Predicting the development of liver cirrhosis by simple modelling in patients with chronic hepatitis C

S. Lens, F. Torres, M. Puigvehi, Z. Mariño, M.-C. Londoño, S. M. Martinez, I. García-Juárez, Á. García-Criado, R. Gilabert, C. Bru, R. Solà, J. M. Sanchez-Tapias, J. A. Carrón & X. Forns

SUMMARY

Background
Data are scarce on the natural history of chronic hepatitis C (CHC) in patients with mild hepatitis C who did not respond to anti-viral therapy.

Aim
To predict the risk of progression to cirrhosis, identifying patients with the more urgent need for therapy with effective anti-virals.

Methods
A cohort of 1289 noncirrhotic CHC patients treated with interferon-based therapy between 1990 and 2004 in two referral hospitals were followed up for a median of 12 years.

Results
Overall, SVR was achieved in 46.6% of patients. Data from a randomly split sample (n = 832) was used to estimate a model to predict outcomes. Among nonresponders (n = 444), cirrhosis developed in 123 (28%) patients. In this group, the 3, 5 and 10-year cumulative probabilities of cirrhosis were 4%, 7% and 22%, respectively, compared to <1% in the SVR-group (P < 0.05). Baseline factors independently associated with progression to cirrhosis in nonresponders were: fibrosis ≥F2, age >40 years, AST >100 IU/L, GGT >40 IU/L. Three logistic regression models that combined these simple variables were highly accurate in predicting the individual risk of developing cirrhosis with areas under the receiving operating characteristic curves (AUC) at 5, 7 and 10 years of ~0.80. The reproducibility of the models in the validation cohort (n = 457, nonresponders = 244), was consistently high.

Conclusions
Modelling based on simple laboratory and clinical data can accurately identify the individual risk of progression to cirrhosis in nonresponder patients with chronic hepatitis C, becoming a very helpful tool to prioritise the start of oral anti-viral therapy in clinical practice.

Aliment Pharmacol Ther 2016; 43: 364–374
INTRODUCTION
Treatment of chronic hepatitis C (CHC) using a combination of pegylated interferon and ribavirin (PR) results in a sustained virologic response (SVR) in ~50% of treated patients. New direct anti-viral therapies (DAAs) have been approved in the U.S and Europe and have become the new standard of care for CHC therapy. Besides increased efficacy, one of the main advantages of these drugs is their excellent safety profile and the possibility of achieving SVR in an all-oral interferon-free combination. Nevertheless, the wide use of such drugs implies a high economical burden so the Health Care Systems of many countries will not afford treating all HCV-infected patients. In this setting, a careful selection of patients is essential, at least during the next few years.

The main goal of viral clearance in patients with chronic hepatitis C is to avoid progression to liver cirrhosis and its complications. Although the progression rate of liver fibrosis in patients with CHC is highly variable, cirrhosis develops in approximately 15–20% of patients over time. A community-based study in Germany in patients with known data of HCV infection provided evidence of disease progression 35 years after infection, with the highest proportion of patients with clinical signs of cirrhosis in the non-SVR group (15%).

A recent study established a risk score to assess long-term mortality among patients with advanced fibrosis; however, due to the slow progression of CHC, large cohorts and long follow-up periods are necessary to reach solid conclusions in patients with mild fibrosis.

The main aim of this study is to identify which factors are related to disease progression in patients with chronic hepatitis C who did not respond to anti-viral therapy and are potential candidates for oral anti-viral treatment. A common question in patients, particularly in those with mild disease who may not fulfill criteria for drug reimbursement, is if receiving anti-viral therapy can be delayed and, more importantly, for how long.

PATIENTS AND METHODS
Patients and study design
This is a retrospective-prospective cohort study performed at two referral centres in Barcelona (Hospital Clinic and Hospital del Mar). The cohort study included 1289 patients with noncirrhotic chronic hepatitis C who received anti-viral therapy between 1990 and 2004. Cirrhosis was excluded clinically and by histological assessment at baseline. Patients with other causes of liver disease (HBV infection, hemochromatosis, autoimmune hepatitis, primary biliary cirrhosis and Wilson’s disease) and those coinfected with the HIV were excluded from the study.

Follow-up started after the last dose of interferon and ended in December 2013, at death or at the last follow-up visit. Physical examination and complete blood testing were performed monthly during anti-viral therapy and 6 months after treatment interruption. Thereafter, all patients underwent at least yearly laboratory tests and an abdominal ultrasound (US) every 2 years. In patients with bridging fibrosis at the time of baseline liver biopsy, or in those who developed cirrhosis during follow-up, laboratory tests and ultrasonographic studies were performed every 6 months and upper gastrointestinal endoscopy every 2 years. All patients provided written informed consent to data handling in accordance with a protocol approved by the local Ethics Committee.

Liver histology
Percutaneous liver biopsies were performed under local anaesthesia by expert radiologists. Specimens were fixed, paraffin-embedded, and stained with haematoxylin-eosin and Masson’s trichrome. Histological grade and stage were determined according to the METAVIR scoring system. Liver fibrosis was considered significant if located beyond the portal tract (F ≥ 2).

Anti-viral treatment
Treatment regimens varied over time. Interferon monotherapy was the standard of care from 1990 to 1996. From 1997 all treated patients received combined treatment with interferon and ribavirin (pegylated alfa-2a or 2b after 2001): 24-week regimens for genotype 2 and 3 HCV-infected patients and 24/48-week regimens for genotype 1 HCV-infected patients. Sustained virological response (SVR) was defined as undetectable serum HCV-RNA 24 weeks after discontinuation of anti-viral treatment. Patients not achieving this criterion were defined as nonresponders.

Ultrasonographic assessments
All abdominal US studies were performed by expert radiologists. The more recent abdominal ultrasound study was reviewed by two expert radiologists (AGC and RG). For this purpose, saved images were recovered and the following characteristics were assessed blindly: presence of surface nodularity, portal vein diameter greater than 12 mm and splenomegaly (spleen size > 12 cm). In patients with ultrasonographic evidence of liver cirrhosis, all previous follow-up ultrasound studies were reviewed.
in order to establish the first in which cirrhosis could be established.

**Outcome measures**
The main aim of our study was to identify the variables independently related to the development of liver cirrhosis in patients with chronic hepatitis who failed to achieve SVR after treatment. Although liver biopsy remains as the gold standard for the diagnosis of liver cirrhosis, repeated biopsies are not regularly performed in patients with chronic hepatitis C since they do not allow for a dynamic approach when evaluating disease progression in patients with CHC. Therefore, the diagnosis of liver cirrhosis during follow-up was based on the presence of at least one of the following criteria: (i) presence of F4 fibrosis stage in a liver biopsy; (ii) presence of portal hypertension, defined as an hepatic venous pressure gradient ≥6 mmHg or presence of gastroesophageal varices in an upper endoscopic study; (iii) presence of at least two signs of cirrhosis (nodular liver surface, portal vein diameter >12 mm, spleen size >12 cm) in two consecutive ultrasound studies. The date of cirrhosis was established as the earliest date at which any of the previous criteria was present. It is important to note that US examinations in patients with chronic hepatitis C are specifically aimed at excluding the presence of cirrhosis and, in patients with evidence of cirrhosis, at excluding the presence of hepatocellular carcinoma (HCC).

The effects of anti-viral therapy on the development of clinical liver decompensation or HCC during follow-up were analysed. Clinical liver decompensation was defined as the occurrence of ascites, spontaneous bacterial peritonitis (SBP), hepatic encephalopathy or variceal bleeding at any time during follow-up.

**Statistical analyses**
Continuous variables are reported as median and interquartile range (percentiles 25–75%) and categorical variables as absolute and relative frequencies. Groups were compared using the Mann–Whitney test for continuous variables and the Fisher’s exact test for the categorical ones. The main endpoint was the occurrence of cirrhosis and clinical liver decompensation during follow-up. The survival function for cirrhosis development was estimated by means of the Kaplan–Meier method. Survival distributions were compared using the log-rank test for the univariate testing, and Cox proportional hazard models were used for the multivariate analyses and to estimate hazard ratios [HR (95% CI)]. Logistic regression models were used to study the predictors of cirrhosis development at 5, 7 and 10 years and of liver events (hepatic decompensation or HCC) at 7 and 10 years in nonresponder patients. Variables with a 10% significance level on univariate testing were included in the multivariate analysis. In selecting the final model, we constructed receiver operating characteristic (ROC) curves for the multivariate models, and then chose the model with the greater area under the curve (AUC). Data from a randomly generated split-sample of 832 patients (2/3) were used to estimate the model, and data from the remaining 457 patients (1/3) were used for validation. Split was performed stratifying by centre and response to therapy. Each of the variables included in the model was analysed to rule out any significant differences between the estimation and the validation cohorts. The analysis was performed with SAS version 9.2 (SAS Institute Inc., Cary, NC, USA). Significance was established at a two-sided P-value of 0.05.

**RESULTS**

**Patients’ characteristics**
The baseline characteristics of the 1289 patients included in the study are summarised in Table 1. Most patients included in this study had mild fibrosis (METAVIR F0-F1) at the time of treatment initiation (76%), whereas only 22% had fibrosis expansion beyond the portal tract (F2) or bridging fibrosis (F3). Sixty-seven (5%) patients did not undergo a liver biopsy or had a biopsy that was considered insufficient for establishing the fibrosis stage, but they were considered noncirrhotic based on clinical, laboratory and US criteria. The presence of any of these criteria was also discarded in patients with histological assessment to exclude liver biopsy sampling error. As expected in our geographical area, most patients (67%) were infected with genotype-1. SVR was achieved in 492 (38%) patients after the first treatment course. One-hundred nine patients obtained SVR after a second treatment course; the remaining 688 (53.4%) individuals were considered nonresponders. Patients were followed up for a median of 12 years (IQR 9–16).

Overall, 207 (16%) patients developed cirrhosis during follow-up. First suspicion of cirrhosis diagnosis was based on US criteria in 174 patients (84%). Importantly, only 13 (2.2%) of those who achieved SVR (n = 601) were classified as cirrhotics during follow-up in the whole cohort. Seven of these patients had bridging fibrosis (F3) at baseline. The remaining six patients (F0–1 at baseline) achieved SVR during a second course of anti-viral therapy 8–10 years after the first treatment, poten-
tially explaining fibrosis progression from baseline. In addition, one patient concomitantly presented alcohol abuse which possibly contributed to the development of cirrhosis. There were no significant differences between the estimation and validation cohort in any of the assessed variables.

**Predictors of cirrhosis in the estimation cohort**

In the estimation cohort (n = 832), the cumulative probabilities of developing cirrhosis among nonresponders (n = 123/444, 28%) were 4%, 7%, 12% and 22%, at 3, 5, 7 and 10 years respectively. For patients who had achieved SVR (n = 7/388), the cumulative probabilities of developing cirrhosis were less than 1% at all time points (Log-rank P < 0.001; Figure 1).

Analysing which variables predicted the occurrence of cirrhosis in nonresponders was of major interest, since cirrhosis developed almost exclusively in this group (Table 2).

Based on multivariate analysis, the only variables identified as independent predictors of cirrhosis were age, fibrosis stage, GGT and AST at baseline (Table 3). APRI, FIB-4 and Forns scores were also independent predictors of cirrhosis development if the individual variables contained in the scores were removed from the analysis (data not shown). As expected, when performing the same analysis in the overall estimation cohort (responders and nonresponders), the same variables were identified as independent predictors for cirrhosis development, as well as failure to achieve virological response.

### Table 1 | Characteristics of patients in the estimation and validation groups (total n = 1289)

| Cohorts                        | Estimation (n = 832)* | Validation (n = 457)* | P-value†  |
|--------------------------------|-----------------------|-----------------------|-----------|
| Age (years)                    | 40 (33–50)            | 40 (33–50)            | 0.96      |
| Sex (males), n (%)             | 533 (64.1%)           | 287 (62.8%)           | 0.67      |
| AST (IU/L)                     | 59 (42–83)            | 53 (40–84)            | 0.20      |
| ALT (IU/L)                     | 102 (69–150)          | 92 (68–153)           | 0.15      |
| GGT (IU/L)                     | 35 (22–56)            | 38 (23–62)            | 0.27      |
| AP (IU/L)                      | 157 (131–190)         | 160 (135–195)         | 0.15      |
| Glucose (mg/dL)                | 93 (86–101)           | 93 (87–101)           | 0.44      |
| Cholesterol (mg/dL)            | 173 (149–198)         | 173 (149–200)         | 0.85      |
| Platelets (×10⁹)               | 193 (163–228)         | 198 (163–233)         | 0.33      |
| APRI                           | 0.68 (0.5–1)          | 0.63 (0.4–1.1)        | 0.22      |
| FIB-4                          | 1.19 (0.8–1.6)        | 1.17 (0.8–1.7)        | 0.36      |
| Forns                          | 4.5 (3.6–5.3)         | 4.46 (3.4–5.6)        | 0.85      |
| HCV genotype                   |                       |                       |           |
| 1a                             | 89 (11.0%)            | 57 (12.8%)            | 0.40      |
| 1b                             | 445 (54.8%)           | 255 (57.0%)           |           |
| 2                              | 51 (6.3%)             | 22 (4.9%)             |           |
| 3                              | 122 (15.0%)           | 63 (14.1%)            |           |
| 4                              | 41 (5.0%)             | 26 (5.8%)             |           |
| Nontypable/unavailable         | 64 (7.9%)             | 24 (5.4%)             |           |
| Liver fibrosis (baseline)      |                       |                       |           |
| Minimal/portal fibrosis (F0/F1)| 612 (78.2%)           | 318 (73.3%)           | 0.22      |
| Fibrosis beyond portal tract (F2)| 94 (11.4%)         | 58 (12.7%)            |           |
| Bridging fibrosis (F3)         | 74 (8.9%)             | 58 (12.7%)            |           |
| Insufficient material/No biopsy| 44 (5.3%)             | 23 (5.0%)             |           |
| SVR (1st treatment) n (%)      | 316 (38.0%)           | 176 (38.5%)           | 0.98      |
| SVR (final treatment) n (%)    | 388 (46.6%)           | 213 (46.6%)           | 0.88      |
| Hospital Clinic n (%)          | 580 (69.7%)           | 316 (69.1%)           | 0.85      |
| Follow-up (years)              | 12 (9–16)             | 12 (9–16)             | 0.73      |
| Cirrhosis on follow-up n (%)   | 130 (15.6%)           | 77 (16.8%)            | 0.58      |
| Decompensation or HCC n (%)    | 50 (6.0%)             | 33 (7.2%)             | 0.41      |
| Death on follow-up n (%)       | 43 (5.2%)             | 26 (5.7%)             | 0.79      |

*Qualitative variables are expressed as n (%) and quantitative variables as median and interquartile range (P25–P75).
†Inferential analysis using the Fisher’s exact test for nominal variables and the Mann–Whitney test for the rest of the variables.
Figure 1 | Survival function for cirrhosis observed in patients achieving SVR and in nonresponders (log-rank P < 0.001).

Table 2 | Baseline characteristics and hazard ratios (95% CI) estimates for the development of cirrhosis among nonresponder patients in the estimation cohort

| Cohorts               | No cirrhosis (n = 321)* | Cirrhosis (n = 123)* | HR† [95% CI] | P-value† |
|-----------------------|-------------------------|----------------------|--------------|----------|
| Age (years)           | 39 (32–50)              | 51 (41–57)           | 1.05 [1.04–1.07] | <0.001   |
| Sex (males), n (%)    | 206 (64.2%)             | 66 (53.7%)           | 1.25 [0.89–1.77] | 0.20     |
| AST (IU/L)            | 56 (40–77)              | 76 (51–105)          | 1.01 [1–1.01]  | <0.001   |
| ALT (IU/L)            | 91 (65–138)             | 109 (68–160)         | 1.01 [1–1.01]  | 0.023    |
| GGT (IU/L)            | 36 (23–62)              | 54 (36–93)           | 1.01 [1–1.01]  | <0.001   |
| AP (IU/L)             | 153 (131–183)           | 179 (139–221)        | 1.01 [1.01–1.01] | <0.001   |
| Glucose (mg/dL)       | 93 (86–100)             | 101 (91–110)         | 1.01 [1.01–1.01] | <0.001   |
| Cholesterol (mg/dL)   | 175 (153–196)           | 172 (154–195)        | 1.01 [1.01–1.01] | 0.590    |
| Platelets (x10⁹)      | 197 (166–231)           | 182 (153–220)        | 1.01 [0.99–1.01] | 0.138    |
| APRI                   | 0.63 (0.4–0.9)          | 0.92 (0.6–1.4)       | 1.39 [1.24–1.56] | <0.001   |
| FIB-4                 | 1.16 (0.8–1.6)          | 1.83 (1.2–2.7)       | 1.39 [1.29–1.50] | <0.001   |
| Forns                 | 4.4 (3.3–5.5)           | 5.5 (4.7–6.6)        | 1.59 [1.41–1.79] | <0.001   |
| HCV genotype          |                         |                      |              |          |
| 1a                    | 54 (17.3%)              | 14 (11.7%)           | 1            | 0.002    |
| 1b                    | 190 (60.9%)             | 92 (76.7%)           | 1.34 [0.78–2.31] |          |
| 2                     | 9 (2.9%)                | 3 (2.5%)             | 0.51 [0.17–1.53] |          |
| 3                     | 19 (6.1%)               | 4 (3.3%)             | 0.35 [0.13–0.96] |          |
| 4                     | 16 (5.1%)               | 1 (0.8%)             | 0.2 [0.03–1.51]  |          |
| Nontypable/unavailable | 24 (7.7%)               | 6 (5.0%)             | 0.5 [0.19–1.3]  |          |
| Liver fibrosis (baseline) |                    |                      |              |          |
| Minimal/portal fibrosis (FO/F1) | 256 (79.7%) | 64 (52%) | 1 | <0.001 |
| Fibrosis beyond portal tract (F2) | 27 (8.4%)   | 25 (20.8%) | 3.16 [1.99–5.02] |          |
| Bridging fibrosis (F3) | 19 (5.9%)              | 24 (20.0%)           | 3.91 [2.51–6.08] |          |
| Insufficient material/no biopsy | 44 (5.3%)   | 23 (5.0%)           | 1.73 [0.83–3.60] |          |
| Follow-up (years)     | 10 (5–14)              | 14 (10–17)           | 0.99 [0.99–1]  | 0.007    |
| HCC development n (%) | 2 (0.6%)               | 21 (17.1%)           | 17.89 [12.4–25.8] | <0.001   |
| Death on follow-up n (%) | 14 (4.4%)    | 21 (17.4%)          | 0.18 [0.11–0.28] | <0.001   |

* Qualitative variables are expressed as n (%) and quantitative variables as median and interquartile range (P25–P75).
† Using Cox-Regression analysis.
Construction and validation of a model to predict the individual risk of developing cirrhosis

In order to construct a model to calculate the individual risk of cirrhosis development, quantitative variables were categorised by the best cut-off point according to the area under the ROC curve. AST, aspartate aminotransferase; GGT, gamma glutamyl transpeptidase.

The following models were constructed (Table 4): (i) Model I, which included all four variables (age, fibrosis stage, AST and GGT); (ii) Model II, which was aimed to assess patients without a liver biopsy and thus included age, AST and GGT; (iii) Model III, which resulted from the combination of fibrosis stage, AST and Forns score.

As shown in Table 5, all models presented similar diagnostic accuracy expressed by ROC analysis and importantly, their value remained very high over time (5 and 10-year prediction from baseline). Harrell’s C-Index [95% CI] for the overall follow-up were calculated: 0.837 [0.696–0.944] (Model I); 0.841 [0.705–0.944] (Model II) and 0.839 [0.695–0.947] (Model III), respectively, which were higher than those for each variable alone. In addition, comparisons between multivariate models using C-Index were not significant (P > 0.4).

A simple score was then constructed by adding up to a constant the value of each variables’ regression coefficient multiplied by 0 or 1, according to the cut-off points described above (Table 6).

As an example, the associated probability of developing liver cirrhosis after 5 years of follow-up in a 60 year-old patient with GGT of 100 IU and AST of 200 IU using Model II would be:

\[
\text{Score} = -6.188 + 2.037 + 1.668 + 1.108 = -1.375
\]

The probability is now derived from the formula:

\[
\text{prob} = \frac{1}{1 + e^{-[-\text{score}]}}; \quad \text{prob} = \frac{1}{1 + e^{-[-1.375]}} = 0.202
\]

The probability of cirrhosis in this individual would be 20% and 33% at 5 and 10 years of follow-up respectively. Using the same model, in a 35 year-old patient, with GGT <40 IU and AST <100 IU the probability of developing cirrhosis would be only 0.2% and 1.2% at 5 and 10 years of follow-up respectively.

When applying the constructed logistic model to estimate the 10-year cirrhosis probability, Kaplan–Meier curves showed a good discriminative value among risk groups. Indeed, using these models nonresponder patients could be stratified into different categories according to their risk of developing liver cirrhosis after 10 years of follow-up. According to models I and III, which are constructed with 4 different variables, patients could be stratified into low (<5%), intermediate (5-20%) and high risk groups (>20%). Model II, combining only 3 categorical variables, accurately stratified patients into a low (<5%) and a high risk group (>5%) for cirrhosis (Figures 2–4).

When assessing the reproducibility of the model for the odds ratio estimates in the validation cohort (244 nonresponders), all models showed a good accuracy with AUC between 0.79 and 0.87 as is shown in Table 5.

Variables predicting liver-related events

During follow-up, 83 patients presented clinical events, including decompensation (n = 42) or HCC (n = 41). In the estimation group, a total of 26 patients developed...
clinical decompensation (20 ascites, 3 variceal bleeding, 1 hepatic encephalopathy, 1 SBP); all of them were nonresponders. HCC was diagnosed in 23 patients; two had previously achieved SVR but had bridging fibrosis at baseline (Table 1). Variables independently associated with the development of clinical decompensation or hepatocellular carcinoma were the same identified for progression to cirrhosis [Tables S1 and S2 (A-B)]. Importantly, the constructed models showed high accuracy for predicting the probability of developing clinical decompensation, with AUCs of 0.84 (model I); 0.80 (model II) and 0.87 (model III) after 10 years of follow-up respectively. The latter data support the strength of these models to predict progression to cirrhosis.

### Table 5 | Assessment of the models in the estimation and validation cohorts

| Estimation cohort* | Validation cohort† |
|-------------------|---------------------|
| **Model** | **Time-point at follow-up** | **Fibrosis stage at baseline** | **Age >40 OR [95% CI]** | **GGT >40 OR [95% CI]** | **AST >100 OR [95% CI]** | **ROC AUC [95% CI]**† | **ROC AUC [95% CI]†** |
| I | 5 years | 5.72 [2.40–13.61] | 5.35 [1.55–18.49] | 4.69 [1.54–14.28] | 2.49 [110–5.63] | 0.882 [0.821–0.942] | 0.859 [0.806–0.911] |
| | 10 years | 2.72 [1.59–4.65] | 3.68 [1.96–6.91] | 5.17 [2.70–9.88] | 1.61 [0.90–2.88] | 0.825 [0.779–0.871] | 0.853 [0.806–0.900] |
| II | 5 years | 7.67 [2.28–25.79] | 5.30 [1.75–16.03] | 3.03 [1.39–6.60] | 0.837 [0.775–0.900] | 0.769 [0.676–0.862] | 0.811 [0.756–0.867] |
| | 10 years | 4.75 [2.59–8.73] | 5.46 [2.93–10.16] | 1.61 [0.93–2.79] | 0.802 [0.758–0.847] | 0.853 [0.806–0.900] |
| III | 5 years | 7.24 [2.88–18.24] | 2.23 [1.61–3.09] | 2.59 [1.10–6.15] | 0.915 [0.881–0.949] | 0.856 [0.770–0.942] |
| | 10 years | 2.85 [1.67–4.87] | 1.72 [1.43–2.07] | 1.91 [1.06–3.46] | 0.797 [0.741–0.853] | 0.871 [0.812–0.930] |

* Multivariate logistic regression and ROC analysis of factors associated with progression to cirrhosis in nonresponder patients (Estimation cohort).
† Assessment of the models for the odds ratio estimates in the validation cohort.
‡ Calculated Harrell’s C-Index [95% CI] for the overall follow-up were: 0.837 [0.696–0.944] (Model I); 0.841 [0.705–0.944] (Model II) and 0.839 [0.695–0.947] (Model III) respectively. C-index [95% CI] for Univariate models were: Fibrosis stage: 0.661 [0.521–0.777], Age >40: 0.645 [0.53–0.74], GGT >40: 0.641 [0.52–0.742], AST >100: 0.605 [0.467–0.72] and Forns: 0.702 [0.558–0.822].

Comparisons between multivariate models using C-Index were not significant (P > 0.4).

### Table 6 | Regression coefficients used to construct the scores and calculate the individual probability of cirrhosis

| Model | Time-point at follow-up | Constant (1) | Fibrosis stage at baseline | Age >40 years | GGT >40 IU | AST >100 IU |
|-------|------------------------|--------------|---------------------------|----------------|-----------|-------------|
|       |                        |              | F2–F3 vs. F0–F1 (0)       | F0–F1 (0) F2–F3 (1) | F0–F1 (0) F2–F3 (1) | F0–F1 (0) F2–F3 (1) | F0–F1 (0) F2–F3 (1) |
| I     | 5 years                | –6.535       | 1.744                     | 1.676          | 1.545     | 0.913       |
|       | 7 years                | –5.009       | 1.471                     | 1.222          | 1.159     | 0.645       |
|       | 10 years               | –4.554       | 1.000                     | 1.301          | 1.642     | 0.479       |
| II    | 5 years                | –6.188       | –                        | 2.037          | 1.668     | 1.108       |
|       | 7 years                | –4.807       | –                        | 1.546          | 1.304     | 0.780       |
|       | 10 years               | –4.430       | –                        | 1.559          | 1.697     | 0.477       |
| III   | 5 years                | –8.897       | 1.980                     | 0.803          | 0.954     |             |
|       | 7 years                | –6.351       | 1.566                     | 0.537          | 0.750     |             |
|       | 10 years               | –5.483       | 1.049                     | 0.542          | 0.648     |             |
DISCUSSION

IFN-based therapies are being replaced by all-oral combinations, at least in those areas of the world in which these molecules are approved and affordable. In this setting, the correct identification of patients at risk of progression to cirrhosis, as well as those with a very low likelihood of progression, is very relevant. However, data on the natural history of patients with mild CHC are scarce, since large cohorts and long follow-up periods are required to assess clinical outcomes.

Evaluation of liver fibrosis is crucial in the assessment of chronic liver disease. However, liver biopsy is an invasive procedure and its accuracy may be limited by sampling error and inter and intra-observer validation.15 Besides, liver biopsy cannot be repeated during follow-up to assess disease progression, as it may be associated with higher morbidity. In recent years, efforts have been made to develop non-invasive methods for fibrosis staging. Some of the surrogate markers of liver fibrosis have shown a good accuracy; however, data on the usefulness of such methods to predict clinical outcomes are scarce as most studies are transversal and others aim to predict liver-related events in patients who already present advanced liver disease.16
The main aim of our study was to develop and validate a simple model to accurately predict individual probabilities to develop liver cirrhosis. We retrospectively assessed a large number of patients mainly with mild stages of fibrosis at baseline, who underwent anti-viral therapy and were prospectively followed up for a median of 12 years. As expected, patients who achieved an SVR had excellent outcomes, with a very low incidence of cirrhosis (<1% at 10 years) and no cases of clinical decompensation. In accordance to previous data, the risk of HCC remained very low after SVR (two patients (0.33%), both with baseline bridging fibrosis). On the contrary, the cumulative probability of developing cirrhosis during follow-up was remarkably high in nonresponders (7% at 5 years and 22% at 10 years), reinforcing the need of tools to accurately predict the risk of fibrosis progression. The clinical variables associated with the development of liver cirrhosis were the presence of significant fibrosis at baseline, older age, and high AST and GGT levels, which have been previously shown to be related to fibrosis progression in patients with CHC.

According to the more recent international guidelines, all patients with chronic hepatitis C infection should be referred and considered for anti-viral therapy. The appropriate drug combination should be chosen depending on HCV genotype and subtype, the severity of liver disease and the availability of the different therapies in each country. IFN-containing regimens have globally been replaced by all-oral, IFN-free therapies with DAAs, at least in those areas of the world where these regimens are approved and their cost is covered. Nevertheless, the huge economical differences between countries make IFN-free therapies not affordable for all Health Care systems. In countries where the indication of DAAs may be restricted because of budget constraints, a careful selection of candidates at risk of disease progression is crucial.

Using baseline variables with an independent predictive value, we constructed three models to forecast individual probabilities for developing liver cirrhosis over time. Model I included AST, GGT, age and fibrosis stage, while model II was simplified by excluding the information provided by liver histology. Model III was generated by including well-known and validated non-invasive fibrosis scores. All three models were equally accurate at estimating the individual risk of progression to cirrhosis among nonresponders in the long-term. Moreover, the classification of nonresponder patients according to the associated baseline probability of developing cirrhosis obtained from the multivariate logistic model was discriminative in our sample throughout the follow-up period: the highest risk group had a probability of developing cirrhosis greater than 20% over 10 years, while the lowest risk group was associated with a very low probability of developing cirrhosis (<5%). The latter may be reassuring (both for doctors and patients) if these individuals are advised to defer therapy.

Figures for models I and III are almost identical as both take into account fibrosis stage either by histological assessment or by Forns index value (Figures 2 and 4). Model II, which is based only in three variables, was accurate at identifying those patients with a very lower risk of progression but did not discriminate between individuals with intermediate and high risk of cirrhosis development (Figure 3). Importantly, the assessment of the repro-
ducibility of the models in the validation cohort showed equally good results even in the long-term follow-up, thus strengthening the statistical power of the models.

Our study has several limitations. The first derives from the lack of paired biopsies in order to establish the diagnosis of cirrhosis, which would have provided further validation to our findings. Nevertheless, we believe that the established criteria for diagnosing liver cirrhosis were those used in routine clinical practice and are very accurate. For those patients in whom the diagnosis relied on US, and in order to exclude inter-observation bias, the US images were recovered and blindly re-assessed by expert radiologists to confirm the diagnosis of cirrhosis. Nonetheless, the fact that the same variables of the models were also associated with clinical decompensation or HCC (outcomes not subjected to inconsistencies) strengthens the criteria used to diagnose cirrhosis in this cohort.

A second limitation is the lack of a baseline transient elastography (TE), which is widely used in Europe to assess the fibrosis stage. However, the method was implemented in our Unit in 2005 and thus, the data was not available in most patients. If a baseline TE might improve the predictability of our current models needs to be assessed in future studies. Third, a selection bias cannot be excluded due to the retrospective nature of the study; our models would need further validation in a prospectively followed cohort.

Finally, in a few years from now and in a decreasing-cost scenario, anti-viral therapy might be offered to the patients even if they have a very low risk of cirrhosis after 10 years.

The strengths of our study are the large cohort of patients with a well-characterised disease and the fact that patients were followed in two large referral centres by an established protocol and the long follow-up period. An additional strength is that besides identifying patients at highest risk of progression of cirrhosis, our models are able to recognise those patients with a remarkably low risk of progression, thus facilitating clinical decisions in routine practice.

In conclusion, sustained virological response to interferon-based therapy in patients with noncirrhotic CHC eliminates liver-related complications in the long term. Among patients with chronic hepatitis C, modelling based on simple laboratory and clinical data is helpful at identifying the individual risks of progression to cirrhosis and could be used in clinical practice to better allocate patients in treatment protocols.

SUPPORTING INFORMATION
Additional Supporting Information may be found in the online version of this article:

Table S1. Variables independently related to the development of clinical decompensation or HCC by Cox-regression analysis in nonresponders.

Table S2A. Multivariate logistic regression and ROC analysis of factors associated with clinical decompensation or HCC development in nonresponder patients.

Table S2B. Regression coefficients used to construct the scores and calculate the individual probability of cirrhosis.

AUTHORSHIP
Guarantor of the article: S. Lens.
Author contributions: All authors have contributed in the study design, its critical revision for important intellectual content; have given final approval of the version to be published, and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the article. S Lens, F Torres and X Forns: analysis and interpretation of the data, drafting the manuscript.

All authors approved the final version of the manuscript.

ACKNOWLEDGEMENTS
Declaration of personal interests: XF has acted as an advisor for Janssen, Abbvie and Gilead and has received unrestricted grant support from Janssen and MSD. JNST has acted as an advisor for MSD. SL has acted as an advisor for Janssen, MSD and Gilead. ZM has acted as an advisor for BMS. ML has acted as an advisor for Janssen, MSD and BMS. RS has acted as an advisor for Janssen, MSD and BMS. JAC has acted as an advisor for Janssen and MSD. The authors declare no other potential personal conflicts of interest.

Declaration of funding interests: Stella Martinez was granted by Fundación Banco Bilbao Vizcaya Argentaria (BBVA). XF received support in part by grants from Instituto de Salud Carlos III (PI11/01907), Ministerio de Economía y Competitividad, co-funded by Fondo Europeo de Desarrollo Regional, Unión Europea, Una manera de hacer Europa. SL was supported by grants from Hospital Clinic and the Fundación BBVA. CIBEREHD is funded by the Instituto de Salud Carlos III.

REFERENCES
1. Manns MP, McHutchison JG, Gordon SC, et al. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. Lancet 2001; 358: 958–65.
2. Pawlotsky JM, Aghemo A, Dusheiko G, et al. EASL Recommendation on treatment of hepatitis C 2015. J Hepatol 2015; 63: 199–236.
3. Zeuzem S, Dusheiko GM, Salupere R, et al. Sofosbuvir and ribavirin in HCV
genotypes 2 and 3. N Engl J Med 2014; 370: 1993–2001.
4. Sulkowski MS, Gardiner DF, Rodriguez-Torres M, et al. Daclatasvir plus sofosbuvir for previously treated or untreated chronic HCV infection. N Engl J Med 2014; 370: 211–21.
5. Lawitz E, Poordad FF, Fang PS, et al. Sofosbuvir and ledipasvir fixed-dose combination with and without ribavirin in treatment-naive and previously treated patients with genotype 1 hepatitis C virus infection (LONESTAR): an open-label, randomised, phase 2 trial. Lancet 2013; 383: 515–23.
6. Lawitz E, Sulkowski MS, Ghalib R, et al. Simeprevir plus sofosbuvir, with or without ribavirin, to treat chronic infection with hepatitis C virus genotype 1 in non-responders to pegylated interferon and ribavirin and treatment-naive patients: the COSMOS randomised study. Lancet 2014; 384: 1756–65.
7. Andreone P, Colombo MG, Enejosa JV, et al. ART-450, ritonavir, ombramovir, and dasabuvir achieves 97% and 100% sustained virologic response with or without ribavirin in treatment-experienced patients with HCV genotype 1b infection. Gastroenterology 2014; 147: 359–65 e351.
8. Seeff LB. Natural history of chronic hepatitis C. Hepatology 2002; 36(Suppl. 1): S35–46.
9. Wiese M, Fischer J, Lobermann M, et al. Evaluation of liver disease progression in the German hepatitis C virus (1b)-contaminated anti-D cohort at 35 years after infection. Hepatology 2014; 59: 49–57.
10. van der Meer AJ, Hansen BE, Fattovich G, et al. Reliable prediction of clinical outcome in patients with chronic HCV infection and compensated advanced hepatic fibrosis: a validated model using objective and readily available clinical parameters. Gut 2014; 64: 322–31.
11. The French METAVIR Cooperative Study Group. Intraobserver and interobserver variations in liver biopsy interpretation in patients with chronic hepatitis C. Hepatology 1994; 20(1 Pt 1): 15–20.
12. Gaiani S, Gramantieri L, Venturoli N, et al. What is the criterion for differentiating chronic hepatitis from compensated cirrhosis? A prospective study comparing ultrasonography and percutaneous liver biopsy. J Hepatol 1997; 27: 979–85.
13. Aube C, Oberti F, Korali N, et al. Ultrasonographic diagnosis of hepatic fibrosis or cirrhosis. J Hepatol 1999; 30: 472–8.
14. Berzigotti A, Ashkenazi E, Reverter E, Abraides JG, Bosch J. Non-invasive diagnostic and prognostic evaluation of liver cirrhosis and portal hypertension. Dis Markers 2011; 31: 129–38.
15. Regev A, Berho M, Jeffer LJ, et al. Sampling error and intraobserver variation in liver biopsy in patients with chronic HCV infection. Am J Gastroenterol 2002; 97: 2614–8.
16. Boursier J, Brochard C, Bertras S, et al. Combination of blood tests for significant fibrosis and cirrhosis improves the assessment of liver-progression in chronic hepatitis C. Aliment Pharmacol Ther 2014; 40: 178–88.
17. Yamashita N, Ohno A, Yamasaki A, et al. Hepatocarcinogenesis in chronic hepatitis C patients achieving a sustained virological response to interferon: significance of lifelong periodic cancer screening for improving outcomes. J Gastroenterol 2014; 49: 1504–13.
18. Rutter K, Stattermayer AF, Beinhardt S, et al. Successful anti-viral treatment improves survival of patients with advanced liver disease due to chronic hepatitis C. Aliment Pharmacol Ther 2015; 41: 521–31.
19. Ghany MG, Lok AS, Everhart JF, et al. Predicting clinical and histologic outcomes based on standard laboratory tests in advanced chronic hepatitis C. Gastroenterology 2010; 138: 136–46.
20. Sheth SG, Flamm SL, Gordon FD, Chopra S. AST/ALT ratio predicts cirrhosis in patients with chronic hepatitis C virus infection. The American journal of gastroenterology 1998; 93: 44–8.
21. Giannini E, Botta F, Fasoli A, et al. Increased levels of gammaGT suggest the presence of bile duct lesions in patients with chronic hepatitis C: absence of influence of HCV genotype, HCV-RNA serum levels, and HGV infection on this histological damage. Dig Dis Sci 2001; 46: 524–9.
22. Younossi Z, Henry L. Systematic review: patient-reported outcomes in chronic hepatitis C—the impact of liver disease and new treatment regimens. Aliment Pharmacol Ther 2015; 41: 497–520.
23. Castaño L, Forns X, Alberti A. Non-invasive evaluation of liver fibrosis using transient elastography. J Hepatol 2008; 48: 835–47.
24. Vergniol J, Boursier J, Coutzac C, et al. Evolution of noninvasive tests of liver fibrosis is associated with prognosis in patients with chronic hepatitis C. Hepatology 2014; 60: 65–76.