A New Combination of Blood Test and Fibroscan for Accurate Non-Invasive Diagnosis of Liver Fibrosis Stages in Chronic Hepatitis C

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OBJECTIVES: **Precise evaluation of the level of liver fibrosis is recommended in patients with chronic hepatitis C (CHC). Blood fibrosis tests and Fibroscan are now widely used for the non-invasive diagnosis of liver fibrosis. Detailed fibrosis stage classifications have been developed to provide an estimation of the liver fibrosis stage from the results of these non-invasive tests. Our aim was to develop a new and more accurate fibrosis stage classification by using new scores combining non-invasive fibrosis tests.**

METHODS: **In all, 729 patients with CHC (exploratory set: 349; validation set: 380) had liver biopsy for Metavir fibrosis (F) staging, and 6 fibrosis tests: Fibroscan, Fibrotest, FibroMeter, Hepascore, FIB-4, APRI.**

RESULTS: ***Exploratory set:*** **Fibroscan and FibroMeter were the independent predictors of different diagnostic targets of liver fibrosis. New fibrosis indexes combining FibroMeter and Fibroscan were thus developed for the diagnosis of clinically significant fibrosis (CSF-index) or severe fibrosis (SF-index). The association of CSF- and SF-indexes provided a new fibrosis stage classification (CSF/SF classification): F0/1, F1/2, F2±1, F2/3, F3±1, F4.** ***Validation set:*** **CSF/SF classification had a high diagnostic accuracy (85.8% well-classified patients), significantly higher than the diagnostic accuracies of FibroMeter (69.7%, $P<0.001$), Fibroscan (63.3%, $P<0.001$), or Fibrotest (43.9%, $P<0.001$) classifications.**

CONCLUSIONS: **The association of new fibrosis indexes combining FibroMeter and Fibroscan provides a new fibrosis stage classification. This classification is significantly more accurate than Fibrotest, FibroMeter, or Fibroscan classifications, and improves the accuracy of the non-invasive diagnosis of liver fibrosis stages to 86% without any liver biopsy.**

SUPPLEMENTARY MATERIAL is linked to the online version of the paper at http://www.nature.com/ajg

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INTRODUCTION

The prognosis of patients with chronic hepatitis C (CHC) is related to the level of fibrosis in the liver (1). Usually, liver fibrosis is evaluated on a biopsy by using semi-quantitative scores that depict the progression of fibrosis according to histological fibrosis stages. Because it has a good prognosis value in patients with CHC (2), histological fibrosis staging is recommended for the clinical management of patients (1). However, liver biopsy is an invasive procedure hampered by sample bias, and histological fibrosis staging has a poor interobserver reproducibility (3).

Several studies have shown that blood fibrosis tests and liver stiffness evaluation by transient elastography (e.g., Fibroscan) are accurate for the non-invasive diagnosis of liver fibrosis in patients with CHC (4–7). However, these studies have mainly evaluated the

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fibrosis tests only for a binary diagnosis of significant fibrosis or cirrhosis. Manufacturers or authors of Fibrotest, FibroMeter, and Fibroscan have developed *fibrosis stage classifications* derived from the results of the fibrosis test for a more precise estimation of the histological fibrosis stage (see **Supplementary Figure S1** online). In a recent study, we have shown that the diagnostic accuracy of those *fibrosis stage classifications* is perfectible (8).

We have recently proposed several methods to improve the non-invasive diagnosis of liver fibrosis. First, we showed that by adapting the marker coefficients of the original test specifically for the diagnosis of cirrhosis, the diagnostic accuracy was significantly increased as compared with the original test (9). Second, we proposed a new statistical method based on "*reliable diagnosis intervals*" (RDIs) that provides a more precise estimation of histological fibrosis stage(s), as compared with the original binary diagnosis. With this method, accuracy was ≥90% (10) and ≥95% (11) depending on the diagnostic target, thus eliminating the need for liver biopsy. However, these RDIs were less precise than Metavir F staging because they included fewer classes; in particular, no/mild fibrosis (F0/1), significant fibrosis (F≥2), and cirrhosis (F4) were not grouped in the same classification. Finally, we showed that a synchronous combination of FibroMeter and Fibroscan results increased the diagnostic accuracy for significant fibrosis as well as for the RDI (12).

The main aim of this study was to improve the non-invasive diagnosis of histological fibrosis stages by creating a new *fibrosis stage classification* that uses new fibrosis indexes combining blood fibrosis tests and Fibroscan results. The secondary aim was to compare the diagnostic accuracy of this new *fibrosis stage classification* with those of Fibrotest, FibroMeter, and Fibroscan classifications.

METHODS

Patients

Exploratory set. Patients with CHC hospitalized for a percutaneous liver biopsy were prospectively enrolled from March 2004 to September 2008 in three tertiary centers in France (Angers, Bordeaux, and Grenoble). Patients with cirrhosis complications (ascites, variceal bleeding, systemic infection, hepatocellular carcinoma) were not included. Blood fibrosis tests and Fibroscan were performed in the week preceding biopsy. All patients gave their informed consent. The study protocol conformed to the ethical guidelines of the current Declaration of Helsinki and received approval from the local Ethics committee.

Validation set. The validation set corresponded to the multicenter population of the FIBROSTAR study promoted by the French National Agency for research in AIDS and hepatitis (13). This study prospectively included 512 patients with CHC. All patients had liver biopsy, blood fibrosis tests, and Fibroscan. Patients included in both the exploratory set and the FIBROSTAR study were excluded from the validation set.

Methods

Histological assessment. Liver fibrosis was evaluated according to Metavir staging. Significant fibrosis was defined as Metavir stages F≥2, severe fibrosis (SF) as Metavir F≥3, and cirrhosis as F4. In the exploratory set, liver fibrosis was evaluated by two senior experts with a consensus reading at Angers, and by a senior expert at Bordeaux and Grenoble. In the FIBROSTAR study, liver fibrosis was centrally evaluated by two senior experts with a consensus reading in cases of discordance. Fibrosis staging was considered as reliable when liver specimen length was ≥15 mm and/or portal tract number ≥8 (14). Liver biopsy was used as the reference for the liver fibrosis evaluations by non-invasive tests.

Fibrosis blood tests. The following blood tests were calculated according to published or patented formulas: Fibrotest (15), FibroMeter (16), Hepascore (17), FIB-4 (18), and APRI (19). All blood assays were performed in the same laboratories of each center, or centralized in the FIBROSTAR study.

Liver stiffness evaluation. Fibroscan (EchoSens, Paris, France) examination was performed by an experienced observer (>50 examinations before the study), blinded for patient data. Examination conditions were those recommended by the manufacturer (5). Fibroscan examination was stopped when 10 valid measurements were recorded. Results (kilopascals) were expressed as the median and the interquartile range (IQR) of all valid measurements. Fibroscan results were considered as reliable when the ratio IQR/result (IQR/median) was <0.21 (20).

Statistical analysis

Quantitative variables were expressed as mean±s.d. The diagnostic cutoffs of fibrosis tests were calculated according to the highest Youden index (sensitivity + specificity − 1), unless otherwise specified.

Fibrosis stage classifications

We evaluated the accuracy of Fibrotest, FibroMeter, and Fibroscan *fibrosis stage classifications* (see **Supplementary Figure S1** online). Fibrotest, Fibroscan, and FibroMeter classifications were those previously published (16,21,22). Fibrotest classification includes eight classes (F0, F0/1, F1, F1/2, F2, F3, F3/4, F4), Fibroscan classification: six classes (F0/1, F1/2, F2, F3, F3/4, F4), and FibroMeter classification: six classes (F0/1, F1, F1/2, F2/3, F3/4, F4).

New fibrosis stage classification

The three-step procedure used to implement the new *fibrosis stage classification* (see **Supplementary Figure S2** online) and precise definitions (see Glossary in the **Supplementary Material** online) are detailed in the **Supplementary Material** online.

First step: new combined fibrosis indexes. To identify the best combination of *single fibrosis tests* for the diagnosis of significant fibrosis, we performed a stepwise binary logistic regression repeated on 1,000 bootstrap samples in the exploratory set. Independent variables tested were the five blood fibrosis tests and Fibroscan. The bootstrap method consists of a repeated sampling (with replacement) from the original entire data set, followed by a stepwise logistic regression procedure in each subsample (1,000 subsamples here). The most frequently (>50%) selected *single*

Table 1. Patient characteristics at inclusion

	All patients	Set		
		Exploratory	Validation	*P*
Patients (*n*)	729	349	380	—
Males (%)	61.3	60.2	62.4	0.531
Age (years)	51.7±11.2	52.1±11.2	51.3±11.2	0.347
Metavir (%)				<0.001
F0	4.0	1.4	6.3	
F1	37.7	30.7	44.2	
F2	25.8	35.5	16.8	
F3	17.6	20.6	14.7	
F4	15.0	11.7	17.9	0.020
Significant fibrosis (%)	58.3	67.9	49.5	<0.001
Reliable biopsy (%)	93.5	92.6	94.2	0.391
Fibroscan result (kPa)	10.0±7.9	9.9±8.1	10.1±7.7	0.755
IQR/median <0.21 (%)	66.9	66.2	67.6	0.700

IQR, interquartile range; kPa, kilopascal.

fibrosis tests among the 1,000 analyses were then included in a single binary logistic regression performed in the whole population of the exploratory set. Using the regression score of this multivariate analysis, we constructed a new *combined fibrosis index* for clinically significant fibrosis called "CSF-index", ranging from 0 to 1. We also constructed *combined fibrosis indexes* for the diagnosis of SF-index and cirrhosis (C-index) using the same process.

Second step: RDIs. RDIs correspond to the intervals of fibrosis test values where the individual diagnostic accuracy is considered sufficiently reliable for clinical practice. This method has been previously described (10) and is detailed in the Glossary in the **Supplementary Material** online. Briefly, we first calculated the 90% negative and positive predictive value thresholds for significant fibrosis of the CSF-index. These two thresholds determined three intervals of CSF-index values: a low interval (from 0 to the 90% negative predictive value threshold) where the non-invasive diagnosis was consequently "F0/1"; a high interval (from the 90% positive predictive value threshold to 1) where the diagnosis was "F≥2"; and an intermediate interval between the two thresholds. The intermediate interval was then divided into two new intervals according to the diagnostic cutoff corresponding to the highest Youden index. In each of these two new intermediate intervals, the non-invasive diagnosis corresponded to the combined Metavir F stages having ≥90% prevalence (e.g. F1/2 for the interval between the 90% negative predictive value threshold and the highest Youden index cutoff). Finally, the four RDI that were obtained provided ≥90% diagnostic accuracy by definition.

We also calculated the RDIs of SF-index and C-index in the same way. Because SF-index was developed for the diagnosis of SF, its 90% negative and positive predictive value thresholds and its highest Youden index cutoff were determined for this diagnostic target. For C-index, we calculated the thresholds for cirrhosis according to the 95% predictive values due to the clinical importance of cirrhosis diagnosis.

Third step: new fibrosis stage classifications. A new *fibrosis stage classification* was derived by associating RDIs for CSF- and SF-indexes. For example, if CSF-index provided a reliable diagnosis of "F≥2" and SF-index a reliable diagnosis of "F2±1", the ensuing diagnosis of the new *fibrosis stage classification* was "F2/3". Another *fibrosis stage classification* was derived by associating RDIs for CSF- and C-indexes.

Statistical softwares were SPSS, version 17.0 (SPSS, Chicago, IL) and SAS 9.1 (SAS Institute, Cary, NC).

RESULTS

Patients

The exploratory and validation sets included 349 and 380 patients, respectively. The characteristics of both sets are detailed in **Table 1**. Among the two sets, 93.5% of liver biopsies were considered as reliable.

Development of new fibrosis stage classifications

Main results are presented here. For more details, see **Supplementary Results** online.

First step: new combined fibrosis indexes . For each diagnostic target of liver fibrosis, Fibroscan and FibroMeter were *single fibrosis tests* the most frequently selected by the stepwise binary logistic regression repeated on the 1000 bootstrap samples. These two fibrosis tests were independent variables in logistic models that ran in the exploratory set and thus provided three new *combined*

Table 2. Accuracy (AUROC±s.d., bold values) of FibroMeter, Fibroscan, and their synchronous combination in new *combined fibrosis indexes*, as a function of diagnostic target and patient group

Diagnostic target	Fibrosis test	Set			All
		Exploratory	Validation	*P*	
Significant fibrosis (F≥2)	FibroMeter	**0.806±0.026**	**0.839±0.022**	0.333	**0.813±0.017**
	Fibroscan	**0.785±0.026**	**0.828±0.022**	0.207	**0.791±0.017**
	CSF-index	**0.835±0.023**	**0.875±0.019**	0.180	**0.846±0.015**
	Comparison (*P*)	—	—	—	—
	FibroMeter vs. Fibroscan	0.513	0.685	—	0.301
	FibroMeter vs. CSF-index	0.027	0.002	—	<0.001
	Fibroscan vs. CSF-index	0.024	0.009	—	<0.001
SF (F≥3)	FibroMeter	**0.776±0.025**	**0.880±0.020**	0.001	**0.829±0.016**
	Fibroscan	**0.816±0.025**	**0.881±0.019**	0.038	**0.847±0.016**
	SF-index	**0.830±0.022**	**0.918±0.017**	0.002	**0.875±0.014**
	Comparison (*P*)	—	—	—	—
	FibroMeter vs. Fibroscan	0.163	0.993	—	0.324
	FibroMeter vs. SF-index	<0.001	<0.001	—	<0.001
	Fibroscan vs. SF-index	0.458	0.014	—	0.019
Cirrhosis (F4)	FibroMeter	**0.814±0.031**	**0.897±0.021**	0.027	**0.861±0.018**
	Fibroscan	**0.878±0.032**	**0.927±0.017**	0.176	**0.905±0.017**
	C-index	**0.890±0.028**	**0.947±±0.014**	0.069	**0.921±0.015**
	Comparison (*P*)	—	—	—	—
	FibroMeter vs. Fibroscan	0.059	0.193	—	0.026
	FibroMeter vs. C-index	<0.001	<0.001	—	<0.001
	Fibroscan vs. C-index	0.511	0.120	—	0.133

AUROC, area under the receiver operating characteristic curve; C, cirrhosis; CSF, clinically significant fibrosis; SF, severe fibrosis.

fibrosis indexes for three diagnostic targets: CSF-index for significant fibrosis, SF-index for SF, and C-index for cirrhosis. CSF-index had a significantly higher area under the receiver operating characteristic curve than its composite tests, that is, FibroMeter or Fibroscan, in the exploratory set (**Table 2**). SF-index and C-index also had higher area under the receiver operating characteristic curves than FibroMeter or Fibroscan in the exploratory set, but the difference was significant only with FibroMeter.

Second step: RDIs. By using the thresholds of 90% predictive values for significant fibrosis and the diagnostic cutoff corresponding to the maximum Youden index, CSF-index provided four RDIs (F0/1, F1/2, F2±1, F≥2), which provided 90.3% diagnostic accuracy. By using the same method for SF, SF-index provided four RDIs (F1±1, F2±1, F3±1, F≥3) with 92.0% diagnostic accuracy. Finally, by using the thresholds of 95% predictive values for cirrhosis, C-index provided three RDIs (F3, F3±1, F4) with 95.1% diagnostic accuracy.

Third step: new fibrosis stage classifications. The first classification (CSF/SF classification) was derived from the association of CSF- and SF-index RDIs (**Table 3**). CSF/SF classification included six classes (F0/1, F1/2, F2±1, F2/3, F3±1, F4) and provided 87.7% diagnostic accuracy in the exploratory set (**Figure 1a**).

The second classification (CSF/C classification) was derived from CSF- and C-index RDIs (**Table 3**). CSF/C classification also included six classes (F0/1, F1/2, F2±1, F2/3, F3±1, F4) and provided 86.5% diagnostic accuracy ($P=0.503$ vs. CSF/SF classification; **Table 4**).

Validation of the new fibrosis stage classifications

The diagnostic accuracies of CSF-index, SF-index, and C-index RDIs were not significantly different between the exploratory and the validation sets, with respectively: 90.3% vs. 86.7% ($P=0.142$), 92.0% vs. 91.5% ($P=0.827$), and 95.1% vs. 94.5% ($P=0.731$). Similarly, diagnostic accuracies of CSF/SF and CSF/C classifications were not significantly different between the two sets (**Table 4**).

In the validation set, CSF/SF classification provided a significantly higher diagnostic accuracy (85.8%) than CSF/C classification and those of *single fibrosis tests* ($P<0.008$; **Table 4**). **Figure 1b** shows the proportion of Metavir fibrosis stages as a function of CSF/SF classification. According to diagnostic accuracy in the validation set, classification ranking was: CSF/SF > CSF/C > FibroMeter > Fibroscan > Fibrotest (**Table 4**).

Figure 2 shows the diagnostic accuracy of each *fibrosis stage classification* as a function of Metavir fibrosis stage in the validation set. Among *single fibrosis tests*, FibroMeter provided the most homogeneous profile with no significant differences among histological fibrosis stages ($P=0.352$). The new CSF/SF and CSF/C classifications provided better profiles than those of *single fibrosis tests*. However, the rate of well-classified patients among cirrhotic patients was significantly higher with CSF/SF classification (94.5%) than with CSF/C classification (67.3%, $P<0.001$).

Table 3. Development in the exploratory set of new *fibrosis stage classifications* derived from the association of the *reliable diagnosis intervals* (RDIs) of *combined fibrosis indexes* (CSF- and SF-indexes, CSF- and C-indexes)

		RDI of CSF-index				
		F0/1	F1/2	F2±1	F≥2	
RDI of SF-index	F1±1	F0/1 (29/32)	F1/2 (84/96)	F1/2 (21/28)	– (0/0)	CSF/SF classification
	F2±1	– (0/0)	– (0/0)	F2±1 (32/32)	F2/3 (32/40)	
	F3±1	– (0/0)	– (0/0)	– (0/0)	F3±1 (94/103)	
	F≥3	– (0/0)	– (0/0)	– (0/0)	F4 (14/18)	
RDI of C-index	F≤3	F0/1 (29/32)	F1/2 (84/96)	F2±1 (57/60)	F2/3 (90/118)	CSF/C classification
	F3±1	– (0/0)	– (0/0)	– (0/0)	F3±1 (35/36)	
	F4	– (0/0)	– (0/0)	– (0/0)	F4 (7/7)	

C, cirrhosis; CSF, clinically significant fibrosis; SF, severe fibrosis.
Gray cells indicate the RDIs of the combined fibrosis indexes. Colored cells indicate the F stages provided by the new fibrosis stage classifications. Figures into brackets are the rates of correctly classified patients in each class of the new fibrosis stage classifications according to liver biopsy results.

Influencing factors

In the whole study population, we performed a stepwise binary logistic regression including age, sex, biopsy length, Metavir F, and IQR/median as independent variables. The rate of well-classified patients by CSF/SF classification was independently associated with the ratio IQR/median (first step, exp(β) = 0,322), Metavir F (second step, exp(β) = 1.370), and age (third step, exp(β) = 0.976).

In the validation set, CSF/SF classification provided 89.5% diagnostic accuracy in patients with IQR/median <0.21 vs. 78.1% in patients with IQR/median ≥0.21 ($P=0.006$). In the subgroup of patients with IQR/median <0.21, CSF/SF classification had the highest diagnostic accuracy ($P=0.006$ vs. other classifications, see **Supplementary Figure S4** online).

DISCUSSION

Because of its important role in guiding patient management, fibrosis staging in chronic viral hepatitis is clinically relevant (2). Blood fibrosis tests and liver stiffness evaluation are now used for the non-invasive assessment of liver fibrosis in CHC. In clinical practice, their results are interpreted by using *fibrosis stage classifications*, which provide an estimation of the histological fibrosis stage. We developed a new and accurate *fibrosis stage classification* for the non-invasive diagnosis of histological fibrosis stages by using an original method that includes several statistical techniques we have previously described: combinations of fibrosis tests (12), scores of blood tests adapted to specific diagnostic target (9,12), and RDI (10,11). This new *fibrosis stage classification* had a very significantly higher diagnostic accuracy than those previously published for *single fibrosis tests*.

Our study has several noteworthy aspects. First, both the exploratory and validation sets had a multicenter design (especially for the validation set). Second, histological examination was performed by expert pathologists and liver specimens were considered as reliable in 94% of the cases. Finally, fibrosis stage prevalence in our validation set was quite similar to the reference prevalence reported in a large cohort of around 33,000 patients with CHC (23). This

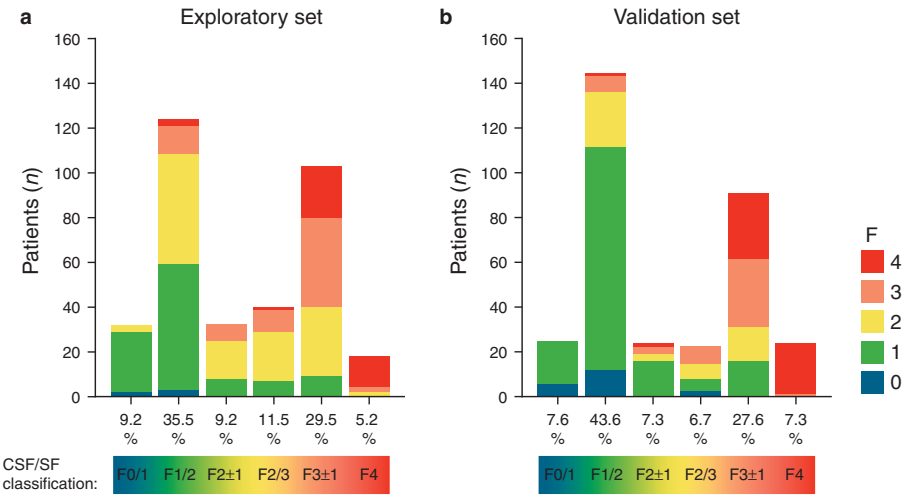

Figure 1. Proportion of Metavir fibrosis (F) stages as a function of clinically significant fibrosis/severe fibrosis (CSF/SF) classification (*x* axis with the rate of patients included in each class in italics), in the exploratory (**a**) and validation (**b**) sets. The bottom line indicates the *fibrosis stage classification*.

Table 4. Diagnostic accuracy (% of correctly classified patients) of *fibrosis stage classifications* as a function of patient group

Classification	Set			
	All	Exploratory	Validation	*P*[a]
CSF/SF	86.7	87.7	85.8	0.461
CSF/C	84.4	86.5	82.1	0.113
FibroMeter	68.7	67.6	69.7	0.550
Fibroscan	58.7	54.4	63.3	0.020
Fibrotest	38.8	33.5	43.9	0.005

C, cirrhosis; CSF, clinically significant fibrosis; SF, severe fibrosis.
[a]Comparison between sets.
Comparison between *fibrosis* stage classifications.
All patients: CSF/SF vs. CSF/C, $P=0.014$; others comparisons, $P<0.001$.
Exploratory set: CSF/SF vs. CSF/C, $P=0.503$; other comparisons, $P<0.001$.
Validation set: CSF/SF vs. CSF/C, $P=0.008$; FibroMeter vs. Fibroscan, $P=0.029$; other comparisons, $P<0.001$.

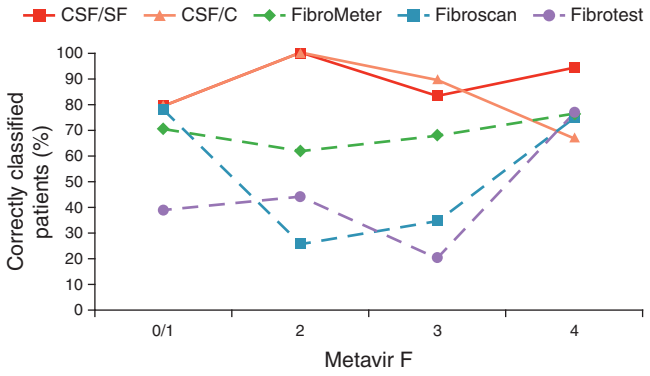

Figure 2. Rates of correctly classified patients by *fibrosis stage classifications* as a function of Metavir fibrosis stages in the validation set. Hatched lines: *single fibrosis tests*, continuous lines: new *fibrosis stage classifications* derived from new *combined fibrosis indexes*. Because of the few number of F0 patients, F0 and F1 were pooled together.

aspect is important as diagnostic accuracy relies on fibrosis stage prevalence (24).

Why a combination of fibrosis tests?

Best candidates for combination. We have previously shown that the best combination among several fibrosis tests evaluated was FibroMeter + Fibroscan (12). However, this study included various causes of chronic liver diseases. The present study confirms these findings in a homogeneous population of patients having CHC. Indeed, the bootstrap method identified FibroMeter and Fibroscan as the best independent predictors for each diagnostic target studied in the exploratory set (see **Supplementary Table S1** online).

The contribution of fibrosis test combination. Our results clearly show several advantages provided by *combined fibrosis indexes*. *Combined fibrosis indexes* increased the accuracy of fibrosis diagnosis: in the validation set and in the whole population, they provided significantly higher area under the receiver operating characteristic curves for significant fibrosis and SF than did *single fibrosis tests* (**Table 2**). *Combined fibrosis indexes* also increased the reliability of fibrosis diagnosis: they provided the highest rates of patients included in the intervals of ≥90% predictive values (see **Supplementary Table S2** online).

RDIs of *combined fibrosis indexes* had similar diagnostic accuracy than those of *single fibrosis tests* (see **Supplementary Results** online). There were thus two options: to associate RDIs of *combined fibrosis indexes* or, more simply, to associate RDIs of *single fibrosis tests*. The association of *single fibrosis tests* RDIs provided discordant results and a more detailed but less accurate *fibrosis stage classification* than the association of *combined fibrosis indexes* RDIs. Thus, *combined fibrosis indexes* improved diagnostic accuracy and erase discrepancy between *single fibrosis tests*.

Combined fibrosis indexes provided more robust *fibrosis stage classifications*. Indeed, despite the significantly different prevalence of histological fibrosis stages between the exploratory and validation sets, diagnostic accuracies of CSF/SF and CSF/C classifications were not significantly different between the two patient sets (**Table 4**). By contrast, the *fibrosis stage classification* derived from the association of the *single fibrosis tests* RDIs showed a dramatic decrease in diagnostic accuracy, from 83% in the exploratory set to 69% in the validation set.

Finally, the new CSF/SF and CSF/C classifications provided significantly higher diagnostic accuracies that the *fibrosis stage classifications* of *single fibrosis tests* (**Table 4**).

Best-performing new fibrosis stage classification

CSF/SF classification provided significantly higher diagnostic accuracy (85.8%) than did the other *fibrosis stage classifications* (**Table 4**). This high diagnostic accuracy was very close to what would be expected for a perfect diagnostic test as evaluated against the imperfect gold standard that is liver biopsy (25). Moreover, the rate of correctly classified cirrhotic patients was significantly higher with CSF/SF classification: 95% vs. 67% with CSF/C classification. Overall, these results suggest that CSF/SF classification is more relevant for use in clinical practice than CSF/C or *single fibrosis tests* classifications.

How does it run in clinical practice?

Determination of CSF/SF classification. The use of CSF/SF classification in clinical practice may seem complex because it requires several calculations. However, after computerization of all steps, the user has only to provide two results (Fibroscan and blood test) to obtain an accurate estimation of the histological fibrosis stage (**Figure 3**).

CSF/SF classification vs. previously published algorithms. Several combinations of fibrosis tests such as SAFE (26) or Bordeaux algorithm (15) have been proposed to improve the non-invasive diagnosis of liver fibrosis in clinical practice. SAFE is accurate (27,28) but has several limits. Indeed, SAFE requires a sequential use of blood fibrosis tests and thus needs to repeat blood samples in patients. Moreover, SAFE includes two algorithms, each one

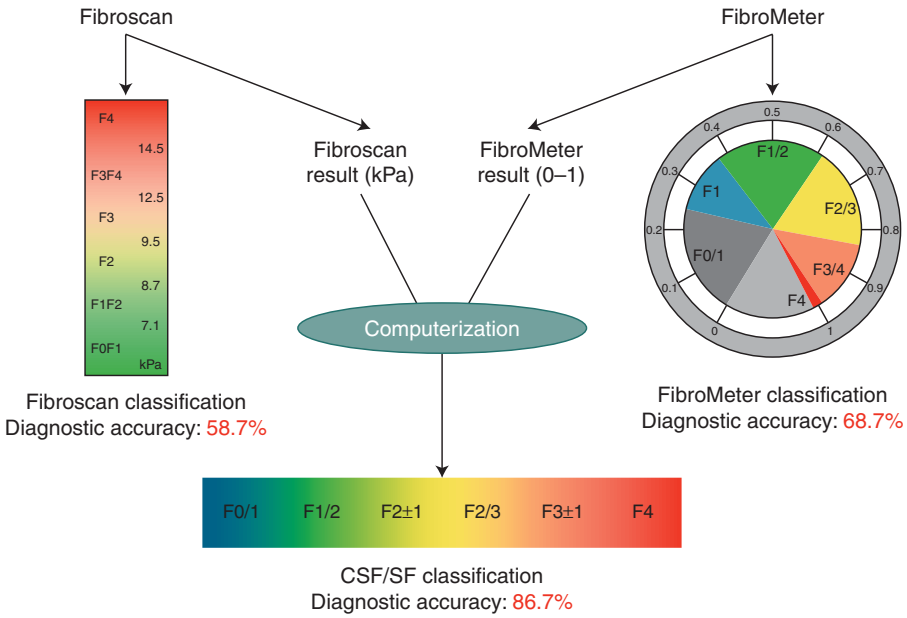

Figure 3. Non-invasive determination of the liver fibrosis stage in clinical practice. Fibroscan or FibroMeter could be used alone: results are interpreted according to their respective fibrosis stage classifications that provide fair/moderate diagnostic accuracy. Otherwise, Fibroscan and FibroMeter results could be introduced in a computerized algorithm to determine the clinically significant fibrosis/severe fibrosis (CSF/SF) classification that provides a high diagnostic accuracy.

providing only a binary diagnosis of significant fibrosis or cirrhosis. Thus, physician must first complete the SAFE for significant fibrosis and then, in case of F≥2 diagnosis, the SAFE for cirrhosis. This finally induces a decrease in the diagnostic accuracy by accumulation of each algorithm errors. Finally, liver fibrosis diagnosis remains undetermined in a large proportion of patients (>50% for the diagnosis of significant fibrosis and 20–25% for cirrhosis), with thus a high rate of liver biopsy required (27,28). The six-class CSF/SF classification circumvents all these limits by providing an accurate estimation of the fibrosis stage in a one-step procedure, without any liver biopsy required.

Castera *et al.* (15) have proposed the Bordeaux algorithm combining blood test (Fibrotest) and Fibroscan. However, because of disagreement between fibrosis tests, it requires liver biopsy in 30% of cases for the diagnosis of significant fibrosis and 20% for cirrhosis (27). The synchronous combination of blood test with Fibroscan into new *combined fibrosis indexes* allows CSF/SF classification to circumvent this limit.

Could CSF/SF classification replace liver biopsy in clinical practice? Liver biopsy is currently the reference procedure for liver fibrosis evaluation in clinical practice (1). However, it is now well established that liver biopsy cannot be considered a "gold standard" because of sampling bias and poor interobserver reproducibility of histological semi-quantitative scores (29). Thus, when a liver biopsy and a non-invasive test give discordant results, it is difficult to know which one is right. Several studies have evaluated discordances between liver biopsy and either blood fibrosis tests (30–32) or Fibroscan results (33). In these studies, discordances attributed to misdiagnoses of non-invasive tests were as frequent as those attributed to liver biopsy errors, suggesting that non-invasive tests were as accurate as liver biopsy for liver fibrosis evaluation in clinical practice. In our study, liver biopsy and CSF/SF classification results were discordant in only 13.3% of cases and we may suppose that a significant part of these discordances could be attributed to liver biopsy errors. The subsequent decrease in diagnostic accuracy of liver biopsy (i.e., <100%) and increase in accuracy of CSF/SF classification (i.e., >86.7%) suggest that both methods most likely provide similar accuracy for the evaluation of liver fibrosis in clinical practice. Other studies with different methodology (e.g., surgical biopsy) will address that issue more completely. Nevertheless, the CSF/SF classification has the advantage of being totally non-invasive; it can thus be repeated at regular intervals, unlike liver biopsy. Finally, although liver biopsy remains the best standard (29), non-invasive tests have other indications, for example, a closer follow-up of patients. In this setting, cirrhotic patients initially misclassified by CSF/SF classification due to compensated cirrhosis will most likely be correctly classified later thanks to a slight progression of liver disease.

Beyond diagnostic accuracy, another crucial point is to compare the cost-effectiveness of liver biopsy and CSF/SF classification for liver fibrosis evaluation. Such a comparison would need to take into account not only numerous cost factors (liver biopsy, blood markers, Fibroscan, resulting antiviral prescriptions, treatments for initially misdiagnosed cirrhosis complications), but also morbidity and mortality due to liver biopsy and liver disease decompensation. The question of cost-effectiveness thus requires further longitudinal studies using clinical events as study end points.

Finally, we have previously shown that blood fibrosis tests adapted to the cause of liver disease provide higher diagnostic accuracy (34).

Continuing in this direction, we have developed the CSF/SF classification in a homogeneous population of patients with a single cause of chronic liver disease, that is, CHC. Future studies may evaluate if combinations of non-invasive tests according to our methodology could replace liver biopsy in other causes of liver disease.

In conclusion, the association of *combined fibrosis indexes* including FibroMeter and Fibroscan provides a new *fibrosis stage classification* for the non-invasive diagnosis of liver fibrosis stages in patients with CHC. This classification has a high and robust diagnostic accuracy, and is significantly more accurate than the previously published *fibrosis stage classifications* of Fibrotest, FibroMeter, and Fibroscan. It now seems possible to implement fully non-invasive management of patients with CHC.

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CONFLICT OF INTEREST

Guarantor of the article: Paul Calès, MD.

Specific author contributions: Planning and conducting the study, collecting and interpreting data, drafting the manuscript, and approving the final draft submitted: Jérôme Boursier; planning and conducting the study, collecting data, drafting the manuscript, and approving the final draft submitted: Victor de Ledinghen and Jean-Pierre Zarski; collecting data: Marie-Christine Rousselet, Nathalie Sturm, Juliette Foucher, Isabelle Fouchard-Hubert, Yves Gallois, Frédéric Oberti, and Nina Dib; planning and conducting the study and collecting data: Vincent Leroy; collecting and interpreting data: Sandrine Bertrais; planning and conducting the study, collecting and interpreting data, drafting the manuscript, approving the final draft submitted, accepts full responsibility for the conduct of the study, has access to the data, and has control of the decision to publish: Paul Calès.

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Study Highlights

WHAT IS CURRENT KNOWLEDGE

- ✓ Precise evaluation of the level of liver fibrosis is recommended in patients with chronic hepatitis C.
- ✓ Liver fibrosis stages are usually determined on a biopsy by using histological semi-quantitative scores of fibrosis.
- ✓ Fibrosis stages classifications derived from blood fibrosis tests or Fibroscan results allow for a non-invasive determination of liver fibrosis stages.

WHAT IS NEW HERE

- ✓ The association of new fibrosis indexes combining FibroMeter and Fibroscan provides a new fibrosis stage classification.
- ✓ This classification is highly accurate for the non-invasive diagnosis of histological fibrosis stages in chronic hepatitis C.
- ✓ The new classification has a significantly higher diagnostic accuracy than those previously published for blood fibrosis tests or Fibroscan.

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