Early virological assessment during telaprevir- or boceprevir-based triple therapy in hepatitis C cirrhotic patients who failed a previous interferon based regimen — The ANRS CO20-CUPIC study

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Summary

Background and objective: To assess within the ANRS CO20-CUPIC cohort whether the viral load (VL) at week 2/week 6 for telaprevir/boceprevir-based triple therapy, respectively, was predictive of sustained virological response (SVR) in patients with hepatitis C virus (HCV) infection and to study the relevance of this measurement to early diagnose drug resistance.

Methods: Observational study of HCV genotype 1 patients with compensated cirrhosis (Child-Pugh A), non-responders to a prior course of interferon (IFN)-based therapy and who started triple therapy. Patients received either 12 weeks of telaprevir in combination with peg-IFN/ribavirin (RBV), then 36 weeks of PEG-IFN/RBV, or 4 weeks of PEG-IFN/RBV, then 44 weeks of PEG-IFN/RBV and boceprevir.

Results: A total of 262 patients were analyzed. For telaprevir-treated patients, 28% had undetectable VL at W2 of whom 81% achieved SVR12 whereas 67% had undetectable VL at W4 of whom 67% achieved SVR12. For boceprevir-treated patients 20% had undetectable VL at W6 and 86% of them achieved SVR12 whereas 36% had undetectable VL at W8 among whom 73% achieved SVR12. Five telaprevir-treated patients had a VL increase between W2 and W4 after a decrease between D0 and W2. Four of them did not achieve SVR12. Similarly, six boceprevir-treated patients had a VL increase between W6 and W8 after a decrease between D0 and W6. Five did not reach SVR12.

Conclusions: The assessment of HCV RNA level after two weeks of triple therapy in cirrhotic non-responder patients is a good predictor of SVR. This assessment was useful to do an early diagnosis of viral breakthrough.

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Introduction

About 130–170 million people are chronically infected with the hepatitis C virus (HCV) worldwide[1], with 9 million infected in the United States and Western Europe [2,3]. During the past decade, the standard of care for HCV treatment was a combination of pegylated interferon (PEG-IFN) plus ribavirin (RBV) allowing a sustained virological response (SVR) rate of about 50% in patients with HCV genotype 1 [4,5]. Response to therapy varied according to genotype with genotype 1 and 4 patients achieving lower SVR rates than patients infected with genotype 2 or 3 (40–50% vs. >80%, respectively) [5,6]. Thus, the low SVR rate in genotype 1 and the poor response rates observed in special populations, such as black patients and cirrhotics [7,8] have driven the development of novel antiviral therapies and the combination of boceprevir or telaprevir—two protease inhibitors (PI) of NS3/NS4A proteins of HCV—with PEG-IFN/RBV has become the new standard of care since the end of 2011 [9,10]. In boceprevir-based triple therapy, results from phase III clinical trials reported 60% of SVR for naive patients (SPRINT-2 study [11]) and 75% and 52% for relapsers and previous partial non-responders respectively (RESPOND-2 study [12]). For telaprevir, SVR was reached in 75% of naive patients (ADVANCE [13] and ILLUMINATE [14] studies) and 83% of previous relapers, 59% of partial non-responders and 29% of previous null responders (REALIZE) [15].

Many studies in the literature including observational studies, clinical trials and meta-analyses, have conclusively shown that a rapid virological response (RVR) defined as an undetectable HCV RNA at week 4 of PEG-IFN/RBV combination therapy was highly associated with SVR in genotype 1 patients [16]. In boceprevir-treated patients, a >1 log_{10} decrease of HCV RNA at week 4 (end of lead-in-phase) is associated with SVR. In SPRINT-2 and RESPOND-2 studies, 88% and 86% of patients with undetectable HCV RNA at week 8 (week 4 of boceprevir) subsequently achieved SVR [11,17]. For triple therapy, the notion of an extended rapid virological response (eRVR), defined as undetectable HCV RNA at week 8 maintained until week 24 for boceprevir, and as undetectable HCV RNA at week 4 maintained until week 12 for telaprevir has been introduced. In telaprevir-treated patients, the REALIZE study conducted in patients previously non-responders to PEG-IFN/RBV combination therapy, reported that the eRVR was the highest predictive factor of SVR [15,18]. Similarly, in the French CUPIC cohort, the virological response at week 4 for telaprevir-treated patients and week 8 for boceprevir-treated patients, was associated with SVR [19]. In a recently published paper, we showed that the positive predictive value (PPV) of eRVR was 87% for telaprevir and 56% for boceprevir [20]. However, the predictive impact of an earlier detection of HCV RNA remains unknown.

French guidelines recommended the measurement of HCV RNA level after two weeks of triple therapy in order to early detect the emergence of resistance mutations [21].

In this study, we assessed whether the viral load at week 2/week 6 for telaprevir/boceprevir-treated patients, respectively, was predictive of SVR and assessed the relevance of this measurement to early detect resistance mutations and to adapt therapeutic strategies.

Material and methods

Patients

The ANRS CO20-CUPIC cohort (ClinicalTrials.gov number NCT01514890) is a national multicenter prospective cohort study conducted in 56 French centres. From February 2011 to April 2012, patients with compensated cirrhosis...
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(Child-Pugh class A) chronically infected with HCV genotype 1, who did not achieve SVR after a prior course of IFN-based therapy and who started triple therapy, were recruited. Initially, only relapsers and partial responders were eligible in the French early access program. Since the approval of both PIs, the inclusion criteria were amended in September 2011 allowing the inclusion of null responders. Patients received either 12 weeks of telaprevir in combination with PEG-IFN/RBV, then 36 weeks of PEG-IFN/RBV, or 4 weeks (lead-in phase) of PEG-IFN/RBV, then 44 weeks of PEG-IFN/RBV and boceprevir, according to the European label.

In the present study, only patients with viral load assessment at week 2 for telaprevir or week 6 for boceprevir were kept for the analysis. Patients with HIV or HBV co-infection, renal insufficiency (defined by creatinine clearance < 50 mL/min) or organ graft were not eligible for inclusion. More details on patients’ inclusion in the CUPIC cohort were given previously [19,22].

HCV RNA level monitoring

HCV RNA levels were measured at baseline and at weeks 2, 4, 6, 8, 12, 16, 24, 36, and 48 of therapy, and 12 weeks after the end of treatment, with a real-time PCR based assay, either COBAS AmpliPrep/COBAS TaqMan (Roche Molecular Systems, Pleasanton, California) with a lower limit of detection of 15 IU/mL, or m2000SP/m2000RT (Abbott Molecular, Des Moines, Illinois), with a lower limit of detection of 12 IU/mL. Both assays have been validated for their accuracy in patients infected with HCV genotype 1 [23,24].

Treatments

Treatment was prescribed at the discretion of each investigator without randomization, which precludes any comparison between the two treatment regimens.

Statistical analyses

Quantitative variables were presented as the mean ± standard deviation. Categorical variables were studied using the two-sided Chi² test whereas quantitative variables were analyzed using t-test. A ROC curve analysis was performed to study the viral load decrease after two weeks of triple therapy as a predictor of SVR. A logistic regression analysis was also conducted to identify factors potentially associated with SVR. Statistical analysis was performed using SPSS v.19.0 for Windows (SPSS Inc., Chicago, Illinois, USA). All statistical tests were two-sided and a P value < 0.05 was considered statistically significant.

Ethical consideration

Written informed consent was obtained from each patient before enrolment. The protocol was conducted in accordance with the Declaration of Helsinki and French law for biomedical research and was approved by the “Ille de France IX” Ethics Committee (Créteil, France).

Results

Patients’ characteristics

Data on 262 patients from the ANRS CO20-CUPIC initiating triple therapy with boceprevir or telaprevir combined with PEG-IFN/RBV were analyzed. Twenty-two patients who received a 4-week PEG-IFN/RBV lead-in phase before telaprevir-based triple therapy were previously excluded. Patients’ characteristics are presented in Table 1. Male gender was predominant in both groups of patients (74.4% for boceprevir and 70.9% for telaprevir) and mean age was 59.3 years (± 8.5) and 58.7 years (± 10.5) for boceprevir and telaprevir, respectively. HCV genotype 1b was more predominant than 1a in both groups of patients (62.0% and 62.3% respectively). Previous treatment status was similar in both groups with about 15–19% of null responders, 42–46% of partial non-responders and 33-37% of relapers.

Patients’ characteristics are similar to those from the overall CUPIC cohort in terms of gender, age, BMI, HCV subtype (1a/1b), previous treatment response and IL28B polymorphism [19,22].

Predictive factors of virological response

The SVR12 rate was 55% for telaprevir and 46% for boceprevir. For telaprevir-treated patients, 28% had undetectable viral load at week 2 of whom 81% achieved SVR12 (positive predictive value, PPV) whereas 67% had undetectable viral load at week 4 of whom 67% achieved SVR12 (Fig. 1A). For boceprevir-treated patients, 20% had undetectable viral load at week 6 and 86% of them achieved SVR12 whereas 36% had undetectable viral load at week 8 among whom 73% achieved SVR12 (Fig. 1B). On the other hand, 55% of patients with a detectable viral load at week 2 and 63% of patients with a detectable viral load at week 6 had no SVR12 (negative predictive values, NPV). For telaprevir-treated patients, univariate analysis indicated that predictive factors of undetectability at week 2 were low initial viral load (P = 0.001) and previous treatment response with 19% of undetectability among NR patients, 20% among partial NR and 41% among relapers (P = 0.012). For boceprevir-treated patients, the only predictive factors of undetectability at week 6 were IL28B genotype (P = 0.050) and previous treatment response with 7% of undetectability among NR patients, 13% among partial NR and 35% among relapers (P = 0.047). For telaprevir-treated patients, a multivariate logistic regression analysis indicated that only initial viral load remained associated with undetectability at week 2 whereas for boceprevir-treated patients, no factor remained associated with undetectability at week 6.

Percentage of viral load decrease between treatment initiation (D0) and week 2/week 6 was calculated. Using a ROC curve analysis, cut-off values which best predicted SVR were then determined. For telaprevir, a 70% decrease of viral load between D0 and week 2 gave the best compromise sensitivity/specificity (AUC = 0.612; P = 0.037; Se = 53%, Spe = 58%) and for boceprevir, a 50% decrease between D0 and week 6 gave the best ratio sensitivity/specificity (AUC = 0.759; P < 0.001; Se = 86%, Spe = 58%). Three patient profiles were
Table 1 Patients’ characteristics at triple therapy initiation.

<table>
<thead>
<tr>
<th>Characteristics (n = 262)</th>
<th>Boceprevir (n = 90)</th>
<th>Telaprevir (n = 172)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male gender n (%)</td>
<td>67 (74.4)</td>
<td>122 (70.9)</td>
</tr>
<tr>
<td>Age, mean ± SD</td>
<td>59.3 ± 8.5</td>
<td>58.7 ± 10.5</td>
</tr>
<tr>
<td>BMI, mean ± SD</td>
<td>26.1 ± 4.2</td>
<td>26.6 ± 4.1</td>
</tr>
<tr>
<td>HCV genotype n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1a</td>
<td>30 (38.0)</td>
<td>58 (37.7)</td>
</tr>
<tr>
<td>1b</td>
<td>49 (62.0)</td>
<td>96 (62.3)</td>
</tr>
<tr>
<td>1 unspecified</td>
<td>11</td>
<td>18</td>
</tr>
<tr>
<td>Previous treatment response n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Null response</td>
<td>13 (15.5)</td>
<td>32 (19.3)</td>
</tr>
<tr>
<td>Partial non-responder</td>
<td>39 (46.4)</td>
<td>69 (41.6)</td>
</tr>
<tr>
<td>Relapser</td>
<td>28 (33.3)</td>
<td>62 (37.3)</td>
</tr>
<tr>
<td>Viral breakthrough</td>
<td>4 (4.8)</td>
<td>3 (1.8)</td>
</tr>
<tr>
<td>Non-available</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>IL28B genotype n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C/C</td>
<td>10 (16.1)</td>
<td>14 (12.2)</td>
</tr>
<tr>
<td>C/T</td>
<td>43 (69.4)</td>
<td>80 (69.6)</td>
</tr>
<tr>
<td>T/T</td>
<td>9 (14.5)</td>
<td>21 (18.2)</td>
</tr>
<tr>
<td>Non-available</td>
<td>28</td>
<td>57</td>
</tr>
</tbody>
</table>

defined according to their viral load decrease at week 2 or week 6, respectively: patients with a decrease of less than 70% (or 50%), patients with a decrease of more than 70% (or 50%), and patients with undetectable viral load at week 2 (or week 6 for boceprevir). Among telaprevir-treated patients, 60% of those with a >70% decrease in viral load at week 2 achieved SVR12 (PPV) whereas 63% of patients with a decrease <70% did not achieve SVR12 (NPV). For

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![Figure 1](image_url)  
**Figure 1** Sustained virological response rate (SVR) according to early HCV RNA undetectability. A. Telaprevir-treated patients. B. Boceprevir-treated patients.

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boceprevir-treated patients, PPV and NPV were 56% and 87%, respectively for a viral load decrease of 50%.

A decision tree using SVR12 as dependent variable was performed including previous treatment response and week 2 virological responses. For telaprevir-treated patients (Fig. 2A), previous treatment response was highly predictive of subsequent treatment response with SVR12 rates varying from 22% in previous null responders, 43% in partial NR, and 81% in relapsers/breakthrough. Among previous null responders, week 2 virological response was a good predictor of SVR12 with 67% of patients undetectable at week 2 reaching SVR12 compared with only 11% of patients detectable at week 2 (P = 0.010). Similarly, among partial NR with undetectable viral load at week 2 (or decrease >70% of initial viral load), 61% achieved SVR12 vs. 24% of those with a decrease <70% (P = 0.006). Among relapsers/breakthrough, the SVR12 rate varied from 68% in patients with viral load decrease <70% at week 2 to 87% in those being undetectable or with a decrease >70% (P = 0.080).

Among boceprevir-treated patients, virological response at week 6 was also associated with SVR12 both in partial NR (P = 0.001) and in relapsers/breakthrough (P = 0.039; see Fig. 2B). In the NR group, the number of patients was too limited to assess the effect of viral load at week 6.

Early detection of viral breakthrough

Among telaprevir-treated patients, five had an increase of their viral load between week 2 and week 4 after a decrease of their initial viral load between D0 and week 2 (Fig. 3A). Four of them did not achieve SVR12 despite a global decrease of their initial viral load between D0 and week 4. Similarly, six boceprevir-treated patients had an increase of their viral load between week 6 and week 8 after a decrease of their initial viral load between D0 and week 6 (Fig. 3B). Five of them did not reach SVR12 despite a global decrease of their initial viral load between D0 and week 8.

Discussion

This sub-analysis of the ANRS CO20-CUPIC cohort focused on the role of the assessment of HCV RNA level two weeks after the introduction of a protease inhibitor with or without a lead-in phase of PEG-IFN/RBV. Despite French guidelines which recommended the measurement of HCV RNA level at week 2 of triple therapy, only about 43% of all CUPIC patients were evaluated at week 2/week 6. However, patients analyzed in our study did not differ from the overall CUPIC cohort, which precludes any selection bias.

During PEG-IFN/RBV double therapy, early HCV RNA undetectability at week 4 was associated with a high positive predictive value of SVR whereas patients with detectable HCV RNA level at week 4 had a low probability of achieving SVR. This early undetectability of HCV RNA was even more strongly associated with SVR than IL28B genotype. With the recent use of more effective DAA, HCV RNA undetectability appears earlier during therapy and most patients have undetectable viral load at week 4 of triple therapy (68% of naive patients for telaprevir and 57% of naive patients for boceprevir) [11,13]. Among these patients more than 80% achieve SVR. Among treatment-experienced patients the proportion of HCV RNA undetectability at week 4 is lower but this undetectability is still associated with SVR. This fast virologic response led us to evaluate the role of viral load assessment at week 2 of triple therapy in order to identify a potential earlier predictive factor of SVR. The positive predictive values of SVR associated with the undetectability at week 2 were high (81% and 86% for telaprevir and boceprevir, respectively) and even higher than those at week 4 of triple therapy (67% and 73% for telaprevir and boceprevir, respectively). Conversely, the negative predictive value was really low (55% and 63%). Viral load assessment at week 2 can thus hardly be used to early detect a non-response to triple therapy and to stop therapy in these patients. Indeed, 45% and 37% of patients treated with telaprevir and boceprevir, respectively and with detectable HCV RNA at week 2 still achieved SVR12.

Using a ROC curve analysis, we determined the thresholds of HCV RNA decrease between D0 and week 2 that could predict SVR12. However, the prognostic value of these thresholds is not high enough and cannot be used for response guided therapy.

A decision tree indicates that among cirrhotic non-responder patients treated with telaprevir, only 11.5% of those with a detectable HCV RNA at week 2 achieved SVR12. Among this subgroup, the too limited number of patients did not allow us to distinguish between those with a viral load decrease < and >70%. However, since patients with cirrhosis have a high likelihood of developing severe side effects while on first generation triple therapy, it is probable that treatment cessation could be considered in previous null responder patients failing to achieve a 70% decrease of viral load at week 2. A similar strategy could be discussed in partial non-responders with an HCV RNA decrease <70% at week 2 since SVR12 is only achieved in 24% of cases. However, in patients with previous relapse or breakthrough with HCV RNA decrease <70% at week 2, virological assessment should be confirmed at week 4. Since this study was conducted on a group of cirrhotic non-responder patients (difficult-to-treat and with characteristics which may impact the initial viral load decrease), our results are not representative of the overall HCV population. Unfortunately, data on IL28B genotype was only available in 73% and 66% of telaprevir- or boceprevir-treated patients, respectively. Nevertheless, as already reported, the impact of IL28B genotype on treatment response seems less important during triple therapy, and more particularly for telaprevir [18].

The CUPIC study has shown that telaprevir- or boceprevir-based triple therapy was associated with a poor tolerance in cirrhotic patients [19,22]. In such patients developing severe side effects and for whom the probability of achieving virological response is limited, early treatment discontinuation could be proposed. In this context, virological assessment at W2 could be useful to limit triple therapy based strategies to patients with high probability of achieving virological response.

The second potential role of HCV RNA assessment at week 2/week 6 of therapy is to detect viral breakthrough early. During phase I and phase II clinical trials, an increase of HCV RNA level was observed just after a quick initial decrease of HCV RNA during the first week of triple therapy suggesting the possible emergence of a new viral strain with PI
resistance mutation. Under DAA pressure, this resistant viral population will increase rapidly and become the major viral population. At the end of therapy, the new viral strain with resistance mutation will become progressively undetectable but can survive until 2 years after the end of treatment. Thus, it was recommended to stop treatment as soon as possible after the emergence of a viral resistance strain [21,25].

In our study, 11 patients had an early viral breakthrough characterized by a decrease of HCV RNA level during the first 2 weeks of triple therapy followed by an increase of HCV RNA level between week 2 and week 4. Nine of these patients did not achieve SVR. If the viral load was not assessed at week 2, this viral breakthrough would not have been detected until week 8 of triple therapy, with as a result, long-term virological, clinical and psychological consequences for the patients.

Even if these situations are rather uncommon, the potential consequences justify the viral load assessment at week 2/week 6. Virological monitoring at week 2/week 6 is thus relevant both for the early detection of resistance mutation allowing early treatment discontinuation, but also for the early assessment of treatment compliance.

However, the use of first generation PI-based triple therapy is today questionable and not anymore recommended by most of current guidelines. Emerging therapies with DAA-based IFN-free regimens may thus limit the interest of our findings. However, because of cost issues, access to these new therapies is still limited in many parts of the world [26] where PI-based therapy may remain a treatment option to treat patients who failed previous PEG-IFN/RBV-based therapy. Moreover, beside IFN-free regimens, the latest EASL recommendations for HCV genotype 1 patients still include PEG-IFN/RBV combined with either sofosbuvir, simeprevir, or daclatasvir [27]. In that respect, the results of our study could provide new clinical meaningful information for a better management of PEG-IFN/RBV-based triple therapy.

In conclusion, the assessment of HCV RNA level two weeks after the introduction of a PI in cirrhotic non-responder patients is a good predictive factor of SVR. This could be useful in patients who had previously failed a PEG-IFN/RBV therapy, or in cirrhotic patients with high risk of side effects. In such patients, treatment could be discontinued in those with a low probability of response. Moreover, HCV RNA assessment at week 2 may be useful to detect early viral resistance.

Figure 2 Decision tree giving the proportion of patients achieving SVR12 according to previous treatment response and viral load at week 2/week 6. In each box, the probability of achieving SVR12 is given. Figures slightly differ from the total number of cases since only patients with information on previous treatment response are included. A. Telaprevir- treated patients. B. Boceprevir-treated patients.
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Figure 3 Patients with early virological rebound. A. Telaprevir-treated patients with virological rebound between W2 and W4. B. Boceprevir-treated patients with virological rebound between W6 and W8.

breakthrough. In such cases, treatment can be withdrawn earlier to prevent the outgrowth of viral resistant strains.

Disclosure of interest
The authors declare that they have no conflicts of interest concerning this article.

Authors’ contribution
FB, VV, CD, PP, FC, and FZ contributed substantially to the conception and design of the study and to the analysis of data.
CH, DL, LA, DS, MB, SM, JPZ, HF, VLR, LS, and JPB contributed substantially to the acquisition and interpretation of data.
All authors contributed to the drafting of the manuscript and to revising it critically for important intellectual content and all approved the final version to be published.
All authors agreed to be accountable for all aspects of the work.

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Appendix A. Supplementary data
Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.clinre.2014.12.007.

References