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Hepatitis C virus RNA levels at week-2 of telaprevir/boceprevir administration are predictive of virological outcome☆

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ABSTRACT

Background: Triple therapy with telaprevir/boceprevir + pegylated-interferon + ribavirin can achieve excellent antiviral efficacy, but it can be burdened with resistance development at failure.
Aims: To evaluate kinetics of hepatitis C virus (HCV) RNA decay and early resistance development, in order to promptly identify patients at highest risk of failure to first generation protease inhibitors.
Methods: HCV-RNA was prospectively quantified in 158 patients receiving pegylated-interferon + ribavirin + telaprevir ($N = 114$) or + boceprevir ($N = 44$), at early time-points and during per protocol follow-up. Drug resistance was contextually evaluated by population sequencing.
Results: HCV-RNA at week-2 was significantly higher in patients experiencing virological failure to triple-therapy than in patients with sustained viral response (2.3 [1.9–2.8] *versus* 1.2 [0.3–1.7] log IU/mL, $p < 0.001$). A 100 IU/mL cut-off value for week-2 HCV-RNA had the highest sensitivity (86%) in predicting virological success. Indeed, 23/23 (100%) patients with undetectable HCV-RNA reached success, *versus* 26/34 (76.5%) patients with HCV-RNA < 100 IU/mL, and only 11/31 (35.5%) with HCV-RNA > 100 IU/mL ($p < 0.001$). Furthermore, differently from failing patients, none of the patient with undetectable HCV-RNA at week-2 had baseline/early resistance.

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Conclusions: With triple therapy based on first generation protease inhibitors, suboptimal HCV-RNA decay at week-2 combined with early detection of resistance can help identifying patients with higher risk of virological failure, thus requiring a closer monitoring during therapy.

1. Introduction

According to the HCV-kinetics model, initially based on interferon (IFN) monotherapy [1], antiviral treatment of chronic hepatitis C leads to a biphasic decay of plasma HCV-RNA. Initially, treatment acts by blocking viral production, determining a very fast first phase of HCV-RNA decline characterized by the clearance of free circulating virions. Afterwards, the progressive clearance of infected cells determines a much slower second phase of viraemia decline. This model was later confirmed also in pegylated IFN (pegIFN), pegIFN + ribavirin (RBV) and in treatments including direct acting antiviral agents (DAAs), such as telaprevir (TVR) [2–6].

With IFN treatment, the dividing line between first and second phase was set at day 2 [1], but in the context of TVR-treatment, viral dynamics are much more rapid and the abovementioned line may be moved backwards [3].

TVR and boceprevir (BOC), approved by the U.S. Food and Drug Administration (FDA) and European Medicines Agency (EMA) in 2011, are the first-generation protease inhibitors (PIs) currently available in clinical practice. Both are administered using a response-guided protocol, in which viral decline determines treatment-duration [7–11]. All guidelines set the first viraemia check-point at week-4. Nevertheless, given the rapid HCV-dynamics during PI-based triple therapy, earlier time-points may be additionally informative on expected treatment outcome, and therefore become useful in clinical practice.

Moreover, a typical feature of HCV is the ability to develop/select resistance associated variants (RAVs) during treatment, as a consequence of potential natural resistance and low genetic barrier of first-generation PIs [12–14]. Virological-failure to TVR and BOC is indeed associated with RAVs development in the vast majority of cases [15–18].

When RAVs are present at baseline, either as major viral population or as minority variants, they could greatly affect viral response to treatment, particularly in monotherapy, determining a suboptimal viral decay and thus further increasing in resistance level [14,19–26]. This point should be taken into account to fully determine the kinetics of HCV-RNA decay.

In the present study, a large heterogeneous population of patients infected with HCV genotype 1 treated with TVR- or BOC-based triple-therapy was analyzed, in order to investigate HCV-kinetics according to patients' complexity in real-life settings. The kinetics of viral response was assessed shortly after PI's start, and was correlated with both clinical outcome and viral genetic background, focusing on baseline/early detection of RAVs. Several cut-offs categorizing early HCV-RNA decay were then evaluated, in order to provide a useful tool for the monitoring of virological response to first-generation PIs in clinical practice.

2. Methods

2.1. Patients

Chronically HCV genotype 1 infected patients, consecutively seen at several Italian clinical centres between January 2011 and August 2013 and starting a triple-therapy based on PegIFN/RBV plus BOC or TVR, were considered for inclusion. Only patients with available treatment outcome were considered for the analysis. Exclusion criteria were age under 18 years and other chronic liver diseases. Patients who stopped triple-therapy early for any other reasons than virological breakthrough or stopping rules were also excluded. Treatment schedules and stopping rules followed TVR/BOC prescribing information [8,9]. The choice between a BOC- or TVR-based regimen was at the investigator's discretion.

This study was conducted in accordance with the principles of the Declaration of Helsinki and approved by the local Ethics Committees. All enrolled patients provided written informed consent.

2.2. Patients monitoring

Fibrosis staging was determined using either Fibroscan® (Echosens, Paris, France), Fibrotest® (Biopredictive, Paris, France) or liver biopsy, and interpreted by an expert pathologist.

HCV-RNA viral load quantification was performed using the COBASAmpliprep/COBASTaqMan HCV quantitative test v2.0 (Roche Diagnostics) or Abbott RealTime HCV assay (Abbott Laboratories, Abbott Park, IL, USA) with lower limit of detection (LLOD) of 15 and 12 IU/mL, respectively. In addition to standard viraemia check-points [7], HCV-RNA was also determined at 48 h, week-1 and week-2 after PI start (TVR or BOC).

Plasma samples were collected and stored at −80 °C after each visit.

2.3. NS3-protease sequencing

Genotypic resistance test (GRT) on NS3-protease sequences (aa 1–181) was performed by an home-made population-sequencing protocol as elsewhere described [12]. Baseline-GRT was performed for 110 patients included in the analysis, on the basis of samples' availability. For 39 patients was also available an additional GRT at early time points, between 48 h and week-4 of triple-therapy.

The following PI RAVs were considered in the analyses: 36AGLM, 41R, 43ISV, 54ASV, 55IA, 80K, 155IKMQT, 156GSTV, 168AEGNTVY and 170AT.

2.4. Statistical analysis

Results are expressed as median values and interquartile range (IQR). Values were compared using the Mann–Whitney *U*-test.

Sensitivity, specificity and positive predictive values were calculated to evaluate the prediction of virological-success in relation to HCV-RNA values after 48 h, 2 weeks and 4 weeks since PI-start. Correlation coefficient between baseline HCV-RNA and HCV-RNA at 48 h and at week-2 of triple therapy was determined using Spearman rank correlation test. A ROC curve analysis was used to determine the optimal HCV-RNA cut-off for treatment outcome prediction.

Linear logistic regression analysis was used to estimate the association between sustained viral response (SVR) and HCV-RNA values at week-2 and week-4 since PI-start, stratified according to the prediction cut-off. HCV-genotype, gender, age, diagnosis of cirrhosis, null-response to previous pegIFN + RBV administrations,