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FibroTest is an independent predictor of virologic response in chronic hepatitis C patients retreated with pegylated interferon alfa-2b and ribavirin in the EPIC[3] program☆

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Background & Aims: EPIC-3 is a prospective, international study that has demonstrated the efficacy of PEG-IFN alfa-2b plus weight-based ribavirin in patients with chronic hepatitis C and significant fibrosis who previously failed any interferon–alfa/ribavirin therapy. The aim of the present study was to assess FibroTest (FT), a validated non-invasive marker of fibrosis in treatment-naive patients, as a possible alternative to biopsy as the baseline predictor of subsequent early virologic (EVR) and sustained virologic response (SVR) in previously treated patients.

Methods: Of 2312 patients enrolled, 1459 had an available baseline FT, biopsy, and complete data. Uni- (UV) and multi-variable (MV) analyses were performed using FT and biopsy.

Results: Baseline characteristics were similar as in the overall population; METAVIR stage: 28% F2, 29% F3, and 43% F4, previous relapsers 29%, previous PEG-IFN regimen 41%, high baseline viral load (BVL) 64%. 506 patients (35%) had undetectable HCV-RNA at TW12 (TW12neg), with 58% achieving SVR. The accuracy of FT was similar to that in naive patients: AUROC curve for the diagnosis of F4 vs F2 = 0.80 (p <0.00001). Five baseline factors were associated (p <0.001) with SVR in UV and MV analyses (odds ratio: UV/MV): fibrosis stage estimated using FT (4.5/5.9) or biopsy (1.5/1.6), genotype 2/3 (4.5/5.1), BVL (1.5/1.3), prior relapse (1.6/1.6), previous treatment with non-PEG-IFN (2.6/2.0). These same factors were associated ($p \leqslant 0.001$) with EVR. Among patients TW12neg, two independent factors remained highly predictive of SVR by MV analysis ($p \leqslant 0.001$): genotype 2/3 (odds ratio = 2.9), fibrosis estimated with FT (4.3) or by biopsy (1.5).

Conclusions: FibroTest at baseline is a possible non-invasive alternative to biopsy for the prediction of EVR at 12 weeks and SVR, in patients with previous failures and advanced fibrosis, retreated with PEG-IFN alfa-2b and ribavirin.

Keywords: Hepatitis C; Non-responder; Relapser; Cirrhosis; Fibrosis; Biomarkers, Treatment failure; Early virologic response.

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† The complete list of the EPIC3 Study Group membership appears in Appendix 1. Study participants were told of investigators' conflicts of interests.

Abbreviations: CI, confidence interval; COPILOT, Colchicine versus PegIntron Long-Term; EPIC[3], Evaluation of PegIntron in Control of Hepatitis C Cirrhosis; EVR, early virologic response; FT, FibroTest; HALT-C, Hepatitis C Antiviral Long-term Treatment against Cirrhosis; HCV, hepatitis C virus; IFN, interferon; LLD, lower limit of detection; OR, odds ratio; PCR, polymerase chain reaction; PEG-IFN, peginterferon; RT-PCR, reverse transcriptase PCR; SVR, sustained virologic response; WBD, weight-based dose.

Introduction

The assessment of fibrosis stage is useful in the treatment of patients with chronic hepatitis C (CHC), both for the decision to treat and in follow-up [1]. Because of the potentially untoward

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complications of liver biopsy, several non-invasive methods have been developed as possible alternatives [2].

FibroTest (FT), a set of non-invasive biomarkers of liver fibrosis and activity, has been extensively validated in patients with CHC [2,3]; with similar diagnostic and prognostic value as a biopsy of 25-mm length [4,5], FT has been approved by the health authority in France as a possible alternative for the initial assessment of fibrosis and cirrhosis in patients with CHC[6,7].

Several studies of FT during standard hepatitis C virus (HCV) treatment in treatment-naive patients have been performed. These studies demonstrated that FT is similar to paired liver biopsies in demonstrating reduced necrosis and fibrosis in sustained virologic responders [5,8–11]. However, no specific study of FT has been performed in previous non-responders to interferon/ribavirin treatment.

The aim of this analysis was to validate the utility of FT as a possible alternative to biopsy for staging fibrosis in non-responders using the first phase of the EPIC-3 trial (Evaluation of PegIntron in Control of Hepatitis C Cirrhosis) – a large, prospective, multiphase clinical program – evaluating the retreatment of patients with CHC with significant fibrosis/cirrhosis in whom previous treatment with non-pegylated or peginterferon (PEG-IFN) alfa plus ribavirin was ineffective (i.e., virologic non-responders or relapsers) [12].

Patients and methods

Patient selection

Patients enrolled in EPIC3 were included in the present FT evaluation if they had 12 weeks of virology (TW12) results available, interpretable baseline FT and liver biopsy. Details of inclusion criteria as well as the results of the first study of the program have been published elsewhere. Twelve patients 18–65 years of age with CHC and significant hepatic fibrosis/cirrhosis (METAVIR score F2, F3, or F4), who failed combination therapy with non-pegylated or PEG-IFN alfa/ribavirin therapy, were eligible to enroll. All patients in this study had previously received a minimum of 12 weeks of combination therapy and did not achieve sustained virologic response (SVR). Patients were categorized according to the previous response (non-responder, relapser, or treatment failure) to combination therapy based on documented HCV-RNA polymerase chain reaction (PCR) results. Non-responders had detectable HCV-RNA at the end of therapy. Relapsers had undetectable HCV-RNA at the end of treatment (EOT) and had subsequent detectable HCV-RNA during post-treatment follow-up. Patients, who did not meet the protocol definition for non-responder or relapser because documentation of HCV-RNA assays did not fulfill these definitions but had detectable HCV-RNA more than 1 week after the end of their previous therapeutic regimen, were designated as treatment failures. These patients likely represented a mixture of relapsers and non-responders. Additional major inclusion criteria were HCV-RNA positivity, hepatic fibrosis documented by historical biopsy showing at least portal fibrosis with few septa (METAVIR score F2), compensated liver disease (Child-Pugh class A), hemoglobin $\geqslant$12 g/dl for women and $\geqslant$13 g/dl for men, absolute neutrophil count $\geqslant$1500/mm^3, platelet count $\geqslant$80,000/mm^3 and body weight of 40–125 kg. Major exclusion criteria included known coinfection with HIV or hepatitis B virus, decompensated liver disease and history of or current hepatocellular carcinoma.

Study design and conduct

The retreatment trial of the EPIC-3 program is a prospective, open-label, clinical trial and was conducted at 133 sites in North America, Europe, Latin America, Taiwan and Australia. Patients received PEG-IFN alfa-2b 1.5 μg/kg/wk and daily weight-based dose (WBD) ribavirin (800 mg for $\leqslant$65 kg; 1000 mg for >65–85 kg; 1200 mg for >85–105 kg; and 1400 mg for >105–125 kg) for up to 48 weeks. Patients with detectable HCV-RNA at treatment week (TW) 12 were offered randomization into maintenance studies. The study was approved by the ethics committee at each participating institution and was conducted according to good clinical practice and the Declaration of Helsinki. All patients provided written informed consent [12].

Assessments

Serum samples and biochemical markers

FibroTest was measured at screening. Serum samples were collected and centrally stored. Samples were blindly assessed without knowledge of any patient characteristics and according to recommended procedures [3,13–15]. FT combined the following five markers: alpha2-macroglobulin, haptoglobin, gamma glutamyl transpeptidase (GGT), total bilirubin, and apolipoprotein A1. Apolipoprotein A1, alpha2-macroglobulin, and haptoglobin were determined using serum samples stored at −80 °C. An automatic nephelometer (Beckman Instruments, Brea, CA, USA, or Dade-Behring, Deerfield, IL, USA) and Roche Diagnostics reagents (Roche Diagnostics, Indianapolis, IN, USA), Siemens Healthcare Diagnostics reagents (Siemens Healthcare Diagnostics, Deerfield, IL, USA), or Beckman Instruments reagents (Beckman Instruments, Brea, CA, USA) were used. The coefficient of variation of all assays was lower than 3%. GGT, alanine aminotransferase (ALT), and total bilirubin were assessed prospectively during the trial period, using Hitachi 747 or 911 automates or Roche Modular Analysers.

ActiTest (AT) combined the same five markers as FT plus ALT. It has a high predictive value for the diagnosis of significant activity features [3,14]. SteatoTest combines the same 6 markers as AT plus aspartate aminotransferase (AST), serum triglycerides, cholesterol, fasting glucose and body mass index. It has a high predictive value for the diagnosis of significant steatosis [16].

Virologic markers

Plasma HCV-RNA was measured at screening, at weeks 12, 24, and 48 of treatment and at 12 and 24 weeks post-treatment. HCV-RNA analyses (TaqMan®; Applied Biosystems, Foster City, CA, USA; lower limit of detection [LLD] for 95% sensitivity of 125 IU/ml) were performed centrally at the Schering-Plough Research Institute (SPRI) Laboratory (Kenilworth, NJ, USA). Samples below the LLD for which a signal was detected were characterized as low positive or detectable; those for which no signal was detected were characterized as negative or undetectable. Confirmatory testing using TaqMan (Quest Nichols Laboratory, San Juan Capistrano, CA, USA) was performed on a subset of samples tested at the SPRI Laboratory. Early virologic response (EVR) was defined as HCV-RNA below the LLD (125 IU/ml) at TW12. The primary efficacy end point of the therapeutic study was SVR, defined as undetectable serum HCV-RNA 24 weeks post-treatment. A secondary efficacy end point was the difference in SVR rates by fibrosis score.

Histological criteria

Pretreatment liver biopsy specimens were scored by a single pathologist using METAVIR criteria [17]. The pathologist was blinded to historical biopsy reports and other clinical data. All the patients were biopsied after their prior failed therapeutic regimen.

Statistical analysis

Calculating FibroTest diagnostic values

The diagnostic values of FT were assessed by the receiver-operating characteristics (ROC) curves, which plot sensitivity versus 1 – specificity [14,18–19]. The respective overall diagnostic values were compared using the area under the ROC curves (AUROCs). The AUROCs were compared to 0.50 (no diagnostic value) and to AUROCs observed in an integrated database of patients with CHC who were never treated (naive population) [14].

Estimates of AUROCs and comparisons between AUROCs used an empirical nonparametric method [18,19]. Two factors are strongly associated with the AUROCs of fibrosis biomarkers and biopsy: the prevalence of the different fibrosis stages that define advanced and non-advanced fibrosis [20,21] and the length of biopsy [21,22].

To address this risk of spectrum bias due to different prevalences of fibrosis stages between studies we used two previously validated method of standardization [20–22]. For the same test, if only F3 and F4 patients are included, AUROCs are mathematically lower than if only F2 and F4 patients are included [20,23]. Using an equal proportion of each fibrosis stage permitted a standardized AUROC expression. In this standard prevalence distribution, the difference between the mean fibrosis stage of advanced fibrosis minus the mean fibrosis stage of non-advanced fibrosis (DANA) is 2.5. The first step of such a standardization is to estimate the relationship between DANA and observed AUROCs (ObAUROC). For each population, DANA is calculated as: mean advanced fibrosis estimated by {[(prevalence F2 $\times$ 2) + (prevalence F3 $\times$ 3) + (prevalence F4 $\times$ 4)]/(prevalence F2 + prevalence F3 + prevalence F4)} minus mean non-advanced fibrosis estimated by [prevalence F1/(prevalence F0 + prevalence F1)]. From the regression formula linking the ObAUROC to DANA, one can calculate an AUROC standardized

(StAUROC) at the DANA value of 2.5. Contrary to the ObAUROC, the StAUROC estimate for a given test for the diagnosis of advanced fibrosis is independent of the prevalence of advanced (F4) and non-advanced fibrosis (F2/F3) stages. We used the previously validated formula in patients with CHC [StAUROC = ObAUROC + (0.1056) (2.5 – ObDANA)]. The corresponding StAUROC for F2 versus F4 (ObDANA = 2) was ObAUROC + (0.1056)(0.5) = ObAUROC + 0.0528. The corresponding StAUROC for F2 versus F3 (ObDANA = 1) was ObAUROC + (0.1056)(1.5) = ObAUROC + 0.1584.

To prevent the risk of considering a short biopsy sample as the gold standard, we used the previously validated adjusted FT AUROC as the gold standard for the given biopsy length (AlAUROC) [21] For instance a biopsy of 16-mm length for the diagnosis of F3 versus F4 has an AUROC of 0.82 versus the gold standard (entire liver) (GsAUROC). The FibroTest AlAUROC = ObAUROC/GsAUROC. An ObAUROC for FT of 0.71, using biopsy of 16 mm, has an A1AUROC = 0.71/0.82 = 0.87.22.

Obuchowski measure

Lambert et al. proposed in order to overcome both spectrum effect and ordinal scale, to use the Obuchowski measure [21]. Furthermore this measure allows to compare two biomarkers with a single test, avoiding appropriate correction for the type I error when comparing two biomarkers for different stages or grades. This measure is a multinomial version of the AUROC. With N categories of the gold standard outcome (histological fibrosis stage) and AUROCst, the estimate of the AUROC of diagnostic tests for differentiating between categories s and t, the Obuchowski measure, is a weighted average of the $N(N-1)/2$ different AUROCst corresponding to all the pairwise comparisons between two of the N categories. Each pairwise comparison has been weighted to take into account the distance between fibrosis stages (i.e., the number of units on the ordinal scale). A penalty function proportional to the difference in METAVIR units between Stages was defined: the penalty function was 0.25 when the difference between stages was 1, 0.50 when the difference was 2, and 1 when the difference was 3. The Obuchowski measure can be interpreted as the probability that the non-invasive index will correctly rank 2 randomly chosen patient samples from different fibrosis stages according to the weighting scheme, with a penalty for misclassifying patients [21].

Prognostic value of FibroTest

Uni- (UV) and multivariable (MV) analyses for SVR were performed using a complete model (genotype, viral load, age, sex and previous PEG-IFN) [12] that also included FT and biopsy. Logistic regression analyses, with SVR as the response variable and key baseline and demographic variables as explanatory variables, were performed to assess the effect of the prognostic factors on SVR rates. Stepwise regression methods were used to build prediction models. In the modeling FT was entered as a continuous variable and biopsy as METAVIR stages. SVR rates were also summarized by baseline METAVIR fibrosis score (F2, F3 and F4), estimated both by FT and biopsy. The Armitage S trend test was used to compare the proportion of patients who attained SVR among these patients. Number Cruncher Statistical Software was used [24].

Diagnostic and prognostic value of ActiTest and SteatoTest

Both the degree of necroinflammation and steatosis were also evaluated using validated biomarkers, ActiTest and SteatoTest, previously validated in naive patients [9,10,16].

The predetermined following cutoffs of FT values were used for the corresponding METAVIR stages: ⩽0.27: F0; ⩽0.48: F1; ⩽0.58: F2; ⩽0.74: F3;and >0.74: F4.

Significant activity was defined as presumed grade A2/A3 (METAVIR scoring system: moderate/severe necroinflammatory activity) according to the 0.52 laboratory predetermined cutoff [3]. The predetermined following cutoffs of AT values were used for the corresponding METAVIR grades: ⩽0.29: A0; ⩽0.52: F1; ⩽0.62: F2;and >0.62: A3.

Significant steatosis was defined as presumed stage S2/S3/S4 (steatosis between 5% and 100%) according to the laboratory predetermined cutoff [16].

Results

Patient characteristics

In all, 2333 patients were screened, 2312 patients were enrolled (treatment and safety population) and 1459 patients (diagnostic population) had available baseline FT, biopsy and complete data to be included in the present study. Four patients were excluded due to un-interpretable FT results (Fig. 1).

Baseline characteristics of the diagnostic population were similar to the safety population (Table 1): 70% male; median age 51 years; 28% with few fibrous septa (METAVIR F2), 29% with many septa (F3) and 43% with cirrhosis (F4) at biopsy; previous relapses 29%; previous PEG-IFN regimen 41%; genotype 1 in 82%; and high (>6×10^5 IU/ml) baseline viral load (BVL) in 64%. The median time difference between FT sampling and historical biopsy was 161 days (95% CI 150–168).

Diagnostic value of FT

The accuracy of FT for the diagnosis of fibrosis in the present population of non-responder patients was similar to that of previous validations in treatment-naive patients for all the observed and adjusted AUROCs, according to prevalence of stages or biopsy length (Table 2) [3,23]. The unique test estimating all pairwise performances using Obuchowski measure was significant versus random performance (Table 2). The mean biopsy length was similar in the present study to that in the previously published integrated database (13 mm vs 16 mm) [14]. The observed AUROCs were 0.75 for the diagnosis between F2 and F4, 0.65 between adjacent stages F2 and F3 and 0.62 between F3 and F4. These

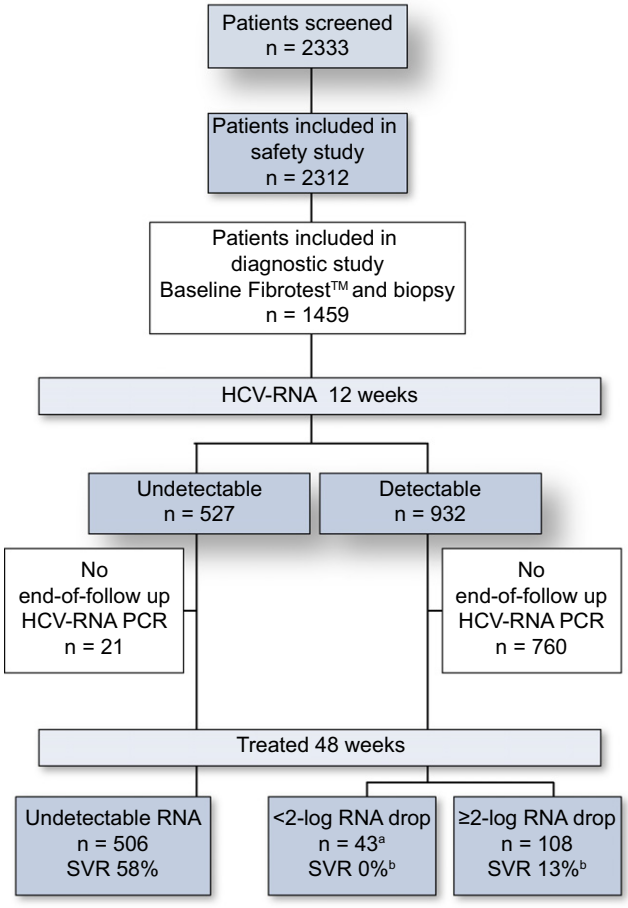

Fig. 1. Flow diagram of patients included in the therapeutic study and in the diagnosis study.

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Table 1. Baseline Patient Characteristics of the "Safety" and "Diagnostic Population".

	Safety population[a] n = 2312	Diagnostic population n = 1459
Male, n (%)	1650 (71)	1015 (70)
Caucasian, n (%)	1932 (84)	
Mean age, y (SD)	49.2 (12.4)	50.9 (8.3)
Fibrosis stage (METAVIR)		
Few septa (F2)	658 (29)	410 (28)
Many septa (F3)	676 (29)	424 (29)
Cirrhosis (F4)	974 (42)	625 (43)
Necroinflammatory activity (METAVIR)		
No activity (A0)	157 (7)	92 (6)
Minimal activity (A1)	1742 (75)	1107 (76)
Moderate activity (A2)	389 (17)	245 (17)
Severe activity (A3)	22 (1)	15 (1)
Mean weight, kg	81.1	80.5
Genotype, n (%)		
1	1859 (80)	1203 (82)
2	75 (3)	47 (3)
3	294 (13)	168 (12)
4	68 (3)	37 (3)
Missing	17 (1)	4 (<1)
Viral load		
≤600,000 IU/ml	853 (37)	524 (36)
>600,000 IU/ml	1451 (63)	935 (64)
Missing	8 (<1)	0 (0)
Previous combination therapy, n (%)		
IFN alfa + ribavirin	1425 (62)	857 (59)
PEG-IFN alfa + ribavirin	865 (37)	602 (41)
No combination therapy	22 (1)	0 (0)
Previous response, n (%)		
Nonresponse	1401 (61)	880 (60)
Relapse	647 (28)	424 (29)
Treatment failure[b]	264 (11)	155 (11)

[a]All patients enrolled in the EPIC[3] trial [12].
[b]There was no significant difference for the characteristics of patients between the safety population and the diagnostic population.

ObAUROCs were equivalent to AUROCs of 0.78–0.81 after standardization on DANA and to AUROCs of 0.72–0.82 after standardization to biopsy length (Table 2). The AUROC for stage F4 vs F2F3 was 0.69 (95% CI 0.66–0.71), higher than random value (p <0.0001).

Virologic response to treatment

Results were comparable to those observed in the overall population [12]. One thousand four hundred and fifty nine patients received PEG-IFN alfa-2b 1.5 μg/kg/wk plus WBD ribavirin (800–1400 mg/days) for 12–18 weeks; 506 (35%) had undetectable serum HCV-RNA at TW12 (TW12neg); 678 (46%) were treated for 48 weeks with 24 weeks' follow-up. In the 1459 patients enrolled (intention-to-treat population), the rate of SVR was 21% (312/1459). The rate of SVR was 58% among the 506 patients who were TW12neg, 13% among 108 patients with detectable but ⩾2-log drop in viral load (all of whom had an HCV-RNA of <750 IU/ml and the majority had <125 IU/ml) and 0% among 43 patients with less than 2-log drop (Fig. 1).

Prognostic value of baseline FT

Baseline fibrosis stage estimated using FT had the same prognostic value as that estimated using biopsy for SVR and for EVR.

As observed in the therapeutic population, five baseline factors were significantly associated with SVR (Table 3A) and EVR (Table 3B) in UV and MV analyses: fibrosis stage estimated using FT or biopsy, genotype 2/3, BVL, prior relapse and previous treatment with non-PEG-IFN.12 Among patients who were TW12neg (n = 506), only three factors remained highly predictive of SVR by MV analysis: fibrosis estimated with FT or by liver biopsy, genotype 2/3 and BVL (Table 3C).

A graded decrease in SVR was observed among all patients (n = 1459), as well as the subset with EVR (n = 506), as the METAVIR fibrosis score increased by FT or biopsy analysis. SVR decreased in the diagnostic population from 40% to 15% (Armitage test S for trend = 78,554; p <0.00001) and from 75% to 52% (S = 8391; p = 0.004) in the 12-week responder subset according to FT score for fibrosis and from 27% to 16% (S = 56,637; p = 0.00002) and from 63% to 48% (S = 9224; p = 0.001) for liver biopsy scoring of fibrosis, respectively (Fig. 2). This trend was also consistent across patients classified as F0 and F1 with FT (Fig. 2). When discordance between baseline fibrosis estimates was entered as a covariate in the model, FT retained a significant predictive value for EVR (odds ratio [OR] = 0.24; p = 0.009 without significance for discordant cases OR = 1.02, p = 0.93) and SVR (OR = 0.13; p = 0.003); however, biopsy scoring had a weaker predictive value for EVR (OR = 0.83; p = 0.03 with significant predictive value for discordant cases OR = 1.67; p = 0.003); a similar trend was observed for SVR and biopsy (OR = 0.67; p = 0.004 with borderline significance for discordant cases OR = 1.41; p = 0.10).

Discordant patients according to FT and biopsy fibrosis estimates

A total of 292 patients (94 presumed F0 and 198 F1 with FT) were suspected to be either false negative of FT or false positive of biopsy (144 F2, 80 F3, and 68 F4 with biopsy).

Comparison between biopsy length, inflammatory scores, and ALT levels for these 292 discordant patients versus the 1167 concordant patients observed: lower METAVIR inflammatory score at biopsy:1.03 (95%CI 0.99–1.09) vs 1.15 (1.12–1.18) (p = 0.001); lower median ALT: 58 UI/L (52–63) vs 94 (91–97); there was no significant difference between biopsy length: median 13 mm (12–14) vs 12 mm (12–12) respectively (p = 0.18).

Diagnostic and prognostic value of baseline biomarkers of activity and steatosis

The accuracy (AUROCs) of ActiTest for the diagnosis of activity grades was significant (p <0.001), but consistently lower in the

Table 2. Diagnostic Value of FibroTest (AUROCs and Obuchowski measures) for the Diagnosis Between Each Fibrosis Stage Observed in the Present Study (Non-responders) and in Previous Integrated Data (Naive Patients).

	FibroTest vs. Biopsy	
Comparison	Nonresponders	Controls, Naive[e]
F4 vs F2		
Number of patients	1035	376
Observed AUROC[a]	0.75 (0.72-0.78)	0.75 (0.70-0.80)
Standardized AUROC[b]	0.80 (0.77-0.83)	0.80 (0.75-0.85)
Adjusted on biopsy length AUROC[c]	0.82 (0.79-0.85)	0.82 (0.77-0.87)
Weighted Obuchowski measure[d]	0.71 (0.68-0.74)	0.84 (0.80-0.86)
F3 vs F2		
Number of patients	834	364
Observed AUROC[a]	0.65 (0.61-0.68)	0.63 (0.58-0.68)
Standardized AUROC[b]	0.81 (0.77-0.85)	0.79 (0.74-0.84)
Adjusted on biopsy length AUROC[c]	0.76 (0.72-0.80)	0.73 (0.68-0.78)
Weighted Obuchowski measure	0.59 (0.56-0.62)	0.63 (0.61-0.65)
F4 vs F3		
Number of patients	1049	234
Observed AUROC[a]	0.62 (0.59-0.66)	0.65 (0.59-0.71)
Standardized AUROC[b]	0.78 (0.75-0.81)	0.81
Adjusted on biopsy length AUROC[c]	0.72 (0.69-0.75)	0.76
Weighted Obuchowski measure	0.58 (0.55-0.61)	0.63 (0.61-0.65)
All pairwise comparisons (Obuchowski measure)	0.63 (0.61-0.65)	0.70 (0.68-0.72)

All AUROCs were significant (p <0.001) versus random AUROC (0.50). There was no significant difference between the AUROCs of non-responders and naive patients. There were lower weighted Obuchowski measures for non-responders compared to controls.

[a]Observed area under the receiver-operating characteristics curves (AUROCs) and 95% confidence interval.

[b]Standardized AUROC preventing spectrum bias for eventual comparisons between studies, corresponding to a standard difference of fibrosis stage of 2.5 METAVIR unit [20].

[c]Gold standard–adjusted AUROC for the given biopsy length preventing the bias of considering biopsy as a gold standard calculated as the ratio between ObAUROC and GsAUROC of 16-mm biopsy versus gold standard (0.82 for F3 vs. F4 and 0.86 for F2 vs. F3) [21].

[d]Obuchowski measure takes into account the spectrum effect (weighted according to difference between stages) and the overall measure takes into account the multiple testing.

[e]Integrated database of naive patients with chronic hepatitis C [14].

present study than in the integrated database of naive patients (Supplementary file 1) [14]. The Spearman correlation coefficient between ActiTest and activity grade at biopsy was 0.17 (p <0.0001), with an obvious spectrum bias among patients with low activity scores (A0/A1) and high activity scores (A2/A3), as 76% of patients had A1 and 17% had A2. Among 76 patients who had a biopsy and ActiTest within 2 months of each other, the AUROC of A2/A3 versus A0/A1 was 0.71 (95% confidence interval [CI], 0.54–0.83) versus 0.60 (95% CI, 0.56–0.64) for those patients with two or more months between their liver biopsy and ActiTest (p = 0.16). There was no prognostic value of METAVIR activity grades either estimated using ActiTest or biopsy (data not shown).

The accuracy (AUROC) of SteatoTest (n = 1415) for the diagnosis of S2/S3/S4 (steatosis between 5% to 100%; prevalence 40%) versus S0/S1 (steatosis less than 5%; prevalence 60%) was 0.68 (95% CI, 0.65–0.71) (p <0.0001 versus AUC of 0.50). This was lower than AUROCs observed in 171 HCV naive patients, performed 40 days apart: 0.80 (95% CI, 0.74–0.86; p = 0.02). The Spearman correlation coefficient between SteatoTest and steatosis grade at biopsy was 0.32 (p <0.0001). Among 76 patients who had a biopsy and SteatoTest less than 2 months apart, the AUROC was 0.77 (95% CI, 0.64–0.85) versus 0.68 (95% CI, 0.65–0.71) among patients tested 2 months or more apart (p = 0.12).

We observed a significantly lower SVR in patients with steatosis using liver biopsy estimate or SteatoTest. The SVR was lower in those with high steatosis (>10%; S2) graded by liver biopsy (18.8% SVR; 106/564) compared to those with steatosis ⩽10% (23.9% SVR; 203/850; p = 0.02). Those with more hepatic steatosis by SteatoTest (>0.57) had an SVR of 18.1% (95/525) compared to SteatoTest <0.57 (24.1% SVR; 214/889; p = 0.009).

Discussion

The results of this study illustrate both the diagnostic and prognostic utility of liver injury biomarkers (FT, ActiTest and SteatoTest) for clinicians and suggest that they could serve as a possible alternative to liver biopsy in patients with CHC for whom previous combination therapy failed. The diagnostic value of FT [2,3], Actitest, [14], and SteatoTest [16] has already been validated in patients naive to HCV treatment. In this study, FT had the same prognostic value as liver biopsy for predicting the

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Table 3A. Prognostic value of FibroTest versus biopsy for early virologic response.

Factor	Univariate Odds Ratio		Multivariate Odds Ratio	
	n = 1459	Significance	n = 1304	Significance
Fibrosis stage				
FibroTest	4.9 (2.9-8.1)	<0.0001	4.2 (2.2-7.9)	<0.0001
Biopsy	1.2 (1.1-1.4)	0.001	1.3 (1.1-1.5)	0.001
Genotype 2/3	11.5 (7.9-16.6)	<0.0001	8.9 (5.8-13.6)	<0.0001
Baseline viral load $<\log_6$	1.9 (1.5-2.4)	<0.0001	1.5 (1.2-2.0)	0.003
Prior relapse (77 missing)	6.2 (4.8-8.0)	<0.0001	6.5 (4.9-8.8)	<0.0001
Previous non-PEG-IFN	1.3 (1.05-1.6)	0.02	2.0 (1.5-2.7)	<0.0001

likelihood of a SVR to treatment for hepatitis C as has been previously observed in naive patients [8–10].

Limitations

The main limitations of this study were the non-simultaneous measurement of the biomarkers and biopsy, the FT was not assessed in all included patients, and the length of biopsy samples was suboptimal.

There were no differences in demographic characteristics, baseline clinical parameters and virologic responses between the patients who had the measurements required to accurately assess FT and thus be included in the present study, and of those patients recruited to the EPIC3 retreatment study (Table 1).

The variability of FT and its components have been extensively investigated. The assays for this study were centralized in two CLIA laboratories (LabCorp, Raritan, NJ, USA and Covance, Indianapolis, IN, USA) following the recommended pre-analytic and analytic procedures [13–15]. Only four (3/1000) patients were excluded because of a high-risk profile of false positive or false negative. The usual main confounders were observed: hemolysis of the sample, acute inflammation and Gilbert syndrome [3,7,13,14].

One disadvantage of employing FT alone versus biopsy to evaluate hepatic fibrosis would be the possible inclusion of patients with additional causes of liver disease (e.g., due to alcohol, nonalcoholic steatohepatitis, or hemochromatosis). However, this risk is reduced because FT has the same diagnostic value in the most common causes of liver diseases [3] and the same prognostic value as biopsy in patients with chronic hepatitis B [25] and alcoholic liver disease [26]. The diagnosis of necroinflammatory activity grades is also possible with ActiTest9 [14], as are steatosis grades using SteatoTest, both for viral or nonviral steatosis [16]. Other biomarkers (HFE gene, transferrin saturation, magnetic resonance imaging) could also provide non-invasive possible alternatives to biopsy for the diagnosis of hemochromatosis [27].

One limitation in the validation portion of this study was the relatively long mean duration between biopsy and serum sampling (161 days). This limitation relates to the use of historical biopsies for studies, which minimized the invasiveness of this trial. This delay between FT and biopsy could be a factor that may explain the lower accuracy observed for AT for the diagnosis of activity grade and for SteatoTest for the diagnosis of steatosis grade in comparison with the accuracy observed in studies of naive patients in which the median interval between biopsy and FT was only 40 days [16]. These features of activity and steatosis are less stable than the fibrosis stage, and this hypothesis is supported by the increase of ActiTest and SteatoTest AUROCs in subpopulations with shorter intervals between biopsy and serologic testing. Despite these limitations (also present for biopsy), this was a unique opportunity to validate these biomarkers as prognostic indicators of subsequent viral clearance in a large population of prior non-responders to antiviral therapy. They are fewer validations of SteatoTest in patients with chronic hepatitis C than for FT [3–11] and ActiTest, [3,9,10,28] and the SteatoTest performance must be confirmed by other studies.

Another limitation of this study is the relatively short length of biopsy sample (16 mm) in comparison with the recommended length of 25 mm [29]. But as most studies of liver biopsy performed in large populations fail to achieve this optimal length [22], it may be argued that using more reproducible serologic

Table 3B. Prognostic value of FibroTest versus biopsy for sustained virologic response.

Factor	Univariate Odds Ratio		Multivariate Odds Ratio	
	n = 678	Significance	n = 601[a]	Significance
Fibrosis stage				
FibroTest	4.5 (2.2-9.0)	<0.0001	5.0 (2.3-11.0)	<0.0001
Biopsy	1.5 (1.2-1.8)	<0.0001	1.6 (1.3-2.0)	<0.0001
Genotype 2/3	4.5 (3.1-6.6)	<0.0001	4.0 (2.6-6.0)	<0.0001
Baseline viral load $<\log_6$	1.7 (1.2-2.3)	0.0009	1.6 (1.2-2.3)	0.005
Prior relapse	1.6 (1.1-2.2)	0.007	1.7 (1.1-2.4)	0.007
Previous non-PEG-IFN	1.4 (0.99-1.9)	0.06	1.6 (1.1-2.3)	0.02

[a]For 77 patients prior type of response (relapse or non-responder) was missing.

Table 3C. Prognostic value of FibroTest versus biopsy for sustained virologic response among patients with early virologic response.

Factor	Univariate Odds Ratio		Multivariate Odds Ratio	
	n = 506	Significance	n = 444[a]	Significance
Fibrosis stage				
FibroTest	3.5 (1.5-8.0)	0.003	4.0 (1.6-10.0)	0.003
Biopsy	1.4 (1.1-1.7)	0.003	1.5 (1.2-1.9)	0.0007
Genotype 2/3	3.0 (2.0-4.6)	<0.0001	2.9 (1.8-4.5)	<0.0001
Baseline viral load $<\log_6$	1.7 (1.2-2.4)	0.004	1.6 (1.08-2.4)	0.02
Prior relapse	0.99 (0.7-1.4)	0.96	1.06 (0.7-1.6)	0.80
Previous non-PEG-IFN	1.3 (0.93-1.9)	0.11	1.3 (0.8-2.0)	0.23

[a]For 62 patients prior type of response (relapse or non-responder) was missing.

estimates of liver injury achieve the same or better results without the attendant risks of a liver biopsy.

Advantages of serologic markers

The main advantages of this study are the large population of non-responder patients, the multicenter, multinational and prospective nature of the population, and the centralized assessment of histology. The analyses of this study were made with an independent and blinded assessment of biomarkers, fibrosis stage, activity and steatosis grades. During the trial, all sera were prospectively stored. In this study we were able to demonstrate the reproducibility of FT's accuracy for the diagnosis of fibrosis stage, the reproducibility of baseline FT for the prediction of SVR and for the first time the demonstration of the prognostic values of biomarkers for EVR as well as the prognostic value of FT in patients with undetectable HCV-RNA at 12 weeks of therapy.

One advantage of biomarkers compared to biopsy assessment is that it enables to both evaluate liver disease severity and to anticipate the treatment outcome in patients with contraindications to biopsy or those who refuse it. Additionally biomarkers can be safely used to provide long-term follow-up of liver disease severity without the constraint of repetition of an invasive procedure. Patients are spared the risks associated with liver biopsy.

An important advantage already demonstrated in naive patients [5,9,10] is that FT is at least as accurate as biopsy for predicting virologic response, either SVR or SVR among patients with EVR. As with treatment-naive patients, baseline METAVIR fibrosis score estimated using biopsy or FT was the second strongest

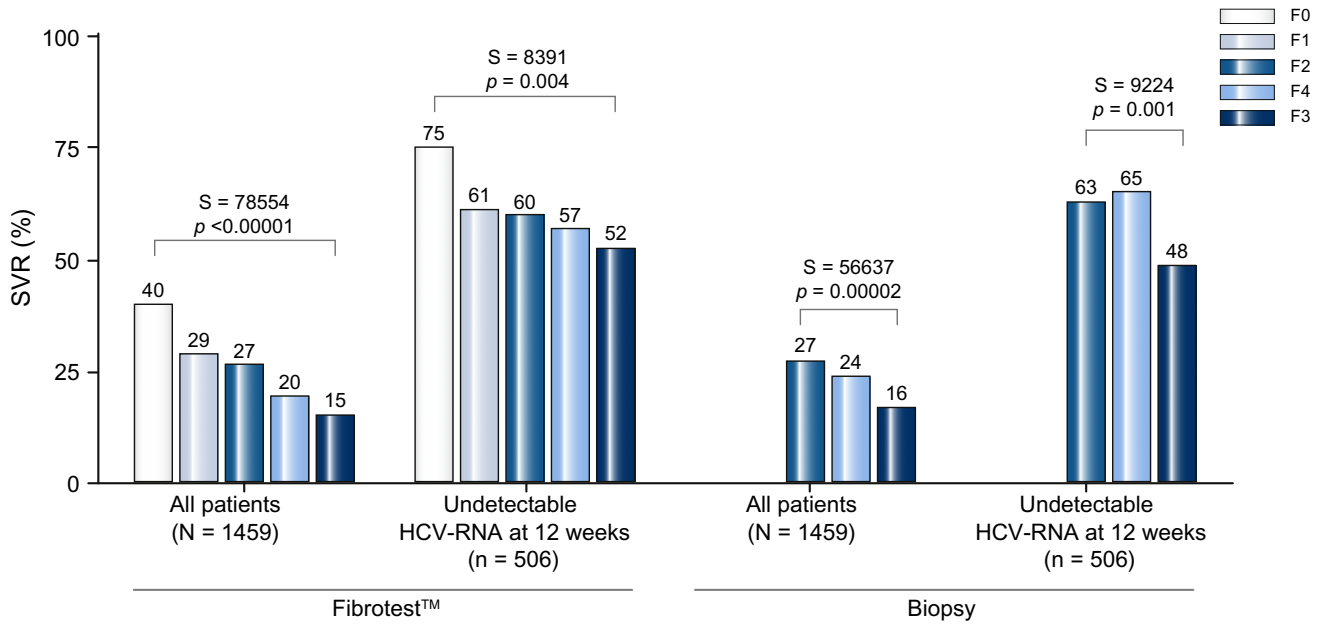

Fig. 2. Sustained virologic response (SVR) rate according to baseline fibrosis stage estimated using either FibroTest or biopsy in overall diagnostic population and in patients with undetectable HCV-RNA at 12 weeks (EVR). When estimated using biopsy, patients with baseline stages F0 (no fibrosis) and F1 (portal fibrosis) were excluded from the overall study. When estimated using FibroTest the baseline fibrosis stage was F0 and F1 in 94 and 198 patients, respectively. These discordant cases could be either false negatives of the FibroTest or false positives of the biopsy. In the absence of a true gold standard, the fact that the virologic responses (either SVR or SVR among EVR) of patients classified as F0 by FT were significantly higher than those classified F2 by biopsy (40% vs 27% for SVR, 75% vs 63% for SVR among EVR) strongly suggest that they could be biopsy false positives.

predictor, after genotype and before BVL, in the present study. These data establish TW12 as a simple and effective point at which to decide on retreatment with PEG-IFN alfa-2b/ribavirin of patients who previously failed interferon alpha/ribavirin treatment. After considering patient and disease factors, including fibrosis stage using FT, patients with HCV-RNA below the LLD at TW12 – those with negative or near negative results – should continue therapy (>50% chance of SVR), whereas others can be spared further drug exposure because the likelihood of attaining an SVR is low. Alternatively, these patients may consider long-term low-dose maintenance therapy [12,30–33].

Although other studies [34,35] have shown that significant fibrosis and cirrhosis are negative predictors of SVR, EPIC3 is the first large study to clearly demonstrate a graded decrease in SVR rates as fibrosis score progresses from F2 to F3 to F4 both using biopsy and FT [12]. Furthermore, as suggested in previous studies [32], it seems possible that FT could be even better than short-length biopsy for the prediction of SVR [4,5,9]. In the present study 302/1459 (20%) patients were classified at baseline as F0 ($n = 94$) or F1 ($n = 198$) by FT and these same patients were classified by biopsy as F2 or F3 or F4 by biopsy. As already demonstrated these discordant cases could be either false negatives of the FT or false positives of the biopsy [4,5,9]. In the absence of a true gold standard, the fact that the virologic responses (either SVR, EVR, or SVR among EVR) of patients classified as F0 by FT were significantly higher than of those classified as F2 by biopsy (40% vs 27% for SVR, 54% vs 42% for EVR, 75% vs 63% for SVR among EVR) strongly suggest that the liver biopsy scores were incorrect. The multivariate analysis reinforced this hypothesis as the knowledge of discordant cases significantly increased the prognostic value of fibrosis staging using biopsy but not using FT.

Despite the smaller number of validations for ST, the present study suggests that similar to findings in treatment-naive patients, steatosis scored by SteatoTest is another predictor of SVR (albeit less accurate) in previously non-responder patients. However, the respective roles of metabolic and viral steatosis as independent prognostic factors need further study [36].

Conclusions

Biomarkers such as FibroTest can be used as a possible alternative to liver biopsy for fibrosis staging and thus simplify the management of patients with CHC who failed their first treatment. As previously suggested in treatment-naive patients, these validated biomarkers should also facilitate the design of trials in non-treatment-naive patients [8,9]. They could also be used as surrogate markers in trials evaluating the risk–benefit of maintenance therapy, without increasing the risk and the cost of repeated liver biopsies but with an increase of power through repeated non-invasive measures of biomarkers.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jhep.2010.06.038.

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