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Monitoring thérapeutique des antiviraux directs pour le traitement de l'hépatite C

Therapeutic monitoring of direct-acting antivirals for the treatment of hepatitis C

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Contents

Abstract

Background. Chronic hepatitis C virus (HCV) infection affects about 71 million people worldwide. Nowadays, the standard therapy of chronic HCV infection is based on direct-acting antivirals (DAAs). DAAs are significantly more effective than pegylated interferon-alfa and ribavirin. Moreover, these drugs have a good tolerance and allow short treatment durations. It is common practice to monitor treatment efficacy with measurements of blood HCV viral load. However, we do not have clear recommendations for this monitoring, based on a model describing viral kinetics under DAA-based treatment. The additional usefulness of DAA concentration monitoring is uncertain.

The aim of our study was to analyze whether HCV RNA profiles during DAA-based therapies predict the final treatment outcome, to assess the adjunctive predictive value of drug concentration and liver function tests monitoring, and to describe clinical and laboratory characteristics of patients with post-treatment relapse.

Methods. We conducted a retrospective observational study with chronic HCV infected patients. All included patients were ≥ 18 years old, treated with DAAs from 2013 to 2017 at CHUV (Lausanne, Switzerland).

Results. We included in the study 202 patients (71% men, mean age 55 years). A sustained virologic response (SVR) was achieved by 193 (95.5%) patients, while 9 (4.5%) patients had a post-treatment relapse. A previous history of hepatocellular carcinoma, HBV co-infection, and IL28B rs12979860 genotype CT were independent predictors of treatment failure. We did not find a relationship between therapy outcome and either HCV RNA, ALT or AST at baseline, at week 2, week 4, or at the end of treatment. The concentrations of sofosbuvir metabolite GS331007 and daclatasvir tended to be lower in patients with post-treatment relapse compared with patients with SVR, however the limited number of patients precludes any firm conclusion.

Conclusions. Beyond known pre-existing prognostic factors, confirmed in our study, there is no indication that the regular monitoring of HCV RNA, AST, and ALT during DAAs treatment could help to predict the sustained virologic response of HCV chronic infection to novel DAAs. The potential role of DAA concentration monitoring deserves to be evaluated in a larger study.

Key words: Hepatitis C virus, direct-acting antivirals, sustained virologic response, HCV RNA, monitoring.

Background

Chronic hepatitis C virus (HCV) infection affects about 71 million people worldwide [1, 2]. There are an estimated 1.75 million new HCV infections each year, with an incidence in European Region and the Eastern Mediterranean Region of about 62 cases per 100 000 population [3]. Switzerland has a prevalence of chronic HCV infection of about 0.5% (about 40 000 patients) [4], placing it among the regions with low prevalence (<1.5%) along with other countries in western and central Europe. In contrast, Central Africa and Central Asia have high prevalence (>3.5%) [5].

About 55–85% of the usually asymptomatic acute HCV infections become chronic, with a 15–30% risk of developing cirrhosis within 20 years [6]. It should be noted that HCV is the cause of more than 400 000 deaths each year, an increase of 22% since 2000. Most of these deaths are related to the development of life-threatening complications such as cirrhosis (about 280 000 deaths) and hepatocellular carcinoma (HCC) (about 120 000 deaths) [6].

Until 2011, the standard treatment of chronic HCV infection had been a double therapy consisting of pegylated interferon-alfa (Peg-IFN) and ribavirin (RBV). But this treatment only yielded a sustained virologic response (SVR) rate of about 50-70% after 48 weeks [7]. The treatment of chronic HCV infection has been revolutionized by the introduction of direct-acting antivirals (DAAs) such as nucleoside and nucleotide NS5B polymerase inhibitors (sofosbuvir), NS5A inhibitors (daclatasvir, ledipasvir), NS3-4A protease inhibitors (grazoprevir), and non-nucleoside NS5B polymerase inhibitors (dasabuvir).

In clinical trials DAAs allow the achievement of SVR in more than 90% of patients after 8-12 weeks of treatment [8]. DAAs are not only significantly more effective than Peg-IFN, they have a better tolerance, while permitting shorter treatment duration and oral administration [9].

Overall, the efficacy of DAA treatment is high. However, a certain proportion of patients has a risk of virological breakthrough or post-treatment relapse. To ensure treatment efficacy and patient compliance, international guidelines recommended a therapeutic monitoring based on measurements of HCV RNA levels in serum or plasma (with a lower limit of detection ≤15 IU/ml). Current guidelines recommend monitoring of quantitative HCV RNA at baseline, between week 2 and week 4, as well as at the end-of-treatment and 12 or 24 weeks after the end of the therapy (to assess SVR12 or SVR24, respectively) [8, 10, 11]. At the same time, the cost of DAA treatment is very high (for example, 12 weeks therapy with ledipasvir/sofosbuvir costs €40 000). Therefore, the monitoring of efficacy and compliance may be cost-effective.

In clinical routine, HCV RNA measurements is not infrequently performed even more often. However, the results of trials are ambiguous towards an association of HCV RNA monitoring during treatment and treatment outcome. Actually, there are models describing the viral kinetics under DAA-based treatment, but their predictive value is questionable [12]. The adjunctive role of drug concentration monitoring and liver function tests monitoring is poorly supported by any evidence.

The aims of our study were to analyze whether HCV RNA profiles during DAA therapies predict the final treatment outcome, to assess the adjunctive predictive value of drug concentration and liver function tests monitoring, and to describe clinical and laboratory characteristics of patients with post-treatment relapse.

Methods

Study population

We conducted a retrospective observational study in chronic HCV infected patients. All patients were ≥ 18 years old and treated for chronic HCV infection with DAAs from 2013 to 2017 at the CHUV (Lausanne, Switzerland). Clinical and laboratory data were obtained from paper medical records and CHUV databases of medical records (Soarian, Archimède, and Molis). We excluded patients who refused the reuse of their personal data, as well as patients with less than 3 determinations of HCV RNA level during the observational period.

To select patients for the study, we first extracted data from the laboratory database Molis (patients with 3 or more available measurements of HCV RNA and liver function from 1 January 2013 to 31 August 2017). First, we selected only chronic HCV patients followed at the Service of Gastroenterology and Hepatology treated between 1 January 2013 and 31 August 2017 with one of the following combinations: sofosbuvir and daclatasvir, sofosbuvir and ledipasvir or sofosbuvir and simeprevir, with or without ribavirine. Secondly, among the patients listed in the Molis extraction, we looked for those having available plasma concentration results for sofosbuvir, its metabolite (GS331007), daclatasvir or ledipasvir. Inclusion and exclusion criteria were also checked. For all these patients we obtained completed clinical, histological, and laboratory data from paper medical records and databases (Soarian, Archimède, and Molis).

This study was approved by the *Commission cantonale d'éthique de la recherche sur l'être humain* (CER-VD, project No 2017-01102).

2. Treatments

We included in our study patients treated with DAAs (sofosbuvir, daclatasvir, ledipasvir, simeprevir), with or without ribavirin. Drug combinations and doses were defined by physicians based on patient characteristics and applicable guidelines at the time of treatment.

3. Study assessments

SVR was defined as undetectable HCV RNA 12 weeks after treatment completion [8, 10]. Post-treatment relapse was defined as confirmed HCV RNA ≥ 15 IU/ml during follow-up in patients having undetectable HCV RNA at the end of treatment [8, 10]. Viral breakthrough was defined as a ≥10 U/ml increase from the nadir of HCV RNA or when HCV RNA ≥15 IU/ml after HCV-RNA was undetectable during the treatment [8, 10]. Non-response patients were defined as those with viral breakthrough, or post-treatment relapse, or with absence of initial decline of HCV RNA.

4. Observations

Clinical data were recorded manually from paper medical records and in CHUV's Soarian and Archimède databases: gender, date of birth, weight, height, date of treatment, treatment duration, treatment response, dose of DAAs, HCV genotype, FibroScan score stiffness, FibroScan score IQR, METAVIR score, Child-Pugh score, previous HCV therapies and number of cures, hepatocellular carcinoma (HCC), HCC treatment, transplantation, hemodialysis, HIV infection, HBV infection, IL28B rs12979860 genotype.

Laboratory data were extracted from database Molis: HCV RNA, leucocytes, erythrocytes, hemoglobin, thrombocytes, prothrombin time, activated

partial thromboplastin time (aPTT), fibrinogen, creatinine, albumin, bilirubin (total and direct), alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), gamma-glutamyltransferase (GGT), alpha-fetoprotein (AFP), HCV mutation (NS5A).

Initially, we had concentrations of drugs measured at the peak or in the middle of the dosing interval or at the trough. Assuming that trough level of drugs is the most informative, we included in our analysis only trough concentrations (i.e. those sampled at least 18 hours after drug administration).

For the determination of the response to treatment, we followed HCV RNA levels before, during, and after treatment for each patient.

5. Statistical Analysis

To describe the data, we used the arithmetic means (± standard deviation or range) for numerical variables and fraction percentages for qualitative variables. The data of HCV RNA were transformed in logarithm of viral load.

To compare the response and non-response groups, we used t-test (when the tested variable could be assumed to follow a normality assumption), Wilcoxon test (when the values could not be assumed to be normally distributed), and Fisher's exact test (for categorical variables). A p-value lower than 0.05 was considered a statistically significant difference in standard tests.

For the prediction of HCV infection relapse, we retained all variables for which a univariate test (Student, Wilcoxon, Fisher) reported a significant or a trend for association ($p \leq 0.1$) and included them (forward stepwise approach) in a logistic regression model for multivariate analysis. Odds ratios (OR) are presented with their 95% confidence intervals.

We used the Pearson correlation coefficient (r) for correlation analysis.

We generated histograms, dot plots, bar plots, and spaghetti plots to illustrate the results.

Statistical analyses were performed using the R version 3 and STATA version 15 software packages.

Results

1. Study population

1.1. Population description

Our initial data extraction from the laboratory database Molis (patients with 3 or more available measurements of HCV RNA and liver function from 1 January 2013 to 31 August 2017) produced data for 940 patients. From those, our selection of patients followed at the Service of Gastroenterology and Hepatology and treated between 1 January 2013 and 31 August 2017 with identified 197 consecutive patients with clinical, histological, and laboratory documentation available. An additional combined search between the Molis extraction and the database for drug concentrations of the Division of Clinical Pharmacology identified 14 additional patients (followed in CHUV services other than Gastroenterology and Hepatology). Among these, available clinical information was found for 5 patients fulfilling our inclusion/exclusion criteria. Thus, we obtained complete data for 202 patients, who were eventually included in our analysis.

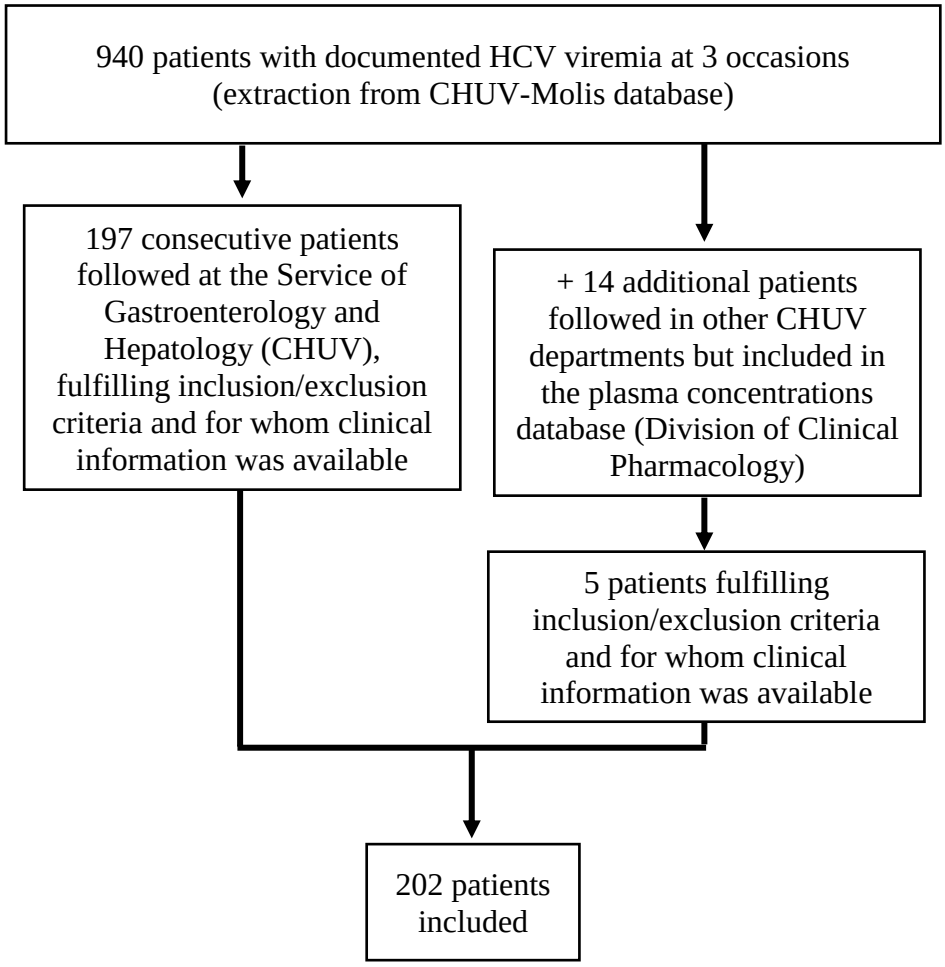
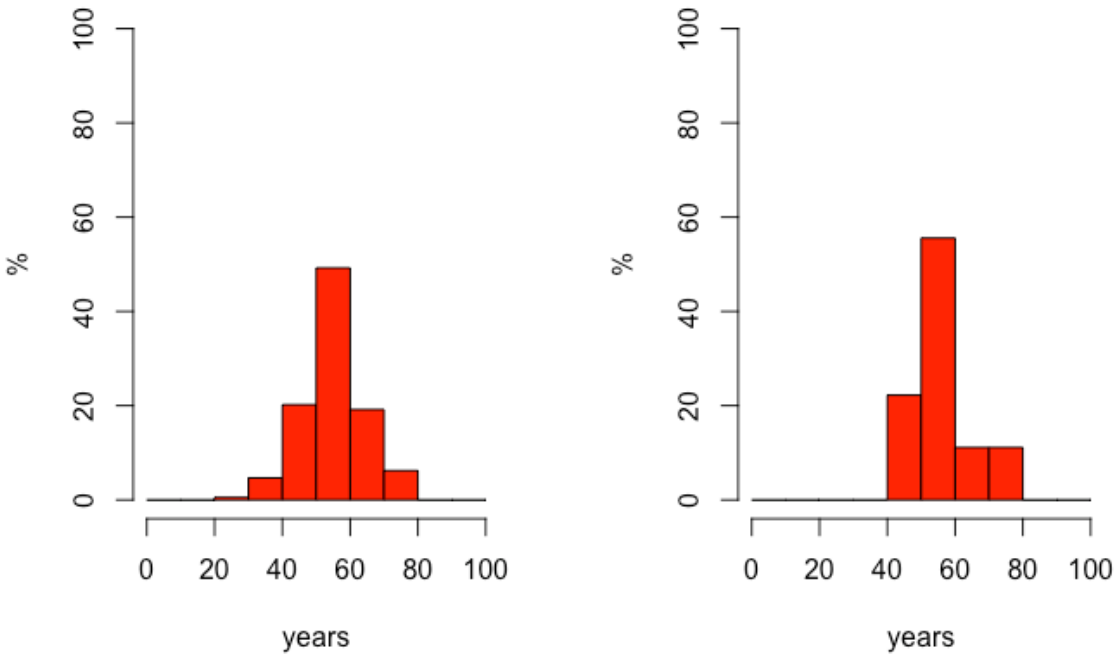


Figure 1: Schema of patient recruitment

1.2. Clinical characteristics of study population

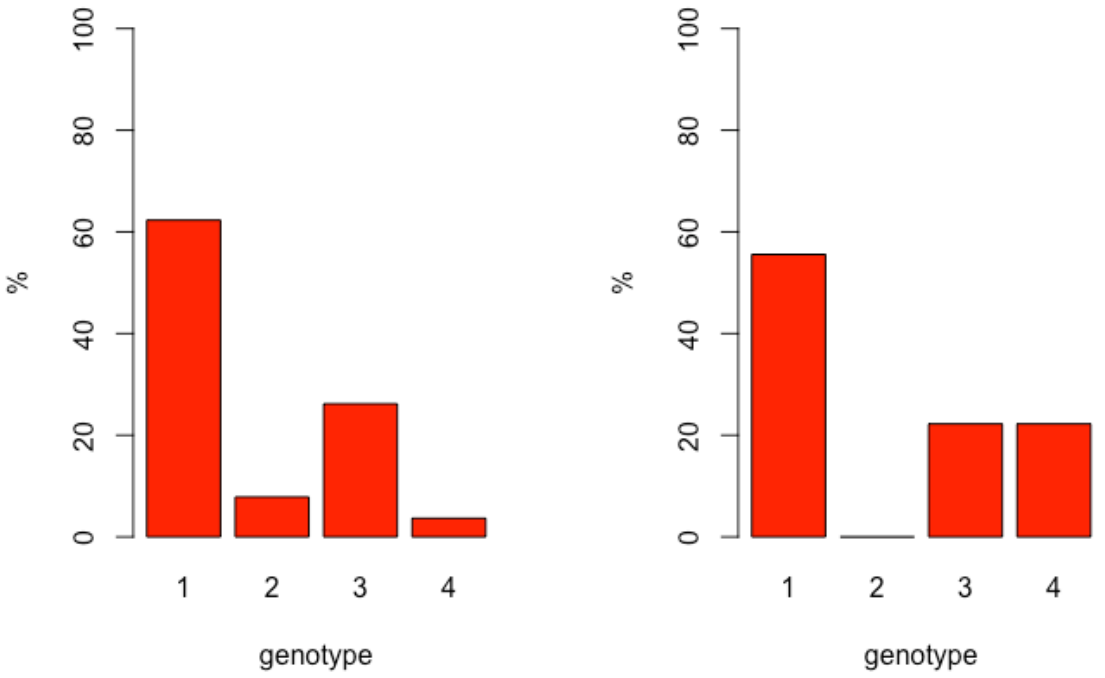
The 202 patients included in the study were 144 men (71%) and 58 women (29%). Their mean age was 55.3±8.9 years (range 25-79). Among them, SVR was achieved by 193 patients (95.5%) irrespectively of the drug combination used. These 193 patients were included in the treatment-respondent group (137 men (71%), mean age 55.3±9.0 years), and the remaining 9 patients in the non-response group (7 men (78%), mean age 55.6±8.0 years). All patients in the non-response group actually achieved HCV RNA suppression under treatment but had a post-treatment relapse.

During our search for individual factors predicting treatment response, we did not find any significant differences between responders and non-responders in age, gender, BMI, HCV genotype, FibroScan score, METAVIR score, previous HCV therapy, hemodialysis, transplantation, HIV and HBV infection, and IL28B rs12979860 genotype. Conversely, in patients belonging to the non-response group, the rate of past HCC was higher than in the responders (56% and 10%, respectively, p=0.002). In addition, the distribution of Child-Pugh score differed significantly between both groups: 89% of patients in the response group had score A, 7% score B, and 4% score C, while in the non-response group they were 67%, 22%, and 11%, respectively (p=0.045). Baseline clinical characteristics of the whole study population are shown in Table 1. Distributions of age, HCV genotype, and Child-Pugh score are represented in Figures 2, 3, and 4.



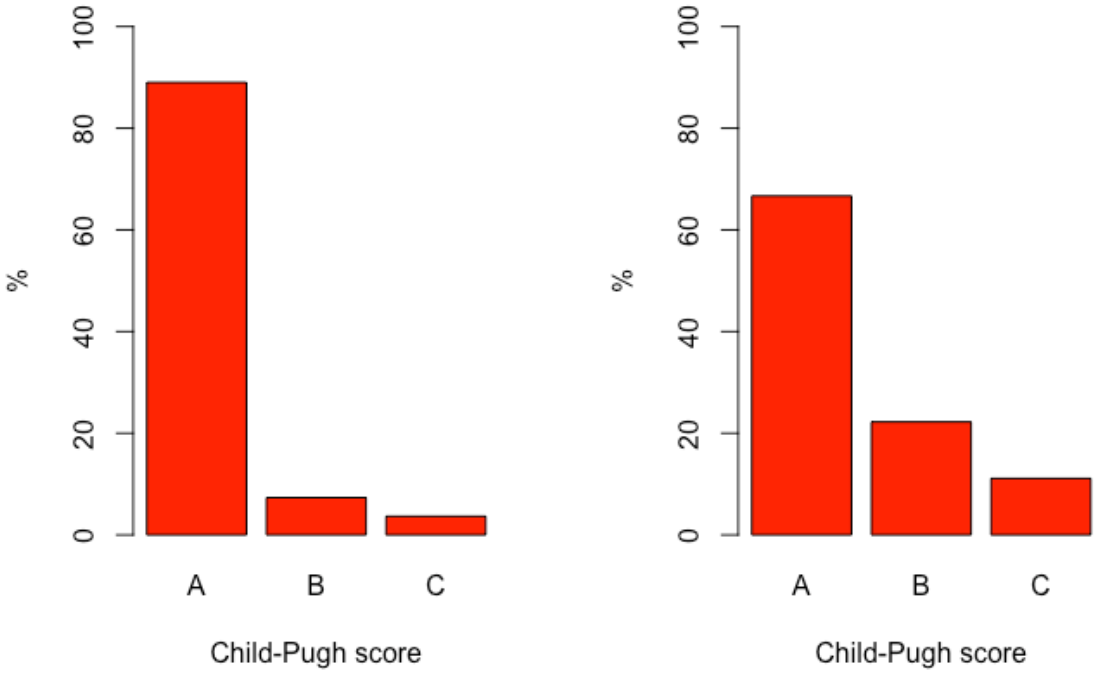
a b

Figure 2: Distribution of age: a. response group, b. non-response group



a **b**

Figure 3: Distribution of HCV genotype: a. response group, b. non-response group



a **b**

Figure 4: Distribution of Child-Pugh score: a. response group, b. non-response group

Table 1: Clinical characteristics of the study population (mean ± standard deviation or n (%))

Characteristic	total	responders	non-responders	p value
Women : men	29% : 71%	29% : 71%	22% : 78%	1
Age (years)	55.3±8.9	55.3±9.0	55.7±8.1	0.90
Weight (kg)	77±15	77±15	77±9	0.94
Height (cm)	173±10	173±10	174±8	0.73
BMI (kg/m^2)	26±4	26±4	26±4	0.96
Treatment duration (weeks)	16±6	16±6	20±6	0.09
HCV genotype 1: 2:	124 (61%) 15 (8%)	119 (62%) 15 (8%)	5 (56%) 0 (0%)	0.14

3: 4:	52 (26%) 9 (5%)	50 (26%) 7 (4%)	2 (22%) 2 (22%)	
FibroScan stiffness score	13.1±9.9	13.2±10.0	8.9±3.7	0.09
FibroScan score IQR	2.1±1.9	2.1±1.8	2.2±2.8	0.97
METAVIR score A	2±1	2±1	2±0	0.07
METAVIR score F	3±1	3±1	3±1	0.12
Child-Pugh score A: B: C:	176 (88%) 16 (8%) 8 (4%)	170 (89%) 14 (7%) 7 (4%)	6 (67%) 2 (22%) 1 (11%)	**0.045**
Past history of HCC	25 (12%)	20 (10%)	5 (56%)	**0.002**
Transplantation	11 (5%)	11 (6%)	0 (0%)	1
Hemodialysis	1 (0.4%)	1 (0.5%)	0 (0%)	1
HIV infection	15 (7%)	15 (8%)	0 (0%)	1
HBV infection	45 (22%)	41 (21%)	4 (44%)	0.11
IL28B rs12979860 genotype CC: CT:	3 (1.5%) 12 (6%)	3 (2%) 10 (5%)	0 (0%) 2 (22%)	0.12
Previous HCV therapy				
Ribavirin	75 (37%)	71 (37%)	4 (44%)	0.72
Peg-IFN	83 (41%)	78 (40%)	5 (56%)	0.53
Boceprevir	2 (0.9%)	2 (1%)	0 (0%)	1
Telaprevir	8 (3.9%)	8 (4%)	0 (0%)	1
Sofosbuvir	2 (0.9%)	2 (1%)	0 (0%)	1

1.3 Laboratory characteristics of study population

We analyzed the laboratory baseline characteristics of patients in both groups. We found significant differences between groups in erythrocytes level (p=0.049), prothrombin time (p=0.0002), creatinine level (p=0.005), ALP level (p=0.04), and bilirubin total level (p=0.02). All laboratory characteristics are described in Table 2.

Table 2: Baseline laboratory characteristics of study population (mean ± standard deviation)

Characteristic	total	response group	non-response group	p value
Leucocytes G/l)	5.7± 2.7	5.9±2.7	4.5±2.8	0.06
Erythrocytes (T/l)	4.2± 1.0	4.3±0.8	3.7±1.0	**0.049**
Hemoglobin (g/l)	131±28	132±28	118±28	0.06
Thrombocytes (G/l)	132±77	133±76	117±111	0.55
aPTT (sec)	49±19	44±19	71±36	0.06
Prothrombin time (%)	77±28	81±28	50±30	**0.0002**
Fibrinogen (g/l)	1.5±0.8	1.5±0.8	1.0±1.0	0.59
Creatinine (µmol/l)	93±61	95±61	77±20	**0.005**

HCV RNA (log IU/ml)	6.58±6.7	6.58±6.7	6.6±6.62	0.92
Albumin (g/l)	38±6	39±6	34±7	0.07
ALT (U/l)	93±71	88±71	153±163	0.18
AST (U/l)	92±89	84±89	194±257	0.15
ALP (U/l)	109±46	97±39	125±44	**0.04**
GGT (U/l)	129±117	131±106	104±93	0.34
AFP (ng/ml)	38±47	25±47	45±42	0.41
Bilirubin total (mmol/l)	27±39	23±39	60±56	**0.02**
Bilirubin direct (mmol/l)	13±21	13±21	22±20	0.19
NS5A mutation	0 (0%)	0 (0%)	0 (0%)	1

2. Treatments

The median duration of antiviral treatment was 16±6 weeks for patients from the response group and 20±6 weeks for patients from the non-response group (p=0.09). All patients included in the study received sofosbuvir. This drug was combined with ledipasvir in most cases (50% of patients in the response group and 78% in the non-response group) and with daclatasvir, simeprevir, and ribavirin. In the response group, patients received a combination of 3 drugs: sofosbuvir + ledipasvir ± ribavirin or sofosbuvir + daclatasvir ± ribavirin. We did not find significant differences between the two groups in the type of drug used or in the drugs combination (Table 3 and 4).

Table 3: Treatment (by drug)

Drug	response group	non-response group	p value
Sofosbuvir	193 (100%)	9 (100%)	1
Daclatasvir	60 (31%)	2 (22%)	0.72
Ledipasvir	111 (58%)	7 (78%)	0.31
Ribavirin	57 (30%)	0 (0%)	0.06
Simeprevir	2 (1%)	0 (0%)	1

Table 4: Treatment (by drug combinations)

Drug combination	response group	non-response group	p value
Sofosbuvir + simeprevir	2 (1%)	0 (0%)	0.77
Sofosbuvir + ribavirin	20 (10%)	0 (0%)	
Sofosbuvir + ledipasvir	96 (50%)	7 (78%)	
Sofosbuvir + ledipasvir + ribavirin	15 (8%)	0 (0%)	
Sofosbuvir + daclatasvir	38 (20%)	2 (22%)	
Sofosbuvir + daclatasvir + ribavirin	22 (11%)	0 (0%)	

3. HCV RNA monitoring during treatment and viral kinetic model

Our dataset contained altogether 1771 HCV RNA determinations, among which 659 indicated detectable levels and 1112 undetectable viral load. The

median number of viral load determinations in our study patients was 8, with an interquartile range (IQR) of 7 to 10, a minimum of 3 and a maximum of 20 (mean ± SD: 8.8 ± 3.2). Still the patients had only a median of 3 (IQR 2-4) detectable levels.

Patients from the non-response group had an average 10.6 determinations, i.e. some 2 determinations more than responders ($p>0.05$); moreover, they had expectedly a higher number of detectable levels (average 6.1 ± 0.9, versus 3.1 ± 0.1 in the responder group).

No virological breakthrough was recorded during the treatment period in any the 202 study patients. However, 9 patients (4.5%) experienced post-treatment relapse.

In most patients HCV-RNA levels were measured at baseline, at week 2, week 4, at the end of treatment, at 12 and at 24 weeks after treatment. The difference between both groups was not significant at baseline ($p = 0.92$) and at week 2, week 4, at the end of treatment ($p>0.05$).

In our analysis, we did not find any relationship between the rate of HCV RNA decrease under treatment and the treatment outcome. By definition, relapsing patients were characterized by post-treatment re-appearance and increase of viral load. We drew spaghetti plots for two patient groups, supported by a local regression fit (Figure 5).

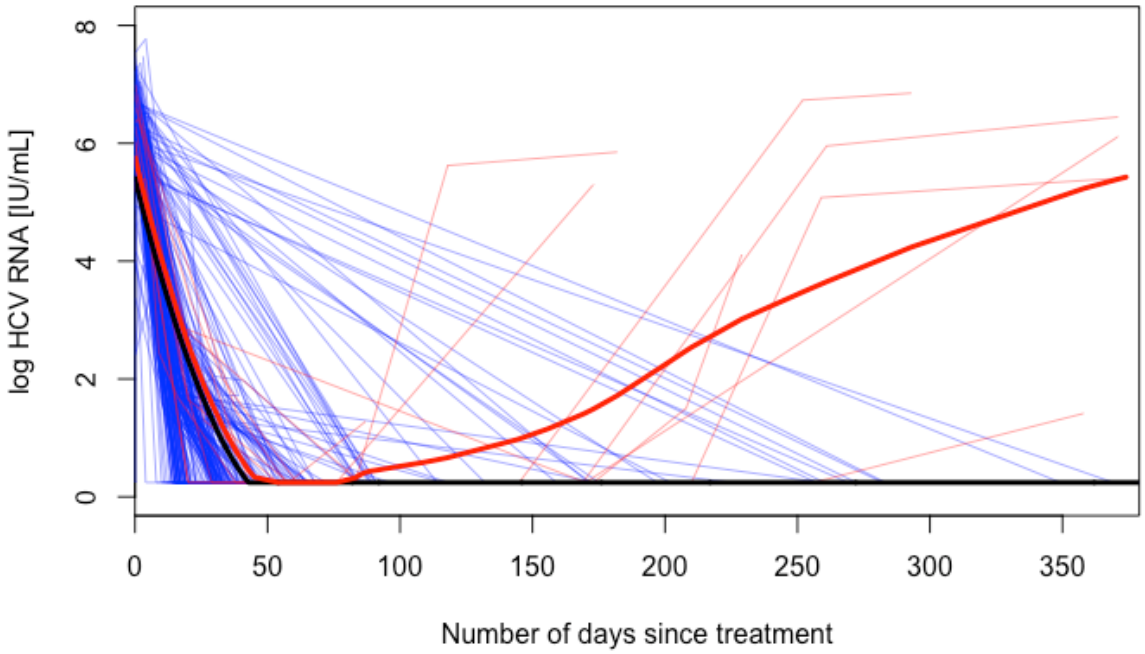


Figure 5: Spaghetti plot of HCV-RNA level during the treatment (blue: response group; red: relapse group)

We found a positive correlation between HCV RNA and ALT (r = 0.47) and between HCV RNA and AST (r = 0.19)

To describe the viral kinetics during treatment, most authors use a "biphasic" virologic response model. This model was published in 1998 for IFN-based treatment [13]. It is described by the following biexponential function

$$V_0 = \begin{cases} V_0, & t \le t_0 \\ V_0[Ae^{-\lambda_1(t-t_0)} + (1-A)e^{-\lambda_2(t-t_0)}], & t > t_0 \end{cases}$$

where

$$\lambda_{1,2} = \frac{c+\delta \pm \sqrt{(c-\delta)^2 + 4(1-\varepsilon)(1-\eta)c\delta}}{2}$$

and

$$A = \frac{\varepsilon c - \lambda_2}{\lambda_1 - \lambda_2}$$

In this model, HCV-RNA initially declines from pre-treatment plateau value (V_0) with rate $\lambda_1 \approx \varepsilon c$; thus the treatment is "potent" ($\varepsilon \approx 1$), viral load declines with a maximum rate equal to c. This declining phase continues until the viral load reaches a value V_1 that reflects the new equilibrium between the viral production and clearance under treatment given by: $V_1 = (1-\varepsilon)\, V_0$. Thus, for instance if ε = 0.99, there will be a rapid decline of 2 $\log_{10}$ of viral load in the first 2 days.

We tried to apply different models to described viral kinetics in study patients. First, we used a "monophasic model"

$$V = V_0 \cdot e^{-\lambda t}$$

where V_0 is the pre-treatment HCV RNA level and λ is the single coefficient of exponential decay.

If we use this mathematical model, we obtain the parameter values: V = 4.949E+06, λ = 0.1588 (residual sum-of-squares: 5.02e+15=. The fitting is shown in Figure 6.

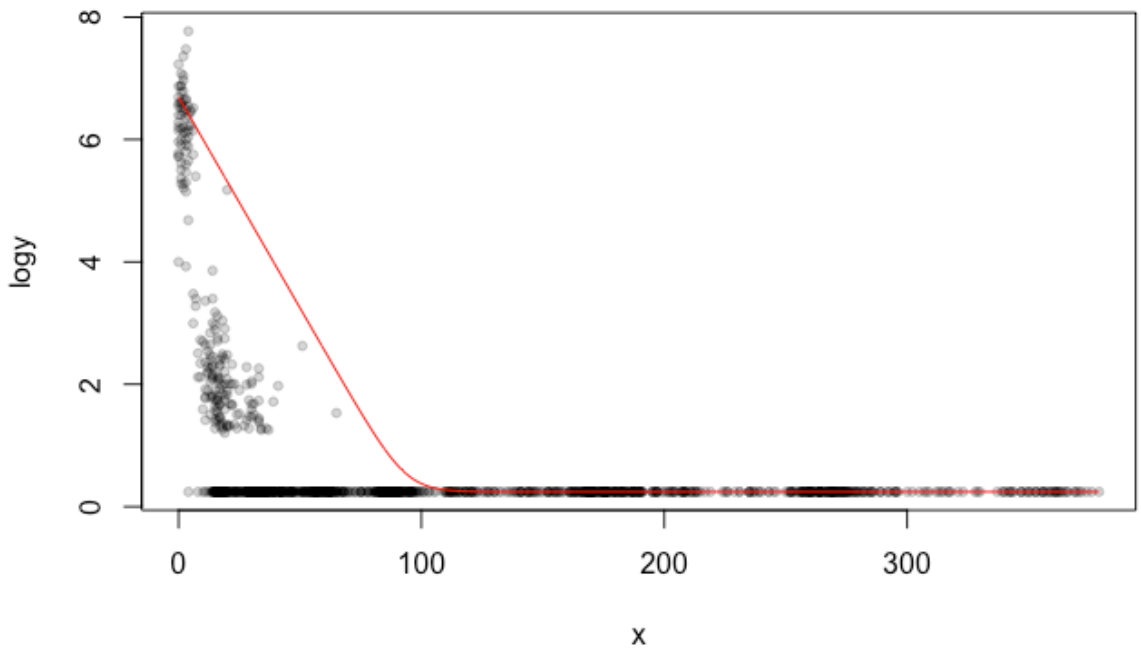


Figure 6: Fit the exponential with model $V = V_0 \cdot \exp(-\lambda t)$

A model that apparently better describes the viral kinetics is a “double exponential model”, where

$$\log(V) = \log(V_0) \cdot e^{-\lambda t}$$

or

$$V = V_0 \cdot \exp(\exp(-\lambda t))$$

If we use this mathematical model, we obtain the following coefficients: $V_0 = 6.9047$, $\lambda = 0.1066$ (residual sum-of-squares: 366.4). The fitting is shown in Figure 7.

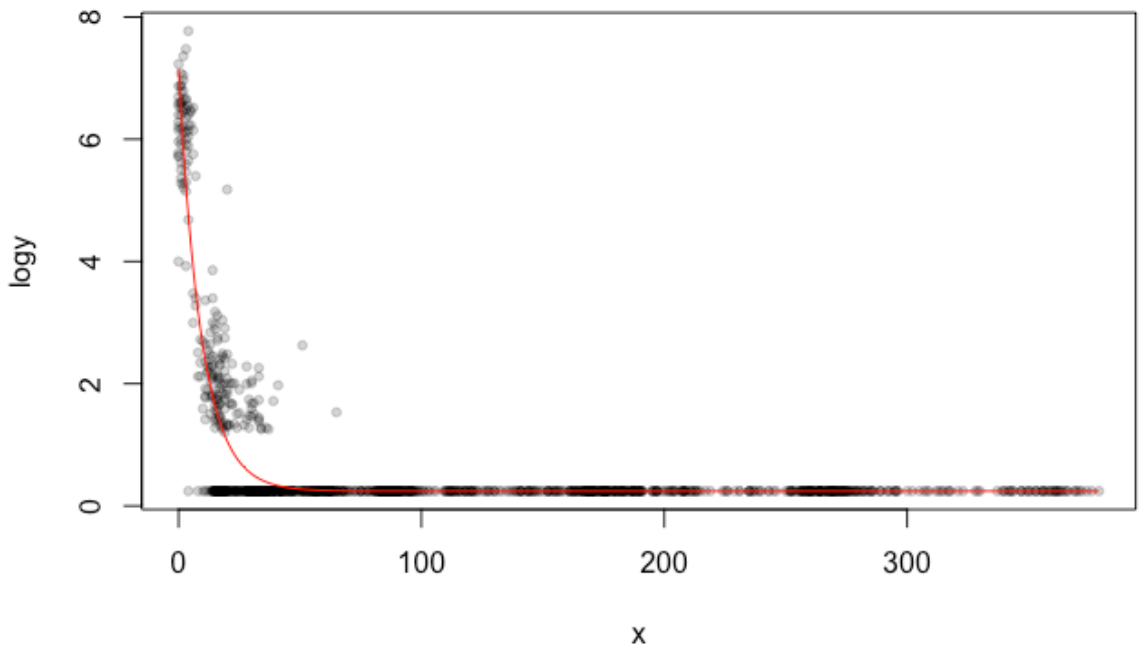


Figure 7: Fit the exponential with model $V = V_0 \cdot \exp(\exp(-\lambda t))$

Still in theory, both these models are less satisfactory than a biexponential model would be; however, the data precluded the adaptation of a biexponential model, because of the abundance of undetectable HCV RNA levels measured, leaving too few values to fit a biexponential equation; in particular, the early treatment period was poorly covered by measurement points. Neither was it

possible to fit a two-levels, hierarchical or mixed-effect model incorporating a between-patient variability on model parameters (V_0, λ). Such a model would certainly have been conceptually quite correct, but the paucity of detectable HCV RNA levels precluded all our attempts for this analysis.

4. Analysis of viral kinetics after end of treatment in relapse group

All patients in relapse group experienced post-treatment relapse, and none shown primary non-response. We described the viral kinetics of these patients after end of treatment. The individual characteristics of these patients are shown in Table 5

Table 5: Treatment duration and time of HCV relapse in treatment failure group

Patient	Treatment	Treatment duration	Time of HCV relapse after end of treatment
Male, 45 years, genotype 1a	Sofosbuvir + ledipasvir	12 weeks	287 days
Male, 57 years, genotype 1b	Sofosbuvir + ledipasvir	24 weeks	93 days
Male, 50 years, genotype 3	Sofosbuvir + daclatasvir	24 weeks	91 days
Male, 45 years, genotype 3	Sofosbuvir + daclatasvir	24 weeks	40 days
Female, 56 years, genotype 4	Sofosbuvir + ledipasvir	24 weeks	84 days
Male, 59 years, genotype 1a	Sofosbuvir + ledipasvir	12 weeks	89 days
Male, 61 years genotype 1a	Sofosbuvir + ledipasvir	16 weeks	6 days
Male, 52 years, genotype 1a	Sofosbuvir + ledipasvir	20 weeks	218 days
Female, 71 years, genotype 4	Sofosbuvir + ledipasvir	12 weeks	3 days

We did not find any correlation between the duration of treatment and the time of relapse. There seemed to be 3 types of behavior: relapse immediately (3-6 days after end of treatment), or about 80-90 days after end of treatment, or more than 6 months after treatment (Figure 8).

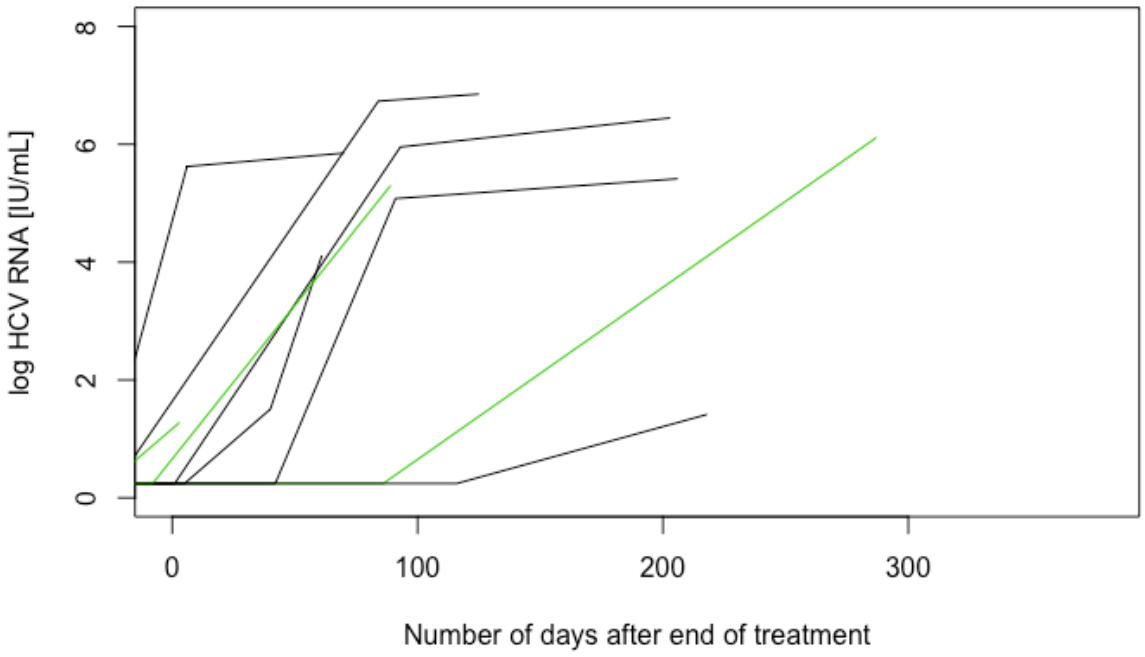


Figure 8: Spaghetti plot of HCV RNA level after end of treatment in non-response group (green: treatment duration 12 or 16 weeks; black: treatment duration 20 or 24 weeks)

5. Liver function tests monitoring

Liver function monitoring (AST and ALT) was usually performed at the same time as HCV-RNA monitoring. At baseline we did not find any significant differences between groups in ALT ($p = 0.18$) or AST levels ($p = 0.15$). The levels of hepatic enzymes were not significantly different at weeks 2, 4 or at the end of treatment ($p>0.05$).

We did not find any relationship between the decrease rate of ALT and AST levels and treatment outcome. We drew spaghetti plots for two patient groups, supported by a local regression fit (Figure 9 and Figure 10).

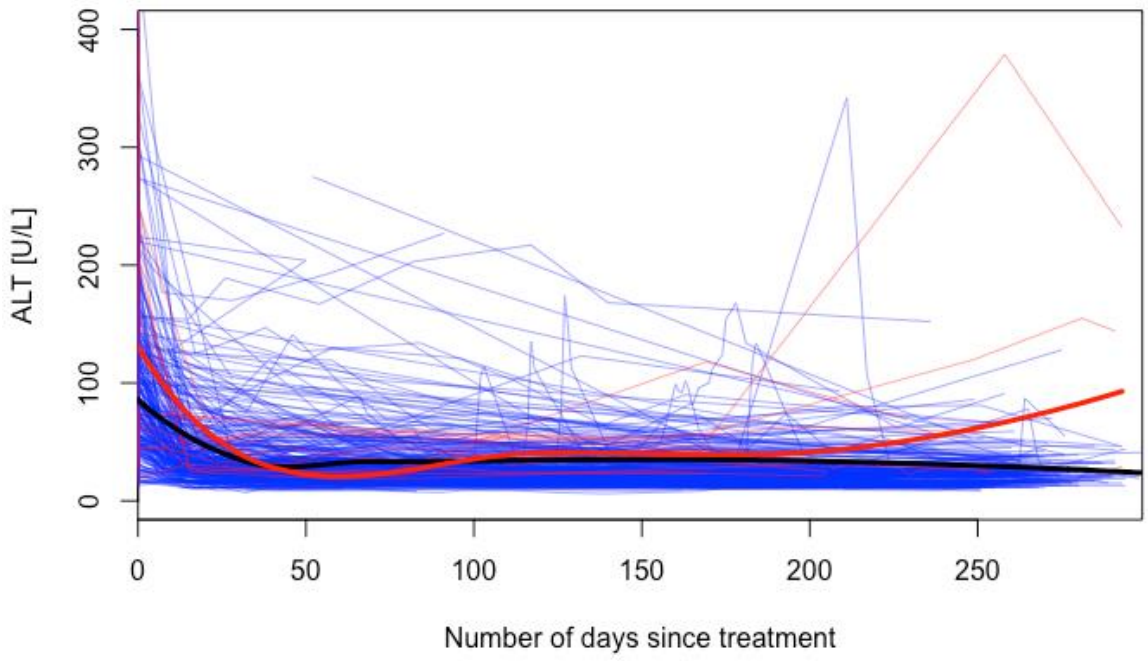


Figure 9: Spaghetti plot of ALT level during the treatment (blue, response group; red, non-response group)

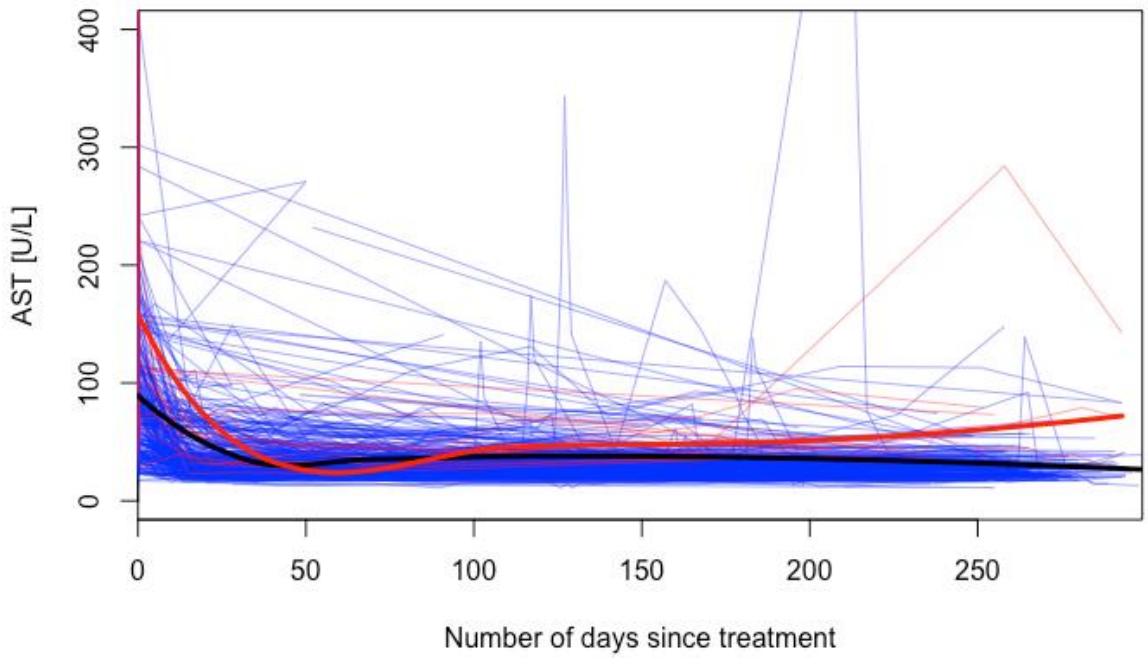
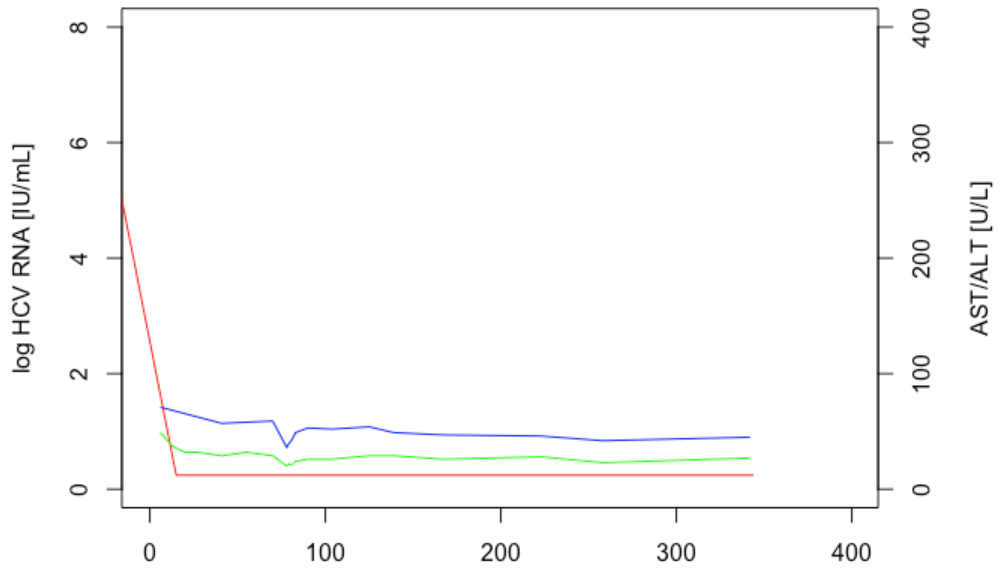
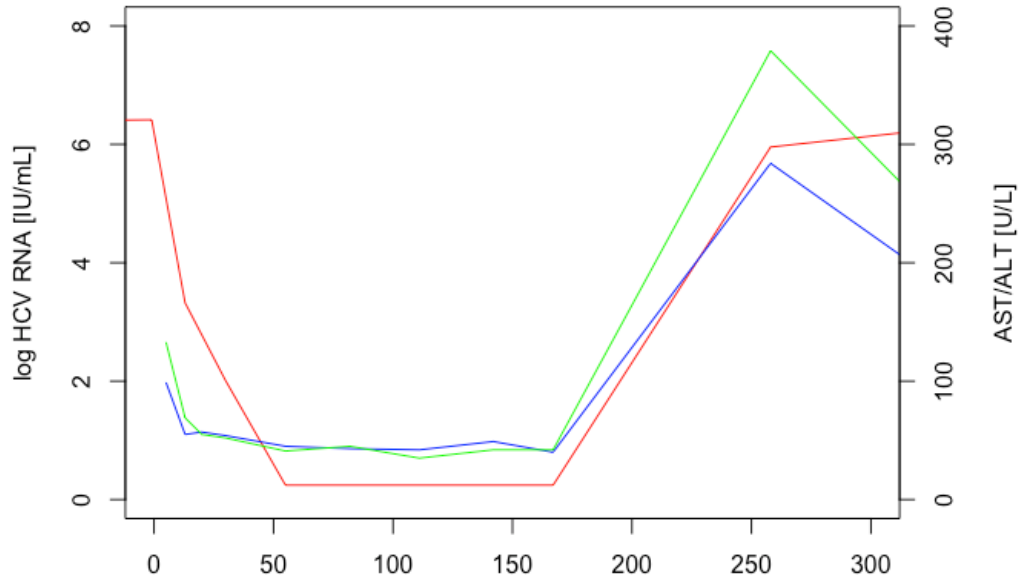


Figure 10: Spaghetti plot of AST level during the treatment (blue, response group; red, non-response group)

Figure 11 shows the viral and liver function tests over the monitoring course in a patient with SVR after 24 weeks of treatment and a patient with treatment relapse after 24 weeks of treatment.



a



b.

Figure 11: Monitoring profiles for HCV RNA (red), ALT (green), and AST (blue) of a representative patient (male, 53 years, genotype 1a) with SVR after 24 weeks of treatment with sofosbuvir + daclatasvir (a) and of a patient (male, 57 years, genotype 1b) with relapse after 24 weeks of treatment with sofosbuvir + ledipasvir (b).

6. Analysis of drug concentration

We analyzed the concentration values of sofosbuvir, its metabolite GS331007, daclatasvir, and ledipasvir. We did not consider the concentrations of drugs measured at the peak or in the middle of the dosing interval. Assuming that the trough levels (measured at the end of the dosing interval) were the most informative ones, we had drug 95 concentration values for 29 patients (85 values for 27 patients in the response group and only 8 values for 2 patients in the non-response group).

The mean concentration of drug in patients was 579±502 ng/ml for GS331007, 640±519 ng/ml for daclatasvir, and 345±212 ng/ml for ledipasvir. In the response group, the mean concentration was 596±521 ng/ml for GS331007, 652±528 ng/ml for daclatasvir, and 347±215 ng/ml for ledipasvir. In the non-response group, the mean concentration was 404±102 ng/ml for GS331007, 376±91 ng/ml for daclatasvir. Taken individually as if they were independent points, these valued would reveal significant differences between groups for GS331007 ($p=0.006$) and daclatasvir ($p=0.03$). Still a more correct analysis taking into account the clustering of concentration values measured on several occasions in the same patients failed to show significant differences. The concentration values are represented in Figure 12 and 13, suggesting graphically a trend for lower levels in non-responders.

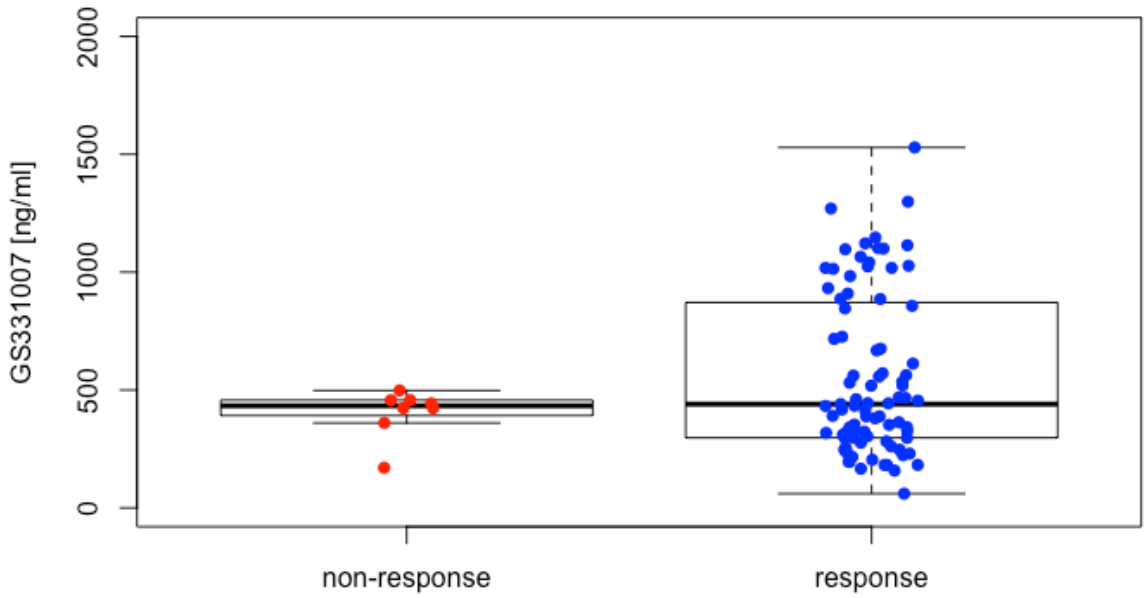


Figure 12: Concentration of GS331007 in non-response and response patients

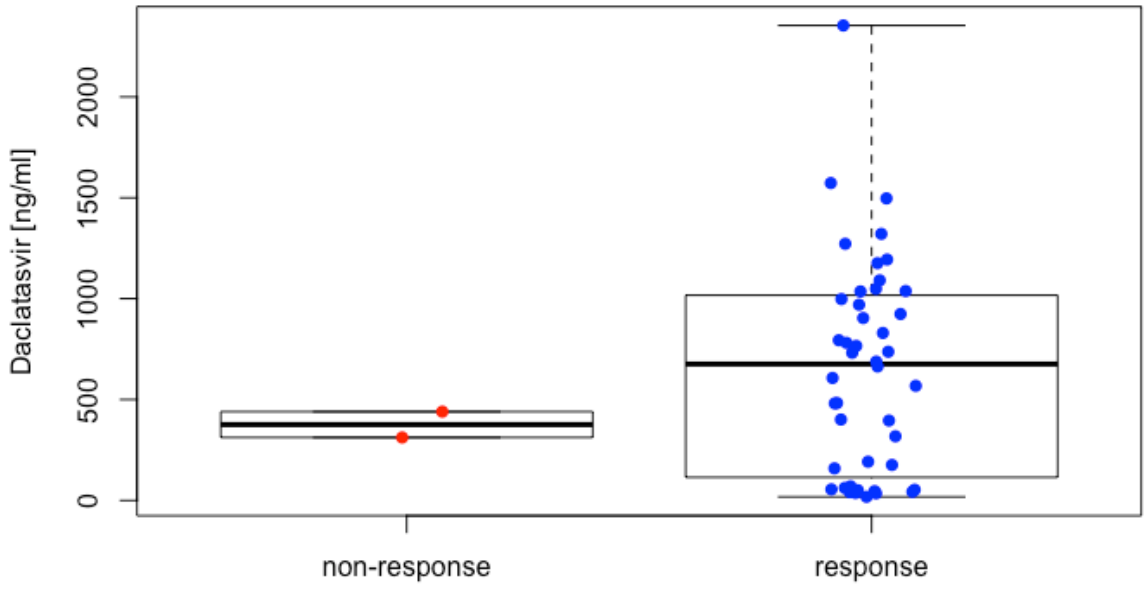


Figure 13: Concentration of daclatasvir in relapse and response patients

7. Prediction of the response to treatment

We used a logistic regression model to predict the response to treatment. We constructed a predictive model using individual covariates (all variables for which a univariate test (Student, Wilcoxon, Fisher) reported a significant change ($p \leq 0.1$)

The multivariate analysis showed that only past HCC ($p<0.0001$; OR, 0.06), HBV infection ($p=0.038$; OR, 0.19), and IL28B rs12979860 genotype CT ($p=0.02$; OR, 0.09) were independent predictors of treatment failure (Table 6).

Table 6: Predictors of the treatment response

Characteristic	Univariate analysis	Multivariate analysis	
	p value	OR (95% CI)	p value
Past HCC	0.002	0.06 (0.01-0.28)	<0.0001
HBV infection	0.11	0.19 (0.04-0.92)	0.038
IL28B rs12979860 genotype CT	0.12	0.09 (0.01-0.69)	0.02

It should be noted that neither hepatic tests, nor drug concentration were predictors of treatment response, either independently or added over the above variables into the model.

Discussion

The primary goal of HCV therapy is to achieve SVR, defined as undetectable HCV RNA 12 weeks (SVR12) or 24 weeks (SVR24) after treatment completion [8, 11]. The modern DAA therapy is associated with high SVR rate (more than 90%). In our study, 95.5% of patients had achieved the SVR.

The characteristics of our population do not markedly differ from large cohorts previously described, i.e. the most frequent HCV genotype is 1 (61%), followed by genotypes 3, 4, and 2; the most frequent Child-Pugh score is A (88%).

In our study, the non-response group is made only of patients with post-treatment relapse. We did not observe patients with immediate non-response or virological breakthrough. According to literature, the rate of virological breakthrough during DAA therapy is very low, about 1-2% [14], and most of non-response cases are explained by post-treatment relapse.

Patients in response and non-response groups had similar clinical characteristics, except for the rate of history of HCC which was significantly higher in the non-response group (56% vs 10%, p=0.002) and a higher prevalence of Child-Pugh scores B and C in non-response patients (33% vs 11%, p=0.045). We did not find statistically significant differences between both groups at baseline in main laboratory parameters such as platelet count, aPTT, AST, ALT, albumin, GGT, AFP, and HCV RNA viral load. At the same time, the level of total bilirubin, ALP, creatinine, and erythrocytes was significantly higher in non-response patients (all $p < 0.05$), in relation with the worse level of liver function captured by the Child score and HCC status.

With regard to predictive factors associated with non-response to therapy, various host and viral variables (e.g., gender, age, race, BMI, insulin resistance, advanced fibrosis stage, HCV genotype, presence of VIH, and viral load) had been well identified and were associated with non-response to Peg-IFN based therapies [15-17]. In patients treated with DAAs, the possible predictors of non-response were older age, cirrhosis, especially Child–Pugh class B and C, low platelet count, HCV genotypes 3 or 1a, elevated serum HCV RNA, prior hepatitis C treatment failure, poor drug adherence, and premature drug discontinuation [18-22].

Multivariate analysis showed that past HCC, HBV infection, and IL28B rs12979860 genotype CT are independent predictors of treatment failure. The

IL28B gene is involved in the immune response to certain viruses, including hepatitis C. People with the CC genotype have a stronger immune response to HCV infection than people with the CT or TT genotypes. It was shown in earlier studies that patients with the CC genotype are two to three times more likely to be cured by Peg-IFN and RBV, regardless of race or HIV status [23]. In the study of Prenner SB et al., the presence of active HCC at the initiation of HCV therapy is significantly associated with DAA treatment failure [24]. Sugiura A et al. showed that patients with history of HCC were independently associated with DAA treatment failure (OR, 3.56) [25]. Regarding on HBV infection status, Yek C et al. found that this infection did not have an impact on DAAs treatment response [26]. In other studies, HBV co-infection (past or current) also did not contribute to HCV therapy outcome [27].

All patients in our study received sofosbuvir. This drug was combined with ledipasvir in most of cases. We did not find significant differences between both groups in the type of drug or drugs combination used.

The initial goal of our study was to analyze whether HCV RNA level profiles during DAA therapy would predict the final treatment outcome. Consistent with already published data on rapid decrease of viral kinetics under DAA therapy, we did not find any relationship between viral kinetics and treatment response. The HCV RNA level at baseline, at week 2, week 4 and at the end of treatment failed to predict treatment response ($p>0.05$). Our results are I line with other studies. For example, a recent analysis by Fourati S et al. found that HCV RNA levels at the end of treatment could not differentiate between patients who achieving SVR or not [28]. In another study published in 2017, monitoring by HCV RNA during treatment with DAAs had only a limited predictive value for SVR, and the authors did not observe significant differences between response and non-response patients at 2 and 4 weeks after the start of treatment [29]. Furthermore, low levels of HCV RNA during treatment or at the end of treatment do not predict a relapse [30, 31], and this represents reasons for

simplification of the monitoring strategy. Similarly, our results do not indicate any usefulness of checking the decay of HCV RNA levels during treatment, as viral suppression seems to be guaranteed in all cases.

International clinical guidelines did not support either the monitoring of hepatic tests like ALT or AST during the HCV therapy. Our results essentially confirm this view. We did not find significant differences between response and non-response groups at baseline, at 2 and 4 weeks after the start of treatment, nor at the end of treatment.

In our study we measured the trough concentration of sofosbuvir, its metabolite GS331007, daclatasvir, and ledipasvir. We found that patients from non-response group had a trend for lower concentration of GS331007 and daclatasvir. We did not find a significant correlation between DAAs plasma concentration and HCV viral load kinetics during treatment. However, in some studies such correlations were reported [32, 33].

Our study has several limitations. First, it is a retrospective and observational analysis. Secondly, we had a limited number of detectable HCV RNA measurements during the treatment, which does not allow to describe the viral kinetics with an appropriate virologic response model. Moreover, we had very limited data about drug concentrations, especially for patients from the non-response group. It is thus impossible to express any strong statement about the potential usefulness of concentration monitoring during treatment with DAAs. There is only a slight signal suggesting that it might have some prognostic interest regarding SVR achievement, still warranting further confirmation.

In conclusion, our results do not support the regular monitoring of HCV RNA, AST, and ALT during treatment. Our results are thus essentially consistent with the last EASL recommendations on treatment of hepatitis C, i.e. that HCV RNA level are to be measured no three occasions only, i.e. at baseline and 12 or 24 weeks after the end of therapy (to assess SVR12 or SVR24, respectively) [8]. A potential role for drug concentration monitoring deserves further investigation.

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