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ORIGINAL ARTICLE

HCV-GenoFibrotest: A combination of viral, liver and genomic (IL28b, ITPA, UGT1A1) biomarkers for predicting treatment response in patients with chronic hepatitis C

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Summary

Background and aim: Three gene polymorphisms, interferon-lambda-3 (*IL28B*), inosinet-riphosphatase (*ITPA*) and bilirubinuridine diphosphate-glucuronosyltransferase (*UGT1A1*) are associated with treatment (interferon and ribavirin) efficacy and adherence in patients with chronic hepatitis C. The hypothesis was that fibrosis stage estimated with FibroTest instead of biopsy was still an independent predictive factor of sustained virologic response (SVR) when these new polymorphisms were assessed.

Methods: Patients receiving standard of care treatment were retrospectively analyzed with determination of *IL28B*, *ITPA*, and *UGT1A1* polymorphisms. Baseline prognostic factors were combined using logistic regression analysis in a training group (157 patients) and validated in avalidation group (79 patients).

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Results: The combination of the five most predictive factors (HCV genotype 2/3, *IL28B* genotype, FibroTest, ActiTest and viral load) in the training population had AUROC for SVR = 0.743 (0.655—0.810; $P < 0.0001$ vs. random), which was validated in the validation population, AUROC = 0.753 (0.616—849; $P = 0.0007$ vs. random, not different from training $P = 0.88$). FibroTest remained significant [OR = 4.20 (2.59—12.50); $P = 0.03$] after assessment of the *IL28B* CC, HCV genotype and viral load.
Conclusion: Fibrosis stage assessed by FibroTest is an independent predictor of SVR, after accounting for the *IL28B* genetic polymorphism. A combination of five baseline biomarkers could simplify the baseline prediction of SVR.

Introduction

One hundred and eighty million individuals worldwide are chronically infected with the hepatitis C virus (HCV) and are at risk for related morbidity and mortality from cirrhosis and hepatocellular carcinoma [1]. Curative antiviral therapy is effective in inducing a sustained virologic response (SVR) in about 60% of cases and may prevent these complications. The current standard of care is pegylated-interferon-alfa (pegIFN-alfa) and ribavirin (RBV) combination therapy and may be associated with considerable toxicity despite increasing use of an ''à la carte'' regimen [2].

The ability to identify individual patients who are likely to respond to this treatment is therefore clinically valuable, and some progress has been achieved recently [3—5]. Both pretreatment viral and host factors have been associated with treatment outcome in HCV [3]. Viral factors include HCV genotype and baseline viral load. The main host factors include interferon-lambda-3 (*IL28B*) polymorphism, hepatic injury (fibrosis and steatosis negatively-associated, necro-inflammatory positively-associated), and ''metabolic'' factors (body mass index [BMI], or insulin resistance) [3,4]. Furthermore, progress has been made in the identification of patients with better tolerance to RBV. A recent breakthrough was the identification of the inosinetriphosphatase (*ITPA*) polymorphism, which helps to identify patients with better tolerance to RBV anemia but without clear independent prognostic value for SVR [5]. Rarely patients with genetic deficiency in UDP-glucuronosyltransferase activity (*UGT1A1* polymorphism, Gilbert's syndrome) are also at risk of severe jaundice due to RBV high increase of unconjugated hyperbilirubinemia [6].

All of these host factors can be determined simply by a blood test except fibrosis stage and steatosis grade, which are classically assessed through liver biopsy. Because of the limitations of biopsy (sampling error, cost and adverse events), non-invasive biomarkers have been developed. The most validated biomarkers, such as FibroTest and Fibroscan, are widely used and have already been validated as alternatives to biopsy in several countries [7—9].

The aim of the present pilot study was to assess the performance of different combinations of blood tests without biopsy for predicting SVR: three genetic tests (*IL28B, ITPA* and *UGT1A1* polymorphisms), two virologic tests (HCV genotype and viral load) and three validated serum biomarkers of liver injury (FibroTest for fibrosis stage, ActiTest for activity grade and SteatoTest for steatosis grade).

Patients and methods

Predetermined design

The main endpoint was that fibrosis stage estimated with FibroTest instead of biopsy was still an independent predictive factor of SVR when the three other main independent factors were assessed: the two ''standard'' factors (genotype and viral load), as well as the recently validated *IL28B* polymorphism.

The secondary endpoints were that the two polymorphisms related to RBV tolerance (*ITPA* and *UGT1A1*), necroinflammatory activity estimated by ActiTest and steatosis estimated by SteatoTest were independent predictors of SVR.

Patients

Two populations of patients with chronic hepatitis C receiving standard of care treatment were retrospectively analyzed, one training group in Pitié-Salpêtrière Hospital, Paris France and one validation group in the National Medical University Hospital, Department of Infectious Diseases, Lviv, Ukraine.

The inclusion and exclusion criteria were the same for both groups. Patients were required to have had their first treatment with pegylated interferon and RBV according to standard recommendations: either pegylated interferon alpha 2b (1.5 microg/kg) or alpha 2a (90 to 180 μg according to weight), combined with RBV (10 mg/kg); the treatment should have been taken for at least 3 months, and for a total duration of 6 months in genotypes 2 and 3, and 12 months in genotypes 1,4 and 5. They should have had measurements done for HCV genotype, viral load and FibroTest according to recommended methods less than one month before treatment, and genetic test measurements done on serum centralized in Cerbalaboratory (Cergy-Pontoise, France). Patients were excluded if they had HBV coinfection or other liver diseases (alcoholic liver disease, non-alcoholic steatohepatitis, autoimmune hepatitis, hemochromatosis), had been treated with pegylated interferon alone or a combination of non-pegylated interferon with RBV, or had been

previously treated. Treatment response was defined as SVR in intention to treat, the end of follow-up being 6 months after the end of treatment.

Biomarkers

Samples were blindly assessed without knowledge of any patient characteristics and according to recommended procedures [8,10—13]. FibroTest combined age and gender with the following five markers: alpha2-macroglobulin, haptoglobin, gamma glutamyltranspeptidase (GGT), total bilirubin, and apolipoprotein A1. Apolipoprotein A1, alpha2-macroglobulin, and haptoglobin were assessed by turbidimetry (Modular and Cobas Integra from Roche Diagnostics, Mannheim, Germany) and by nephelometry (BNII from Dade-Behring-Siemens Healthcare Diagnostics, Deerfield, IL, USA) and using manufacturer reagents (Roche Diagnostics, Mannheim, Germany, and Siemens Healthcare Diagnostics, Deerfield, IL, USA) and Diagam reagents (Ghislenghien, Belgium) for alpha2-macroglobulin turbidimetric assay. The coefficient of variation of all assays was lower than 3%. GGT, alanineaminotransferase (ALT), aspatateaminotransferase (AST), total bilirubin, total cholesterol, triglycerides and fasting glucose were assessed, using Hitachi 917, Modular and Cobas Integra analyzers (Roche Diagnostics, Mannheim, Germany).

ActiTest combined the same five markers as FT plus ALT. It has a high predictive value for the diagnosis of significant activity features [8,11,14]. SteatoTest combines the same six 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 [13].

Virologic markers

HCV viral loads were measured using Abbott M2000sp/rt HCV RealTime assay (Abbott, Rungis, France). HCV genotypes were determined by partial sequencing of NS5B gene and comparison of the obtained sequence with reference strains by phylogenic analysis as previously described [15].

Genetic markers

Patients were analyzed for polymorphisms of *IL28B* (rs12979860), *ITPA* (rs1127354 and rs7270101) and *UGT1A1* polymorphism by studying serum circulating free DNA after extraction by using the NucliSENS®easyMag® automated system.

IL28B and *ITPA* genotypes were determined by melting curve analysis of hybridization probes using the LightCycler®480 Instrument (Roche Diagnostics, Meylan, France) in a similar manner than previously described [16]. Briefly, 5 μl DNA sample was amplified in a final volume of 20 μl, using the LC480 Genotyping Master (Roche Diagnostics, Meylan, France), containing 0.5 μM of each primer, 0.25 μM of each probe. Cycle conditions were as follows: denaturation step for one cycle, 95 °C for 8 min, amplification of the target DNA for 45 cycles at 95 °C for 10 s, 58 °C for 10 s and 72 °C for 15 s, melting curve analysis for one cycle at 95 and 45 °C for 2 min each and then ramping to 75 °C with a continuous fluorescence acquisition (1/°C). Characteristics and sequences of the primers and probes (Sigma-Aldrich, Lyon, France) are available upon request. Genotypes were defined as CC, CT or TT for *IL28B,* CC, AC or AA (minor allele = A) for *ITPA* rs1127354 and AA, AC or CC (minor allele = C) for *ITPA*rs7270101. The functional promoter TATA box polymorphism of the *UGT1A1* gene was analyzed by fragment analysis in a similar manner than Baudhuin et al. [17]. Briefly, amplification was carried out in a 20 μl reaction mixture containing 1X buffer, 1.25 mM MgCl2, 1 mM each dNTP, 0.125 μM each primer (Sigma-Aldrich, Lyon, France) and 1.25 U of FastStartTaq DNA polymerase (Roche Diagnostics, Meylan, France). After an initial denaturation step at 95 °C for 8 min, samples were amplified for 30 cycles of denaturation at 95 °C for 30 s, annealing at 60 °C for 30 s, and an extension at 72 °C for 1 min. The reaction products were then run on a ABI PRISM 3130 Genetic Analyzer and analyzed with the GeneMapper v4.5 software (Applied Biosystems, Courtaboeuf, France). Genotypes were defined as *UGT1A1**1, *UGT1A1**28, *UGT1A1**36 or *UGT1A1**37 at homozygous or heterozygous status. *UGT1A1**28 and *UGT1A1**37 genotypes were considered to be associated with reduced level of UGT1A1 transcription compared to the *UGT1A1**1 wild type genotype. Characteristics and sequences of the primers (Sigma-Aldrich, Lyon, France) are available upon request.

Statistical methods

Statistical analysis used Fisher's exact test, the chi-square test, Student's *t*-test, Mann-Whitney test, and multiple logistic regression for the multivariate analysis [18].

The multivariate analysis was based on the predetermined hypothesis of independent prognostic value of HCV genotype, three patient's genetic markers (IL28B, ITPA and UGT1A1), fibrosis stage, activity grade, and steatosisgrade. The main endpoint was that fibrosis stage estimated with FibroTest instead of biopsy was still an independent predictive factor of SVR when the other main factors were taking into account including the recently validated *IL28B* polymorphism.

After identification of independent factors, a sensitivity analysis was performed using population (validation vs. training) as a covariate. The diagnostic values of the markers alone or in combination were assessed using sensitivities, specificities, positive (PPVs) and negative (NPVs) predictive values, and the area under the receiver operating characteristics curves (AUROCs). Corresponding METAVIR fibrosis stages, activity grades and steatosis grades were calculated according to previously determined and validated cutoffs [11,13]. AUROCs were calculated using the empirical nonparametric method according to Delong et al. [19] and compared using the method of Zhou et al. [20]. For all analyses, two-sided statistical tests were used; a *P*- value of 0.05 or less was considered significant. Number Cruncher Statistical Systems 2003 software (NCSS, Kaysville, Utah, USA) was used for all analyses [18].

The study was realized in accordance with the Helsinki declaration. Patents were informed and gave a written con-

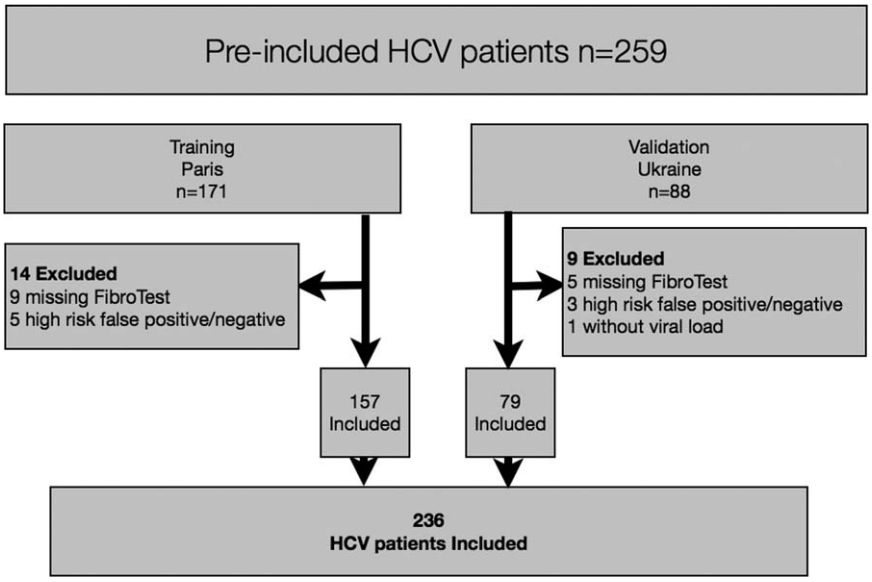

Figure 1 Patients included and excluded.

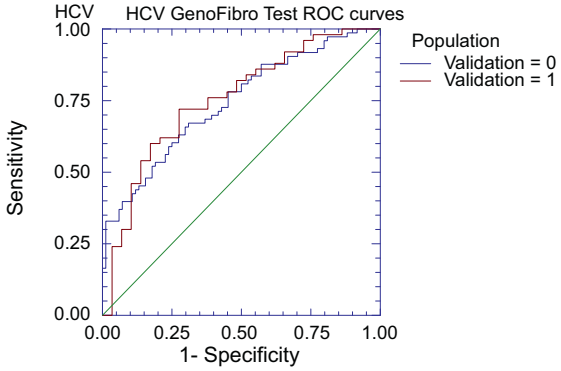

Figure 2 Receiver Operating Characteristics (ROC) curves of baseline HCV GenoFibroTest in training and validation populations for the prediction of SVR. The AUROC for SVR in the training population =0.743 (0.655-0.810; $P < 0.0001$ vs. random), not different ($P = 0.88$) from the AUROC in the validation population = 0.753 (0.616-849; $P = 0.0007$ vs. random).

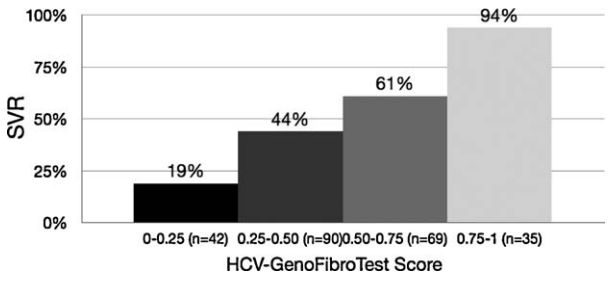

Figure 3 Sustained virological response according to HCV Geno-FibroTest.

sent and the study was approved by ethical committee (CPP Île de France III).

Results

Patients included

Out of a total of 259 pre-included cases and sera, 23 were excluded and 236 were included, with 157 in the training group and 79 in the validation group (Fig. 1). The characteristics of included patients are shown in Table 1.

The two groups were not significantly different in terms of the three genetic polymorphisms, the prevalence of easy-to-treat genotypes 2 and 3, viral load, gender, and the prevalence of advanced fibrosis. Compared with the training group, the validation group was younger, had more metabolic risk factors (higher BMI and higher fasting glucose) and a higher SVR. The SVR was 47% in the training population and 63% in the validation population.

Factors associated with SVR

Baseline factors associated with SVR in multivariate analysis are shown in Table 2. In all patients, five factors were significantly associated with SVR: HCV genotype 2/3, CC *IL28B* genotype, FibroTest, ActiTest and viral load. The main endpoint was reached, as FibroTest remained significant (OR = 4.20; 2.59—12.50; $P = 0.03$) after assessment of the *IL28B* CC genotype, HCV genotype and HCV viral load. The same trends with these five factors were observed in both the validation and training groups, with HCV genotype 2/3 and CC *IL28B* genotype being the most predictive. *ITPA* deficiency and the absence of *UGT1A1* deficiency were not associated with SVR. When the population group (validation vs. training) was entered in a multivariate model with the five identified predictive factors, the results were the same: odds ratio (OR) for HCV genotype

Table 1 Characteristics of patients included in the training and validation groups.

Baseline characteristics	Training group	Validation group	*P* Value
N	157	79	< 0.0001
Age	46 (44—49)	41 (37—45)	< 0.0001
Age > 40 years	121 (77%)	43 (54%)	0.0004
Male gender	93 (59%)	47 (59%)	0.97
Low Alcohol consumption[a]	78 (80%)	not available	not available
Ethnicity			
Caucasian	120 (76%)	79 (100%)	<0.0001
Sub-Saharan	28 (18%)	0 (0%)	
Asian	9 (6%)	0 (0%)	
BMI[b]	24 (23—25)	27 (26—29)	<0.0001
BMI >25	46 (34%)	31 (76%)	<0.0001
HCV RNA (log 10 IU/mL)	5.8 (5.7—6.0)	5.7 (5.6—5.9)	0.06
HCV RNA > 600,000 IU/mL	82 (52%)	39 (49%)	0.68
HCV genotype			Too small sample
Genotype 1	89 (57%)	65 (82%)	
Genotype 2	15 (10%)	3 (4%)	
Genotype 3	21 (13%)	11 (14%)	
Genotype 4/5	32 (20%)	0 (0%)	
Easy to treat G2/G3	36 (23%)	14 (18%)	0.36
ALT (IU/L)	60 (53—71)	102 (68—136)	0.002
Fasting glucose	4.7 (4.6—4.9)	5.5 (4.7—5.7)	0.0003
FibroTest	0.45 (0.36—0.56)	0.44 (0.33—0.63)	0.40
Presumed fibrosis stage			0.02
F0	49 (31%)	22 (30%)	
F1	31 (20%)	20 (25%)	
F2	14 (9%)	6 (8%)	
F3	43 (27%)	10 (13%)	
F4	20 (13%)	21 (27%)	
Advanced fibrosis (F2F3F4)	77 (49%)	37 (48%)	0.75
ActiTest	0.42 (0.33—0.49)	0.59 (0.50—0.75)	0.004
Presumed activity grade			0.04
A0	48 (31%)	16 (20%)	
A1	48 (31%)	17 (22%)	
A2	10 (6%)	9 (11%)	
A3	51 (32%)	37 (47%)	
Advanced activity (A2A3)	61 (38%)	46 (58%)	0.005
SteatoTest	0.33 (0.26—0.40)	0.50 (0.39—0.55)	
Presumed steatosis grade			0.03
S0	60 (58%)	13 (32%)	
S1	23 (22%)	14 (34%)	
S2	12 (12%)	6 (15%)	
S3S4	9 (9%)	8 (19%)	
Advanced Steatosis (S2S3S4)	21 (21%)	14 (34%)	0.08
IL28B genotype frequency			0.46
genotype CC	52 (33%)	28 (35%)	
genotype CT	75 (48%)	41 (52%)	
genotype TT	30 (19%)	10 (13%)	
ITPA deficiency			
None	107 (68%)	47 (60%)	0.06
+	32 (20%)	16 (20%)	
++	18 (12%)	13 (16%)	
+++	0 (0%)	3 (4%)	
*UGT1A1*genotype			0.07
None	71 (45%)	24 (30%)	
Heterozygote	65 (41%)	39 (50%)	

Table 1 (*Continued*).

Baseline characteristics	Training group	Validation group	*P* Value
Homozygote	21 (13%)	16 (20%)	
Sustained Virologic Response	73 (47%)	50 (63%)	0.01

[a] Alcohol consumption was missing in 139 cases. Low alcohol consumption was defined as a maximum of 20 g/day for female and 30 g/day for male.
[b] BMI missing in 48 cases.

2/3 = 5.78 (2.63—12.69; $P < 0.0001$); low HCV viral load = 1.79 (1.01—3.19; $P = 0.047$); *IL28B* type CC = 4.39 (2.09—9.22; $P < 0.0001$); low FibroTest =4.33 (1.21—15.47; $P = 0.02$); high ActiTest = 3.44 (0.99—11.9; $P = 0.051$); and no significant independent association of the ''population'' factor with SVR: OR = 1.78 (0.94—3.34; $P = 0.07$). In order to identify which confounding factor among the five significant factors could explain the better SVR observed in the validation group vs. the training group in univariate analysis (Table 1), five ''2—factor models'' were constructed with the population factor and each of the five predictive factors: the univariate OR of the validation group was 1.98 (1.14—3.45; $P = 0.02$), decreasing the most when *IL28B* CC genotype was introduced: OR = 1.67 (0.94—2.96; $P = 0.08$); otherwise, the population factor was still significant when HCV genotype, viral load, FibroTest or ActiTest were introduced (data not shown).

There was no difference in the median FibroTest result according to the genetic markers. For the *IL28B* genotype: CC = 0.45 (0.35—0.62) vs. CT = 0.48 (0.35—0.58;

Table 2 Multivariate Logistic Regression Models for Sustained Virologic Response (SVR).

Model 1	Training group		Validation group		All patients	
N	157		79		236	
	Odds Ratio (95% CI)	*P* Value	Odds Ratio (95% CI)	*P* Value	Odds Ratio (95% CI)	*P* Value
Virus						
HCV genotype 2/3 vs. Others	6.22 (2.54—15.23)	< 0.0001	3.43 (0.58—20.5)	0.18	5.69 (2.59—12.50)	< 0.0001
HCV RNA < 600,000 vs. > 600,000 IU/mL	1.73 (0.85—3.51)	0.13	2.51 (0.81—7.73)	0.11	1.91 (1.07—3.40)	0.03
Genetic						
CC *IL28B* genotype	3.32 (1.29—8.53)	0.01	8.95 (2.16—37.1)	0.003	4.84 (2.59—12.50)	< 0.0001
ITPA deficiency vs. none	1.16 (0.54—2.48)	0.70	2.25 (0.73—6.92)	0.16	1.48 (0.80—2.72)	0.21
No *UGT1A1* deficiency	2.02 (0.67—6.07)	0.21	0.35 (0.08—1.47)	0.35	0.94 (0.43—2.06)	0.88
Liver injury						
FibroTest[a]	3.41 (0.73—16.07)	0.12	8.10 (0.73—90.00)	0.07	4.20 (1.18—14.90)	0.03
ActiTest[b]	2.24 (0.53—9.49)	0.27	7.39 (0.63—86.02)	0.11	3.89 (1.12—13.44)	0.03
SteatoTest[c]	0.80 (0.07—9.44)	0.86	90.84 (0.07—1000)	0.21	1.48 (0.17—12.7)	0.72
Area Under the ROC Curve	0.73	< 0.0001	0.82	< 0.0001	0.75	< 0.0001
Model R Squared	0.13	< 0.0001	0.23	< 0.0001	0.15	< 0.0001

[a] A decrease of FibroTest was associated with an increase of the response rate.
[b] An increase of ActiTest was associated with increase of the response rate.
[c] Not available in 53 patients in the training group and in 38 patients in the validation group, and the analysis was performed in a subset of 183 patients without missing data.

$P = 0.57$) and vs. TT = 0.41 (0.26—0.52). For ITPA insufficiency: no insufficiency = 0.50 (0.38—0.59) vs. insufficiency = 0.42 (0.32—0.51; $P = 0.31$). For the *UGT1A1* genotype: homozygous 0.55 (0.38—0.67) vs. heterozygous 0.42 (0.32—0.52; $P = 0.24$) vs. wild—type 0.51 (0.35—0.61; $P = 0.47$).

Construction of a multivariate assay, the HCV—GenoFibroTest

The combination of the five most predictive factors (HCV genotype 2/3, *IL28B* genotype, FibroTest, ActiTest and viral load), called HCV—GenoFibroTest, enabled a logistic regression formula with significant diagnostic performances to be constructed in the training population, which was then validated in the validation population, despite some characteristic differences. The equation in the training population was HCV—GenoFibroTest = 0.1217 + 0.9324*ActiTest —1.1804*FibroTest + 1.4587*(Geno23 = 1) —1.0435*(IL28B = ''CT'') —1.8742*(IL28B = ''TT'') + 0.7389*(Viral Load < 600,000 IU/mL = 1). The AUROC for SVR in the training population was 0.743 (0.655—0.810; $P < 0.0001$ vs. random), which did not differ significantly ($P = 0.88$) from the AUROC in the validation population [= 0.753 (0.616—849; $P = 0.0007$ vs. random)] (Fig. 2).

The overall diagnostic performances according to cutoffs are detailed in Table 3. An 83% positive predictive value was obtained at a cutoff of 0.66, and an 87% negative predictive value at a cutoff of 0.20; these two extreme response groups correspond to 33% of the overall population [(23 + 54)/236] (Fig. 3).

Discussion

This study demonstrates that FibroTest as an alternative to biopsy for liver fibrosis staging is an independent predictive factor for SVR in patients with chronic hepatitis C. The study confirms the independent and highly significant predictive value of *IL28B* CC genotype for SVR in two European populations with different genotypes. In this relatively small sample size population, *ITPA* and *UGT1A1* polymorphisms were not associated with SVR.

FibroTest

Several studies of FibroTest during standard HCV treatment in treatment—naive patients have been performed [9,21—25]. FibroTest was similar to paired liver biopsies in demonstrating reduced necrosis and fibrosis in sustained virologic responders [9]. The independent prognostic value of FibroTest observed in the present multivariate analysis for SVR in 236 patients, OR = 4.20 (1.18—14.90; $P = 0.03$), was similar to that observed in 601 retreated patients, OR = 5.0 (2.3—11.0; $P < 0.0001$) [26]. The present study is however the first to show the performance of FibroTest when *IL28B* polymorphism is assessed.

IL28B

This study confirms the performance of *IL28B* CC genotype for predicting SVR at baseline, as already demonstrated in patients infected with genotype 1 in naive patients [4], or with genotype 2 and 3 in retreated patients [27]. In the present study, as in the others, the level of performance of *IL28B* type was impressive—always stronger than that of viral load or fibrosis stage and when multiple genotypes were present at the same levels as genotype 2/3 versus genotype 1. As already observed in patients in the US (of African or Caucasian origin), *IL28B* polymorphism can now be used to explain previously odd SVR differences between European populations. In the present study, the significantly higher SVR in the Ukrainian validation population compared with the Parisian training population could not be explained by differences in genotype 2/3, viral load, or fibrosis stage, and the validation population OR became non-significant only after adjustment of IL28B genotypes. Contrary to recent observations, we found no significant association between fibrosis estimated by FibroTest, ActiTest and GGT with *IL28B* genotypes [28]. Larger studies are needed to assess these combinations in order to take into account the numerous confounding factors.

ITPA genotype

As in the Thompson et al. study, ITPA-insufficiency genotype was not associated with a higher SVR in the present study [5]. Larger studies are needed to assess whether there is no impact on SVR, particularly in older patients or in patients with cirrhosis who are at higher risk of anemia.

UGT1A1

Despite a few clinical observations, no study had thus far assessed the impact of Gilbert's syndrome on SVR or on RBV treatment compliance [6]. Treatment of chronic hepatitis C with type I interferons and RBV can be associated with exacerbation of hepatitis, and sometimes, liver decompensation. Deterding et al. reported two patients with chronic HCV infection who experienced a severe increase inbilirubin levels up to 17 times the upper limit of normal value in the absence of hepatic function deterioration during therapy with pegylated-interferon and RBV [6]. The genetic disposition for Gilbert's syndrome explained the adverse events and enabled the therapy to be continued, leading to a sustained clearance of chronic hepatitis C infection. Since one patient had already become jaundiced during a lead-in treatment period with RBV monotherapy, it was suggested that hyperbilirubinemia during combination therapy is primarily caused by RBV rather than by effects of interferon alpha UGT1A1 activity [6]. In the present study, there were no cases of highly elevated bilirubinemia declared during treatment or at baseline.

ActiTest

We also confirmed that elevated necroinflammatory activity as assessed byActiTest had an independent predictive value

Table 3 Predictive values for SVR of HCV-GenoFibroTest, a combination of 5 biomarkers: HCV genotype, HCV viral load, *IL28B*genotype, FibroTest and ActiTest.

Biomarkers	Cutoff	Sensitivity	Specificity	Positive predictive value	Negative predictive value
5 Factors (HCV-GenoFibroTest)					
	0.20	0.98 (120/123)	0.18 (20/113)	0.56 (120/213)	0.87 (20/23)
	0.50	0.61 (75/123)	0.64 (84/113)	0.70 (75/104)	0.64 (84/132)
	0.66	0.36 (45/123)	0.92 (104/113)	0.83 (45/54)	0.57 (104/182)
Isolated factor[a]					
HCV Genotype	2/3 vs. others	0.32	0.90	0.78	0.55
Viral Load	HCV RNA < 600,000 vs. > 600,000 IU/mL	0.54	0.56	0.57	0.53
*IL28B*genotype	CC vs. others	0.32	0.88	0.74	0.54
FibroTest	< 0.48	0.54	0.51	0.55	0.51
ActiTest	> 0.52	0.57	0.52	0.57	0.52

Prevalence of SVR was 0.52 among 236 patients included.
[a] Published prognostic factors and predetermined standard cutoffs.

for SVR. This factor had not been clearly identified in initial studies on predictive factors of SVR [2]. Better SVRs in patients with higher baseline ALT levels have been recently observed in the large prospective IDEAL study [3] and in transplanted patients with high histological activity grades at baseline biopsy [29].

SteatoTest and metabolic factors

In the present study, low SteatoTest results were not significantly associated with SVR. Because of the number of missing data and the fact that there were only 35 cases with advanced steatosis (> 33%), no conclusion can be drawn for this negative finding. In a larger population, the absence of histological steatosis was associated with SVR in naive patients [3] as well as in retreated patients both using liver biopsy estimate and SteatoTest [26]. Furthermore, the prognostic value of steatosis must be analyzed separately in genotype 3 and genotypes non-3. In the present study, this was not possible due to the lack of power with a total of only 33 patients with genotype 3. Therefore more patients are needed to assess the possible prognostic value of SteatoTest and other metabolic biomarkers.

Combination of prognostic biomarkers

The constructed combination of the five most predictive factors (HCV genotype 2/3, *IL28B* genotype, FibroTest, ActiTest and viral load), known as HCV-GenoFibroTest, obtained good prognostic performanceswith3 zones: high risk (more than 87% SVR) in 10% of patients; a very low risk of SVR (less than 13% SVR) in 23% of patients; and an intermediate risk group (47% SVR). The limitation of such a combination is obviously the large intermediate zone. Many factors remain unknown as demonstrated by the AUROC of 0.75, far from 100%, and the squared correlation coefficient of the regression analysis around 15%.

Other weaknesses

This was a retrospective study and despite the two populations included for training and validation, a new prospective validation study is necessary. Only HCV and IL28B genotypes were independently associated with SVR in training and validation groups separately. However, the patients were consecutive cases with available samples, and data were blindly analyzed. All factors, including the genetic tests, had already been validated as prognostic factors by independent authors. The two populations were heterogeneous; few patients with genotype 4 and 5 were included and there were no patients with genotype 6.

As previously demonstrated, the dynamics of virologic response using viral load measurements at 4 and 12 weeks rapidly improved the predictive values and the possibility of tailored duration of treatment. The prognostic performance of combination should now be compared at 4 and 12 weeks of treatment.

Another weakness of the present study is the absence of a prospective assessment of the adherence to RBV and interferon treatment, which could explain the up to 15%

SVR difference [30]. Further studies must assess the utility of using pharmacogenomic tests such as *ITPA* and *UGT1A1* polymorphisms.

Conclusion

Fibrosis stage assessed by FibroTest is an independent predictor of SVR, after including assessment of the *IL28B* genetic polymorphism. A combination of five baseline non-invasive biomarkers (HCV genotype 2/3, *IL28B* genotype, FibroTest, ActiTest and viral load) could simplify the baseline prediction of virologic response in patients with chronic hepatitis C.

Conflict of interest statement

Thierry Poynard is the inventor of FibroTest and has a capital interest in Biopredictive, the company marketing FibroTest. The patent belongs to Assistance Publique—Hôpitaux de Paris.

Mona Munteanu and Yen Ngo are full employees of Biopredictive.

Jean Marc Costa and Jean Dominique Poveda are full employees of Pasteur Cerba, a company marketing FibroTest.

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