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Impact of HCV kinetics on treatment outcome differs by the type of real-time HCV assay in NS3/4A protease inhibitor-based triple therapy

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ABSTRACT

Repeated measurement of the HCV RNA level is essential for properly monitoring treatment efficacy. The aim of this study was to determine the utility of two HCV real-time assays in the evaluation of the impact of hepatitis C virus (HCV) kinetics on the outcome of triple therapy with NS3/4A protease inhibitors (PIs), telaprevir or simeprevir. This study consisted of 171 Japanese patients infected with HCV genotype 1. All 3266 serum samples taken during and post treatment were tested with both the COBAS AmpliPrep/COBAS TaqMan (CAP/CTM) HCV Test v2.0 and the Abbott RealTime (ART) HCV Test. Of the 2597 samples undetectable (lower limit of detection [<LOD]) for HCV RNA by the CAP/CTM assay from the on and post treatment, 400 (15.4%) (369 detectable/less than the lower limitation of quantification [<LLOQ] and 31 quantifiable) were detectable by the ART assay. HCV RNA < LOD within the first four weeks by ART was associated with sustained virological response (SVR) for the difficult-to-treat group that included patients with advanced fibrosis or prior partial/null response. In contrast, for the non-difficult-to-treat group, almost all of the late responders by ART achieved SVR, unlike by CAP/CTM. Despite HCV RNA being once < LOD by ART, 33.1% patients experienced the reappearance of residual HCV RNA (detectable/<LLOQ) during treatment. This event in the first 12 weeks (with PI-treatment period) was not related to treatment failure, however, relapse was observed in all patients with a reappearance of residual HCV RNA after 12 weeks (without PI-treatment period). The superior ability to detect low-level HCV RNA by ART could be useful for predicting SVR by difficult-to-treat patients in the early period and relapse in the late period.

1. Introduction

Analysis of hepatitis C virus (HCV) kinetics plays an invaluable role in evaluating the efficacy of antiviral therapy for chronic HCV infection (Jensen et al., 2006; Mangia et al., 2008). In particular, repeated measurement of the HCV RNA level contributes to the prediction of treatment outcome and to decisions on the treatment duration (response-guided therapy) of pegylated interferon-alpha (PEG-IFNα) plus ribavirin therapy (Ferenci et al., 2008; Sarrazin et al., 2010). New treatment regimens with direct-antiviral agents (DAA) have brought about great change in the treatment of chronic HCV infection, with sustained virological response (SVR) rates having been greatly improved by triple therapy with a DAA and PEG-IFNα/ribavirin (Jacobson et al., 2011; Kowdley et al., 2013; Ogawa et al., 2013a; Manns et al., 2014). Early HCV kinetics are strongly associated with SVR, and assessing the HCV kinetics remains essential.

Real-time PCR-based assays of variable sensitivity and accuracy are commonly used in clinical practice. They include assays by Roche Molecular Systems, the COBAS AmpliPrep/COBAS TaqMan (CAP/CTM) HCV Test, the COBAS TaqMan HCV Test for use with the High-Pure System (HPS), and the Abbott RealTime (ART) HCV Test. In recent reports, including ours, good correlations were observed between CAP/CTM and ART and HPS and ART in terms of HCV RNA quantification (Ikezaki et al., 2011; Ogawa et al., 2013b; Fevery et al.,

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2014; Maasoumy et al., 2014b; Taylor et al., 2014). However, the time HCV RNA became undetectable (lower limit of detection [<LOD]) by the ART assay was significantly later than that by the CAP/CTM and HPS assays in NS3/4A protease inhibitor (PI)-based triple therapy (Ogawa et al., 2013b; Fevery et al., 2014; Maasoumy et al., 2014b). Although the discordance of these assays in the detection of low-level HCV RNA is thought to be important for predicting treatment outcome and for developing the criteria for response-guided therapy and stopping rules, limited clinical data are available that evaluate these important aspects of DAA-based antiviral regimens.

This study was carried out to compare the performance characteristics of the ART and CAP/CTM v2.0 real-time HCV RNA assays for the analysis of the treatment outcome of patients with HCV genotype 1 who underwent PI (telaprevir or simeprevir)-based triple therapy.

2. Patients and methods

2.1. Patients

This study consisted of 175 consecutive Japanese patients who were enrolled between December 2011 and March 2014 and assigned, not randomized, to a PI treatment group, either telaprevir or simeprevir. Eligible patients were aged 20 years and older with confirmed chronic HCV genotype 1 infection. Exclusion criteria included positivity for antibody to human immunodeficiency virus ($n = 3$) or positivity for hepatitis B surface antigen ($n = 0$), lost to follow up ($n = 1$), clinical or biochemical evidence of hepatic decompensation ($n = 0$), and any non-HCV related liver disease, such as autoimmune hepatitis or primary biliary cirrhosis ($n = 0$). After exclusions, the data of 171 patients was available for analysis.

The study was conducted in accordance with the ethics principles of the 2008 Declaration of Helsinki and was approved by the Ethics Committee of Kyushu University hospital. Written informed consent was obtained from all patients before enrollment.

2.2. Clinical and laboratory assessment

Clinical parameters were measured by standard laboratory techniques at a commercial laboratory (SRL Laboratory, Tokyo, Japan). Body mass index was defined as the body mass divided by the square of the height, which is universally expressed in units of kg/m^2. The estimated glomerular filtration rate was calculated based on the modification of diet in renal disease formula. Blood samples were obtained at baseline, every week from weeks 1–12, at 4-week intervals thereafter until the end of treatment, then at 4, 8, 12, and 24 weeks after the end of treatment. Interleukin-28B (IL28B) genotype was determined by polymerase chain reaction amplification and sequencing of the rs8099917 single nucleoside polymorphism (SNP).

Hepatic fibrosis status was assessed by biopsy or transient elastography (FibroScan). Liver biopsy was performed within one month before the initiation of treatment. The minimum length of the liver biopsy was 15 mm, and at least 10 complete portal tracts were necessary for inclusion. For each specimen, the stage of fibrosis was established according to the METAVIR score. We defined advanced fibrosis as METAVIR F3-4 or FibroScan >10.3 kPa (Ogawa et al., 2009), which was used if a liver biopsy result was not available.

Prior treatment response to PEG-IFNα and ribavirin was categorized as follows: Relapse, relapse of serum HCV RNA after treatment of patients whose HCV RNA level was undetectable at the end of treatment and the re-appearance of HCV RNA at any time during treatment after virological response (breakthrough): Non-virological response (NVR), HCV RNA was detectable during treatment, including both partial (a more than 2 log_{10}IU/mL decrease in the HCV RNA level from baseline to week 12) and null (a decrease in the HCV RNA level of less than 2 log_{10}IU/mL at week 12) responses.

2.3. Determination of the HCV RNA level

A total of 3266 serum samples obtained from the 171 patients were tested in parallel with both the CAP/CTM v2.0 (Roche Diagnostics, Tokyo, Japan) and ART (Abbott Molecular, Des Plaines, IL) assays. Each of the specimens was frozen to −80 °C within two hours of collection. HCV RNA was determined as per the manufacturers' recommendations. Sample preparation for CAP/CTM was done using the Cobas AmpliPrep and Cobas TaqMan instruments, and for ART with the Abbott m2000sp and m2000rt instruments.

CAP/CTM v2.0 assay results are reported as < LOD, detectable but less than the lower limitation of quantification (detectable/<LLOQ), or absolute numbers (quantifiable results: ≥15 IU/mL) (Zitzer et al., 2013). The range for linear quantification is between 15 and 6.9×10^7 IU/mL. ART assay results are reported as < LOD, detectable/<LLOQ, or absolute numbers (quantifiable results: ≥12 IU/mL) (Chevaliez et al., 2009). The range of linear quantification is between 12 and 10^8 IU/mL.

To minimize the possibility of contamination, the sample preparation, nucleic acid extraction, and PCR setup were done in separate rooms from that in which amplification and detection were done. The CAP/CTM system incorporates uracil-*N*-glycosylase contamination control in the master mix, and three replicates at each concentration (high positive, low positive, and negative controls) were tested simultaneously by both assays for every HCV RNA measurement.

2.4. Antiviral treatment and treatment outcome

Patients received a combination treatment of telaprevir (Telavic; Mitsubishi Tanabe Pharma Co., Osaka, Japan) (750 mg three times a day after meals, orally) or simeprevir (Sovriad; Janssen Pharmaceutical K.K., Tokyo, Japan) (100 mg once daily, orally), PEG-IFNα2b (PegIntron; MSD K.K., Tokyo, Japan) (1.5 μg/kg once-weekly subcutaneous injections) and ribavirin (Rebetol; MSD) (600–1000 mg daily based on bodyweight, orally) for 12 weeks, followed by an additional 12 weeks of PEG-IFNα2b and ribavirin alone. The method of dose adjustment of each drug was as described previously (Ogawa et al., 2015b).

Treatment outcome was categorized as SVR, HCV RNA < LLOQ by both CAP/CTM and ART at week 24 after the end of treatment or rapid virological response (RVR), HCV RNA < LOD at week 4.

2.5. Statistical analysis

Statistical analyses were conducted using SPSS Statistics version 22.0 (IBM SPSS Inc, Chicago, IL, USA). Baseline continuous data are expressed as median (first-third quartiles) and categorical variables are reported as frequencies and percentages. Univariate analyses were done using the Chi-square or Fisher's Exact tests, as appropriate. Variables with $P < 0.10$ in univariate analysis were evaluated using multivariate logistic regression to identify variables significantly associated with SVR. The results are expressed as odds ratios (OR) and their 95% confidence interval (CI). We used Bland–Altman plot analysis to compare the HCV RNA levels from CAP/CTM and ART. The linear correlation between viral load by CAP/CTM and the difference of viral load by CAP/CTM and ART were assessed by Spearman rank-order correlation coefficient. A *P* value less than 0.05 was regarded as statistically significant in all analyses.