Clinical efficacy of the highly sensitive hepatitis C virus RNA quantitative assay in patients with relapse following interferon-based therapy with second-generation direct-acting antivirals

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Abstract. For refractory chronic hepatitis C, interferon (IFN)-based triple-agent combination therapy with second-generation direct-acting antivirals (DAAs) has been established as the standard treatment method. The rate of decrease in the viral load and the negative conversion of hepatitis C virus (HCV) RNA in the early phase following treatment initiation are considered important factors for predicting the therapeutic outcome. In the present study, the Roche Cobas AmpliPrep/COBAS TaqMan (CAP/CTM) HCV v2.0 assay and the AccuGENE m-HCV RNA quantitative assay [Abbott RealTime HCV (ART) assay] were analyzed for their clinical efficacy and ability to predict therapeutic outcomes in the early phase in patients with relapse following IFN-based second-generation DAA therapy. Of the 56 patients who received IFN-based second-generation DAA therapy since December 2013, 6 achieved an end-of-treatment response (ETR), but subsequently experienced relapse. In these 6 patients, fluctuations in viral loads in the early phase detected by the CAP/CTM and ART assays were compared. At 4 weeks after treatment initiation, 4 of the 6 patients were diagnosed as negative by the CAP/CTM assay, whereas 2 of these 4 patients were not identified as negative by the ART assay. Of the 2 patients, one was signal-positive with an HCV RNA load <1.08 Log IU/ml, and the other patient had a viral load of 1.12 Log IU/ml. At 8 weeks after treatment initiation, 1 patient was found to be negative by the CAP/CTM assay, but signal-positive with a viral load <1.08 Log IU/ml by the ART assay. From 4 to 8 weeks after treatment initiation, 3 of the 6 patients appeared to be discrepant cases. In conclusion, of the 6 patients who achieved an ETR, 4 were determined to have achieved a rapid virological response (RVR) by the CAP/CTM assay, but may not have actually become negative. The ART assay is highly sensitive, has a wide measurement range, may be suitable for monitoring HCV RNA loads, and is expected to have an important role in providing a predictive marker for early therapeutic outcomes. In discrepant cases in which no RVR is proved by either assay, it was assumed important to consider continuation of treatment and to attempt to achieve a sustained virological response.

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Introduction

At present, for refractory chronic hepatitis C, interferon (IFN)-based triple-agent combination therapy with second-generation direct-acting antivirals (DAAs) has been established as the standard treatment method. In IFN-based therapy, negative conversion of hepatitis C virus (HCV) RNA in the early phase following treatment initiation is considered an important factor for predicting treatment outcomes (1-4).

Recently, HCV-RNA measuring systems that are more sensitive compared to conventional HCV-RNA qualitative assays have been developed and reported to be clinically useful (5,6).

The AccuGENE m-HCV RNA quantitative assay [Abbott RealTime HCV (ART) assay], which was developed as a highly sensitive HCV RNA quantitative assay by Abbott Laboratories (Abbott Park, IL, USA), has a minimum detection sensitivity of 12 IU/ml and enables the quantification and detection of lower viral loads compared to that allowed by the conventional Cobas TaqMan HCV quantitative assay [Cobas AmpliPrep/Cobas TaqMan (CAP/CTM) assay], developed by Roche Diagnostics (Basel, Switzerland).

In the present study, these two highly sensitive HCV-RNA quantitative assays were compared and analyzed for their ability to predict therapeutic outcomes in patients with relapse following triple-agent combination therapy with pegylated interferon (PEG-IFN), ribavirin (RBV) and simeprevir (SMV).

Subjects and methods

Study design. Between December 2013 and October 2015, patients received 100 mg/day SMV *per os* (Sovriad; Janssen Pharmaceutical K.K., Tokyo, Japan), combined with weekly

subcutaneous injections of 1.5 μg/kg of PEG-IFN α2b (Peg-Intron with weekly subcutaneous and *per os* administration of 600-1,000 mg/day of RBV (Rebetol; MSD) in accordance with the prescribing information for 12 weeks followed by PEG-IFN α2b and RBV between weeks 12 and 24.

Dose reductions or discontinuation of SMV, PEG-IFN and RBV were according to the judgment of the treating physicians. Patients were followed up for ≥24 weeks after the final treatment administration to assess the sustained virological response (SVR).

SVR24 was defined as undetectable serum HCV RNA levels at 24 weeks after the end-of-treatment (EOT).

Patients. The present study included 6 patients with genotype 1 chronic hepatitis C with a high viral load who achieved an EOT response (ETR) but subsequently experienced a relapse.

Assays. The CAP/CTM assay, which is the first-generation COBAS® assay developed by Roche Diagnostics, and the ART assay, developed by Abbott Laboratories, were used for the measurements, following the manufacturer's protocols.

Viral kinetics in the HCV-RNA loads detected by these two assays were compared and analyzed at each measurement point on treatment weeks 2, 4, 8, 12, 16, 20 and 24, at EOT and at 24 weeks after EOT. A negative response signifies no detection of HCV RNA.

Results

Patient characteristics. The patient clinical characteristics are shown in Table I. The mean age was 73.8±6.5 years, and 3 patients (50%) were male and 3 (50%) were female. The interleukin-28B major allele was present in 1 patient, and the minor allele was present in the others.

Assay diagnosis. At 4 weeks after treatment initiation, 4 of the 6 patients were diagnosed as negative by the CAP/CTM assay, whereas 2 of these 4 patients were not found to be negative by the ART assay. Of the 2 patients, one was signal-positive with an HCV RNA load <1.08 Log IU/ml, and the other patient had a viral load of 1.12 Log IU/ml. At 8 weeks after treatment initiation, 1 patient was found to be negative by the CAP/CTM assay, but signal-positive with a viral load <1.08 Log IU/ml by the ART assay of one patient. From 4 to 8 weeks after treatment initiation, 3 of the 6 patients appeared to be discrepant cases (Table II).

Table I. Clinical characteristics.

Case no.	Age, years	Gender	IL-28B allele	Previous type of response
1	72	Male	Major	SOC relapse
2	66	Male	Minor	SOC null response
3	77	Female	Minor	SOC relapse
4	85	Female	Minor	SOC relapse
5	71	Female	Minor	Naïve
6	72	Male	Minor	SOC relapse

IL-28B, interleukin-28B; SOC, standard of care (peginterferon/ribavirin).

Table II. Comparison of the viral kinetics between the ART and CAP/CTM assays.

		Viral kinetics value, Log IU/ml		
Case no.	Assay	Pre-treatment	4 weeks	8 weeks
1	ART	6.3	ND	ND
	CAP/CTM	6.2	ND	ND
2	ART	6.2	<1.08	ND
	CAP/CTM	6.2	<1.2	ND
3	ART	5.9	1.37	<1.08
	CAP/CTM	6.1	<1.2	ND
4	ART	6.1	1.12	ND
	CAP/CTM	6.1	ND	ND
5	ART	6.3	<1.08	ND
	CAP/CTM	6.2	ND	ND
6	ART	7.1	ND	ND
	CAP/CTM	7.2	ND	ND

ART, Abbott RealTime hepatitis C virus (HCV) assay (AccuGENE m-HCV RNA quantitative assay assay); CAP/CTM, Cobas AmpliPrep/Cobas TaqMan assay (Cobas TaqMan HCV quantitative assay); ND, not detected.

Discussion

Antiviral therapy for chronic hepatitis C was initiated with IFN alone in the 1990s. Until recently, standard treatment for chronic HCV genotype 1 infection was PEG-IFN in combination with RBV (7,8).

A combination therapy with PEG-IFN and RBV was subsequently applied to patients with genotype 1 chronic hepatitis C with a high viral load.

Novel drug classes, including inhibitors of the NS3/S4 protease of HCV polyprotein (protease inhibitors), have recently become available (9-11).

Of these, telaprevir (TVR) was the first to be approved in Japan for the treatment of chronic hepatitis C. In a clinical trial of TVR triple combination therapy (TVR, PEG-IFN and RBV) for 24 weeks in Japan, rapid reductions in the serum HCV RNA levels were observed with an SVR rate of ~70% (12,13).

Subsequently, SMV, a second-generation protease inhibitor, was included in the standard treatment regimen.

SMV is a second-generation NS3/NS4 protease inhibitor (14). The QUEST 1 and QUEST 2 phase 3 clinical trials demonstrated SVRs of 80 and 81%, respectively, in patients treated with SMV triple combination therapy (SMV, PEG-IFN, and RBV). Similar results have been reported in phase 3 clinical trials conducted in Japan (15-17).

As the factors determining the effects of PEG-IFN/RBV combination therapy, which is the standard treatment strategy for refractory chronic hepatitis C, the time required for the negative conversion of HCV RNA has attracted attention.

Serial measurement of HCV-RNA loads and assessment of the rates of decrease in viral loads enable clinicians to make decisions regarding changes in treatment duration or to predict therapeutic outcomes.

It is essential to predict therapeutic outcomes in order to more accurately determine the time required for viral clearance or to more precisely understand viral kinetics in the treatment of chronic hepatitis C.

In the present study, HCV-RNA loads were serially measured following treatment initiation by 2 types of quantitative polymerase chain reaction assays in patients with chronic hepatitis C who received PEG-IFN/RBV/SMV triple-agent combination therapy, and the results of the two assays were compared and analyzed.

At 4 weeks after treatment initiation, 4 of the 6 patients were diagnosed as negative by the CAP/CTM assay, whereas 2 of these 4 patients were not found to be negative by the ART assay; 1 was signal-positive with an HCV-RNA load <1.08 Log IU/ml and the second had a viral load of 1.12 Log IU/ml. At 8 weeks after treatment initiation, one patient was found to be negative by the CAP/CTM assay, but signal-positive with a viral load <1.08 Log IU/ml by the ART assay. From 4 to 8 weeks after treatment initiation, 3 of the 6 patients appeared to be discrepant cases. Of the 6 patients who achieved an ETR, 4 were determined to have achieved a rapid virological response (RVR) by the CAP/CTM assay but may not have actually become negative.

As aforementioned, in patients who achieved an RVR but were found to have an extremely low HCV-RNA load during subsequent treatment, it is assumed that the HCV-RNA loads may serve as a predictive factor for relapse.

A recent study showed that in patients who become negative for HCV RNA at or after 12 weeks of treatment, the rate of complete response can be improved by the use of PEG-IFN/RBV combination therapy for 72 weeks (18). Although further studies with a larger sample size are required, the use of the highly sensitive HCV-RNA quantitative assay may improve the prediction of therapeutic outcomes. In discrepant cases showing slightly delayed negative conversion as determined by this assay, the patients should be regarded as late responders; the continuation of the PEG-IFN/RBV combination therapy may lead to further improvement of the therapeutic effects in such cases.

Although therapeutic outcomes have recently been improved, more accurate test methods are required to predict these therapeutic outcomes.

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