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Non-invasive fibrosis tests to predict complications in compensated post-hepatitis C cirrhosis

Short title: non-invasive fibrosis tests and cirrhosis

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Non-invasive fibrosis tests to predict complications in compensated post-hepatitis C cirrhosis

ABSTRACT

Background and Aims: Markers predicting complications of post-hepatitis C cirrhosis are needed. We asked whether changes in noninvasive markers of fibrosis can predict liver-related complications.

Methods: This was a case-controlled study using a prospective national cohort (ANRS-CO12-CIRVIR) of 1323 HCV-infected patients with compensated cirrhosis: 97 patients who developed liver-related complications such as hepatocellular carcinoma or hepatic decompensation (cases) matched in age, sex and follow-up duration were compared with 257 patients without complications (controls). Actitest/Fibrotest™, Inflameter/Fibrometer™, ELF™ and Fibroscan™ were performed at baseline and yearly. Samples based on Propensity score matching were built and mixed linear models performed. Outcomes in a sustained virological response (SVR) negative population and a SVR-positive population were also described.

Results: At baseline, all characteristics of patients were similar between the groups. All fibrosis tests were statistically higher for cases compared to controls, Fibroscan™ excepted: Fibrotest™: 0.83 ± 0.13 vs 0.77 ± 0.16 ; Fibrometer™: 0.93 ± 0.07 vs 0.90 ± 0.11 ; ELF™: 11.4 ± 1.0 vs 11.0 ± 1.2 ($P < 0.02$). The mean follow-up was 5.7 ± 1.9 years. Over a 3-year period, the significant difference in fibrosis marker values between cases and controls remained constant; with a trend toward a decrease in inflammation markers in controls, independent of SVR status.

Conclusions: Baseline noninvasive serum fibrosis and inflammation markers were significantly higher in patients developing a complication than in controls. During the follow-up only inflammatory markers decreased in controls, but not in cases, and thus could potentially be used to predict the occurrence of complications in cirrhotic patients.

Keywords: Noninvasive markers; hepatocellular carcinoma; HCV infection; hepatic decompensation.

Abbreviations

HCV	Hepatitis C Virus
HCC	hepatocellular carcinoma
ANRS	French agency for research on AIDS and viral hepatitis
MELD	Model of End Stage Liver Disease
LSM	noninvasive liver stiffness measurement "Fibroscan™"
US	Ultrasound
AFP	serum alpha-fetoprotein
AASLD	American Association for the Study of Liver Diseases
SVR	sustained virological response
AST	aspartate aminotransferase
ALT	alanine aminotransferase
GGT	gamma-glutamyl transferase
CRB	Liver Disease Biobank

INTRODUCTION

Chronic hepatitis C is an endemic disease affecting millions of individuals worldwide [1]. HCV infection is typically accompanied by no conspicuous symptoms but liver damage imperceptibly progresses and may result in cirrhosis over a couple of decades [1] with the risk of developing hepatocellular carcinoma (HCC) or hepatic decompensation [2,3]. Large prospective Western cohorts have shown a high incidence of HCC [4]; reaching close to 7% at 5 years in the French ANRS CO12 CirVir multicenter cohort [5]. However experience shows that not all cirrhotic patients have the same risk of developing HCC or decompensation suggesting that HCC screening for certain subsets of patients with cirrhosis may not be cost-effective [6]. Some scores such as the Child Pugh score or the Model of End Stage Liver Disease (MELD) score are correlated with a prognosis of advanced cirrhosis [7,8] and help physicians to manage cirrhotic patients. Several factors have been shown to be independently associated with a higher risk of HCC in cirrhotic patients, especially some host factors (age, sex, and diabetes), detectable HCV RNA, and disease factors including liver failure or portal hypertension [9]. The degree of liver fibrosis is also known to be important [10]. Until recently, this could only be assessed by liver biopsy, an invasive procedure with the possibility of life-threatening complications. However, liver biopsy does not satisfy quality criteria as a surrogate marker because of its complication rate, sampling error, intra- and inter-observer variability, expense and patient reluctance to undergo serial monitoring [11,12].

In the past decade, noninvasive markers of fibrosis have been developed as an alternative to liver biopsy for evaluating the severity of chronic liver disease. Fibrotest™ (Biopredictive, Paris, France), Fibrometer™ (Echosens, Paris, France) especially the latest 3G version, and ELF™ (Siemens, New York, USA) scores are the most common validated noninvasive blood tests for estimating the degree of liver fibrosis in chronic hepatitis C [13-16]. Liver stiffness measurement (LSM) by transient elastography (Fibroscan™ device, Echosens, France) is an alternative physical method to estimate the degree of liver fibrosis and has been validated in chronic hepatitis C. In addition, inflammation scores combined with fibrosis tests have been developed for Fibrotest and Fibrometer (Actitest and Inflameter respectively). Moreover, the correlation between noninvasive tests and fibrosis stage is at its best in cirrhotic patients [14-18].

The aim of this study was to evaluate the ability of any changes in blood test values and LSM as predictors of developing HCC or of hepatic decompensation (ascites, portal hypertension or encephalopathy) in a prospective cohort of patients with histologically proven Child-Pugh A post-hepatitis C cirrhosis.

PATIENTS AND METHODS

The study was sponsored and funded by the ANRS (France REcherche Nord & sud Sida-HIV Hépatites). The protocol was approved by a regional Ethics Committee (Comité de Protection des

Personnes, Aulnay-sous-Bois, France) and complied with the ethical guidelines of the 1975 Declaration of Helsinki. All patients gave their written informed consent to be included in the cohort.

Study design

Cases being rare; we conducted a nested case control study in the prospective French multicenter ANRS 012 CirVir cohort (CirVir). This cohort has been previously described [5,10-13,19-21]. The study case-control population was constituted between March 2006 and July 2012 from the 1822 patients who were enrolled in the CirVir cohort during this period. As case frequency is low (around 5%), this design allowed not only enough cases for the statistical analyses but also a sufficient number of blood tests on a realistically sized sample.

Considering the initial assumptions and taking into account the multiple comparisons (bilateral tests): 1 case to 1 or 2 controls, a power of 90% and an alpha risk of 0.001, the necessary number of subjects was 66 cases and 132 controls. Allowing for 15% of missing data, we would need to include 76 cases and 152 controls. This outcome was feasible with potential recruitment via the CirVir cohort. Considering the statistical analysis plan (creation of a sample issuing from propensity score matching and analysis using of 3 linear models) the power of the analysis remained above 80%.

Data source

Patients included in the CirVir cohort were recruited by 35 French clinical centers. Pre-inclusion assessments included clinical and biological parameters and an ultrasound (US) examination. The selection criteria were: a) age over 18 years; b) histologically-proven cirrhosis; c) serum positive for HBsAg or HCV antibodies; d) absence of previous complications of cirrhosis (particularly ascites, gastrointestinal hemorrhage or HCC); e) Child-Pugh class A; f) absence of severe uncontrolled extra-hepatic disease resulting in an estimated life expectancy of less than 1 year.

A total of 1,822 cirrhotic patients were included. Among them, 151 were subsequently excluded from analysis after review of individual data, due to either non-compliance with inclusion criteria (n=142) or consent withdrawal (n=9). Among the 1,671 cirrhotic patients, 1,323 had HCV-related compensated cirrhosis and constituted the final study population. For analysis, the reference date was 31 December 2015. At that date, the median duration of follow-up was 58.2 months [36.6; 79.0].

As previously described [5,10-13,19-21], patients were seen by physicians every 6 months. A US examination was performed every 6 months. In the event of a focal liver lesion detected by US, a diagnostic procedure was performed according to the 2005 AASLD guidelines [22] updated in 2011 [23]. Regular endoscopic surveillance was also performed. All events occurring during follow-up, liver-related or not, based on information obtained from the medical files of patients in each center, were also recorded [24]. All treatments, including antiviral therapy, were recorded at inclusion and any modification during follow-up was noted.

Antiviral treatment and viral replication

Since inclusions mainly took place before 2012 and the analyses of data were conducted in January 2016, most antiviral therapies conducted during follow-up were interferon-based. After 2011, genotype 1 patients could also receive either 12 weeks of telaprevir and boceprevir according to the European recommendations. In France, since February 2014, sofosbuvir-containing regimens have become progressively available for cirrhotic patients and are prescribed and reimbursed for all genotypes. The primary efficacy outcome was sustained virological response (SVR) at the end of a 12-week follow-up period without treatment [25].

In our study we chose to define SVR patients only as those with SVR at inclusion in the cohort. For the controls, we used the date of an event in the matching cases.

Case and control selection

Cases were defined as all individuals in the cohort developing a complication such as HCC or decompensation (ascites or gastrointestinal bleeding due to portal hypertension), or encephalopathy during the follow-up. The date of the first event was regarded as the index-date for each case. Controls were defined as all individuals in the cohort not developing a complication during the same period. Up to 4 controls (minimum: 1:2) were matched with each case based on age, sex, center and duration of follow-up. The date of inclusion in the cohort and in the study was the same. The main characteristics of patients (cases and controls) were recorded at inclusion in the cohort.

Moreover, to obtain proper comparability between cases and controls, a propensity score match was built, using a logistic regression based on age, sex, follow-up duration, platelet count and GGT level. A caliper distance of 0.1 was chosen to select a maximum of two neighbors in controls for every case.

Noninvasive markers

Sequential serum samples were collected by all 35 centers according to the CirVir protocol at the patient's inclusion in the cohort, then once annually, stored frozen and analyzed in a central laboratory (place). The following fibrosis parameters were calculated: Actitest/Fibrotest™, Inflammeter/Fibrometer™ (3G), and ELF™. All blood tests were performed blindly and independently of occurrence of complications. LSM was performed in each center by a senior operator. LSM was considered as reliable when 10 measurements were realized and the interquartile range among the measurements was less than 30% [15].

Covariates studied

The ANRS 012 CirVir cohort dataset contains the usual clinical and biochemical parameters at entry in the cohort and study (sex, age, body mass index [BMI], diabetes, arterial hypertension, dyslipidemia, metabolic syndrome, prothrombin time, serum bilirubin level, serum albumin level, platelet count, liver enzymes [aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma-glutamyltransferase (GGT), alpha-fetoprotein (AFP)], serum level, oesophageal varices, HCV genotype, HCV viral load, human immunodeficiency virus [HCV-HIV co-infection], histological lesions of steatohepatitis, and ultrasonography (US) at baseline. Missing biological data were determined using frozen serum samples provided by the Liver Disease Biobank (CRB - location of Biobank). For

all patients, past and present alcohol or tobacco consumption was quantified and recorded at inclusion. Excessive alcohol consumption was defined according to WHO criteria: more than 2 drinks/day for females and more than 3 drinks/day for males during at least 8 years. Past medical history was also recorded. In particular, the senior hepatologist at each center in charge of a given patient decided whether or not metabolic syndrome (met S) was present based on clinical guidelines and/or histological examination of the liver biopsy.

At each center patient information was recorded in a computer database by a clinical research associate specifically assigned to the ANRS CO12 CirVir cohort. Data quality was monitored through routine analysis of the entered data.

Statistical analysis

According to the STROBE guidelines [26], we have used well-suited methods for the analysis of this case control study. Indeed, conditional logistic regression, which takes into account matching, was used for descriptive analysis (baseline characteristics) with an alpha risk of 5% [27]. Multivariate analyses taking into account the time period and the non-independence of data (mixed model) were then performed.

All analyses were performed using STATA 13 (Stata Corp., College Station, TX, USA) and R version 3.3.0 package rpart.

RESULTS

Two hundred and nineteen patients (86 cases, 133 controls) were included in the sample after propensity score matching.

Baseline Characteristics

Baseline characteristics of patients at inclusion in the CirVir cohort are shown in Table 1. No difference was observed between cases and controls regarding the main risk factors: sex, age, antiviral response status, geographic origin, etiology of cirrhosis, interval between the diagnosis of hepatitis C and inclusion in the cohort, interval between the diagnosis of cirrhosis by liver biopsy and inclusion in the cohort, mode of contamination, presence of co-morbidities (diabetes, hypertension, dyslipidemia, alcohol consumption), past or present antiviral treatment, bilirubin concentration, GGT level, platelet count, prothrombin time, and albumin level. In our sample, the rate of positive SVR at baseline wasn't statistically different between the control and case groups: 21.5% and 10.5% respectively ($p=0.12$).

Follow-up of patients:

The overall number of adverse events was 124 including 58 HCC (47%), 2 cholangiocarcinoma (1.5%) and 38 ascites (30.5%), 16 digestive hemorrhages (13%), and 10 encephalopathies (8%), as well as comorbidities unrelated to the liver disease (particularly 28 irritable bowel syndrome).

Concerning occurrence of the first event; 86 patients had one or more events during follow-up: 7 patients (8.1%) developed a complication during the first year, 24 (27.9%) during the second year, 22 (25.6%) in the third year, 12 (14.0%) in the fourth year and 21 after the fourth year (24.4%). The most usual first event was HCC (54; 62.8%), ascites (24; 27.9%), and digestive hemorrhage (n=8; 9.3%). Figure 1 shows the cumulative probability of occurrence of a first event.

Whatever the non-invasive test, the AUROCs at baseline (M0) were 0.640 for ELF™, 0.643 for Fibrotest™, 0.634 for Fibrometer™, 0.629 for Actitest™, 0.614 for Inflammeter™ and 0.545 for LSM.

Changes in the values of noninvasive markers during the follow-up period are shown in Tables 2A, 2B (SVR negative population) and 2C (SVR positive population). In the overall population (Table 2A), all noninvasive biomarker values (ELF™, Fibrotest™, Actitest™, Inflammeter™ and Fibrometer™) were significantly higher in cases than in controls throughout the 3-year follow-up period ($p < 0.05$). These results were similar in the SVR negative population (Table 2B) except for Fibrometer™ and Inflammeter™ which lost significance ($p = 0.06$). For LSM no significant difference was observed between cases and controls at baseline and during the follow-up. Although trends were maintained in the SVR positive population (Table 2C) the small size of the sample precluded significant differences.

During the 3-year follow-up, while Actitest™ and Inflammeter™ decreased in controls and not in cases, the between-group differences were not significant.

DISCUSSION

In patients with post-hepatitis C cirrhosis, cohort studies conducted in Western countries have reported 5-year cumulative incidences of HCC at close to 17% and of liver decompensation at 10.8% in the absence of SVR [4]. The incidence of HCC in HCV patients is usually higher than that in HBV patients and occurs earlier than other complications, as reported by Sangiovanni et al. in 2006 [28]. Indeed, subsequent studies have shown that viral eradication is associated with a dramatic decrease in the incidence of HCC and also of decompensation [20,29-31]. Until recently, the prognostic markers validated for use in chronic hepatitis C have been the histological staging of fibrosis in biopsy specimens, clinical symptoms and blood tests, and the Child-Pugh score or MELD score for patients with cirrhosis, but rarely noninvasive tests of fibrosis [32-38]. Nevertheless, several studies have shown that among the latter both Fibrotest™ and LSM can predict survival and have better prognostic values than liver biopsy [17,37]. Fibrotest™ has a better performance than the FIB-4 and APRI [17] scores, but other biomarkers such as Fibrometer™ or ELF™ have not been evaluated. These tests have been essentially used to predict liver fibrosis severity and survival of patients with chronic hepatitis [17,37], but rarely to specifically predict the occurrence of HCC or the decompensation of cirrhosis [33,35]. Thus, the purpose of our study was to evaluate the prognostic value of the different blood tests (Fibrotest™, Actitest, Fibrometer™, Inflammeter™ and ELF™) in these indications. Indeed, we looked at whether it would be informative to annually repeat noninvasive tests for fibrosis over 3

years to determine if modifications in tests during follow-up provided any additional prognostic information, according to SVR status.

Our first finding was that at study entry, the performance of tests evaluated by AUROCs was poorer than that observed in retrospective and prospective studies of cirrhotic patients [14,16,18] without any statistical difference between them. This disparity could be due to differences in inclusion criteria, particularly the requirement for liver biopsy for all patients in some studies, and in our study the requirement for a Child-Pugh score, eliminating more severe cirrhotics. Noninvasive blood test levels were significantly higher in cases than in controls, whatever the test used and throughout the 3-year period. These results confirmed the relationship between high values in blood test results and the risk of complications in patients with post-hepatitis C cirrhosis [17]. For example, a Fibrotest™ score ≥ 0.85 or a Fibrometer™ result ≥ 0.90 at entry seems to be associated with a high risk of complications. These results were observed in the whole study population, including in non-SVR patients. For LSM the number of patients tested at baseline and during the follow-up period was inferior to those having blood tests and was probably too small to demonstrate any changes.

The second message was that the longitudinal study of noninvasive fibrosis markers during the 3 years of follow-up was uninformative regarding the risk of developing a complication. These results were observed in the whole study population and in both SVR negative and positive patients. In contrast, inflammatory markers decreased in the SVR positive population but the difference was not significant, probably due to the small size of this sub-population. We know that in patients with a SVR, the risk of developing a complication, especially HCC or hepatic decompensation, decreases [6,10,20,29-31] although it still persists [29-31]. Virological eradication is sometimes taken as evidence for an improvement in clinical symptoms [38], histological improvement in inflammation and a slow regression of fibrosis [39,40]. An earlier analysis, i.e., before 5 years of follow-up, would not have identified significant differences [39,40]. A reduction in histological inflammation or a decrease in activity are usually the first events occurring after the disappearance of the virus and come before any regression in fibrosis, which is usually observed a long time after viral eradication, and in less than 5% of cirrhotic patients. Indeed the risk of progression of fibrosis and the occurrence of “new-cirrhosis”, even in patients with SVR, exists [41]. Our results suggest that a 3-year follow-up visit is sufficient to detect any decrease in inflammation but is inadequate to detect any regression in fibrosis and patients must be followed up for longer than 3 years with the hope for changes in noninvasive fibrosis test values as reported by Poynard et al. [41] using Fibrotest™ and LSM (Fibroscan™). A recent study has suggested that a more pertinent combination to predict the absence of HCC occurrence could be albumin level plus LSM $< 10\text{kPa}$ at 1 year after viral eradication [42]. However markers of inflammation could be more relevant to precociously predict a risk of hepatocellular carcinoma in cirrhotic patients and could be used to follow this population after viral eradication, providing an alternative to repeated ultrasonography [43].

In conclusion, non-invasive inflammatory markers and not fibrosis tests seem be useful in the follow-up of patients with post-hepatitis C cirrhosis, especially for the early recognition of those at risk of developing HCC.

REFERENCES

- [1] Liang TJ, Rehermann B, Seef LB, Hoofnagle JH. Pathogenesis, natural history, treatment, and preventing of hepatitis C. *Ann Intern Med* 2000; 132: 296-305.
- [2] El Serag HB. Epidemiology of viral hepatitis and hepatocellular carcinoma. *Gastroenterology* 2012; 142: 1264-73.
- [3] Webster DP, Klenerman P, Dusheiko GM. Hepatitis C. *Lancet* 2015; 385: 1124-35.
- [4] Fattovich G, Stroffolini T, Zagni I, Donato F. Hepatocellular carcinoma in cirrhosis : incidence and risk factors. *Gastroenterology* 2004; 127: S35-S50
- [5] Trinchet JC, Bourcier C, Chaffaut C, Ait Ahmed M, Allah S, Marcellin P et al. Complications and competing risks of death in compensated viral cirrhosis (ANRS CO12 CirVir prospective cohort). *Hepatology* 2015; 62: 737-50.
- [6] Morgan RL, Baack B, Smith BD, Yartel A, Pitas M, Falck-Ytter Y. Eradication of hepatitis C virus infection and the development of hepatocellular carcinoma : a meta-analysis of observational studies. *Ann Intern Med* 2013; 158: 329-37.
- [7] Kamath PS, Kim WR. The model for end-stage liver disease (MELD). *Hepatology* 2007; 45: 757-805.
- [8] Pugh RN, Murray-Lyon IM, Dawson JL. Transsection of the oesophagus for bleeding oesophageal varices. *Br J Surg* 1973; 60: 646-9.
- [9] Ganne-Carrié N, Layese R, Bourcier V, Cagnot C, Marcellin P, Guyader D et al. Nomogram for individualized prediction of hepatocellular carcinoma occurrence in hepatitis C virus cirrhosis (ANRS CO 12 CirVir). *Hepatology* 2016; 64: 1136-47.
- [10] Yoshida H, Shiratori Y, Moriyama M, Anakawa , Ide T, Sata M et al. Interferon therapy reduces the risk for hepatocellular carcinoma: national surveillance progress of cirrhotic and non-cirrhotic patients with chronic hepatitis C in Japan. IHIT Study Group. Inhibition of hepatocarcinogenesis by Interferon therapy. *Ann Intern Med* 1999; 134: 174-81.
- [11] Bedossa P, Dargere D, Paradis V. Sampling variability of liver fibrosis in chronic hepatitis C. *Hepatology* 2003; 38: 1449-57.
- [12] Dienstag JL. The role of liver biopsy in chronic hepatitis C. *Hepatology* 2002; 36: S152-S160.
- [13] Cales P, Oberti F, Michalak S, Hubert-Fouchard I, Rousselet MC, Konate A et al. A novel panel of blood markers to assess the degree of liver fibrosis. *Hepatology* 2005; 42: 1373-81.
- [14] Castera L, Vergniol J, Foucher J, Le Bail B, Chanteloup E, Haaser M et al. Prospective comparison of transient elastography, Fibrotest, APRI, and liver biopsy for the assessment of fibrosis in chronic hepatitis C. *Gastroenterology* 2005; 128: 343-50.
- [15] Friedrich-Rust M, Rosenberg W, Parkes J, Hermann E, Zeuzem S, Sarrazin C. Comparison of ELF, Fibrotest and Fibroscan for the non-invasive assessment of liver fibrosis *BMC Gastroenterology* 2010; 10: 1-8.
- [16] Zarski JP, Sturm N, Guechot J, Paris A, Zafrani ES, Asselah T et al. Comparison of nine blood tests and transient elastography for liver fibrosis in chronic hepatitis C: the ANRS H CEP-23 study. *J Hepatol* 2012; 56 : 55-62

- [17] Vergniol J, Foucher J, Terrebonne E, Bernard PH, Le Bail B, Merrouche W et al. Noninvasive tests for fibrosis and liver stiffness predict 5-year outcomes of patients with chronic hepatitis C. *Gastroenterology* 2011; 140: 1970-9.
- [18] Foucher J, Chanteloup E, Vergniol J. Diagnosis of cirrhosis by transient elastography (FibroScan): a prospective study. *Gut* 2006; 55: 403-8.
- [19] Carrat F, Nahon P, Duclos-Vallée JC, Pageaux GP, Fontaine H, Pol S, ARNS Collaborative study group on hepatocellular carcinoma (ARNS C022 HEPATHER, C012 CirVir and C023 CUPILT cohorts. Reply to "A strong message is needed to address the issue of HCC recurrence after DAA therapy" *J Hepatol* 2016; 65: 1269-70.
- [20] Nahon P, Bourcier C, Layese R, Audureau E, Cagnot C, Marcellin P et al. Eradication of hepatitis C virus infection in patients with cirrhosis reduces risk of liver and non-liver complications. *Gastroenterology* 2017; 152 : 142-56.
- [21] Nahon P, Lescat M, Layese R, Bourcier V, Talmat N, Allah S et al. Bacterial infection in compensated viral cirrhosis impairs 5-years survival (ANRS C012, CirVir prospective cohort. *Gut* 2017; 66 : 330-41.
- [22] Bruix J Sherman M. Management of hepatocellular carcinoma. *Hepatology* 2005; 42 : 1208-36.
- [23] Bruix J, Sherman M. Management of hepatocellular carcinoma: an update. *Hepatology* 2011; 53 : 1020-2.
- [24] de Franchis R. Revising consensus in portal hypertension: report of the Baveno V consensus workshop on methodology of diagnosis and therapy in portal hypertension. *J Hepatol* 2010; 53: 762-82
- [25] Sarrazin C, Hezode CC, Zeuzem S. Antiviral strategies in hepatitis C virus infections. *J Hepatol* 2012;56 (Suppl 1):S88-S100
- [26] von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP for the STROBE Initiative. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement: Guidelines for Reporting Observational Studies. *Ann Intern Med.* 2007;147(8):573-577
- [27] Kleinbaum DG, Klein M. Analysis of Matched Data Using Logistic Regression. In: *Logistic Regression. Statistics for Biology and Health.* 2010 Springer, New York, NY
- [28] Sangiovanni A, Prati GM, Fasani D, Ronchi G, Romeo R, Manini M et al. The natural history of compensated cirrhosis due to hepatitis C virus: a 17-year cohort study of 214 patients. *Hepatology* 2006; 43: 1303-10.
- [29] Bruno S, Stroffoloni T, Colombo M, Bollani S, Benvegna L, Mazzella G et al. Sustained virological response to interferon-alfa is associated with improved outcome in HCV-related cirrhosis: a retrospective study. *Hepatology* 2007; 45: 578-87.
- [30] Cardoso AC, Moucari R, Figueiredo-Mendes C. Impact of peg interferon and ribavirin therapy on hepatocellular carcinoma: incidence and survival in hepatitis C patients with advanced fibrosis. *J Hepatol* 2010; 52: 652-7.
- [31] Van Der Meer AJ, Veldt BJ, Feld JJ, Wedemeyer H, Dufour JF, Lammert F et al. Association between sustained virological response and all-cause mortality among patients with chronic hepatitis C and advanced hepatic fibrosis. *JAMA* 2012; 308: 2584-93.

- [32] Chang KC, Wuy Y, Hung CH, Lu SN, Lee CM, Chiu KW et al. Clinical-guide risk prediction of hepatocellular carcinoma development in chronic hepatitis C patients after interferon-based therapy. *Br J Cancer* 2013; 109: 2481-8.
- [33] Lee K, Sinn DH, Gwak GY, Cho HC, Jung SH, Paik YH et al. Prediction of the risk of hepatocellular carcinoma in chronic hepatitis C patients after sustained virological response by aspartate aminotransferase to platelet ratio index. *Gut Liver* 2016; 10: 796-802.
- [34] Nunes D, Fleming C, Offner G. Non invasive markers of liver fibrosis are highly predictive of liver-related deaths in a cohort of HCV-infected individuals with and without HIV infection. *Am J Gastroenterol* 2010; 105: 346-53.
- [35] Wu CK, Chan KC, Hung CH, Tsen PL, Lu SN, Chen H, et al. Dynamic alfa-foetoprotein, platelets and AST-to-platelet ratio index predict hepatocellular carcinoma in chronic hepatitis C patients with sustained virological response after antiviral therapy. *J Antimicrob Chemother* 2016; 71: 1943-7.
- [36] Zeng QL, Li B, Zhang XX, Chen Y, Fu YL, Lu J et al. Clinical model for predicting hepatocellular carcinomas in patients with post-sustained virological responses of chronic hepatitis C. A case control study. *Gut Liver* 2016; 10: 955-61.
- [37] Ngo Y, Munteanu M, Messous D, Charlotte F, Imbert-Bismuth F, Thabut D, et al. A prospective analysis of the prognostic value of biomarkers (Fibrotest™) in patients with chronic hepatitis C. *Clin. Chem* 2006; 52: 1887-96.
- [38] Ellis EL, Mann DA. Clinical evidence for the regression of liver fibrosis. *J Hepatol* 2012; 56: 1171-80
- [39] Poynard T, Moussali J, Munteanu M, Thabut D, Lebray P, Rudler M et al. Slow regression of liver fibrosis presumed by repeated biomarkers after virological cure in patients with chronic hepatitis C. *J Hepatol* 2013; 59 : 675-83.
- [40] George SL, Bacon BR, Brunt EM, Mihindukulasuriya KL, Hoffman J, Di Bisceglie AM. Clinical, virologic, histologic, and biochemical outcomes after successful HCV therapy: a 5-year follow-up of 150 patients. *Hepatology* 2009; 49: 729-38.
- [41] Poynard T, McHutchison J, Manns M, Trepo C, Lindsay K, Goodman Z et al. Impact of pegylated interferon alfa-2b on liver fibrosis in patients with chronic hepatitis C. *Gastroenterology* 2002;122:1303-13.
- [42] Pons M, Rodriguez-Tajes S, Esteban JI, Marino Z, Vargas V, Lens S et al. Non-invasive prediction of liver related events in HCV compensated advanced chronic liver disease patients after oral antivirals. *J Hepatol* 2019; *in press* DOI: 10.1016/j.jhep.2019.10.005
- [43] Trinchet JC, Chaffaut C, Bourcier V, Degos F, Henrion J, Fontaine H et al. Ultrasonography surveillance of hepatocellular carcinoma in cirrhosis. A randomized trial comparing 3 and 6-month periodicities. *Hepatology* 2011; 54: 198-207

Table 1: Characteristics of patients (controls and cases) at entry in the study with matching on age, sex, follow-up duration, platelet count and GGT.

Characteristics of patients at inclusion (D0)	Controls (N=133)	Cases (N=86)	p-value
Age (years) m \pm 1 SD	58.1 \pm 9.5	57.3 \pm 9.6	NS
Sex (Man, %)	63.9%	65.1%	NS
Geographic origin (Africa-Asia / Europe)	18.3 / 81.7	24.0 / 76.0	NS
Delay between HCV infection and inclusion in CIRVIR (m \pm 1 SD)	9.3 y \pm 6.2	10.1 y \pm 6.1	NS
Mode of contamination (%):			
Transfusion	30.8	34.9	
IVD abuse	26.3	19.8	NS
Other	23.3	27.9	
Unknown	19.6	17.4	
Diabetes (%)	21.1	22.5	NS
HTA (%)	24.8	30.2	NS
Dyslipidemia (%)	4.5	4.7	NS
Past alcohol consumption (%)	26.8	29.4	NS
Past or present antiviral treatment (%)	97.0	96.5	NS
Bilirubin (μ mol/L)	14.2 \pm 8.3	16.7 \pm 8.1	NS
Platelet count (giga/L)	132.7 \pm 54.5	120.0 \pm 58.5	NS
GGT (UI/L)	148.1 \pm 98.0	152.9 \pm 121.5	NS
PT (%) m \pm 1 SD	87.4% \pm 12.1	83.9% \pm 11.0	NS
Creatinine (μ mol/L)	73.0 \pm 15.1	74.3 \pm 17.6	NS
Positive RVS (%)	21.5	10.5	NS
Albumin (g/L)	41.6 \pm 4.1	40.4 \pm 4.4	NS

m: mean +/- one standard deviation*

Table 2.A: mean values of all noninvasive fibrosis tests in cases and controls at Day (D) 0 and during the follow-up (3 years) with matching on age, sex, follow-up duration, platelet count and GGT.

		D0 Mean ± 1 SD (N)	Year1 Mean ± 1 SD (N)	Year2 Mean ± 1 SD (N)	Year3 Mean ± 1 SD (N)	p-value case vs control *
ELF™	Controls*	11.0±1.2 (126)	10.8±1.3 (92)	10.7±1.2 (87)	10.6±1.2 (68)	0.02
	Cases	11.4±1.0 (81)	11.4±1.3 (66)	11.7±1.4 (58)	11.5±1.2 (48)	
Fibrotest™	Controls*	0.77±0.16 (133)	0.77±0.16 (94)	0.76±0.17 (88)	0.76±0.17 (69)	0.01
	Cases	0.83±0.13 (86)	0.85±0.13 (67)	0.85±0.13 (61)	0.85±0.12 (48)	
Fibrometer™ cirr	Controls*	0.90±0.11 (132)	0.88±0.13 (83)	0.86±0.14 (74)	0.86±0.15 (58)	0.01
	Cases	0.93±0.07 (85)	0.93±0.10 (53)	0.94±0.06 (53)	0.93±0.08 (37)	
Actitest™	Controls*	0.55±0.27 (133)	0.51±0.28 (94)	0.49±0.28 (88)	0.47±0.28 (69)	0.02
	Cases	0.64±0.22 (86)	0.63±0.24 (67)	0.60±0.23 (61)	0.59±0.26 (48)	
Fibrometer™ inf	Controls*	0.73±0.16 (132)	0.72±0.17 (83)	0.69±0.19 (74)	0.70±0.17 (58)	0.03
	Cases	0.78±0.12 (85)	0.77±0.13 (53)	0.77±0.13 (53)	0.75±0.13 (37)	
ASAT	Controls*	73.7±47.7 (133)	80.5±108.1 (96)	72.0±61.2 (88)	63.0±45.5 (69)	0.20
	Cases	85.1±47.6 (86)	90.1±55.4 (67)	87.3±57.9 (61)	93.6±71.0 (48)	
Fibroscan™**	Controls*	20.4±12.5 (64)	19.8±12.8 (34)	20.0±11.9 (27)		0.63
	Cases	22.9±11.6 (41)	23.4±11.4 (25)	21.8±12.4 (19)		

* Mixed linear model adjusted on time and interaction with case/control group for fixed effects, and on individuals for random effect.

**Not all patients had noninvasive liver stiffness measurement(s) (LSM) by transient elastography: Fibroscan™

Table 2.B: mean values of all noninvasive fibrosis tests in cases and controls at Day (D) 0 and during the follow-up (3 years) with matching on age, sex, follow-up duration, platelet count and GGT. SVR negative exclusively.

		D0 Mean ± 1 SD (N)	Year1 Mean ± 1 SD (N)	Year2 Mean ± 1 SD (N)	Year3 Mean ± 1 SD (N)	p-value case vs control *
ELF™	Controls*	11.1±1.1 (96)	11.1±1.2 (70)	11.0±1.1 (66)	11.0±1.0 (50)	0.02
	Cases	11.5±1.0 (72)	11.6±1.2 (60)	11.9±1.4 (51)	11.7±1.2 (42)	
Fibrotest™	Controls*	0.80±0.14 (102)	0.80±0.15 (71)	0.81±0.14 (68)	0.82±0.12 (51)	0.03
	Cases	0.84±0.12 (77)	0.86±0.12 (61)	0.87±0.12 (54)	0.86±0.12 (42)	
Fibrometer™ cirr	Controls*	0.91±0.10 (102)	0.91±0.10 (62)	0.89±0.11 (56)	0.92±0.09 (42)	0.06
	Cases	0.93±0.07 (77)	0.93±0.11 (48)	0.94±0.06 (48)	0.93±0.08 (31)	
Actitest™	Controls*	0.59±0.25 (102)	0.55±0.26 (71)	0.56±0.26 (68)	0.57±0.25 (51)	0.04
	Cases	0.66±0.22 (77)	0.64±0.23 (61)	0.63±0.22 (54)	0.62±0.25 (42)	
Fibrometer™ inf	Controls*	0.74±0.16 (102)	0.74±0.16 (62)	0.73±0.18 (56)	0.75±0.14 (42)	0.06
	Cases	0.78±0.12 (77)	0.78±0.13 (48)	0.77±0.13 (48)	0.76±0.13 (31)	
ASAT	Controls*	78.1±48.6 (102)	89.8±119.9 (73)	82.4±63.9 (67)	75.1±47.2 (51)	0.27
	Cases	89.5±48.2 (77)	94.3±55.8 (61)	93.3±58.7 (54)	99.9±73.4 (42)	
Fibroscan™**	Controls*	20.9±11.5 (42)	20.3±13.1 (27)	21.1±11.8 (21)		0.53
	Cases	23.1±12.1 (38)	24.4±11.3 (23)	22.5±12.4 (18)		

* Mixed linear model adjusted on time and interaction with case/control group for fixed effects, and on individuals for random effect.

**Not all patients had noninvasive liver stiffness measurement(s) (LSM) by transient elastography: Fibroscan™

Table 2.C: mean values of all noninvasive fibrosis tests in cases and controls at Day (D) 0 and during the follow-up (3 years) with matching on age, sex, follow-up duration, platelet count and GGT. SVR positive exclusively.

		D0 Mean ± 1 SD (N)	Year1 Mean ± 1 SD (N)	Year2 Mean ± 1 SD (N)	Year3 Mean ± 1 SD (N)	p-value case vs control
ELF™	Controls*	10.5±1.5 (27)	10.0±1.2 (21)	9.7±0.9 (19)	9.5±1.1 (18)	0.93
	Cases	10.5±1.0 (9)	10.0±0.9 (6)	10.1±0.3 (7)	10.1±0.6 (6)	
Fibrotest™	Controls*	0.67±0.17 (28)	0.66±0.18 (21)	0.60±0.20 (18)	0.61±0.19 (18)	0.22
	Cases	0.75±0.16 (9)	0.75±0.15 (6)	0.74±0.11 (7)	0.73±0.12 (6)	
Fibrometer™ cirr	Controls*	0.85±0.14 (27)	0.80±0.17 (19)	0.75±0.15 (17)	0.70±0.17 (16)	0.09
	Cases	0.94±0.03 (8)	0.96±0.03 (5)	0.95±0.05 (5)	0.93±0.06 (6)	
Actitest™	Controls*	0.43±0.31 (28)	0.33±0.26 (21)	0.22±0.19 (18)	0.20±0.14 (18)	0.78
	Cases	0.45±0.16 (9)	0.46±0.24 (6)	0.33±0.10 (7)	0.36±0.14 (6)	
Fibrometer™ inf	Controls*	0.69±0.19 (27)	0.62±0.19 (19)	0.57±0.17 (17)	0.56±0.16 (16)	0.40
	Cases	0.75±0.10 (8)	0.76±0.10 (5)	0.75±0.12 (5)	0.71±0.12 (6)	
ASAT	Controls*	57.4±41.8 (37)	44.3±33.9 (21)	30.3±14.1 (19)	28.7±6.9 (18)	0.36
	Cases	47.3±15.2 (9)	47.8±27.3 (6)	41.3±17.9 (7)	49.7±24.2 (6)	
Fibroscan™	Controls*	18.7±15.0 (20)	18.3±12.2 (7)	16.0±12.3 (6)		0.94
	Cases	19.8±0.9 (3)	12.0±2.8 (2)	9.1 (1)		

* Mixed linear model adjusted on time and interaction with case/control group for fixed effects, and on individuals for random effect.

**Not all patients had noninvasive liver stiffness measurement(s) (LSM) by transient elastography: Fibroscan™.

Figure Legend**Figure 1. Occurrence of events during follow-up with a 95%CI**

