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Assessment of liver fibrosis before and after antiviral therapy by different serum marker panels in patients with chronic hepatitis C

Running title: Serum marker panels in chronic hepatitis C

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ABSTRACT

Background/Aims: Liver biopsy is the reference standard to assess liver fibrosis in chronic hepatitis C (CHC). We validated and compared the diagnostic performance of noninvasive tests for prediction of liver fibrosis severity and assessed changes in extracellular matrix (ECM) markers after antiviral treatment. ***Methods:*** The performance of Forns´score, APRI, FIB-4 index, and ELF (Enhanced Liver Fibrosis) score was validated in 340 patients who underwent antiviral therapy. These scores were determined 24 weeks after treatment in 161 patients. ***Results*:** Forns´ score, APRI, FIB-4 and ELF score showed comparable diagnostic accuracies for significant fibrosis (AUROC 0.83, 0.83, 0.85 and 0.81, respectively). To identify cirrhosis, FIB-4 index showed a significantly better performance over APRI and ELF score (AUROC 0.89 vs. 0.83 and 0.82, respectively). ELF score decreased significantly in patients with sustained virologic response (SVR) (p<0.0001) but remained unchanged in non-responders. Non-1 HCV genotype, baseline lower HCV RNA, glucose, hyaluronic acid and higher cholesterol levels were independently associated with SVR**.** ***Conclusions:*** Simple panel markers and ELF score are accurate at identifying significant fibrosis and cirrhosis in CHC. A decrease in ELF score after antiviral treatment reflects the impact of viral clearance in hepatic ECM and probably in the improvement of liver fibrosis.

Keywords: cirrhosis, ELF score, extracellular matrix markers, Forns´ score, significant fibrosis

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Introduction

An estimated 170 million persons worldwide are chronically infected with hepatitis C virus (HCV) (1), a leading cause of chronic liver disease, which may eventually lead to cirrhosis and end-stage liver disease (2).

Current antiviral treatment for CHC has significant side effects and has a far from optimal efficacy, particularly in patients with genotype 1 (3, 4). Thus, identification of significant liver fibrosis stage is essential to establish the timing of antiviral treatment (5). Furthermore, the diagnosis of cirrhosis not only establishes the need for antiviral treatment but is crucial for identifying those patients in whom screening for gastroesophageal varices and hepatocellular carcinoma is mandatory. Finally, assessment of the effect of antiviral treatment on liver fibrosis is another desirable end point for evaluation of the efficacy of therapy. Liver biopsy is classically considered the reference standard to assess the extent of fibrosis, though it is associated with risk of complications and has limitations due to observer variability and sampling error (6-8). Thus, several routine laboratory tests combined in scores and indices such as Forns'score, APRI index and FIB-4 index, have been developed and validated as useful noninvasive and inexpensive tools to accurately detect significant fibrosis or cirrhosis in clinical practice (9-12). Furthermore, a panel of 5 markers (α2-macroglobulin, haptoglobin, apolipoprotein A1, gammaglutamyl transpeptidase and total bilirubin), that is commercialized as FibroTest, has also been validated in the detection of fibrosis and in the evaluation of response to interferon based therapy (13, 14).

More recently, transient elastography has become a useful, rapid and reproducible novel method to assess liver fibrosis through the measurement of liver stiffness. It has been shown to have good diagnostic performance when combined with FibroTest (15, 16).

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In addition, some serum markers that reflect the dynamics of fibrosis, involving extracellular matrix (ECM) synthesis or degradation mainly by hepatic stellate cells (HSC) (17), such as aminoterminal propeptide of type III procollagen (PIIINP), hyaluronic acid (HA), tissue inhibitor of matrix metalloproteinase type 1 (TIMP-1) and YKL-40, have been studied individually or in combination in the detection of the severity and progression of hepatic fibrosis and in the follow-up of changes in relation to antiviral treatment (18-22). A panel of such markers (PIIINP, HA and TIMP-1) combined with age, originally reported as the European Liver Fibrosis (OELF) score (23), was shown to be specific and sensitive in the evaluation of liver fibrosis in chronic liver diseases of different etiology (24-26). **A longitudinal assessment of this validated predictive score (ELF) after antiviral treatment is lacking in the literature. Since the markers included in this score are directly related to the fibrogenesis process, the evaluation in this setting could confirm their ability in monitoring liver fibrosis regression.**

The aims of the present study were (1) to validate and compare the diagnostic performance of several noninvasive tests in the prediction of liver fibrosis severity in a large cohort of patients with CHC from a single center, and (2) to assess the relationship between changes in serum ECM markers and virologic response to antiviral therapy.

Patients and methods

Patient population

This is a cohort study that included 340 consecutive patients with CHC who underwent antiviral treatment at our institution between August 2001 and November 2007. **The same protocol (including number of visits, blood tests and serum sampling) was used during the study period.** The diagnosis of CHC was established by the presence of hepatitis C virus (HCV) RNA using polymerase chain reaction assays. All patients underwent a pretreatment

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liver biopsy within 6 months prior to the initiation of therapy. Patients with human immunodeficiency virus or hepatitis B virus co-infection, or with other causes of chronic liver disease were not included.

Antiviral treatment was the standard of care, with weekly pegylated interferon alfa-2a (180ug) or alfa-2b (1.5 ug/kg) plus ribavirin (0.8-1.2 g daily) for 24 or 48 weeks, according to HCV genotype. The use of hematopoietic growth factors, epoetin alfa or darbepoetin and filgrastim, was allowed to treat anemia or neutropenia, respectively**.** Sustained virologic response (SVR) was defined by undetectable serum HCV RNA by qualitative polymerase chain reaction assay (Cobas Amplicor, HCV Roche, v 2.0, detection limit 50 IU/mL) at 24 weeks after the end of therapy.

All patients provided written informed consent to data handling according to a protocol approved by the ethical committee of our Institution.

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Liver Histology

Percutaneous liver biopsies were performed under local anesthesia and ultrasound guidance with a Tru-Cut 14 gauge needle (Angiomed, Bard, Karlsruhe, Germany) by expert radiologists. Specimens were fixed in formalin, embedded in paraffin and stained with hematoxylin-eosin and Masson´s trichrome. **A minimum length of 10 mm and the presence of 6 portal tracts were required for diagnosis**. Histological grade and stage were determined according to METAVIR scoring system (27) by the same pathologist (M.B.), **who was blinded for patients' data**. Liver fibrosis was considered significant when it spread out of the portal tract (stages 2, 3 or 4).

Routine laboratory tests

Baseline blood samples were collected on the day of antiviral treatment initiation, as well as at the end of treatment and the end of follow-up on a protocol basis. Laboratory tests included complete blood cell counts, HCV RNA serum concentration, HCV genotype, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyl transpeptidase (GGT) and cholesterol. These parameters were used to calculate Forns´score, APRI index and FIB-4 index at baseline and at 24 weeks after treatment, as previously described (9-12).

Serum markers of ECM assays

Fasting serum samples (collected at baseline and at 24 weeks after antiviral treatment) were stored at - 80 º C until assayed for levels of HA, TIMP-1 and PIIINP. ECM assays were determined using a random access automated clinical immunochemistry analyzer that performs magnetic separation enzyme immunoassay tests (Immuno 1^{TM}, Siemens Healthcare Diagnostics, Tarrytown, NY). **Each method has a set of six calibrators. The HA and TIMP-1 methods use a cubic-through-zero curve-fitting algorithm and the PIIINP method uses a weighted-cubic-through-zero fit to construct calibration curves. Rates are measured for the six calibrators.** Serum levels of ECM markers were expressed in ng/mL. The mean reference values of PIIINP, TIMP-1 and HA, obtained from 60 healthy subjects (47% males, median age 44 years) were 6.13 ± 2.9, 636.4 ± 108.4 and 38.8 ± 36.9, respectively. The intra- and inter-assay coefficients of variation were < 3.8 % and < 5.8 %, respectively**.** Patient values were entered into the ELF (Enhanced Liver Fibrosis) algorithm, where the original score was simplified by removing age (unpublished observations by Parkes et al., recently validated in the detection of fibrosis in patients with nonalcoholic fatty liver disease) (24), and the

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results expressed as discriminant scores. **Investigators performing the laboratory tests were blinded for patients' clinical and histological data.**

Statistical Analysis

Patients' baseline characteristics are given as mean ± SD**,** median or proportion, as appropriate. The diagnostic accuracy of the different methods was analyzed by constructing receiver operating characteristic (ROC) curves and calculating the area under the curves (AUROCs). **We assessed the impact of the prevalences of the fibrosis stages defining nonadvanced and advanced fibrosis on the observed AUROCs (obAUROC) by using a previously suggested method of standardization (28). Firstly, we calculated the difference between advanced and nonadvanced mean fibrosis stage (DANA) according to a uniform distribution with a prevalence of 0.20 for each of the 5 stages in METAVIR units (uniform DANA= [2+3+4/3]-[1+0/2] = 2.5) or to the observed prevalence distribution in our cohort (natural DANA). The regression between the AUROCs of the different tests for the diagnosis of advanced fibrosis versus the DANAs resulting of different combinations of fibrosis stages allows to estimate the AUROCs from DANAs. The resulting regression equation was: AUROC = constant coefficient + (DANA regression coefficient) (DANA). The formula to calculate the adjusted AUROC according to our observed fibrosis prevalence was: ObAUROC + (DANA regression coefficient) (natural DANA - Observed DANA). The formula to calculate the adjusted AUROC according to a uniform DANA of 2.5 was: ObAUROC + (DANA regression coefficient) (2.5 – Observed DANA).**

The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), likelihood ratios (LR) and diagnostic odds ratios **(DOR)** were determined, using hepatic fibrosis stage as determined by liver biopsy as the reference. **DOR measures the overall**

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diagnostic test´s accuracy by dividing the LR+ by the LR-. Chi-square and *t* tests were used to analyze categorical and continuous variables, respectively. The Wilcoxon matched pairs signed-rank test was used to evaluate changes between baseline and end of follow-up scores. Logistic regression analysis was used to test for associations between variables and the type of virologic response. A two-tailed *p* value of less than 0.05 was considered statistical significant.

Statistical analyses were performed with SPSS software (version 14.0, SPSS Inc, Chicago, IL) and the comparison of AUROC values was carried out by the DeLong et al method (29).

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Results

Characteristics of patients

Baseline clinical, laboratory and virologic characteristics of the 340 patients are shown in Table 1. The mean age of the patients was 47.7 years and 63.8% were male. The vast majority of patients (73.9%) were infected with HCV genotype 1. **Mean biopsy length was 15 mm (range 10-30 mm), with 55% of specimens > 15 mm, 16% > 20 mm and 1% > 25mm**. **Mean number of portal tracts was 9.** The stage of liver fibrosis was distributed as follows: F0, n= 34 (10%); F1, n= 77 (22.6 %); F2, n= 74 (21.8%); F3, n= 31 (9.1%); F4, n= 124 (36.4%). The prevalence of significant fibrosis (F ≥ 2) in this cohort was 67.3%. **Diabetes mellitus was present in 8.5% of the patients.**

Performance of noninvasive tests

The results for the overall accuracy of the different tests for significant fibrosis and cirrhosis are presented as ROC curves in figures 1 and 2, respectively. The AUROCs of Forns´ score, APRI, FIB-4 index and the ELF score had similar diagnostic accuracies for significant

fibrosis as assessed by DeLong´s method. FIB-4 index was significantly better than APRI and the ELF score for the diagnosis of cirrhosis, as assessed by non parametric analysis of AUC values (Table 2). **The performance of all the tests improved only for the diagnosis of cirrhosis in patients with a biopsy length > 20 mm.** Standardization of AUROCs to address spectrum effect was evaluated for the different tests. **The observed AUROCs for significant fibrosis for Forns´score, APRI, FIB-4 and ELF test ranged from 0.70 to 0.94; 0.67 to 0.95; 0.70 to 0.96 and 0.68 to 0.88, respectively. Standardized AUROCs according to the uniform and naturally observed prevalences of fibrosis stages for these tests were 0.82 and 0.83; 0.831 and 0.832; 0.85 and 0.85; 0.80 and 0.81, respectively.**

The estimates of sensitivity, specificity and negative and positive predictive values according to the cutoff values originally reported for diagnosis of significant fibrosis**,** severe fibrosis (F3-4) and cirrhosis for each test are shown in table 3.

By using Forns´score for the prediction of absence or presence of significant fibrosis (F2 or greater), 64 of 89 (71.9%) patients with a value lower than 4.2 had non significant fibrosis. Applying the high cutoff of this score (>6.9), 101 of 109 (92.6%) had significant fibrosis. Only 8 patients with non significant fibrosis were misclassified. Forty-nine percent were correctly classified and liver biopsy could be avoided in 58% of patients.

By APRI, 56 of 76 (73.7%) patients with a value of 0.5 or lower did not have significant fibrosis. Among those with an index higher than 1.5, 107 of 115 (93%) patients did have significant fibrosis. Using these cutoff values, absence or presence of significant fibrosis was correctly identified in 48% of patients, and liver biopsy could be avoided in 56%. For the outcome of cirrhosis, 159 of 181 (87.9%) patients with an APRI value of 1 or lower did not have cirrhosis. Only 22 of 124 (17.7%) with cirrhosis were incorrectly classified. For those with an APRI value higher than 2, 61 of 81 (75.3%) had cirrhosis and only 20 of 216 (9%)

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without cirrhosis were incorrectly classified. Using these cutoff values, absence or presence of cirrhosis can be correctly identified in 65% of patients, avoiding liver biopsy in 77% of patients.

By applying FIB-4 index, 118 of 131 patients (90%) with a value below 1.45 did not have severe fibrosis (F3-4). Eighty-three of 100 patients (83%) with an index higher than 3.25 had severe fibrosis. With both thresholds, 59% of patients were correctly identified and liver biopsy could be avoided in 68% of patients.

When using the ELF score, we determined two threshold values to recognize the absence or presence of significant fibrosis. Below a cutoff value of -0.45, 58 of 80 patients (73%) did not have significant fibrosis. Above a cutoff value of 1.07, 108 of 119 (91%) patients had significant fibrosis. With these thresholds we could correctly classify 49 % of patients, and liver biopsy could be avoided in 59% with a minimal diagnostic error. For the outcome of cirrhosis, 114 of 127 patients (90%) with a cutoff value below 0.06 did not have cirrhosis. For those patients with an ELF score higher than 1.73, 65 of 86 (76%) had cirrhosis. With these thresholds absence or presence of cirrhosis could be correctly identified in 53%, and liver biopsy could be avoided in 63%.

Baseline ECM markers as predictors of virologic response

One hundred and sixty-two patients achieved a sustained virologic response while 178 patients did not. Among the latter, 62 patients had undetectable HCV-RNA at the end of treatment but relapsed during follow-up. Baseline clinical characteristics according to virologic response are shown in Table 4. Sustained virologic responders were more likely to have lower baseline levels of GGT, glucose, and HCV RNA, higher levels of cholesterol, higher platelet count, non-1 HCV genotype, and less severe fibrosis. The ELF score and two

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of its components, HA and TIMP-1, were also significantly lower in sustained virologic responders.

Multivariate logistic regression analysis was used to test which baseline variables could predict virologic response. In the final model, HCV RNA viral load (odds ratio (OR), 0.45; 95% CI 0.32-0.65, *p*= 0.0001), cholesterol (OR, 1.01; 95% CI 1.01-1.02, *p*= 0.0001), glucose (OR, 0.98; 95% CI 0.97-0.99, *p*= 0.004), HCV genotype 1 (OR, 0.42; 95% CI 0.24 –0.76, *p*= 0.004) and serum HA (OR, 0.99; 95% CI 0.99 –1.00, *p*= 0.008) were all independent predictors of SVR. A sub-analysis of patients with HCV genotype 1 showed that the same variables were independent predictors of SVR.

Score changes according to virologic response

Among the 340 patients included in this study, 161 had serum samples available for measurement of matrix markers at 24 weeks of follow-up after treatment (limited availability of reagents precluded testing of all samples). Baseline characteristics of this subset of patients are shown in Table 1. Eighty-five patients achieved sustained virologic response and seventy-six, including 39 who relapsed, did not.

Changes in the mean ELF score and its components are summarized in Table 5. At 24 weeks after therapy the ELF score decreased significantly in patients who achieved sustained virologic response but remained unchanged in those who did not. The mean ELF score did not change significantly in relapsers (data not shown). A significant decrease of all of the components of the ELF score was observed in sustained virologic responders whereas HA and PIIINP remained unchanged and TIMP-1 increased in non sustained responders.

The individual changes in the ELF score according to virologic response and to the severity of liver fibrosis at baseline are shown in Figure 3. On an individual basis, the ELF score decreased in most sustained virologic responders but in only a minority of non sustained

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virologic responders, in most of whom the ELF score remained unchanged or increased. The decrease of the ELF score was more marked in patients with more advanced liver fibrosis, who showed higher mean ELF scores at baseline.

As expected, Forns´score, APRI and FIB-4 index decreased significantly in patients who achieved sustained virologic response (Table 5). This is mainly due to the normalization of their respective components (particularly AST and ALT).

Discussion

The use of routine hematological and biochemical parameters combined in panels such as Forns´ score, APRI or FIB-4 index, is an "indirect", easy and inexpensive approach to identify patients with significant fibrosis and cirrhosis. The Forns´ score was developed and validated in a population where only 25% of patients had significant hepatic fibrosis (9) while the prevalence of significant fibrosis in the present cohort was much higher (67 %). This difference may explain why this test performed better in the present cohort than in the original study to rule in significant fibrosis, with a PPV higher than 90%, but not to exclude significant fibrosis. Similarly, the PPV for significant fibrosis obtained in this study with APRI ≥ 1.5, compared favorably with that reported in the original study (93% vs. 88%), although the NPV at the 0.5 cutoff point was lower (74 % vs. 86%) (10). Similar results were shown with the FIB-4 index. For the outcome of cirrhosis, all scores performed well but FIB-4 showed a significantly better accuracy as compared to APRI and ELF.

The use of scores including direct fibrogenesis markers may be an advantage in certain situations, such as in patients with CHC who undergo antiviral therapy. **Since the markers included in such scores are directly related to the fibrogenesis process, their assessment might prove useful to monitor liver fibrosis regression, a well documented finding in individuals achieving a sustained virological response (30, 31).** In previous studies,

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significant declines from baseline of PIIIP and YKL-40 were also noted at week 72 in patients with an SVR compared with in NRs (22).

The main purpose of this study, was to assess the performance of a score based on fibrogenesis markers (ELF score), due to its potential utility to evaluate changes during follow-up or after treatment. **The testing algorithm is patented but is not yet commercially available in most countries. In our cohort, the ELF score yielded a good accuracy in the diagnosis of liver fibrosis and cirrhosis, with similar accuracy to that of simple panels. Moreover, our** study showed a significant decrease of the mean serum concentration of ECM markers in patients who had eliminated the virus, which did not occur in non responders or in relapsers. As expected, analysis of the combined ELF score produced similar results. Analysis of changes in individual patients showed that the ELF score decreased in most sustained virologic responders, particularly in those with more severe fibrosis at baseline, whereas the ELF scores increased or remained unchanged in most non-sustained virologic responders. The significant decline in ECM markers experienced only by sustained responders probably reflects a decrease in liver fibrogenesis once the primary cause, HCV virus, is cleared, with eventual normalization of the imbalance between degradation and synthesis of liver collagen. These conclusions are in agreement with the improvement in liver fibrosis observed in sustained virological responders from large clinical trials where a follow-up liver biopsy was available (30, 31).

The results of our study are in accordance with recent reports evaluating the effect of anti-HCV treatment using other non-invasive methods. **A comparison of HCV FibroSURE (or FT-AT) and FIBROSpect II (HA, TIMP-1 and α_2 macroglobulin) during a phase 2b clinical trial with albinterferon alfa-2b plus ribavirin noted a significant decline in the score values in patients with SVR compared with in nonresponders (32). Another study performed a longitudinal evaluation of FT-AT with HA as a comparative**

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reference in CHC patients treated with IFN monotherapy; the authors observed a significant decrease of FT-AT in those who obtained SVR versus NR and relapsers, but with no significant changes noted in HA (33). In a more recent study, a comparison of the effect of antiviral therapy on FT and FibroScan between treated and untreated patients, showed a significant decrease of FT at the end of follow-up for those patients who obtained SVR or relapsed (34).

In our study, the significant increase in serum TIMP-1 levels observed at the end of follow-up in non-sustained virologic responders may indicate that fibrosis is progressing in these patients. Indeed, other reports found a similar TIMP-1 increase following interferon alfa therapy in nonresponder patients (35-37). TIMP-1 protects collagen from fibrolysis by the matrix metalloproteinases and also inhibits the apoptosis of hepatic stellate cells (38). In experimental models, overexpression of TIMP-1 was associated to enhanced fibrosis, supporting the hypothesis that inhibition of matrix degradation may contribute to progression of fibrosis (39).

We also observed significant post-treatment changes of Forns' score, APRI and FIB-4 tests. However several components of these tests, such as serum cholesterol, platelet counts and particularly transaminases, that are not directly involved in hepatic fibrogenesis or fibrolysis, may change under antiviral therapy, particularly in responders.

Of interest, by multivariate analysis, HA, a component of the ELF score showed an association with sustained virologic response. Previous studies have shown that HA levels reflect an increased production of this marker by hepatic stellate cell as well as a decreased removal from circulation, which depends on the uptake by specific receptors in hepatic sinusoidal endothelial cells (40, 41). Higher HA levels and lower probability of virologic response could reflect dysfunction of endothelial sinusoidal cells that is present in patients with more advanced liver fibrosis, another independent predictor of virologic response.

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Our study has several limitations. First, the lack of a follow-up liver biopsy, which prevented us to directly assess the effect of treatment on liver fibrosis. Second, the short period of time that elapsed between baseline and follow-up evaluations. Since liver fibrosis decreases progressively after a sustained virologic response (42), the evaluation period of the study might have been too short to detect additional effects. Third, the proportion of patients with a biopsy size >20 mm was suboptimal. Finally, although this is a cohort study, ECM assays were performed on stored serum samples which were not available for all included patients.

In summary, this study of a large cohort of patients with CHC confirms that both indirect fibrosis tests and measurement of ECM serum markers, included in the ELF score, are accurate to predict the severity of fibrosis. ECM markers and the composite ELF score significantly decreased in sustained virologic responders but remained unchanged in non sustained responders, suggesting that these markers may be useful as a non-invasive means to assess the effects of antiviral therapy on hepatic fibrosis and fibrogenesis. **The potential utility of the ELF test in this setting as compared with other commercially available patented markers would require extensive validation and a cost-effective analysis.**

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Abbreviations:

CHC, chronic hepatitis C; ECM, extracellular matrix; significant fibrosis, F≥ 2; ELF, Enhanced Liver Fibrosis; APRI, AST to platelet ratio index; PIIINP, aminoterminal propeptide of pro-collagen III; HA, hyaluronic acid; TIMP-1, tissue inhibitor of metalloproteinase type 1; ROC, receiver operating characteristic curve; AUROC, area under the ROC curve; **obAUROC, observed AUROC;** SVR, sustained virologic response; HCV, hepatitis C virus; HSC, hepatic stellate cells; AST, aspartate aminotransferase; ULN, upper limit of normal; ALT, alanine aminotransferase; GGT, gamma glutamyl transpeptidase; severe fibrosis, F≥ 3; Se, sensitivity; Sp, specificity; PPV, positive predictive value; NPV, negative predictive value; LR, likelihood ratio; DOR, diagnostic odds ratio; OR, odds ratio.

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Tables

Table 1. Baseline characteristics of the patients

Variable	Entire cohort n = 340	Follow-up ELF score n = 161[a]	*P* value
Demographics			
Age (yrs)	47.7 ± 10.6	48.1 ± 10.7	0.54
Male sex	217 (63.8)	102 (63.4)	0.87
Body mass index (kg/m^2)	25.4 ± 3.6	25.1 ± 3.5	0.07
Laboratory			
AST/ULN	2.15 ±1.64	2.2 ±1.8	0.51
ALT/ULN	2.94 ± 2.5	3.1 ± 3.0	0.28
GGT/ULN	2.03 ± 1.81	1.9 ± 1.7	0.06
Platelet count (10^3/mm^3)	180.5 ± 72.1	179.0 ± 73.6	0.15
Cholesterol (mg/dL)	177.9 ± 39.2	184.5 ± 44.1	0.005
Virology			
HCV RNA $\log_{10}$ (IU/mL)	5.6 ± 0.7	5.9 ± 0.7	0.001
HCV genotype			0.2
1	251 (73.9)	111 (69)	
2	26 (7.6)	15 (9.3)	
3	45 (13.2)	28 (17.4)	
4	18 (5.3)	7 (4.3)	
Length of biopsy core (mm)	15 ± 3.9	15 ± 4.1	0.15
Fibrosis Stage			0.96
F0	34 (10)	13 (8.1)	
F1	77 (22.6)	37 (23.0)	
F2	74 (21.8)	40 (24.8)	
F3	31 (9.1)	16 (9.9)	
F4	124 (36.4)	55 (34.2)	

[a] 161 patients in whom ELF score was determined before and after antiviral therapy

Results are means ± standard deviation or n (%)

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Table 2. Overall accuracy of Forns´ Score, APRI, FIB-4 Index and the ELF Score in predicting significant fibrosis, severe fibrosis and cirrhosis (n=340)

Non-invasive test	Significant fibrosis AUC (95% CI)	F ≥ 3 AUC (95% CI)	Cirrhosis AUC (95% CI)
Forns' Score	0.83 (0.78-0.87)	0.85 (0.81-0.89)	0.87 (0.83-0.91)
APRI	0.83 (0.79-0.88)	0.86 (0.82-0.90)	0.86 (0.82-0.90)
FIB-4 Index	0.85 (0.81-0.89)	0.87 (0.83-0.91)	0.89 *(0.85-0.92)
ELF Score	0.81 (0.76-0.86)	0.83 (0.79-0.87)	0.82 (0.78-0.87)

* p <0.05 vs. APRI and ELF Score by DeLong et al method

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Table 3. Diagnostic accuracy of non-invasive tests in the prediction of significant fibrosis (F≥ 2), severe fibrosis (F ≥ 3) and cirrhosis (F4)

End point	All patients N= 340	Fibrosis Stage n (%)		Se %	Sp %	PPV %	NPV %	LR +ve %	LR -ve %	DOR
F≥ 2	**n (%)**	**F0-1 n=111**	**F ≥ 2 n=229**							
Forns' Score										
<4.2	89 (26.2)	64 (57.7)	25 (10.9)	89	58	81	72	2.1	0.2	10.5
>4.2	251 (73.9)	47 (42.3)	204 (89.1)							
<6.9	231 (68)	103 (92.8)	128 (55.9)	44	93	93	45	6.3	0.6	10.5
>6.9	109 (32.1)	8 (7.2)	101 (44.1)							
APRI										
≤0.5	76 (22.4)	56 (50.5)	20 (8.7)	91	51	79	74	1.9	0.2	9.5
>0.5	264 (77.6)	55 (49.6)	209 (91.3)							
≤1.5	225 (66.2)	103 (92.8)	122 (53.3)	47	93	93	46	6.7	0.6	11.2
>1.5	115 (33.8)	8 (7.2)	107 (46.7)							
ELF Score										
≤ -0.45	80 (23.5)	58 (52.2)	22 (9.6)	90	52	80	73	1.9	0.2	9.5
> -0.45	260 (76.5)	53 (47.7)	207 (90.4)							
≤ 1.07	221 (65)	100 (90.1)	121 (52.8)	47	90	91	45	4.7	0.6	7.8
> 1.07	119 (35)	11 (9.9)	108 (47.2)							
F ≥ 3		**F0-2 n=185**	**F ≥ 3 n=155**							
FIB-4 Index										
<1.45	131 (38.5)	118 (63.8)	13 (8.4)	92	64	74	90	2.6	0.1	26
>1.45	209 (61.5)	67 (36.2)	142 (91.6)							
<3.25	240 (70.6)	168 (91)	72 (46.5)	54	91	83	77	6	0.5	12
>3.25	100 (29.4)	17 (9.2)	83 (53.5)							
F 4		**F0-3 n=216**	**F 4 n=124**							
APRI										
≤1	181(53.2)	159 (73.6)	22 (12.2)	82	74	64	88	3.2	0.2	16
>1	159 (46.8)	57 (16.8)	102(82.2)							
≤2	259 (76.2)	196 (90.7)	63 (50.8)	49	91	75	76	5.4	0.6	9
>2	81 (23.8)	20 (9.3)	61 (49.2)							
ELF Score										
≤0.06	127 (37.4)	114 (52.8)	13 (10.5)	90	53	52	90	1.9	0.2	9.5
>0.06	213 (62.6)	102 (47.2)	111 (89.5)							
≤1.73	254 (74.7)	195 (90.3)	59 (47.6)	52	90	76	77	5.2	0.5	10.4
>1.73	86 (25.3)	21 (9.7)	65 (52.4)							

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Table 4. Clinical characteristics according to virologic response in the entire cohort

Variable	Sustained responders (n = 162)	Non-sustained responders (n = 178)	*P* value
Demographics			
Age (yrs)	46.7 ± 10.8	48.7 ± 10.2	0.07
Sex (male)	103 (64.0)	115 (64.6)	0.74
Body mass index (kg/m^2)	25.5 ± 3.7	25.4 ± 3.5	0.93
Laboratory			
AST/ULN	2.10 ± 1.84	2.20 ± 1.47	0.10
ALT/ULN	3.20 ± 3.10	2.72 ± 1.79	0.23
GGT/ULN	1.63 ± 1.40	2.37 ± 2.10	0.000
Platelet count (10^3/mm^3)	189.2 ± 70.0	172.3 ± 74.6	0.03
Cholesterol (mg/dL)	185.7 ± 40.7	170.5 ± 36.4	0.000
Glucose (mg/dL)	86.6 ± 16.6	100.3 ± 40.0	0.001
Virology			
HCV RNA $\log_{10}$ (IU/mL)	5.6 ± 0.8	6.0 ± 0.6	0.000
Genotype 1	105 (64.8)	146 (82.0)	0.000
Fibrosis Stage			0.04
F0	17 (10.7)	17 (9.6)	
F1	42 (26.4)	34 (19.1)	
F2	36 (22.2)	38 (21.3)	
F3	18 (11.1)	14 (7.9)	
F4	49 (30.3)	75 (42.1)	0.02
ELF Score	0.42 ± 1.31	0.79 ± 1.40	0.02
HA (ng/ml)	146.8 ± 186.3	226.5 ± 290	0.03
TIMP-1 (ng/ml)	836.8 ± 290.0	916.3 ± 286.1	0.002
PIIINP (ng/ml)	9.9 ± 6.7	11.5 ± 9.7	0.06

Data are expressed as number (%), mean ± SD

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Table 5. Baseline and end of follow-up ELF Score and individual markers, Forns´Score, APRI and FIB-4 Index in responder and non-responder patients

Variable	Sustained responders (n = 85)		*P* value	Non-responders (n = 76)		*P* value
	Baseline	End of follow-up		Baseline	End of follow-up	
ELF Score	0.43 ± 1.27	-0.12 ± 1.1	<0.000	0.63 ± 1.4	0.57 ± 1.46	0.80
HA (ng/ml)	135.1 ± 151.5	82.6 ± 105.2	0.000	209.1 ± 293.1	201.6± 279.9	0.70
TIMP-1 (ng/ml)	860.7 ± 272.6	759.1 ± 606.4	0.000	897.3 ± 275.6	919.6± 604.4	0.04
PIIINP (ng/ml)	9.8 ± 6.8	7.5 ± 7.6	0.000	10.3 ± 6.6	10.3± 8.5	0.16
Forns` Score	5.3 ± 2.2	4.6 ± 2.3	0.000	6.2 ± 2.2	6.2 ± 2.3	0.8
APRI	1.7 ± 1.6	0.49 ± 0.36	0.000	1.7 ± 1.5	1.5 ± 1.8	0.02
FIB-4 Index	2.6 ± 2.2	1.9 ± 1.4	0.000	3.2 ± 2.5	3.4 ± 3.5	0.6

Data expressed as mean ± SD

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Legends for figures

Figure 1. ROC curves of Forns' Score, APRI, FIB-4 Index, and the ELF Score for predicting the presence of significant hepatic fibrosis.

Figure 2. ROC curves of Forns' Score, APRI, FIB-4 Index, and the ELF Score for predicting the presence of cirrhosis.

Figure 3. ELF scores according to virologic response and fibrosis stage for baseline and 24 week follow-up samples. (A): F0-1 and sustained virologic response. (B): F0-1 and non-sustained virologic response. (C): F2-4 and sustained virologic response. (D): F2-4 and non-sustained virologic response. The black squares indicate the mean values of the ELF scores at each time points.

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Acknowledgements

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Statement of interests

Authors´ declaration of personal interests

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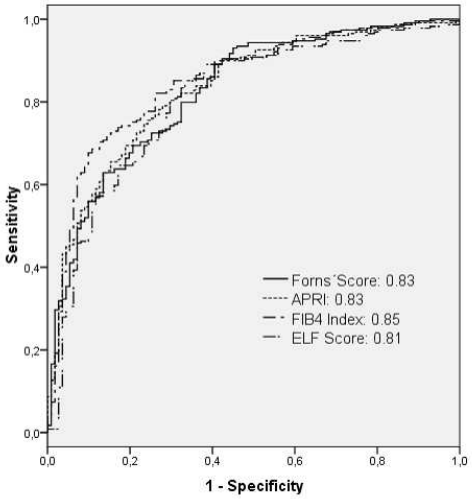

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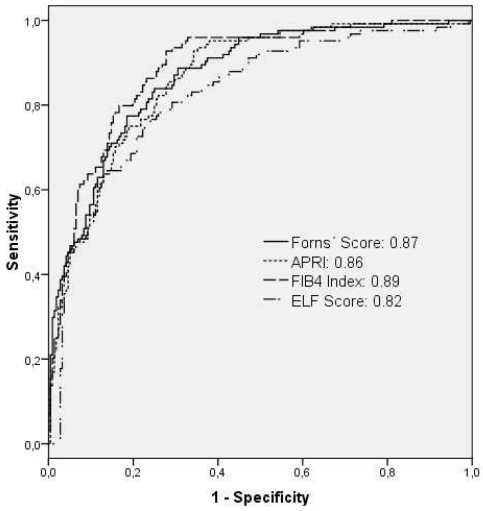

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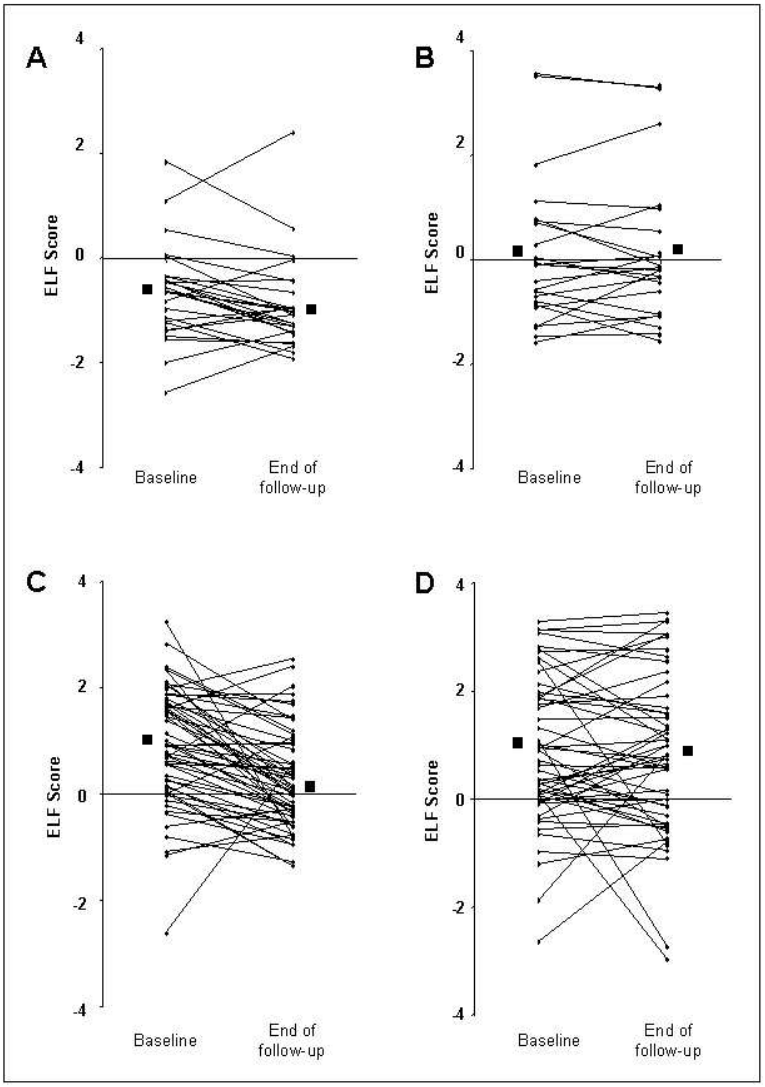

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1

STARD checklist for reporting of studies of diagnostic accuracy

(version January 2003)

Section and Topic	Item #		On page #
TITLE/ABSTRACT/ KEYWORDS	1	Identify the article as a study of diagnostic accuracy (recommend MeSH heading 'sensitivity and specificity').	2
INTRODUCTION	2	State the research questions or study aims, such as estimating diagnostic accuracy or comparing accuracy between tests or across participant groups.	3, 4
METHODS			
Participants	3	The study population: The inclusion and exclusion criteria, setting and locations where data were collected.	4
	4	Participant recruitment: Was recruitment based on presenting symptoms, results from previous tests, or the fact that the participants had received the index tests or the reference standard?	4
	5	Participant sampling: Was the study population a consecutive series of participants defined by the selection criteria in item 3 and 4? If not, specify how participants were further selected.	4
	6	Data collection: Was data collection planned before the index test and reference standard were performed (prospective study) or after (retrospective study)?	4, 5
Test methods	7	The reference standard and its rationale.	5
	8	Technical specifications of material and methods involved including how and when measurements were taken, and/or cite references for index tests and reference standard.	5, 6, 7
	9	Definition of and rationale for the units, cut-offs and/or categories of the results of the index tests and the reference standard.	5, 6, 7
	10	The number, training and expertise of the persons executing and reading the index tests and the reference standard.	5, 6,7
	11	Whether or not the readers of the index tests and reference standard were blind (masked) to the results of the other test and describe any other clinical information available to the readers.	5, 6, 7
Statistical methods	12	Methods for calculating or comparing measures of diagnostic accuracy, and the statistical methods used to quantify uncertainty (e.g. 95% confidence intervals).	6, 7
	13	Methods for calculating test reproducibility, if done.	
RESULTS			
Participants	14	When study was performed, including beginning and end dates of recruitment.	4
	15	Clinical and demographic characteristics of the study population (at least information on age, gender, spectrum of presenting symptoms).	22
	16	The number of participants satisfying the criteria for inclusion who did or did not undergo the index tests and/or the reference standard; describe why participants failed to undergo either test (a flow diagram is strongly recommended).	8,11
Test results	17	Time-interval between the index tests and the reference standard, and any treatment administered in between.	4, 5
	18	Distribution of severity of disease (define criteria) in those with the target condition; other diagnoses in participants without the target condition.	22
	19	A cross tabulation of the results of the index tests (including indeterminate and missing results) by the results of the reference standard; for continuous results, the distribution of the test results by the results of the reference standard.	23, 24
	20	Any adverse events from performing the index tests or the reference standard.	
Estimates	21	Estimates of diagnostic accuracy and measures of statistical uncertainty (e.g. 95% confidence intervals).	23,24
	22	How indeterminate results, missing data and outliers of the index tests were handled.	7, 8
	23	Estimates of variability of diagnostic accuracy between subgroups of participants, readers or centers, if done.	7, 8
	24	Estimates of test reproducibility, if done.	

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DISCUSSION	25	Discuss the clinical applicability of the study findings.	12-15

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