

Liver stiffness diminishes with antiviral response in chronic hepatitis C.

Christophe Hézode, Laurent Castéra, Françoise Roudot-Thoraval, Magali Bouvier-Alias, Isabelle Rosa, Dominique Roulot, Vincent Leroy, Ariane Mallat, Jean-Michel Pawlotsky

► **To cite this version:**

Christophe Hézode, Laurent Castéra, Françoise Roudot-Thoraval, Magali Bouvier-Alias, Isabelle Rosa, et al.. Liver stiffness diminishes with antiviral response in chronic hepatitis C.: Liver Stiffness Kinetics on HCV Therapy. *Alimentary Pharmacology and Therapeutics*, Wiley, 2011, 34 (6), pp.656-663. <10.1111/j.1365-2036.2011.04765.x>. <inserm-00623261>

HAL Id: inserm-00623261

<http://www.hal.inserm.fr/inserm-00623261>

Submitted on 13 Sep 2011

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

**Liver Stiffness Diminishes with Antiviral Response in
Chronic Hepatitis C**

Journal:	<i>Alimentary Pharmacology & Therapeutics</i>
Manuscript ID:	APT-0306-2011.R2
Wiley - Manuscript type:	Original Scientific Paper
Date Submitted by the Author:	n/a
Complete List of Authors:	Hezode, Christophe Castéra, Laurent Roudot-Thoraval, Françoise Bouvier-Alias, Magali Rosa, Isabelle ROULOT, Dominique; Hopital jean verdier, Liver unit Leroy, Vincent Mallat, Ariane Pawlotsky, Jean-Michel; Hopital Henri Mondor - Université Paris XII, Virology
Keywords:	Hepatology, Hepatitis C < Hepatology, Liver fibrosis < Hepatology, Viral hepatitis < Hepatology

Liver Stiffness Diminishes with Antiviral Response in Chronic Hepatitis C

Christophe Hézode,^{1,2*} Laurent Castéra,^{3*} Françoise Roudot-Thoraval,^{2,4} Magali Bouvier-
Alias,^{2,5} Isabelle Rosa,⁶ Dominique Roulot,⁷ Vincent Leroy,⁸ Ariane Mallat,^{1,2}
and Jean-Michel Pawlotsky^{2,5}

¹Department of Hepatology and Gastroenterology, Hôpital Henri Mondor, Université Paris-Est, Créteil, France; ²INSERM U955, Créteil, France; ³Department of Hepatology and Gastroenterology; Hôpital Haut-Lévêque et Hôpital Saint-André, Université Victor Segalen Bordeaux II, Bordeaux, France; ⁴Department of Public Health, Hôpital Henri Mondor, Université Paris-Est, Créteil; ⁵National Reference Center for Viral Hepatitis B, C and Delta, Department of Virology, Hôpital Henri Mondor, Université Paris-Est, Créteil, France; ⁶Department of Hepatology and Gastroenterology, Centre Hospitalier Intercommunal, Créteil, France; ⁷Department of Hepatology and Gastroenterology, Hôpital Avicenne, Université Paris 13, Bobigny, France; ⁸Department of Hepatology and Gastroenterology, Hôpital de la Tronche, University of Grenoble, Grenoble, France

*These 2 authors equally contributed to the work

RUNNING HEAD: Liver Stiffness Kinetics on HCV Therapy

Corresponding author: Prof. Jean-Michel Pawlotsky, MD, PhD, Department of Virology, Hôpital Henri Mondor, 51 avenue du Maréchal de Lattre de Tassigny, 94010 Créteil, France. Tel: +33-1-4981-2827; Fax: +33-1-4981-4831. E-mail: jean-michel.pawlotsky@hmn.aphp.fr

ABSTRACT

Background: Transient elastography measures liver stiffness, which correlates with the hepatic fibrosis stage and has excellent accuracy for the diagnosis of cirrhosis in patients with chronic hepatitis C.

Aim: To prospectively assess the kinetics of liver stiffness in treated patients with chronic hepatitis C and compare them with the viral kinetics on treatment and with the final outcome of therapy.

Methods: 91 patients with chronic hepatitis C with significant fibrosis (>7.0 kPa) at baseline were included. They received therapy with pegylated interferon- α and ribavirin. The kinetics of liver stiffness were characterized during therapy and thereafter by means of Fibroscan[®], and compared with the virological responses at weeks 4, 12, 24, end of treatment and 12 and 24 weeks after.

Results: A significant liver stiffness decrease was observed during therapy, which continued after treatment only in patients who achieved a sustained virological response. In this group, the median intra-patient decrease relative to baseline at the end of follow-up was -3.4 kPa, vs -1.8 kPa in the patients who did not achieve an SVR. Similar dynamics were observed in cirrhotic and non-cirrhotic patients. In multivariate analysis, only the SVR was associated with long-term improvement of liver stiffness (odds ratio: 3.10; 95% confidence interval: 1.20-8.02, $p=0.019$).

Conclusions: In patients with advanced fibrosis at the start of therapy, liver stiffness is significantly reduced during treatment, but improvement continues off treatment only in patients who achieve a sustained virological response. Liver stiffness

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

assessment earlier than 6 months after the end of therapy does not appear to be clinically meaningful.

For Peer Review

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52

Chronic infection with hepatitis C virus (HCV) is the leading cause of chronic liver disease in Europe and the United States. Chronic hepatitis C is responsible for substantial morbidity and mortality related to liver cirrhosis and its complications, including hepatocellular carcinoma.¹ Nowadays, HCV is becoming the first cause of primary liver cancer and is the main indication for liver transplantation in industrialized countries.¹ The current standard treatment of chronic hepatitis C is a combination of pegylated interferon (IFN)- α and ribavirin.² With this treatment, a sustained virological response, defined as an undetectable HCV RNA 24 weeks after the end of therapy, is achieved in approximately 40%-50% of patients infected with HCV genotype 1 and in 80% of those infected with genotypes 2 and 3.³⁻⁵

31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52

The hepatic fibrosis stage is the principal predictor of liver disease progression and drives treatment indications.² Liver biopsy examination has traditionally been considered the reference method for staging liver fibrosis. It is recommended in recent Clinical Practice Guidelines for treatment decision in patients with chronic hepatitis C.² However, the accuracy of liver biopsy has been questioned, as sampling errors and intra- and inter-observer variability may lead to under- or over-staging.⁶⁻⁹ In addition, liver biopsy is an invasive procedure with rare, but potentially life-threatening, complications.¹⁰⁻¹³ These limitations have stimulated the search for noninvasive approaches for liver disease severity assessment, including serological markers and methods based on ultrasonography, such as transient elastography.¹⁴⁻¹⁷

53
54
55
56
57
58
59
60

Transient elastography by means of the Fibroscan[®] (Echosens, Paris, France) measures liver stiffness. It can be performed at the bedside with immediate results and

1
2
3 has been reported to be rapid, user-friendly and reproducible.¹⁸ Liver stiffness has been
4
5 shown to correlate with the hepatic fibrosis stage and to have excellent accuracy for the
6
7 diagnosis of cirrhosis in patients with chronic hepatitis C.¹⁹⁻²² Thus far, transient
8
9 elastography has been used and validated essentially in cross-sectional studies,
10
11 whereas the kinetics of liver stiffness in patients with chronic hepatitis C receiving
12
13 antiviral therapy have not been characterized in prospective longitudinal studies.
14
15

16
17 The goal of this study was to prospectively assess the kinetics of liver stiffness in
18
19 patients with chronic hepatitis C treated with pegylated IFN- α and ribavirin and to
20
21 compare them with the HCV RNA level kinetics on treatment and with the final outcome
22
23 of therapy.
24
25
26
27
28

29 **PATIENTS AND METHODS**

30 31 32 33 34 **Patients**

35
36 This multicentre, prospective study was conducted in 5 French hospitals between
37
38 January 2005 and March 2007. One hundred and five patients with chronic hepatitis C,
39
40 i.e. patients with chronically elevated serum alanineaminotransferase (ALT) levels and
41
42 detectable serum anti-HCV antibodies and HCV RNA, were included. All of them were
43
44 treatment-naïve and the main inclusion criterion was significant fibrosis, defined as liver
45
46 stiffness >7.0 kilopascals (kPa) with the Fibroscan[®] at baseline.²⁰ The exclusion criteria
47
48 were: a coinfection with hepatitis B virus or human immunodeficiency virus, a daily
49
50 alcohol intake >30 g, decompensated liver disease or hepatocellular carcinoma, liver
51
52 transplantation, and a failed or unreliable liver stiffness measurement.²³
53
54
55
56
57
58
59
60

1
2
3 The study protocol conformed to the ethical guidelines of the 1975 Helsinki
4 declaration and the French regulations on clinical trials, and was approved by the
5 Institutional Review Board (Comité de Protection des Personnes d'Ile-de-France IX). The
6 patients were enrolled after giving their written informed consent.
7
8
9
10
11
12
13

14 **Antiviral therapy**

15
16
17 The 105 patients received standard-of-care therapy with either pegylated IFN- α 2a
18 (Pegasys[®], Hoffman-La Roche, Basel, Switzerland), 180 μ g once weekly, or pegylated
19 IFN- α 2b (PegIntron[®], Schering-Plough, Kenilworth, New Jersey), 1.5 μ g/kg once weekly,
20 and ribavirin, 1.0-1.2 g/day according to body weight below or above 75 kg, respectively,
21 in patients infected with genotypes 1 and 4, or 0.8 g/day in patients infected with HCV
22 genotypes 2 and 3. Treatment duration was 48 weeks in patients infected with HCV
23 genotypes 1 and 4 (with a stopping rule at week 12 if the HCV RNA decline was less
24 than 2 Log₁₀ international units (IU)/mL), and 24 weeks in patients infected with HCV
25 genotypes 2 and 3.
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42

43 **Monitoring schedule and definition of virological responses**

44 ALT levels, HCV RNA levels and liver stiffness were evaluated at baseline, at
45 weeks 4, 12, and 24 of therapy in all patients, at week 48 of therapy in patients infected
46 with HCV genotypes 1 and 4, and 12 and 24 weeks after treatment withdrawal in all
47 patients. On treatment, virological responses were defined as follows: rapid virological
48 response (RVR): undetectable HCV RNA at week 4; early virological response (EVR):
49 undetectable HCV RNA at week 12; end-of-treatment response (EOTR): undetectable
50
51
52
53
54
55
56
57
58
59
60

1
2
3 HCV RNA at the end of treatment, i.e. week 24 or 48 in patients infected with HCV
4
5 genotypes 2-3 and 1-4, respectively. The sustained virological response (SVR) was
6
7 defined as an undetectable HCV RNA 24 weeks after the end of therapy.
8
9

10 11 12 **HCV RNA level measurement**

13
14
15 HCV RNA levels were measured centrally by means of the *m2000_{SP}/m2000_{RT}*
16
17 real-time PCR platform (Abbott Molecular, Des Plaines, Illinois), according to the
18
19 manufacturer's instructions. The assay has been shown to accurately quantify HCV RNA
20
21 levels regardless of the HCV genotype.²⁴ Its lower limit of detection is 12 IU/mL.
22
23 Undetectable HCV RNA at the different time points was thus defined as an HCV RNA
24
25 level <12 IU/mL.
26
27
28
29
30

31 32 **Liver stiffness measurement**

33
34 Liver stiffness measurements were performed with the FibroScan® device, as
35
36 previously described.¹⁴ Ten validated measurements were performed for each patient.
37
38 The success rate was calculated as the number of validated measurements divided by
39
40 the total number of measurements. The results were expressed in kilopascals. The
41
42 median value was considered representative of the elastic modulus of the liver. Only
43
44 procedures with at least ten successful acquisitions, a success rate of at least 60% and
45
46 an interquartile range (IQR) of less than 30% of the median value were considered
47
48 reliable.¹⁸ Patients with baseline liver stiffness values above 13 kPa were considered to
49
50 have cirrhosis.²¹ Long-term improvement of liver stiffness was defined as a decrease of
51
52 at least 30% of median values 6 months after treatment withdrawal relative to baseline,
53
54 as the manufacturer allows fluctuations of 30% of the IQR relative to the median value.¹⁸
55
56
57
58
59
60

Statistical analysis

Results are expressed as the mean \pm 1 standard deviation for normally distributed variables (age, body mass index [BMI]) and as the median with IQR (1st and 3rd quartiles) for variables with asymmetric distribution (ALT, HCV RNA level and liver stiffness). Intra-group comparisons were made using Wilcoxon's test for paired data. Categorical data were expressed as numbers and percentages, and compared by means of the Chi-square test or Fisher's exact test where appropriate. The relationship between quantitative data was tested by linear regression analysis.

The factors associated with long-term improvement of liver stiffness were tested by univariate analysis (chi-square test, Mann and Whitney test). Variables with a p value ≤ 0.10 were then tested in a logistic regression model by a forward step-by-step procedure. Odds ratios (OR) and their 95 % confidence intervals (CI) were inferred from the model. A p value ≤ 0.05 was considered significant.

RESULTS

Patient characteristics and disposition

Among the 105 patients prospectively included in the study, 8 were lost to follow-up and an additional 6 patients did not complete follow-up (more than 2 missing time points) and were excluded from the analysis. These 14 patients did not differ from the other treated patients for age, gender, BMI, frequency of cirrhosis, HCV genotype, and baseline HCV RNA level, ALT level, and liver stiffness.

1
2
3 Overall, 91 treated patients were analyzed. There were 63 men and 28 women,
4
5 and their mean age was 52.4 ± 11.6 years. Their characteristics are shown in Table 1.
6
7 Among the 91 patients, 2 were treated for 12 weeks only (one patient who did not
8
9 achieve a 2-Log_{10} HCV RNA drop at week 12 and one who achieved an RVR and
10
11 stopped therapy at week 12); 47 patients infected with genotype 2 or 3 were treated for
12
13 24 weeks; and 42 patients infected with genotype 1, 4 or 6 were treated for 48 weeks.
14
15 An RVR and an EVR were observed in 31 patients (34.1%) and 25 patients (27.5%),
16
17 respectively. An SVR was observed in 59 patients (64.8%).
18
19
20
21
22
23

24 **Median liver stiffness changes according to the virological response**

25
26 Table 2 shows the median intra-patient changes in liver stiffness relative to
27
28 baseline in the patients who achieved or did not achieve an SVR. No significant
29
30 relationship was found between liver stiffness changes and the RVR, EVR or EOTR
31
32 (data not shown). A significant liver stiffness decrease was observed during pegylated
33
34 IFN- α and ribavirin administration in the patients who achieved an SVR, as well as in
35
36 non-sustained virological responders (median changes at the end of treatment relative to
37
38 baseline: -2.0 kPa, $p < 0.001$, and -2.9 kPa, $p = 0.02$, respectively) (Figures 1A and 1B,
39
40 Table 2). After treatment, liver stiffness continued to significantly decrease relative to
41
42 end-of-treatment in the patients who achieved an SVR (median change: -0.7 kPa,
43
44 $p = 0.008$), resulting in a median intra-patient decrease relative to baseline at the end of
45
46 follow-up of -3.4 kPa ($p < 0.001$) (Figure 1B and Table 2). In contrast, in patients who did
47
48 not achieve an SVR, liver stiffness increased after the end of treatment (median change:
49
50 $+0.8$ kPa, $p = 0.59$), resulting in a median intra-patient decrease of liver stiffness relative
51
52
53
54
55
56
57
58
59
60

1
2
3 to baseline of -1.8 kPa ($p=0.03$) (Figure 1A and Table 2). However, as shown in Figure
4
5
6 2, in spite of the global trends, there were important differences in liver stiffness kinetics
7
8 between individual patients.
9

10 11 12 **Liver stiffness changes according to the virological response in cirrhotic** 13 14 **patients**

15
16
17 When cirrhotic and non-cirrhotic patients were considered separately, liver
18
19 stiffness values were always significantly higher in the former than in the latter, but the
20
21 same dynamics as in the global analysis were seen. They are presented in Table 3.
22
23

24 25 26 **Liver stiffness changes according to ALT changes**

27
28
29 No relationship was observed between liver stiffness and ALT changes during
30
31 and after therapy, regardless of the virological outcome of treatment (data not shown).
32
33

34 35 36 **Variables associated with long-term improvement of liver stiffness**

37
38
39 Long-term improvement of liver stiffness was defined as a decrease of median
40
41 liver stiffness between baseline and end of follow-up of 30% or more. Long-term liver
42
43 stiffness improvement was observed in 38 patients. Table 4 shows the factors that
44
45 predicted liver stiffness improvement in univariate analysis. In multivariate analysis, only
46
47 the SVR was associated with long-term improvement of liver stiffness, with an odds ratio
48
49 of 3.10 (95% confidence interval: 1.20-8.02, $p=0.019$).
50
51
52
53

54 55 **DISCUSSION**

56
57
58
59
60

1
2
3 Successful therapy with IFN- α or pegylated IFN- α with or without ribavirin has
4 been shown to be associated with significant histological improvements in studies in
5 which patients underwent paired liver biopsies.²⁵⁻²⁹ However, follow-up liver biopsy is not
6 part of the routine management of patients with chronic HCV infection receiving antiviral
7 therapy.² Therefore, non-invasive methods appear to be better suited to the monitoring
8 of liver disease outcomes during and after therapy. Given its simplicity, high acceptability
9 by patients and intrinsic performance.^{19-21, 30, 31} transient elastography is an appropriate
10 tool for the longitudinal follow-up of fibrosis changes in treated HCV-infected patients, as
11 shown in the present study.
12
13
14
15
16
17
18
19
20
21
22
23

24 In this multicentre study conducted in expert centres for transient elastography
25 and HCV virology, we characterized the dynamics of liver stiffness in patients with
26 significant fibrosis at baseline (defined as liver stiffness >7.0 kPa with the Fibroscan[®])
27 treated with pegylated IFN- α and ribavirin. Liver stiffness and HCV RNA kinetics were
28 assessed at multiple time points during and after therapy and compared. We observed
29 that liver stiffness significantly decreased on treatment both in patients who did and did
30 not achieve an SVR. However, liver stiffness continued to significantly decrease after the
31 end of treatment only in patients who achieved an SVR. On treatment, no relationship
32 was found with RVR, EVR or EOTR. Overall, both patients who achieved and those who
33 did not achieve an SVR experienced a significant decrease of liver stiffness at the end of
34 follow-up relative to baseline, but this decrease was greater in the sustained virological
35 responders ($p=0.11$). These results are in keeping with a previous observation in
36 patients who were assessed at the end of treatment and thereafter.³² The use of mean
37 values for a non-normally distributed variable such as liver stiffness was however
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 questionable in this study, as median values better reflect the distribution and extreme
4 values. In another study,³³ a significant liver stiffness decrease was reported at the end
5 of follow-up in patients treated with pegylated IFN- α and ribavirin, regardless of the final
6 virological outcome of therapy (SVR or non-SVR). No on-treatment monitoring was
7 available, and liver stiffness at baseline significantly differed among the study groups.
8 However, the latter was also the case in our study, with median values of 12 kPa
9 (interquartile range, IQR: 9.6-24.7) in non-SVR vs 10 kPa (8.2-14.1) in SVR patients.
10
11
12
13
14
15
16
17
18
19

20 Overall, our prospectively generated data suggest that the cure of HCV infection
21 is associated with a significant reduction of liver stiffness, as measured by transient
22 elastography. This result may appear surprising as transient elastography is claimed to
23 assess essentially the fibrotic component of liver lesions, while treatment has been
24 mostly associated with reduction of the inflammatory reaction in the liver. Two non-
25 mutually exclusive hypotheses can be raised to explain this result: (i) liver stiffness is
26 also influenced by the local inflammatory reaction; (ii) fibrosis significantly regresses on
27 treatment and thereafter in patients who achieve an SVR. The first hypothesis may
28 appear unlikely as no relationship was observed between liver stiffness changes and
29 ALT kinetics; however, ALT elevations do not always accurately reflect liver
30 inflammation. On the other hand, SVR was the sole predictor of long-term liver stiffness
31 improvement; nevertheless, SVR is also associated with an improvement of liver
32 inflammation. Therefore, this hypothesis cannot be definitively ruled out. Indeed, others
33 have reported an overestimation of liver stiffness in patients with liver inflammation
34 reflected by high ALT levels.^{15, 34-37} In addition, a significant decrease of liver stiffness
35 was observed during therapy, followed by an increase after treatment withdrawal, in the
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 patients from this study who did not clear HCV infection. Histological data might have
4
5 helped resolve this question. However, liver biopsy can no longer be performed during
6
7 or after antiviral treatment for ethical reasons. Therefore, this question will remain
8
9 unanswered.
10

11
12 Our hypothesis concerning liver stiffness changes in patients who achieved an
13
14 SVR witnessing fibrosis regression is in keeping with the reported improvement of
15
16 histological fibrosis in a similar population who underwent paired liver biopsies.²⁵⁻²⁹ It is
17
18 also in keeping with the lower incidence of clinical outcomes, including liver failure,
19
20 variceal bleeding or hepatocellular carcinoma, in patients with cirrhosis who achieved an
21
22 SVR.³⁸⁻⁴¹ However, although patients with cirrhosis who achieved an SVR experienced a
23
24 significant improvement of their liver stiffness 6 months after the end of treatment in our
25
26 study, their median liver stiffness values remained significantly higher than in patients
27
28 without cirrhosis who achieved an SVR. In this context, the fact that the risk of
29
30 developing HCC remains substantial in cirrhotic patients who achieved an SVR.^{26, 38, 40} is
31
32 not surprising.
33
34
35
36
37

38
39 In conclusion, the results of this prospective, multicentre study show that transient
40
41 elastography can be used to monitor liver stiffness changes during and after antiviral
42
43 therapy in patients with chronic hepatitis C. On average, in patients with advanced
44
45 fibrosis at the start of therapy (>7.0 kPa), liver stiffness is significantly reduced during
46
47 pegylated IFN- α and ribavirin administration. The significant reduction of liver stiffness
48
49 continues off treatment only in patients who achieve an SVR. This probably denotes
50
51 fibrosis regression, although it cannot be ruled out that improvements in the
52
53 inflammatory reaction in these patients may also influence liver stiffness changes. These
54
55
56
57
58
59
60

1
2
3 findings suggest that assessing liver stiffness by means of transient elastography at
4
5 baseline and 6 months after treatment in patients who achieve an SVR is useful to
6
7 assess the global response to antiviral therapy, establish a prognosis and serve as a
8
9 basis for subsequent follow-up in patients with advanced fibrosis, especially those with
10
11 cirrhosis. In the latter, post-treatment liver stiffness assessments should not be
12
13 substituted to the recommended periodic surveillance of hepatocellular carcinoma
14
15 occurrence based on ultrasound examination and α -fetoprotein level measurement.⁴²
16
17
18 Our data also suggest that liver stiffness assessment earlier than 6 months after the end
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

REFERENCES

1. Thomas DL, Seeff LB. Natural history of hepatitis C. *Clin Liver Dis* 2005;9:383-98.
2. Ghany MG, Strader DB, Thomas DL, Seeff LB. Diagnosis, management, and treatment of hepatitis C: an update. *Hepatology* 2009;49:1335-74.
3. Fried MW, Shiffman ML, Reddy KR, et al. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. *N Engl J Med* 2002;347:975-82.
4. Hadziyannis SJ, Sette H, Jr., Morgan TR, et al. Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. *Ann Intern Med* 2004;140:346-55.
5. 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.
6. Bedossa P, Dargère D, Paradis V. Sampling variability of liver fibrosis in chronic hepatitis C. *Hepatology* 2003;38:1449-57.
7. Maharaj B, Maharaj RJ, Leary WP, et al. Sampling variability and its influence on the diagnostic yield of percutaneous needle biopsy of the liver. *Lancet* 1986;i:523-5.
8. Regev A, Berho M, Jeffers LJ, et al. Sampling error and intraobserver variation in liver biopsy in patients with chronic HCV infection. *Am J Gastroenterol* 2002;97:2614-8.
9. Rousselet MC, Michalak S, Dupre F, et al. Sources of variability in histological scoring of chronic viral hepatitis. *Hepatology* 2005;41:257-64.

- 1
2
3 10. Castera L, Negre I, Samii K, Buffet C. Pain experienced during percutaneous liver
4 biopsy. *Hepatology* 1999;30:1529-30.
- 5
6
7 11. Cadranet JF, Rufat P, Degos F. Practices of liver biopsy in France: results of a
8 prospective nationwide survey. *Hepatology* 2000;32:477-81.
- 9
10
11 12. Castera L, Negre I, Samii K, Buffet C. Patient-administered nitrous oxide/oxygen
12 inhalation provides safe and effective analgesia for percutaneous liver biopsy: a
13 randomized placebo-controlled trial. *Am J Gastroenterol* 2001;96:1553-7.
- 14
15
16
17 13. Bravo AA, Sheth SG, Chopra S. Liver biopsy. *N Engl J Med* 2001;344:495-500.
- 18
19
20
21 14. Sandrin L, Fourquet B, Hasquenoph JM, et al. Transient elastography: a new
22 noninvasive method for assessment of hepatic fibrosis. *Ultrasound Med Biol*
23 2003;29:1705-13.
- 24
25
26
27 15. Pinzani M, Vizzutti F, Arena U, Marra F. Technology Insight: noninvasive
28 assessment of liver fibrosis by biochemical scores and elastography. *Nat Clin Pract*
29 *Gastroenterol Hepatol* 2008;5:95-106.
- 30
31
32
33 16. Castera L. Transient elastography and other noninvasive tests to assess hepatic
34 fibrosis in patients with viral hepatitis. *J Viral Hepat* 2009;16:300-314.
- 35
36
37
38 17. Castera L, Pinzani M. Non-invasive assessment of liver fibrosis: are we ready?
39 *Lancet* 2010;375:1419-20.
- 40
41
42
43 18. Castera L, Forns X, Alberti A. Non-invasive evaluation of liver fibrosis using
44 transient elastography. *J Hepatol* 2008;48:835-47.
- 45
46
47
48 19. Ziol M, Handra-Luca A, Kettaneh A, et al. Noninvasive assessment of liver fibrosis
49 by measurement of stiffness in patients with chronic hepatitis C. *Hepatology*
50 2005;41:48-54.
- 51
52
53
54
55
56
57
58
59
60

- 1
2
3 20. Castera L, Vergniol J, Foucher J, et al. Prospective comparison of transient
4 elastography, Fibrotest, APRI, and liver biopsy for the assessment of fibrosis in
5 chronic hepatitis C. *Gastroenterology* 2005;128:343-50.
6
7
- 8
9
10 21. Friedrich-Rust M, Ong MF, Martens S, et al. Performance of transient elastography
11 for the staging of liver fibrosis: a meta-analysis. *Gastroenterology* 2008;134:960-
12 74.
13
14
- 15
16
17 22. Castera L, Le Bail B, Roudot-Thoraval F, et al. Early detection in routine clinical
18 practice of cirrhosis and oesophageal varices in chronic hepatitis C: comparison of
19 transient elastography (FibroScan) with standard laboratory tests and non-invasive
20 scores. *J Hepatol* 2009;50:59-68.
21
22
- 23
24
25 23. Castera L, Foucher J, Bernard PH, et al. Pitfalls of liver stiffness measurement: a
26 5-year prospective study of 13,369 examinations. *Hepatology* 2010;51:828-835.
27
28
- 29
30
31 24. Chevaliez S, Bouvier-Alias M, Pawlotsky JM. Performance of the Abbott real-time
32 PCR assay using m2000sp and m2000rt for hepatitis C virus RNA quantification. *J*
33 *Clin Microbiol* 2009;47:1726-32.
34
35
- 36
37
38 25. Marcellin P, Boyer N, Gervais A, et al. Long-term histologic improvement and loss
39 of detectable intrahepatic HCV RNA in patients with chronic hepatitis C and
40 sustained response to interferon-alpha therapy. *Ann Intern Med* 1997;127:875-81.
41
42
- 43
44
45 26. Shiratori Y, Imazeki F, Moriyama M, et al. Histologic improvement of fibrosis in
46 patients with hepatitis C who have sustained response to interferon therapy. *Ann*
47 *Intern Med* 2000;132:517-24.
48
49
- 50
51
52 27. Poynard T, McHutchison J, Manns M, et al. Impact of pegylated interferon alfa-2b
53 and ribavirin on liver fibrosis in patients with chronic hepatitis C. *Gastroenterology*
54 2002;122:1303-13.
55
56
57
58
59
60

- 1
2
3 28. Camma C, Di Bona D, Schepis F, et al. Effect of peginterferon alfa-2a on liver
4 histology in chronic hepatitis C: a meta-analysis of individual patient data.
5
6 *Hepatology* 2004;39:333-42.
7
- 8
9
10 29. Everson GT, Balart L, Lee SS, et al. Histological benefits of virological response to
11 peginterferon alfa-2a monotherapy in patients with hepatitis C and advanced
12 fibrosis or compensated cirrhosis. *Aliment Pharmacol Ther* 2008;27:542-51.
13
14
- 15 30. Fraquelli M, Rigamonti C, Casazza G, et al. Reproducibility of transient
16 elastography in the evaluation of liver fibrosis in patients with chronic liver disease.
17
18 *Gut* 2007;56:968-73.
19
- 20 31. Boursier J, Konate A, Gorea G, et al. Reproducibility of liver stiffness measurement
21 by ultrasonographic elastometry. *Clin Gastroenterol Hepatol* 2008;6:1263-9.
22
23
- 24 32. Ogawa E, Furusyo N, Toyoda K, Takeoka H, Maeda S, Hayashi J. The longitudinal
25 quantitative assessment by transient elastography of chronic hepatitis C patients
26 treated with pegylated interferon alpha-2b and ribavirin. *Antiviral Res* 2009;83:127-
27
28 34.
29
- 30 33. Vergniol J, Foucher J, Castera L, et al. Changes of non-invasive markers and
31 FibroScan values during HCV treatment. *J Viral Hepat* 2009;16:132-40.
32
33
- 34 34. Coco B, Oliveri F, Maina AM, et al. Transient elastography: a new surrogate
35 marker of liver fibrosis influenced by major changes of transaminases. *J Viral*
36
37 *Hepat* 2007;14:360-9.
38
- 39 35. Arena U, Vizzutti F, Corti G, et al. Acute viral hepatitis increases liver stiffness
40 values measured by transient elastography. *Hepatology* 2008;47:380-4.
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

- 1
2
3 36. Oliveri F, Coco B, Ciccorossi P, et al. Liver stiffness in the hepatitis B virus carrier:
4 a non-invasive marker of liver disease influenced by the pattern of transaminases.
5
6 *World J Gastroenterol* 2008;14:6154-62.
7
- 8
9
10 37. Vizzutti F, Arena U, Romanelli RG, et al. Liver stiffness measurement predicts
11 severe portal hypertension in patients with HCV-related cirrhosis. *Hepatology*
12 2007;45:1290-7.
13
14
- 15 38. Bruno S, Crosignani A, Facciotto C, et al. Sustained virologic response prevents
16 the development of esophageal varices in compensated, Child-Pugh class A
17 hepatitis C virus-induced cirrhosis. A 12-year prospective follow-up study.
18 *Hepatology* 2010;51:2069-76.
19
20
- 21 39. Bruno S, Stroffolini T, Colombo M, et al. Sustained virological response to
22 interferon-alpha is associated with improved outcome in HCV-related cirrhosis: a
23 retrospective study. *Hepatology* 2007;45:579-87.
24
25
- 26 40. Mallet V, Gilgenkrantz H, Serpaggi J, et al. Brief communication: the relationship of
27 regression of cirrhosis to outcome in chronic hepatitis C. *Ann Intern Med*
28 2008;149:399-403.
29
30
- 31 41. Veldt BJ, Heathcote EJ, Wedemeyer H, et al. Sustained virologic response and
32 clinical outcomes in patients with chronic hepatitis C and advanced fibrosis. *Ann*
33 *Intern Med* 2007;147:677-84.
34
35
- 36 42. Bruix J, Sherman M. Management of hepatocellular carcinoma. *Hepatology*
37 2005;42:1208-36.
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 **FIGURE LEGENDS**
4
5
6
7

8 **Figure 1.** Median (IQR) intra-patient liver stiffness changes relative to baseline at
9 different time points on treatment and after the end of therapy. (A) Patients who did not
10 achieve an SVR. (B) Patients who achieved an SVR.
11
12
13
14

15
16
17 **Figure 2.** Individual liver stiffness changes during and after treatment in patients who did
18 not achieve an SVR and in patients who achieved an SVR. EOT: end of treatment; EOF:
19 end of follow-up.
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Table 1. Baseline characteristics of the 91 patients.

	Treated (N = 91)
Male gender [n (%)]	63 (69.2)
Age (yrs) [mean±SD]	52.4±11.6
BMI (kg/m ²) [mean±SD]	24.9±3.5
ALT (IU/mL) [median (IQR)]	101 (63-153)
HCV genotype [n (%)]	
1-4-6	49 (54)
2-3	42 (46)
HCV RNA level (Log ₁₀ IU/mL) [median (IQR)]	5.8 (5.2-6.2)
Liver stiffness (kPa) [median (IQR)]	11.1 (8.4-16.3)
Cirrhosis, i.e. liver stiffness ≥13.1 kPa [n (%)]	33 (36.3)

Table 2. Median (IQR) intra-patient liver stiffness changes relative to baseline on treatment and thereafter in patients who did and did not achieve an SVR. Treatment duration was 24 weeks in patients infected with HCV genotype 2 or 3, 48 weeks in patients infected with HCV genotype 1, 4 or 6. P values are for intra-patient liver stiffness changes relative to baseline.

	Time point	SVR (N = 59)		No SVR (N = 32)	
		Liver stiffness change	p	Liver stiffness change	p
On treatment	Week 4	-1.0 (-3.4 - +0.3)	0.001	-1.3 (-5.1 - +0.4)	0.01
	Week 12	-2.2 (-4.0 - +0.2)	<0.001	-1.1 (-3.8 - +0.2)	0.05
	End of treatment	-2.0 (-3.6 - +0.5)	<0.001	-2.9 (-5.0 - -0.5)	0.02
Post-treatment	Follow-up week 12	-2.4 (-4.2 - +1.0)	<0.001	-1.4 (-3.6 - +1.1)	0.08
	Follow-up week 24	-3.4 (-4.7 - -1.1)	<0.001	-1.8 (-4.3 - +0.6)	0.03

Table 3. Median (IQR) intra-patient liver stiffness changes relative to baseline in patients with or without cirrhosis who did or did not achieve an SVR. Treatment duration was 24 weeks in patients infected with HCV genotype 2 or 3, 48 weeks in patients infected with HCV genotype 1, 4 or 6. P values are for intra-patient liver stiffness changes relative to baseline.

	Time point	Cirrhosis				No cirrhosis			
		SVR (n=19)	p	No SVR (n=14)	p	SVR (n=40)	p	No SVR (n=18)	p
On treatment	Week 4	-4.1 (-8.6 - 0)	0.019	-4.7 (-16.9 - -0.5)	0.01	-0.7 (-2.1 - +0.4)	0.024	-1.0 (-2.3 - +1.4)	0.41
	Week 12	-4.0 (-5.9 - +0.7)	0.025	-1.7 (-12.0 - +0.1)	0.035	-1.9 (-3.0 - -0.8)	0.001	-0.6 (-2.6 - +1.9)	0.51
	End of treatment ^a	-4.0 (-7.6 - +1.3)	0.059	-4.1 (-10.0 - -0.2)	0.07	-1.6 (-3.0 - +0.5)	0.001	-2.6 (-4.5 - -0.8)	0.13
Post-treatment	Follow-up week 12	-3.3 (-13.4 - +2.5)	0.20	-2.3 (-9.7 - +1.6)	0.25	-2.3 (-3.8 - -0.8)	<0.001	-0.8 (-2.3 - +1.2)	0.32
	Follow-up week 24	-6.4 (-8.5 - -1.6)	0.001	-2.5 (-9.8 - +1.4)	0.14	-2.7 (-3.9 - -0.7)	<0.001	-1.2 (-3.2 - +0.7)	0.11

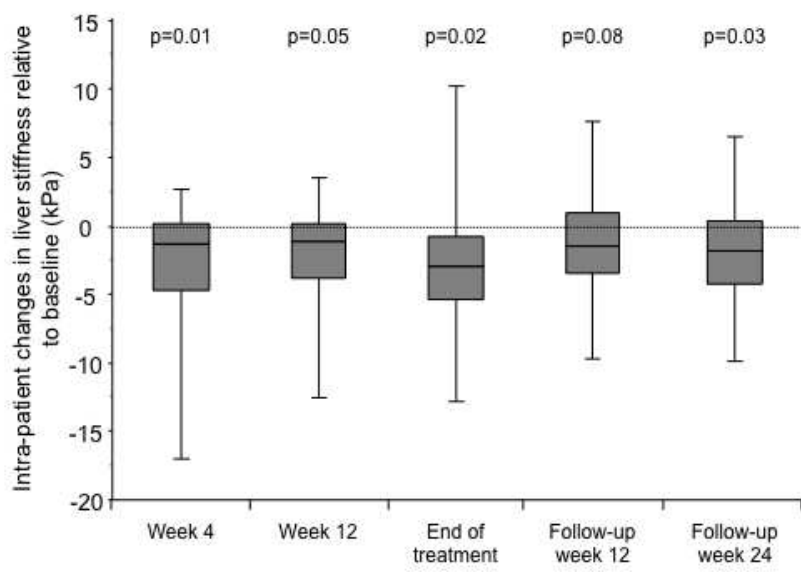
Table 4. Univariate analysis of variables associated with long-term liver stiffness improvement, defined as a liver stiffness decrease $\geq 30\%$ relative to baseline 24 weeks after the end of therapy.

	Liver stiffness change <30% (N = 53)	Liver stiffness change $\geq 30\%$ (N = 38)	p
Age (years)	54.2 \pm 12.3	49.2 \pm 10.2	0.17
Male gender [n(%)]	39 (73.6)	24 (63.2)	0.29
Baseline BMI (kg/m ²)	25.2 \pm 3.7	24.5 \pm 3.3	0.19
HCV genotype [n (%)]			
Genotype 1-4-6	32 (65.3)	17 (34.7)	0.14
Genotype 2-3	21 (50.0)	21 (50.0)	
Baseline ALT level (IU/L) [median (IQR)]	110 (70-162)	91 (60-136)	0.34
Baseline liver stiffness (kPa) [median (IQR)]	10.6 (7.8-16.3)	11.4 (9.1-15.8)	0.28
Baseline HCV RNA level (Log ₁₀ IU/mL) [median (IQR)]	5.9 (5.3-6.3)	5.8 (5.2-6.3)	0.69
Cirrhosis, i.e. liver stiffness >13 kPa [n (%)]	19 (35.8)	14 (36.8)	0.92
Treatment duration			
≤ 24 weeks	24 (52.2)	22 (47.8)	0.24
>24 weeks	29 (64.4)	16 (35.6)	
HCV RNA level 24 weeks after treatment (Log ₁₀ IU/mL) [median (IQR)]	0.85 (0.78-5.89)	0.78 (0.78-0.85)	0.027
Virological response [n (%)]			
No SVR	24 (75.0)	8 (25.0)	0.017
SVR	29 (49.2)	30 (50.8)	

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

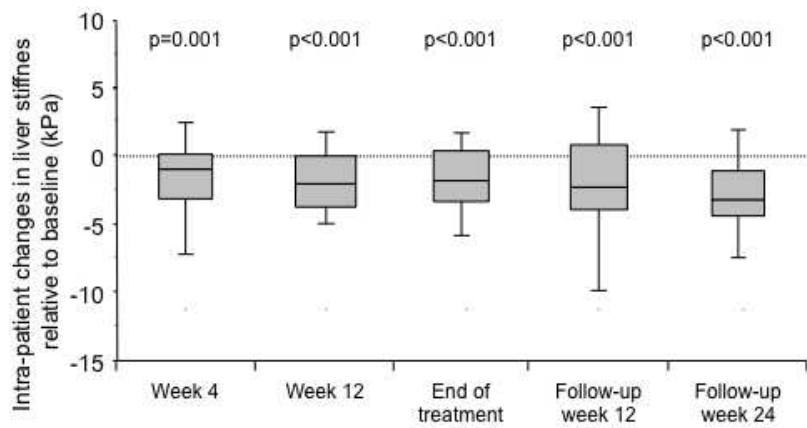
Figure 1A

A. Treated patients who did not achieve an SVR



190x254mm (72 x 72 DPI)

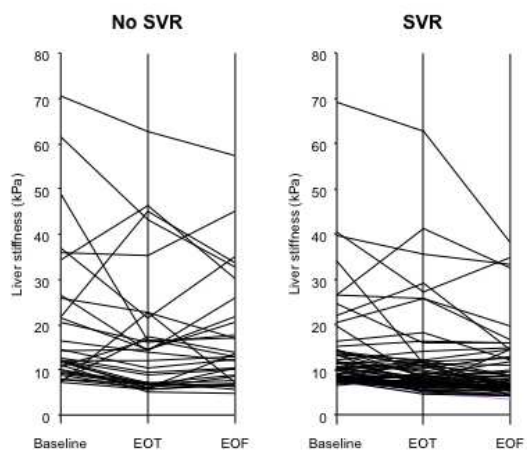
Figure 1B

B. Treated patients who achieved an SVR

190x254mm (72 x 72 DPI)

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Figure 2



254x190mm (72 x 72 DPI)

Review