most accurate fibrosis tests in our study. FM^{VCTE}, which is a combination of the blood markers of FM and the results of VCTE, gave even greater diagnostic accuracy, as it had already done in the setting of chronic hepatitis C where it was developed (10). Because the pathophysiological processes of liver fibrosis are the same whatever the type of liver injury, this suggests that biomarkers directly and closely linked to this lesion are of interest interest in all chronic liver diseases. FM^{VCTE} does however require both blood sampling and VCTE examination. That aspect could represent a limitation for feasibility in clinical practice considering the few VCTE devices available for the large population of patients with NAFLD requiring evaluation. We therefore decided to develop a sequential algorithmic approach starting with a single fibrosis test, either FIB-4 or VCTE. This has 2 advantages. First, as shown by our results, advanced fibrosis can be ruled out in a large proportion of patients with only the first-line test (FIB-4 or VCTE), with no need to continue to the FM^{VCTE} step. Second, physicians can choose the algorithm that is best suited to the locally available resources. When available, VCTE is very attractive as a first-line procedure because it gives an immediate result after a quick and easy-to-perform examination, and thus enables decisions during the consultation. In contrast, the advantage of FIB-4 is that it induces no additional cost as serum aminotransferase and platelet counts are part of the basic liver evaluation. In both cases, should the entry result be indeterminate, moving on to the second step in the algorithm requires performing the FM^{VCTE}, which is the best-performing non-invasive test. In this context, using FM^{VCTE} instead of VCTE alone reduced the need for liver biopsy by a further 30%, emphasizing the value of this test as

a second-line procedure in our study algorithms. As FM^{VCTE} rules advanced fibrosis in or out in half of the patients who reach the second step of the algorithm, the final rate of required liver biopsy is very low, around 20% in our work.

The present study performed in a large population of patients with NAFLD further validates our previously published reliability criteria for VCTE examination (26). Indeed, we confirmed here that an IQR/M ratio >0.30 is associated with a significant decrease in diagnostic accuracy, but only in patients with increased liver stiffness. Thus, it appears that reliability criteria based only on IQR/M without consideration for the level of liver stiffness erroneously exclude reliable examinations and artificially increase the rate of unreliable examinations.

Petta *et al.* recently proposed a combination of non-invasive tests in NAFLD but, in addition to VCTE, they only had simple blood tests in their dataset (28). In their work, they found that NFS and FIB-4 as first-line tests had insufficient 70-75% sensitivity and thus recommended in their final algorithm to perform the second-line VCTE even when the simple blood tests gave negative results. This required the use of VCTE in 90% of the cases, which would seem to decrease the utility of the first-line evaluation with blood tests. The EASL guidelines algorithm is a combination of fibrosis tests based on a pragmatic approach and literature results (11, 12). The guidelines algorithm starts with NFS or FIB-4 used with age-specific cut-offs recently published (23). In the subgroup of patients aged ≥65 years, our results showed that these cut-offs did increase specificity, but at the price of a dramatic decrease in sensitivity. When considering the whole population, the age-specific cut-offs decrease the sensitivities of NFS and FIB-4 to respectively 72.6% and 66.8%. Added to the false-negative results of the second-line procedure, the overall sensitivity of the guidelines algorithm was insufficient, around 50-

70%. The guidelines algorithm also recommends considering liver biopsy to confirm the diagnosis of advanced fibrosis when the non-invasive tests are positive. It seems to us that this very strict attitude could be refined, since some fibrosis tests can reach an excellent 90% PPV in a significant proportion of patients (29). Our FIB-4-FM^{VCTE} and VCTE-FM^{VCTE} algorithms circumvents these limitations. First, our algorithmic approach demonstrates that using an accurate test as a first-line procedure helps to rule out advanced fibrosis in a large proportion of patients while maintaining high sensitivity. Second, our approach shows it is possible to rule in advanced fibrosis with very good PPV and thus no need for a confirmatory liver biopsy. Finally, our approach provides better diagnostic accuracy and a lower rate of liver biopsy requirement than the EASL algorithm.

For use as a first-line procedure, VCTE or specialized blood tests are more expensive than simple tests. However, they are also more specific, which can reduce the need of, and therefore the costs linked to second-line evaluations. This is especially the case for liver biopsy, which is a very expensive procedure. Further studies evaluating and comparing the cost-effectiveness of the different strategies will help to identify those best suited to clinical practice. Our FIB-4-FM^{VCTE} and VCTE-FM^{VCTE} algorithms are still limited by the need for liver biopsy in a small subgroup of patients. Magnetic resonance elastography was recently shown to have excellent diagnostic accuracy for liver fibrosis evaluation in chronic liver diseases (30). It would be of great interest to evaluate the use of this technology as a potential third-line exam in our algorithms, to reduce even further the need for liver biopsy in NAFLD patients.

Given their sequential approach, the FIB-4-FM^{VCTE} and VCTE-FM^{VCTE} algorithms could help organize the patient pathway between physicians involved in the

management of patients with NAFLD (diabetologists, general practitioners…) and specialized hepatologists, in order to facilitate the identification of patients with advanced liver disease requiring a specific management while avoiding unnecessary referrals of patients with mild liver disease. Because our algorithms were developed in a population coming from tertiary care centers, their use in less selected populations requires further independent validation. Our study focused on the diagnosis of advanced F3/4 fibrosis because it represents the subgroup of patients with impaired prognosis. A recent meta-analysis has shown that prognosis in NAFLD starts to decline as soon as F2 stage (2). In addition, many ongoing therapeutic trials in NAFLD target patients with NASH and F2/3 fibrosis, so called “fibrotic NASH” in the latest European guidelines (3, 31). Non-invasive tests able to diagnose fibrotic NASH will therefore be of great interest once the new drugs for NAFLD will be approved. In this context, we have recently developed the MACK-3, a blood test combining AST, HOMA and CK18, with high accuracy for the diagnosis of fibrotic NASH (15). On the other hand, cirrhosis represents the highest-risk subgroup with recommendation for the screening of hepatocellular carcinoma. When considering AUROC of fibrosis tests, data accumulated in the literature show very good accuracy for the diagnosis of cirrhosis (6). How to interpret the results of fibrosis tests to diagnose cirrhosis in NAFLD remains however to be determined.

In conclusion, the FIB-4-FM^{VCTE} and the VCTE-FM^{VCTE} algorithms are highly accurate solutions for the non-invasive diagnosis of advanced fibrosis in NAFLD. These algorithms propose either VCTE or a simple blood test as the first-line procedure, therefore providing all physicians with a solution to identify the patients who develop advanced NAFLD disease, and who therefore are candidate for inclusion in therapeutic trials and who will benefit from treatment with the new drugs when they will be available

on the market. These algorithms should be now validated for case finding of advanced liver fibrosis in diabetology or primary care settings.

Financial support [H1]

Grant support received from University Hospital of Angers.

Conflict of interest [H1]

J.B. and P.C. report consulting activities with Echosens. All other authors report no conflict of interest.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions [H1]

Jerome Boursier designed the study.

	Study design	**Data acquisition**	**Analysis**	**Drafting/ critical revision**
J. Boursier	X	X	X	X
M. Guillaume		X		X
V. Leroy		X		X
M. Irlès		X		
M. Roux			X	
A. Lannes		X		

J. Foucher		X		
F. Zuberbuhler		X		
C Delabaudière		X		
J. Barthelon		X		
S. Michalak		X		
J.B. Hiriart		X		
J.M. Péron		X		
T. Gerster		X		
B. Le Bail		X		
J. Riou			X	
G. Hunault			X	
W Merrouche		X		
F. Oberti		X		
L. Pelade		X		
I. Fouchard		X		
C. Bureau		X		
P. Calès		X		X
V. de Ledinghen		X		X

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Table 1. Patient characteristics at inclusion.

BMI: body mass index; NAS: NAFLD activity score; VCTE: vibration controlled transient elastography (Fibroscan)

Table 2. AUROCs and Obuchowski indexes of non-invasive fibrosis tests (see Table S2 for pairwise comparisons)

VCTE: vibration controlled transient elastography (Fibroscan)

Table 3. Rate of patients with advanced F3/4 fibrosis as a function of the interquartile range/median (IQR/M) ratio of VCTE examination

VCTE: vibration controlled transient elastography (Fibroscan)

Table 4. Diagnostic accuracy of study algorithms based on single tests or stepwise combinations in the validation set

DA: diagnostic accuracy (%); Se: sensitivity (%); Spe: specificity (%); NPV: negative predictive value (%); PPV: positive predictive value (%); -LR: negative likelihood ratio; +LR: positive likelihood ratio; OR: odd ratio; 2^{nd} test: rate of patients requiring the second-line fibrosis test (%); LB: rate of patients requiring liver biopsy (%); NFS: NAFLD fibrosis score; FM: FibroMeter^{V2G}; VCTE: vibration controlled transient elastography (Fibroscan); FM^{VCTE}: FibroMeter^{VCTE}

[a]See Fig. s1a. Fibrosis tests are used with their 2 thresholds calculated in the derivation set (NFS: -1.669 and 0.927; FIB-4: 1.04 and 2.67; FM:0.26 and 0.77; VCTE: 8.0 and 15.7 kPa). Liver biopsy is performed in case of result in the grey zone between the 2 thresholds

[b]See Fig. s1b. Fibrosis test are used with their 2 thresholds calculated in the derivation set. The second test is used in case of result in the grey zone of the first test, liver biopsy is performed in case of result in the grey zone of the second test

Table 5. Comparison of the FIB-4-FM^{VCTE} and the VCTE-FM^{VCTE} algorithms with the EASL guidelines algorithm

DA: diagnostic accuracy (%); Se: sensitivity (%); Spe: specificity (%); NPV: negative predictive value (%); PPV: positive predictive value (%); -LR: negative likelihood ratio; +LR: positive likelihood ratio; OR: odd ratio; 2nd test: rate of patients requiring the second-line fibrosis test (%); LB: rate of patients requiring liver biopsy (%); FM^{VCTE}: FibroMeter^{VCTE}; VCTE: Vibration Controlled Transient Elastography (Fibroscan); EASL: European Association for the Study of the Liver; NFS: NAFLD fibrosis score; FT: Fibrotest; HS: Hepascore

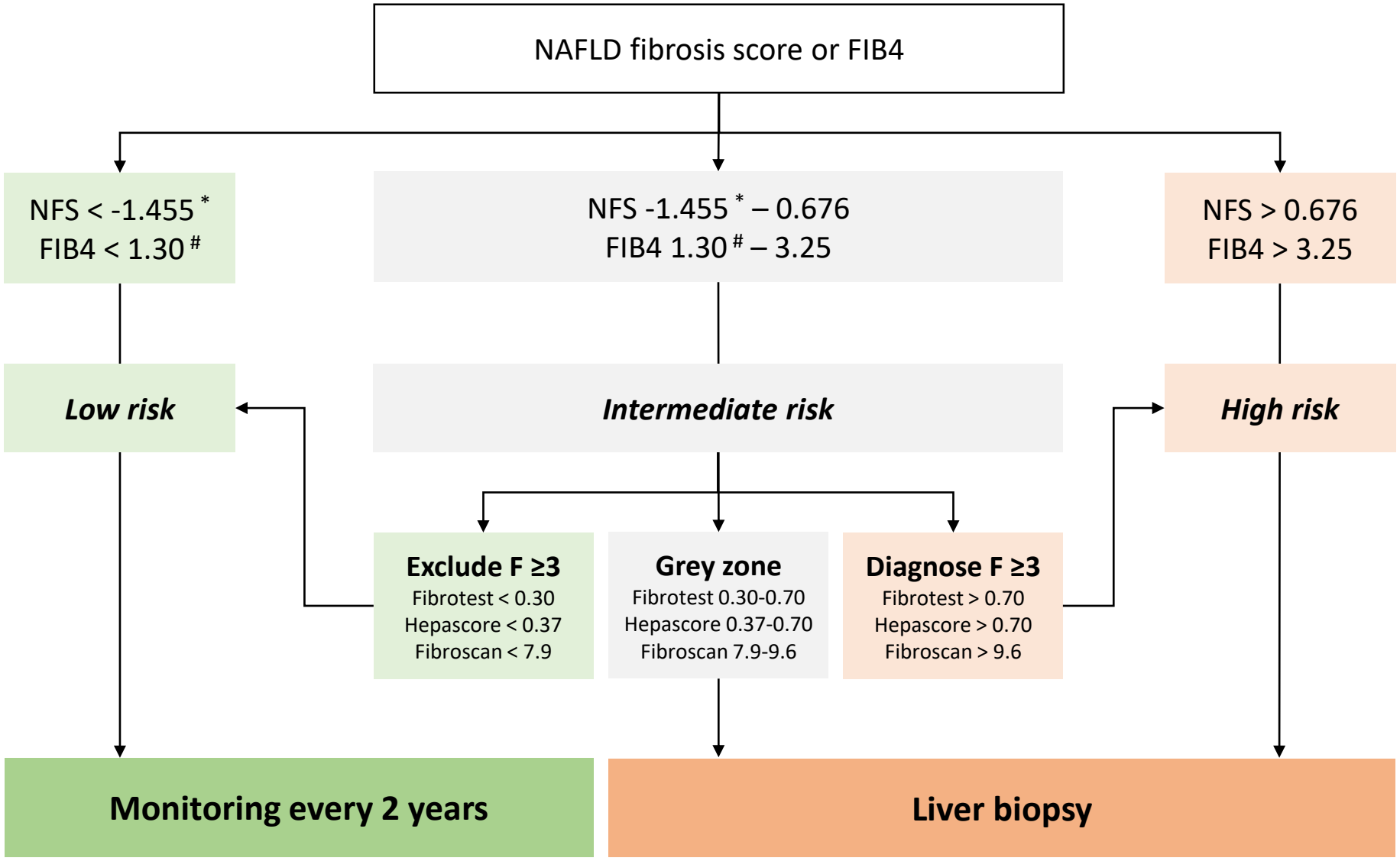
[#]See Fig. 2; [&] See Fig. 1; [§] See Fig. S2

Comparison of study algorithms *vs.* EASL or modified EASL algorithms:

[a]*p* <0.050 *vs.* others (except EASL NFS-VCTE: *p* = n.s.); [b]*p* <0.001 *vs.* others; [c]*p* = 0.033 *vs.* modified EASL FIB-4-VCTE; [d]*p* <0.001 *vs.* others (excepted *vs.* modified EASL NFS-VCTE: *p* = n.s.); [e]p ≤0.002 *vs.* others; [f]p ≤0.001 *vs.* others (excepted *vs.* EASL NFS-VCTE: *p* = n.s.); [g]*p* <0.010 *vs.* modified EASL NFS-VCTE

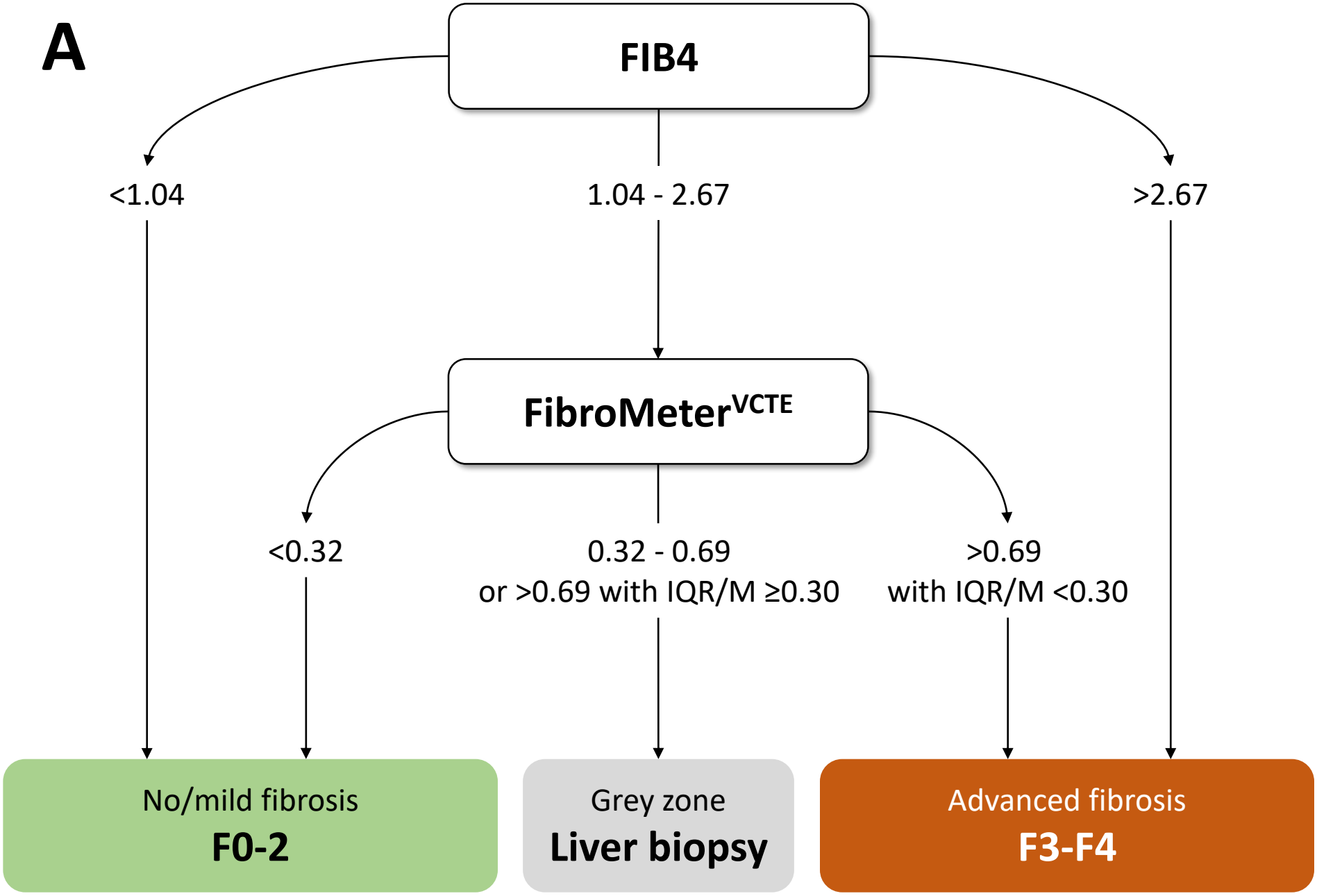
Fig. 1. Diagnostic algorithm proposed by the European Association for the Study of the Liver to noninvasively assess advanced liver fibrosis in NAFLD patients (11, 12).

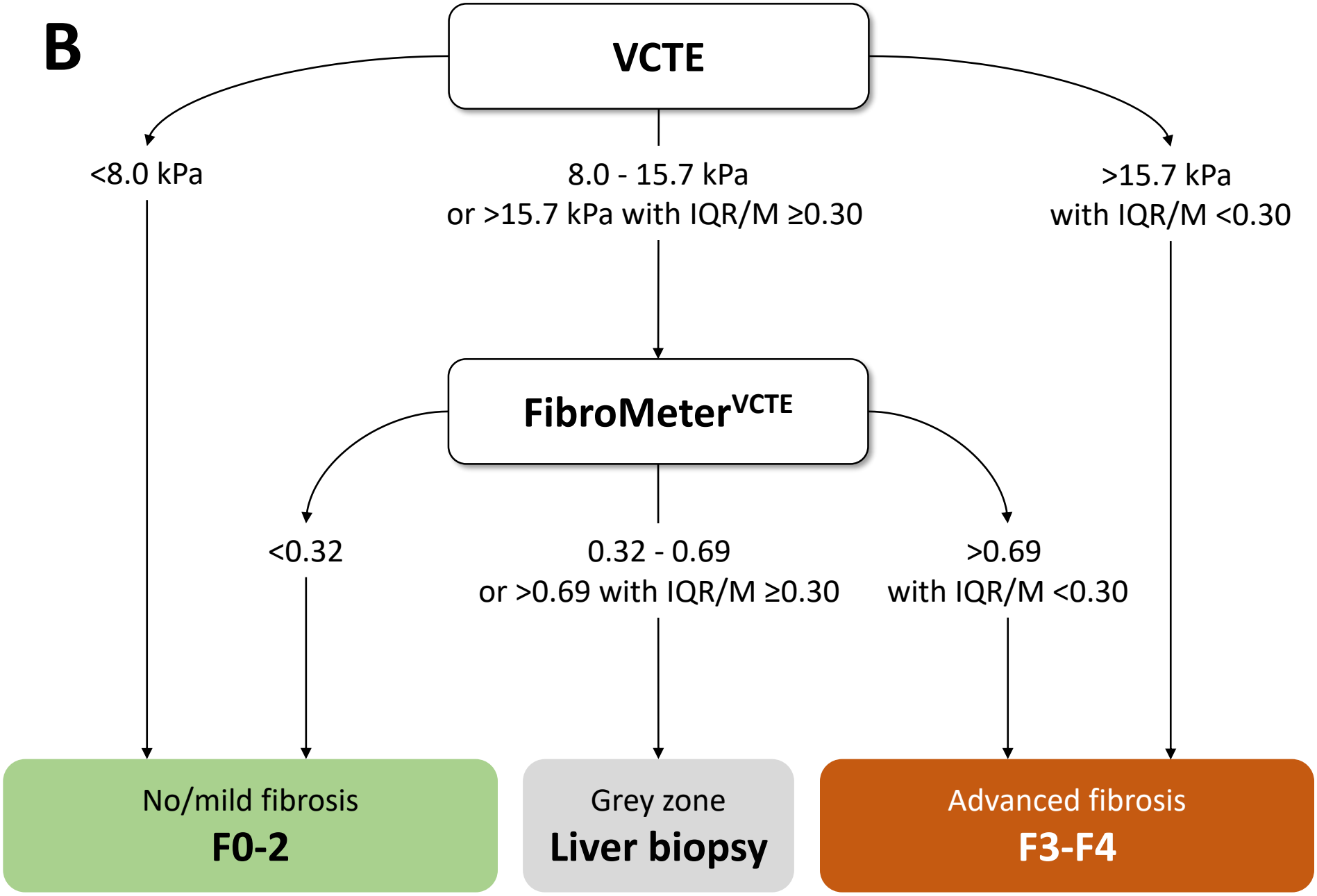
Fig. 2. FIB-4-FM^{VCTE} and VCTE-FM^{VCTE} algorithms.

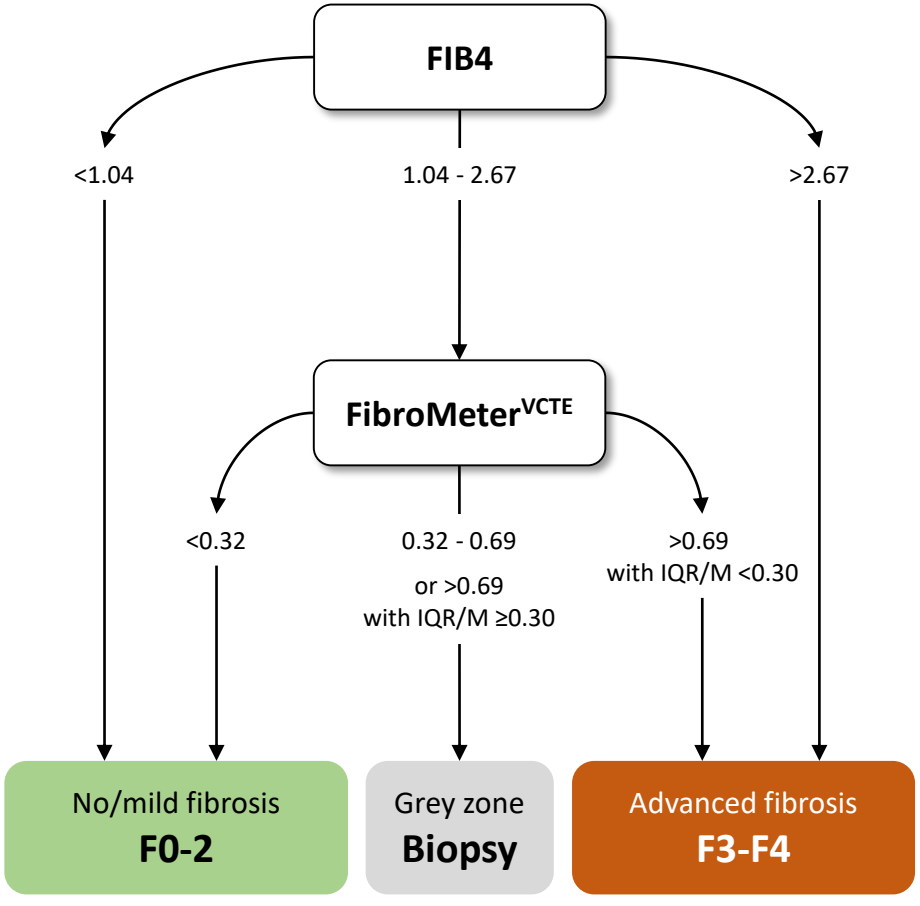


* NFS threshold: -1.455 in patients <65 years old, 0.12 in patients ≥65 years old

FIB4 threshold: 1.30 in patients <65 years old, 2.0 in patients ≥65 years old


A
FIB4
<1.04
1.04 - 2.67
>2.67
FibroMeter^VCTE
<0.32
0.32 - 0.69
or >0.69 with IQR/M ≥0.30
>0.69
with IQR/M <0.30
No/mild fibrosis
F0-2
Grey zone
Liver biopsy
Advanced fibrosis
F3-F4


B
VCTE
<8.0 kPa
8.0 - 15.7 kPa
or >15.7 kPa with IQR/M ≥0.30
>15.7 kPa
with IQR/M <0.30
FibroMeterVCTE
<0.32
0.32 - 0.69
or >0.69 with IQR/M ≥0.30
>0.69
with IQR/M <0.30
No/mild fibrosis
F0-2
Grey zone
Liver biopsy
Advanced fibrosis
F3-F4



1st line simple blood test or vibration controlled transient elastography (VCTE)

2nd line combinatory test (blood markers and VCTE)

90% diagnostic accuracy for advanced fibrosis

85% sensitivity; 90% specificity
90% negative predictive value
85% positive predictive value
20% liver biopsy