Research Article

EASL EUROPEAN ASSOCIATION FOR THE STUDY OF THE LIVER | JOURNAL OF HEPATOLOGY

Slow regression of liver fibrosis presumed by repeated biomarkers after virological cure in patients with chronic hepatitis C

Thierry Poynard[1,*], Joseph Moussalli[1], Mona Munteanu[2], Dominique Thabut[1], Pascal Lebray[1], Marika Rudler[1], Yen Ngo[2], Vincent Thibault[1], Helmi Mkada[3], Frederic Charlotte[1], Françoise Imbert Bismut[3], Olivier Deckmyn[2], Yves Benhamou[1], Marc Antoine Valantin[4], Vlad Ratziu[1], Christine Katlama[4], and the FibroFrance-GHPS group

[1]*Assistance Publique Hôpitaux de Paris (AP-HP), University Pierre Marie Curie (UPMC), Liver Center, Paris, France;* [2]*BioPredictive, Paris, France;* [3]*Service de Biochimie Métabolique, Groupe Hospitalier Pitié-Salpêtrière (GHPS), AP-HP, Paris, France;* [4]*Infectious Disease Unit, GHPS, AP-HP, Paris, France*

Background & Aims: Chronic hepatitis C is both a virologic and fibrotic disease and complications can occur in patients with sustained virologic response (SVR) with residual fibrosis. Due to the limitations of repeated biopsies, no studies have assessed the dynamic of fibrosis before and after treatment. Using biopsy as reference, FibroTest™ has been validated as a biomarker of fibrosis progression and regression, with similar prognostic values. The aim was to estimate the impact of SVR on the dynamic of fibrosis presumed by FibroTest™.

Methods: In a prospective cohort, the main end point was the 10-year regression rate of fibrosis, defined as a minimum 0.20 decrease in FibroTest™, equivalent to one METAVIR stage.

Results: A total of 933 patients with both repeated FibroTest™ and transient elastography were included. At 10 years, among the 415 patients with baseline advanced fibrosis, 49% (95% CI 33–64%) of the 108 SVR had a regression, which was greater than in the 219 non-responders [23% (14–33%; p <0.001 *vs.* SVR)] and not lower than in the 88 non-treated [45% (10–80%; p = 0.39 *vs.* SVR)] patients. In all 171 SVR, cirrhosis regressed in 24/43 patients, but 15 new cirrhosis cases occurred out of 128 patients, that is only a net reduction of 5.3% [(24–15) = 9/171); (2.4–9.8%)]. Four cases of primary liver cancer occurred in SVR [4.6% (0–9.8)], and 13 in non-responders [5.6% (1.5–9.8); p = 0.07].

Conclusions: In patients with chronic hepatitis C, and as presumed by FibroTest™, virological cure was associated with slow regression of fibrosis 10 years later, a disappointing 5% decrease in cirrhosis cases, and a remaining 5% risk of primary liver cancer.

Keywords: Cirrhosis; FibroTest™; FibroSure; Elastography.
Received 31 March 2013; received in revised form 29 April 2013; accepted 8 May 2013; available online 24 May 2013
* Corresponding author. Address: 47–83 Boulevard de l'Hôpital, 75651 Paris, France. Tel.: +33 1 42 16 10 22.
E-mail address: tpoynard@teaser.fr (T. Poynard).

Introduction

Chronic hepatitis C (CHC) is both a virologic and fibrotic disease [1], with mortality resulting mainly from the complications of cirrhosis [2]. Therefore, a sustained virologic response (SVR) is considered the first step toward reducing future HCV mortality [3]. However, complications, such as primary liver cancer (PLC), can occur in patients who have recovered from the hepatitis C virus but continue to have residual fibrosis [4], even 13 years after SVR [5].

Due to the limitations of repeated biopsies, few studies have been done on the long-term outcomes of fibrosis using repeated biopsies in SVR [6–10] (Supplementary File 1). In 2002, we analyzed the larger study, including 1094 SVR with a 2-year interval between biopsies and discussed the need of non-invasive biomarkers for longer follow-up [8]. The study with longer mean interval between biopsies (4-year) included only 60 SVR with 12 advanced fibrosis [9].

Assessment of fibrosis dynamics can now be achieved through the validation of biomarkers such as FibroTest™ (FT) approved in France by health authorities in CHC as an alternative to liver biopsy for staging fibrosis [11,12]. Furthermore, FT was also validated *vs.* biopsy for assessing fibrosis progression rate (FPR) [7,13], fibrosis regression rate (FRR) [7,14], predicting mortality [15,16] and cost-effectiveness [17].

Since 1997, we have proposed the use of FT in all CHC patients followed in our hospital [17], including co-infected patients with HIV [19], and we set up a cohort (FIBROFRANCE-DOSVIRC) to estimate the dynamic of fibrosis. The specific goal of the present analysis was to estimate the impact of SVR on 10-year FR in comparison with non-responders (NR) and non-treated patients (NT).

Materials and methods

Cohort design

The details of this cohort were already published for first analyses on a subpopulation of 537 cases with baseline FT-biopsy permitting analyses of discordances [20] and validation of FT prognostic value [15]; details are described in Supplementary File 2.

Research Article

A specific questionnaire (200 items) was used at baseline and during follow-up for patient characteristics including factors associated with FP [21]. CHC and virological response were defined using standard diagnostic criteria: detectable serum HCV-RNA for more than 6 months. SVR was defined as undetectable HCV-RNA at the end of treatment and for at least 6 months afterwards. The cohort conformed to the ethical guidelines of the 1975 Declaration of Helsinki, was declared and approved by the French health authorities, and used an approved signed informed consent.

Specific criteria of inclusion and exclusion in the "Paired fibrosis estimates population"

Patients were included if they had a chronic hepatitis C, PCR positive, with at least 2 interpretable estimates of liver fibrosis (biopsy, FibroTest™ or FibroScan®) with at least a 6-month interval between 2 estimates. Patients with HIV co-infection were included as well as patients at risk of liver steatosis due to alcohol consumption or metabolic disorder.

Patients were excluded if they had other chronic liver diseases, including HBV-DNA PCR positive, a liver transplantation before the period of follow-up, spontaneous clearance of HCV-RNA, non-reliable or missing FT or TE, or less than a 6-month interval between fibrosis estimates.

All patients were followed in our clinic; we scheduled follow-up visits and non-invasive biomarker assessment at least every 2 years. Liver stiffness measurement by FibroScan® (TE) was introduced in 2005. Patients analyzed in the present study had a minimum of 2 interpretable estimates of liver fibrosis (biopsy, FT or TE) done at least 6 months apart. For cirrhotic patients, ultrasonography was scheduled every 6 months and endoscopy every 2 years.

Estimates of fibrosis

FT includes serum α_2-macroglobulin, apolipoprotein A1, haptoglobin, total bilirubin, and γ-glutamyl-transpeptidase, adjusted for age and gender. FT scores range from 0 to 1.00. The FT components were analyzed according to published recommendations [22].

TE was performed by experienced hepatologists according to published recommendations using the M probe of FibroScan® and results were expressed in kilopascals (kPa) [23].

Patients had liver biopsies done, mainly before 2007, and then after 2007 in case of discordance between FT and TE [11]. One experienced pathologist (FC), unaware of the biomarkers, evaluated the stage of fibrosis according to the METAVIR scoring system [24].

HCV-RNA was assessed by sensitive PCR methods with gradually improving sensitivities over time and ranging from 50 to 12 IU/ml.

Statistical analysis

FRR, the main end point, was defined as the percentage of patients who had achieved a significant decrease of fibrosis during follow-up. A significant FRR was defined as a minimum 0.20 FT decrease between the first and last FT measurement, equivalent to 1 METAVIR stage (or 1.53% area of fibrosis) [25]. FRR was assessed among patients with at least an advanced fibrosis (AdF) (stage F2, F3 or F4) at baseline, predetermined as an FT >0.48 [18].

The secondary end points focus on cirrhosis, the major source of complications. In patients without cirrhosis at baseline, we assessed the progression rate from non-cirrhotic stages to cirrhosis [F4PR (FT $\geqslant$0.74)] during follow-up. In patients with cirrhosis at inclusion, we assessed the rate of significant decrease of fibrosis (0.20 FT), which could be a significant surrogate marker of mortality and morbidity [15,16] and the cirrhosis regression rate [F4RR (FT <0.74 FT)]. The F4PR was also assessed from birth (or from date of infection when known) to baseline, which enabled to quantify the impact of SVR on the natural history of FP [13,26].

The following clinical end points were assessed to associate the FRR to morbidity and mortality: the 10-year overall survival (no death or liver transplantation) and survival without PLC and liver-related complications, using hospital and national mortality files (Supplementary File 2).

FRR was calculated from the date of the first FT to the date of the last FT. Clinical survival was calculated from the first FT to the date of death, transplantation or complications according to the circumstances. We used time-dependent methods to estimate the liver FR. The cumulative FRR at 10 years used the Kaplan Meir method and cumulative hazard function. Hazard function (HR) is the probability that a subject experiences the event of interest (in this case, regression or progression of fibrosis from one stage to a lower or higher stage) during a small time interval given that the individual has survived up to the beginning of the interval. Comparison used using log-rank test, and proportional hazard regression multivariate analysis [13,26]. To avoid overestimation of patient's effect, all cases contributed only to one group; NT were patients never treated during the whole follow-up; NR were patients who never achieved SVR. To take into account the timing of FT and dates of treatments, the interval between first FT and first treatment as well as between last treatment and last FT was included in multivariate analyses. The prognostic value of FT, TE and biopsy was assessed using the AUROC. Overall survivals were compared to survival expected in the French population, matched for age, sex, and follow-up period, as previously described [15]. Statistical analyses used Number Cruncher Statistical Systems software, Kaysville, Utah [27].

Results

Between September 1997 and June 2012, 1271 CHC were pre-included in the paired fibrosis estimates population. A total of 338 patients were excluded, mainly due to non-reliable or missing repeated FT/TE (Fig. 1). A total of 933 patients with paired FT and paired TE were included in the analysis; 171 (18%) were SVR, 424 (45%) NR, and 338 (36%) NT; 59% were male, 49 years of age, 68% Caucasians, and infected by genotype 1 in 62% of the cases. As presumed by FT, 415 (45%) patients had baseline AdF and 170 (18%) cirrhosis.

The main difference between included and non-included subjects was the prevalence of HIV-co-infection (25% *vs.* 9%; p <0.0001) (Table 1 and Supplementary File 3).

The median follow-up was 6.3 years for any pairs of fibrosis estimates (range 0.5–18.2 years), 5.3 for FT, 3.0 for TE, and 1.0 for biopsies. A mean of 4 HCV-RNA detections, all-negative, were performed per patient in the SVR group.

Regression and progression

The main end point was assessed in the 415 patients with AdF; 49% of the 108 SVR (95% CI, 33–64) had regression at 10 years, which was higher than in the 219 NR [23% (14–33; p <0.001 *vs.* SVR)] and not different than in the 88 NT [45% (10–80%; p = 0.39 *vs.* SVR)] (Fig. 2).

Dynamics of fibrosis (hazard function plots) according to baseline stage and virologic response to treatment are given in Fig. 3. As expected, the FRR (p <0.001) in all SVR (Fig. 3E) was only due to the regression in SVR patients with AdF (Fig. 3A). The progression in all patients (Fig. 3F) was mostly due to the progression of NT patients (p <0.001) with (Fig. 3B) or without (Fig. 3D) AdF. There was no significant difference between progression of SVR and NR (p = 0.07).

Factors associated with FR

The SVR status was associated with FRR in multivariate analysis, [Hazard rate (HR) = 4.94 (2.59–9.44), p = 0.0001]. All other factors [body mass index (BMI), metabolic factors, the severity of immunosuppression if HIV co-infection (NADIR CD4), and the maximum declared consumption during follow-up of alcohol, cannabis and tobacco] were not significantly associated with FR in univariate analysis or in multivariate analyses when the following factors were taken into account: age, gender, baseline FT value, and timing between FT and treatments (Table 2 and Supplementary File 4).

Progression to cirrhosis and regression in cirrhosis (Table 3)

In the 171 SVR, before baseline 43(25%) patients progressed to cirrhosis and this rate (HR = 0.19; 0.11–0.26) was not reduced