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Independent validation of the Enhanced Liver Fibrosis (ELF) score in the ANRS HC EP 23 Fibrostar cohort of patients with chronic hepatitis C

Jérôme Guéchot[1,*], Candice Trocmé[2], Jean-Charles Renversez[2], Nathalie Sturm[3,4], Jean-Pierre Zarski[3,5] and the ANRS HC EP 23 Fibrostar Study Group[a]

[1] Pôle de Biologie, Hôpitaux Universitaires Est Parisien, site Saint-Antoine, AP-HP, Paris, France
[2] Laboratoire de Biochimie des Enzymes et des Protéines, Département de Biochimie, Pharmacologie et Toxicologie, Pôle de Biologie, CHU de Grenoble, Grenoble, France
[3] Unité INSERM/UJF U823 IAPC Institut Albert Bonniot, Grenoble, France
[4] Département d'Anatomie et de Cytologie Pathologiques, Pôle Biologie, CHU de Grenoble, France
[5] Clinique Universitaire d'Hépato-Gastroentérologie, Pôle DIGIDUNE, CHU de Grenoble, France

Abstract

Background: The Enhanced Liver Fibrosis (ELF) score combining serum hyaluronan, N-terminal peptide of type III procollagen and tissue inhibitor of metalloproteinase-1, was reported as relevant in predicting liver fibrosis in chronic liver disease and proposed as an alternative to liver biopsy.
Methods: We evaluated the ELF score in a cohort of chronic hepatitis C (CHC) patients included in a multicenter prospective study (ANRS HC EP 23 Fibrostar) using commercial reagents, different from those developed by the manufacturer of the Siemens ELF™ test.
Results: In 512 CHC, the ELF score, using ROC curves, showed good predictive performances for severe fibrosis [AUROC=0.82; 95% confidence interval (CI) 0.78–0.86] and for cirrhosis (AUROC=0.85; 95% CI 0.81–0.90), but slightly lower for significant fibrosis (AUROC=0.78; 95% CI 0.74–0.82). The Obuchowski measure (0.81) showed that the

[a]The ANRS HC EP 23 Fibrostar Study Group
Sponsor:
French National Agency for Research on aids and viral hepatitis (ANRS), Paris.
Scientific Committee:
Coordinators: J.-P. Zarski, Grenoble; M. Vaubourdolle, Paris;
Hepatologists: J.-P. Bronowicki, Lille; P. Calès, Angers; A. Mallat, Créteil; P. Mathurin, Lille;
Pathologists: N. Sturm, Grenoble; E.S. Zafrani, Créteil;
Biologists: B. Poggi, Lyon; J. Guéchot, Paris;
Methodologists and administrators: J.L. Bosson; A. Paris;
ANRS: L. Allain, Paris.
Methodologists and administrators:
A. Bechet, J.-L. Bosson, A. Paris, A. Plages, S. Royannais, Centre Hospitalier Universitaire de Grenoble
Biologists:
R.-C. Boisson, Centre Hospitalier Lyon Sud, Hospices Civils de Lyon
M.-C. Gelineau, B. Poggi, Hôtel Dieu, Hospices Civils de Lyon
J.-C. Renversez, C. Trocmé, Centre Hospitalier Universitaire de Grenoble
J. Guéchot, E. Lasnier, M. Vaubourdolle, Hôpital Saint-Antoine, AP-HP, Paris
H. Voitot, Hôpital Beaujon, AP-HP, Paris
A. Vassault, Hôpital Necker, AP-HP, Paris
A. Rosenthal-Allieri, Centre Hospitalier Universitaire de Nice
A. Lavoinne, F. Ziegler, Centre Hospitalier Universitaire de Rouen
M. Bartoli, C. Dorche, C. Lebrun, Centre Hospitalier de Chambéry
A. Myara, Growwupe Hospitalier Paris Saint-Joseph, Paris

*Corresponding author: Dr. Jérôme Guéchot, Hôpital Saint Antoine 184, rue du Faubourg Saint Antoine 75571 PARIS Cedex 12, France
E-mail: jerome.guechot@sat.aphp.fr
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F. Guerber, A. Pottier, Laboratoire Elibio-Groupe Oriade, Vizille, La Mure
Hepatologists:
V. Leroy, J.-P. Zarski, Centre Hospitalier Universitaire de Grenoble
A. Poujol-Robert, R. Poupon, Hôpital Saint-Antoine, AP-HP, Paris
A. Abergel, Centre Hospitalier Universitaire de Clermont-Ferrand
J.P. Bronowicki, Hôpital de Brabois, Centre Hospitalier Universitaire de Nancy
S. Metivier, J.P. Vinel, Hôpital Purpan, Centre Hospitalier Universitaire de Toulouse
V. De Ledinghen, Hôpital Haut Levêque, Centre Hospitalier Universitaire de Bordeaux
O. Goria, Centre Hospitalier Universitaire de Rouen
M. Maynard-Muet, C. Trepo, Hôtel Dieu, Hospices Civils de Lyon
Ph. Mathurin, Centre Hospitalier Universitaire de Lille
H. Danielou, D. Guyader, Hôpital Pontchaillou, Centre Hospitalier Universitaire de Rennes
O. Rogeaux, Centre Hospitalier de Chambéry
S. Pol, Ph. Sogni, Hôpital Cochin, AP-HP, Paris
A. Tran, Hôpital De l'Archet, Centre Hospitalier Universitaire de Nice
P. Calès, Centre Hospitalier Universitaire d'Angers
T. Asselah, P. Marcellin, Hôpital Beaujon, AP-HP, Clichy
M. Bourlière, V. Oulès, Hôpital Saint Joseph, Assistance Publique-Hôpitaux de Marseille
D. Larrey, Centre Hospitalier Universitaire de Montpellier
F. Habersetzer, Centre Hospitalier Universitaire de Strasbourg
M. Beaugrand, Hôpital Jean Verdier, AP-HP, Bondy
Pathologists:
N. Sturm, Centre Hospitalier Universitaire de Grenoble
E.-S. Zafrani, Hôpital Henri Mondor, AP-HP, Créteil

ELF score globally performed as a marker of liver fibrosis. The ELF score predicted significant fibrosis (cut-off=9.0) with a sensitivity of 0.86, a specificity of 0.62, a positive predictive value (PPV) of 0.80 and a negative predictive value (NPV) of 0.70. For extensive fibrosis (cut-off=9.33), sensitivity was 0.90, specificity was 0.63, PPV was 0.73 and NPV was 0.85. For cirrhosis (cut-off=9.35), sensitivity was 0.83, specificity was 0.75, PPV was 0.44 and NPV was 0.95.

Conclusions: This study confirms the ELF score performance as an index to predict liver fibrosis or cirrhosis in CHC. The ELF test, using validated reagents, could be added to the health authorities approved non-invasive tests in assessing fibrosis as surrogate to liver biopsy.

Keywords: blood marker; diagnostic accuracy; ELF test; hepatitis C; liver fibrosis.

Introduction

Clinical management of chronic hepatitis C (CHC) is largely depending on the extent of liver fibrosis. Liver histological examination of the liver biopsy, although having some estimated disadvantages, was considered as the reference method to assess the fibrosis stage and was until now recommended in the majority of patients (1). However, it is an invasive procedure responsible for severe complications in about 0.5% of cases (2) and its accuracy in scoring liver fibrosis is limited by sampling heterogeneity and inter- and intra-observer variations (3, 4). Various non-invasive markers of liver fibrosis have been recently developed and were shown as interesting alternative to liver biopsy in order to evaluate the severity of liver fibrosis in patients with chronic liver diseases (5, 6). Three indices resulting of the combination of blood markers of liver fibrosis, Fibrotest® (7), Fibrometer® (8), and Hepascore® (9), have a good diagnostic accuracy in patients with CHC for discriminating mild fibrosis to severe fibrosis and for assessing cirrhosis. Recently, the Haute Autorité de Santé (HAS, the French National Authority for Health) has evaluated the benefit of the available methods and considered that in adult patients with untreated CHC without any comorbidity, these three validated biological diagnostic tests (10) have shown sufficient interest to be approved by the health authorities for the inscription to reimbursement. The authority stated that another algorithm, the European Liver Fibrosis Group (ELFG) score (11), has shown a potential interest but has not been fully validated, particularly in the diagnosis of cirrhosis and that further independent studies are needed to validate this test (12).

A multicenter prospective and independent study, designed to evaluate and to compare the diagnosis performance of published tests, definitely confirmed the importance of these non-invasive markers to assess liver fibrosis in CHC. Fibrometer®, Hepascore®, and Fibrotest® performed better than all other blood tests and the diagnostic performances of ELFG score were not far from the most performing tests (13). The original ELFG score combines a panel of three serum biomarkers involved in the synthesis and degradation of extracellular matrix, hyaluronic acid (HA), aminoterminal peptide of type III procollagen (PIIINP) and tissue inhibitor of metalloproteinase-1 (TIMP-1), with age. The ELFG score was recently simplified by removing age and a new algorithm, the Enhanced Liver Fibrosis (ELF) score, was developed (14) using the proprietary assays developed for Siemens ELF™ test (Siemens Healthcare Diagnostics Inc., Tarrytown, NY, USA) introduced onto the market this year.

To confirm the clinical interest of the simplified ELF score, we evaluated its diagnostic accuracy for the prediction of significant fibrosis, severe fibrosis and cirrhosis in the cohort of CHC patients included in the ANRS HC EP 23 Fibrostar multicenter prospective study using METAVIR histological fibrosis stage as reference (15). The diagnostic performance of the simplified ELF score was compared to the original ELFG score and to other serum tests validated as marker of liver fibrosis. Furthermore ELF score was assessed independently both of the authors and of the manufacturer using other commercial assay kits than its own.

Materials and methods

The study was approved by the Committee for protection of persons of Grenoble (France). Informed consent was obtained from each patient.

Patients

Between November 2007 and July 2008, 19 academic centers prospectively enrolled in a cohort study designed to compare different biological blood markers of liver fibrosis and transient elastography (Fibroscan™) 590 untreated patients with CHC (anti-HCV antibodies positive and RNA-HCV positive) referred for evaluation, including liver biopsy.

Patients with associated coinfection, chronic viral hepatitis B (HBsAg positive) or HIV, with other liver disease (drug hepatitis, Wilson disease, hemochromatosis, autoimmune hepatitis, alcohol consumption >30 g/day for men and >20 g/day for women, primary biliary cirrhosis, α-1 antitrypsine deficiency), or with severe systemic diseases were excluded. Patients with antiviral therapy during the 6 months preceding the inclusion or with immunosuppressive therapy were also excluded.

Liver pathological examination

Histological analysis was independently performed by two senior pathologists, academic experts in liver pathology, without knowledge of any clinical and biological data except that patients had chronic hepatitis C. To be considered as adequate for scoring, the liver biopsies had to measure at least 15 mm and/or contain at least 11 portal tracts except for cirrhosis for which no limitation was required. Fibrosis was assessed on red Sirius stained sections according to the semi quantitative Metavir scoring system, on a five-point scale (F0=no fibrosis, F1=portal fibrosis without septa, F2=few septa, F3=numerous septa without cirrhosis, F4=cirrhosis) (15). In case of discrepancies, slides were simultaneously reviewed by the two pathologists using a multi-pipe microscope in order to reach a consensus.

Blood samples

Fasting blood samples were collected by veinipuncture at <2 months away from the liver biopsies. The same kinds of tubes from the same lots were used for all the patients (BD Vacutainer, type Z, Becton-Dickinson, Plymouth, UK).

Each of the biological parameters included in the ELF score were measured in a single laboratory using serum samples immediately separated and fractioned in fractions of 0.5 mL in 1.5 mL screw cap micro tubes (Sarstedt, Nümbrecht, Germany). All the fractions were immediately frozen and stored at –80°C until the assays were undertaken. The transport of samples from the hepatology centers to the laboratory were achieved in carbonic ice by a specialized transporter (AreaTime Logistics, Cergy Pontoise, France).

All the biological tests were processed blindly without knowledge of the clinical and histological data.

ELF score

ELF score biochemical assays The serum HA was assayed using a latex agglutination method that can be applied to general clinical chemistry analyzers (HA detection reagent, Latex method, Wako, Osaka, Japan) using an AU640 analyzer (Beckman Coulter, Brea, CA, USA) as previously described (16). The serum PIIINP was assayed using a radio immunoassay (P3NP RIA kit, Orion Diagnostica, Espoo, Finland), and the serum TIMP-1 was assayed using an Elisa kit (TIMP-1 Biotrak ELISA, GE Healthcare Lifesciences, Chalfont St. Giles, UK) as previously described (17).

ELF score calculation The ELF score was computed from the results by using the simplified algorithm with reference to Metavir classification of liver biopsy, published by Parkes et al. (14): ELF score=–7.412+[ln HA (ng/mL)×0.681]+[ln PIIINP (ng/mL)×0.775]+[ln TIMP1 (ng/mL)×0.494]+10.

ELFG score calculation The ELFG score was computed from the results by using the simplified algorithm with reference to Metavir classification of liver biopsy, published by Rosenberg et al. (11): ELFG score=–{0.014 ln [age (years)]}+{0.616 ln [hyaluronic acid (ng/mL)]}+{0.586 ln [PIIINP (ng/mL)]}+{0.472 ln [TIMP1 (ng/mL)]}–6.38.

Fibrotest

The serum parameters of Fibrotest® were strictly measured according to the technical recommendations of the authors, using the methods used to develop the test as described in the original publication (7). Specific protein assays [α_2-macroglobulin (A2M), haptoglobin, apolipoprotein A1] were measured by immunonephelemetric methods using a BN2 analyzer (Siemens Healthcare Diagnostics, Deerfield, USA). γ-Glutamyltransferase (GGT), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities (IFCC methods at 37°C with pyridoxal phosphate for ALT and AST) and total bilirubin were measured using an Hitachi 917 analyzer (Roche Diagnostics, Mannheim, Germany) with reagents from the manufacturer and a CFAS (Calibrator for Automated Systems, Roche Diagnostics) calibration. Calculations of the scores were purchased from Biopredictive (Paris, France).

Hepascore

An OLYMPUS AU640 (Olympus Diagnostic Systems, Tokyo, Japan) analyzer was used for simultaneous assays of the parameters included in the Hepascore®. Serum HA levels were assayed using a latex agglutination method (HA detection reagent, Latex method, Wako, Osaka, Japan). A2M levels were measured using an immunoturbidimetric assay (α-2-Macroglobulin kit, DakoCytomation, Glostrup, Denmark). GGT activities (IFCC method at 37°C) and total bilirubin concentrations were assayed using Olympus reagents with CFAS calibration (Roche Diagnostic). The Hepascore was computed from the results by using the model previously published by Adams et al. (9): Hepascore=y/(1+y) with y=exp {–4.185818–[0.0249 age (years)]+[0.7464 sex (M=1, F=0)]+[1.0039 A2M (g/L)]+[0.0302 HA (μg/L)]+[0.0691 bilirubin (μmol/L)]–[0.0012 GGT (IU/L)]}.

Statistical analysis

GraphPad Prism computer software was used for statistical analysis (GraphPad Software, La Jolla, CA USA). Quantitative variables are expressed as mean (SD) or median (range) as specified. The Kruskal-Wallis analysis of variance was used to compare the ELF score results according to the histological scores of fibrosis and the Spearman rank correlation to assess the relationship between ELF score and histological degree of fibrosis. A p-value of <0.05 was considered statistically significant.

Receiver-operator characteristic (ROC) curves were built to visualize the discriminating performance of the ELF score considering liver biopsy as the reference. Areas under the ROC curves (AUROC) were calculated to quantify the ability of the test to discriminate between fibrosis grades. The optimal cut-offs were calculated by maximizing the sum of sensitivity plus specificity.

Given the ordinal scaling of histological fibrosis, the Obuchowski measure was used to estimate the overall diagnostic performance of the ELF test and to compare it with the diagnostic performances of other tests. This measure can be used as an estimator for test accuracy when the gold standard is not binary but graded on an ordinal scale (18). Since no assumptions on the distribution of the gold standard are needed, it is insensitive to the distribution of fibrosis stages in the study sample and was recently recommended as a multinomial version of the AUROC to assess diagnostic accuracy of liver fibrosis tests without the spectrum bias due to the prevalence of the different stages of liver fibrosis in the studied cohort of patients (19). The Obuchowski measures were calculated online (http://www.info.univ-angers.fr/~gh/wstat/obu_f.php, by courtesy of Dr. G. Hunault, Angers University, Angers, France) using the observed prevalence for each group of patients and a penalty function that was proportional to the difference in METAVIR units as recommended (19).

Results

Patient characteristics

Because of insufficient liver tissue (n=42), previous interferon therapy (n=5), coexisting liver disease due to chronic HBV infection (n=9), excessive alcohol consumption (n=5), immunosuppressive treatment (n=1), non-confirmed HCV positive status (n=3), or incomplete data (n=13), the final study cohort included 512 patients, 306 male (59.8%) and 206 female (40.2%). The characteristics of the patients were summarized in Table 1.

Liver histology

The length of liver biopsies was 25.1±8.8 mm (mean±SD) and longer than 25 mm in 49.8%. Metavir stages distribution

Table 1 Characteristics of the 512 studied patients.

	Median	Range
Age, years	50	18–79
Weight, kg	70	39–135
Height, m	1.70	1.48–1.97
BMI, kg/m^2	24.3	15.4–49.2
Bilirubin, µmol/L	10.5	2.4–54.8
ALT, IU/L	69	12–594
AST, IU/L	49	11–280
GGT, IU/L	61	9–858
Platelet count, g/L	213	52–474
Prothrombin time, %	99	61–100
Hyaluronic acid, ng/mL	34	5–920
PIIINP, ng/mL	4.5	1.4–69.7
TIMP-1, ng/mL	160	4.9–653

was F0 in 34 (6.6%), F1 in 231 (45.1%), F2 in 92 (18.0%), F3 in 79 (15.4%) and F4 in 76 (14.8%) patients.

Assessment of liver fibrosis using the ELF score

Figure 1 shows box plot for ELF scores according to the Metavir stages of liver fibrosis. ELF score increased with histological stage of liver fibrosis with significant differences between groups ($p<0.0001$, Kruskall-Wallis test). There was a significant correlation between Metavir fibrosis stage and ELF score [$r=0.554$ (95% confidence interval CI 0.489–0.613), $p<0.0001$, Spearman's rank correlation].

The ROC curve analyses showed the diagnostic performances of the automated ELF score to discriminate significant fibrosis (F≥2), severe fibrosis (F≥3) and cirrhosis (F4) in patients with CHC (Figure 2). The areas under the ROC curves (Table 2) showed good performances (AUROC higher than 0.80) for severe fibrosis (AUROC=0.82; 95% CI 0.74–0.82) and for cirrhosis (AUROC=0.85; 95% CI 0.81–0.90), but slightly lower diagnostic performances for significant fibrosis (AUROC=0.78; 95% CI 0.74–0.82). AUROC were not significantly different when using the original ELFG score,

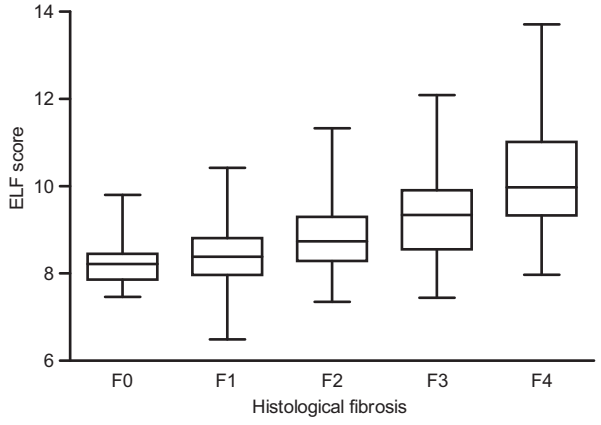

Figure 1 ELF score (median, quartiles, range) according to the Metavir fibrosis stages.

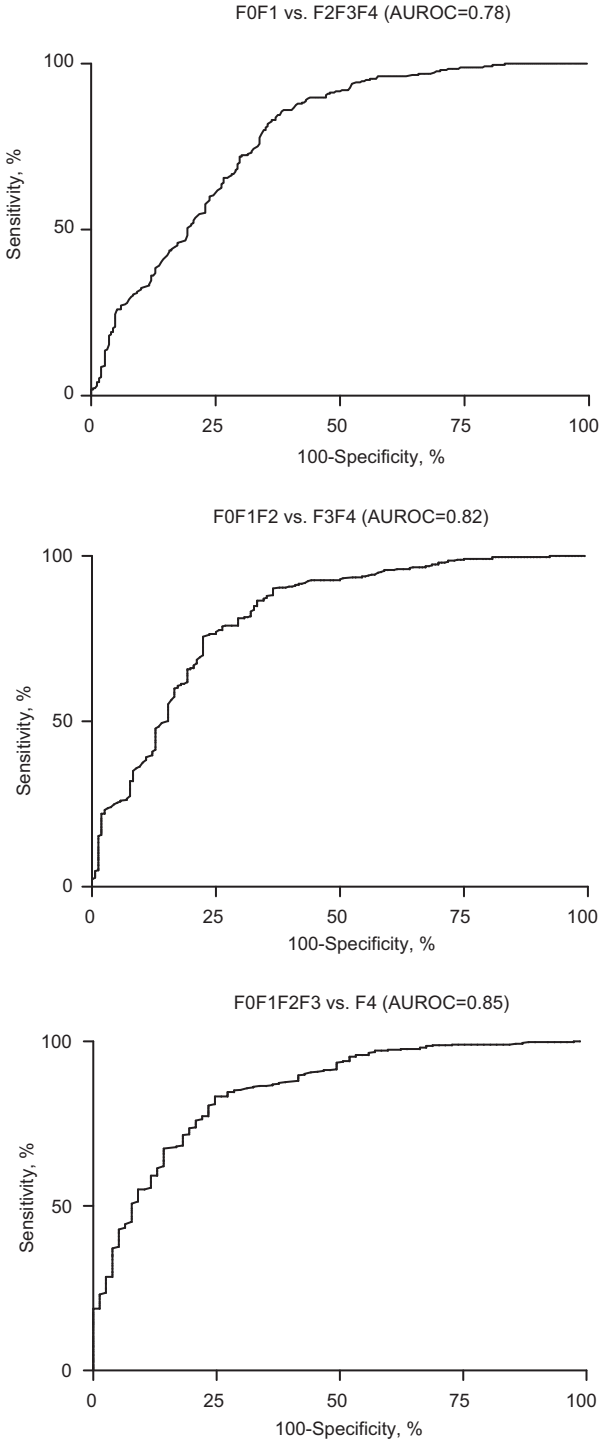

Figure 2 ROC curves for ELF score predictive value of significant fibrosis (≥F2), severe fibrosis (≥F3) and cirrhosis (F4) in patients with chronic hepatitis C.

respectively: 0.780 (CI 0.74–0.82) for diagnostic of significant fibrosis; 0.82 (CI 0.78–0.86) for diagnostic of extensive fibrosis and 0.85 (CI 0.81–0.90) for diagnostic of cirrhosis.

The performances of the ELF score for identification of significant fibrosis, severe fibrosis or cirrhosis are shown in Table 3.

Table 2 Area under ROC curves (95% CI) for ELF score according to fibrosis stage. Comparison with previous studies.

	Parkes et al. (14)				This study
	Original cohort	Cohort 1	Cohort 2	Cohort 3	
Patients number	921	87	173	87	512
Fibrosis stage					
Scheuer F0/F1/F2/F3/F4, %	24/36/13/15/12				
METAVIR F0/F1/F2/F3/F4, %		10/33/32/10/14			7/45/18/15/15
Ishak 0/1/2/3/4/5/6, %			20/15/117/12/7/11/17	1/16/27/21/8/16/10	
For significant fibrosis diagnosis					
AUROC	0.79	0.74	0.83	0.87	0.78
(95% CI)	(0.76–0.82)	(0.63–0.84)	(0.76–0.89)	(0.80–0.95)	(0.74–0.82)
For severe fibrosis diagnosis					
AUROC	0.83	0.84	0.86	0.89	0.82
(95% CI)	(0.80–0.86)	(0.74–0.94)	(0.80–0.92)	(0.83–0.96)	(0.78–0.86)
For cirrhosis diagnosis					
AUROC	0.86	0.90	0.8	0.89	0.85
(95% CI)	(0.83–0.89)	(0.81–0.98)	(0.81–0.93)	(0.82–0.96)	(0.81–0.90)

Comparison of the ELF score with other serum markers of liver fibrosis

The Obuchowski measure was 0.81 (95% CI 0.78–0.84) showing that ELF score globally performed as a biochemical marker of liver fibrosis. In the same 512 patients, the diagnostic accuracy of the simplified ELF score was equivalent to the original ELFG score (Obuchowski measure: 0.81; p=0.292). The comparisons with other serum marker of liver fibrosis showed that ELF score had better diagnostic accuracy than hyaluronic acid serum level (Obuchowski measure: 0.78; p=0.006) but slightly less performed than Fibrotest® (Obuchowski measure: 0.82; p=0.292) or Hepascore® (Obuchowski measure: 0.83; p=0.053).

Discussion

The prognosis of chronic liver diseases is closely related to the development of liver fibrosis which commonly occurs as the disease progresses. In chronic hepatitis C, liver fibrosis has to be evaluated both as a prognostic index and as a criterion in the treatment decision. Furthermore, when cirrhosis, the end-stage consequence of progressive fibrosis, is evidenced, the assessment of the risk of severe complications occurrence, including ascites, variceal bleeding, encephalopathy, and hepatocellular carcinoma, has important clinical and therapeutical implications.

With the specific aim of replacing pathological examination of liver biopsy, various non-invasive tests of liver fibrosis have been developed for monitoring patients with CHC infection (5–14). These include routinely available laboratory tests, such as liver-associated chemistries, A2M, platelet count, and prothrombin time, as well as specific serum markers of fibrosis, such as serum hyaluronic acid, PIIINP, matrix metalloproteinases and their inhibitors.

Until now, there are no FDA approved tests and the American recommendations (20, 21) have considered that currently available non-invasive tests may be useful in defining the presence or absence of advanced fibrosis in persons with chronic hepatitis C infection, but should not yet replace the liver biopsy in routine clinical practice. The French National Authority for Health has considered that in chronic untreated hepatitis C adult patients with no co-morbidities three biological tests have been validated as non-invasive procedures for liver fibrosis evaluation and/or cirrhosis diagnostic [Fibrotest® (7), FibroMeter® (8) and Hepascore® (9)] and recommended (10) to use one of them or liver biopsy or transient elastography [FibroScan™ (22)] as first-line test. In France, these four tests have recently received this year the agreement for reimbursement by the mandatory health insurance in this indication. About the ELFG score, the health authority and the recommendations have specified that this non-invasive test has shown a potential clinical benefit in the same indication but with insufficient scientific

Table 3 ELF score performance for identification of significant fibrosis, severe fibrosis or cirrhosis in patients with chronic hepatitis C.

	For significant fibrosis	For severe fibrosis	For cirrhosis
Cut-off	9.0	9.33	9.35
Sensitivity (95% CI)	0.86 (0.81–0.90)	0.90 (0.87–0.93)	0.83 (0.79–0.66)
Specificity (95% CI)	0.62 (0.55–0.68)	0.63 (0.55–0.71)	0.75 (0.64–0.84)
Positive predictive value	0.80	0.73	0.44
Negative predictive value	0.70	0.85	0.95
Youden index	0.47	0.53	0.59

data to consider it as totally validated, requiring complementary and independent confirmation studies (12, 23). Since complementary and independent confirmation studies were required, we have chosen to evaluate the ELF score in a large cohort (n=512) of patients with CHC included in a prospective controlled study. Using the simplified ELF score did not alter the diagnostic performance of the test since it was equivalent using the original ELFG score. However, the ELF score performs slightly less than Fibrotest® or Hepascore® as previously shown for the ELFG score (13). Since it is well documented that the variability of liver biopsy is not negligible, and it was shown that the histological staging of needle biopsy specimens is impaired both by variation in the severity of the diseases in different parts of the liver and by observer variability (3, 4) the rigorous pathological examination of liver biopsies was a criterion of this study. All patients had liver biopsies of good sizes (>15 mm and/or >10 portal tracts with a mean length of 25 mm) reviewed by two independent senior hepato-pathologists, enough for optimizing the histopathological analysis as previously shown (24). In comparison, other cohorts for simplified ELF score validation (14) included less patients (n=87, 173, 87) without criterion of liver biopsies size (Table 2).

Our results confirm previous data shown with the original ELFG score (11) and with the simplified ELF score in chronic hepatitis C (14), or in chronic liver diseases of various etiologies (24). The Obuchowski measure, an adjusted-on-fibrosis-stages distribution AUC, shows that the ELF score globally performs as a biochemical marker of liver fibrosis. It can be interpreted as the probability that the ELF score will correctly rank two randomly chosen patient samples from different fibrosis grades. The areas under the ROC curves showed good performances (AUROC higher than 0.80) for severe fibrosis and for cirrhosis diagnostics. Slightly lower diagnostic performances were shown for significant fibrosis diagnostic (AUROC=0.78) as in the original cohort (AUROC=0.79) and another study (AUROC=0.78) (14, 25). Globally, the diagnostic performances of the simplified ELF score, as those of the original ELFG score, are only slightly lower the diagnostic performances of Fibrometer®, Hepascore®, and Fibrotest®, the most performing tests and noticeably better than HA, APRI, Forns' or FIB-4 (13).

Since the diagnosis of cirrhosis is important the high NPV for excluding cirrhosis (95%) could be useful in clinical practice to avoid a number of liver biopsies.

We used commercial assays for serum measurements of the ELF score components (HA, PIIINP, TIMP-1) independently of the proprietary assays developed by Siemens Healthcare Diagnostics Inc., which were not yet marketed at the time of the study. Our results show the robustness of the ELF score since it confirms its diagnostic value with other assay methods. While the methods we used for TIMP 1 and PIIINP are sophisticated, time consuming and onerous, only HA assay can be automatized using general clinical chemistry analyzers, an advantage of the ELF score is that it can be totally automated using the captive reagents for the Siemens ELF™ test that necessitate a single analyzer and only one serum sample, like it is possible for the Hepascore® (26) or for the Fibrotest® (27) but not for the scores including platelets count or prothrombin time like the Fibrometer®, APRI, FIB-4 or Forns' score. However, since ELF score components are not specific of liver fibrosis, more studies are needed to determine the best use of the test.

In conclusion, our results show that ELF score parameters can be assayed with other validated reagents than those originally used and confirm the diagnostic value of the simplified ELF score in evaluating the liver fibrosis in patients with chronic hepatitis C. This test might be added to the non-invasive biological tests approved by the health authorities in this indication.

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Conflict of interest statement

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