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Early phase viral kinetics of chronic hepatitis C patients receiving telaprevir-based triple therapy: A comparison of two real-time PCR assays

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ABSTRACT

Monitoring hepatitis C virus (HCV) kinetics during antiviral treatment is recommended for determining the best form of treatment management. We compared the measurement of HCV RNA by two Real-time PCR assays during the first 12 weeks phase of telaprevir in combination with pegylated interferon α2b and ribavirin treatment for chronic hepatitis C patients. The viral kinetics of 65 patients with HCV genotype 1b was assessed. HCV RNA was tested at baseline, on day 3, and every week from 1 to 12 by both the first-generation Roche COBAS® AmpliPrep/COBAS® TaqMan® HCV (CAP/CTM) assay and the Abbott RealTime HCV (ART) assay. A total of 910 serum samples were obtained from the 65 patients. Of these, 168 (28.5%) of the 590 samples HCV RNA negative by CAP/CTM were positive by ART. In contrast, 17 (3.9%) of the 439 samples HCV RNA negative by ART were positive by CAP/CTM. The rates of HCV RNA negativity by ART at weeks 3, 4, and 5 were significantly lower than those by CAP/CTM (21.5% vs. 50.8%, 36.9% vs. 70.8% and 44.6% vs. 81.5%; $P < 0.001$, $P < 0.0001$ and $P < 0.05$, respectively). Although the ART is superior for the determination of HCV RNA negativity, the predictive value of detectable HCV RNA for non-sustained virological response (non-SVR) by CAP/CTM is higher than by ART at weeks 4, 6, and 8. We also found that 16 (24.6%) by CAP/CTM and 28 (43.1%) by ART had a reappearance of residual HCV RNA during the telaprevir treatment period. However, the reappearance of residual HCV RNA was not associated with non-SVR. In conclusion, a significant difference was found between the two real-time PCR assays for the assessment of virological response based on undetectable HCV RNA.

1. Introduction

Chronic hepatitis C virus (HCV) infection represents a serious issue for the 130–170 million infected people globally (Perz et al., 2006). For the past decade, pegylated interferon (PEG-IFN) α and ribavirin (RBV) treatment has been associated with a significant increase in the rate of sustained virological response (SVR), and SVR or relapse had a significantly lower risk than non-virological response for the incidence of hepatocellular carcinoma (HCC) (Ogawa et al., 2013). However, the rate of SVR by patients infected with HCV genotype 1 remains low at 40–50% (Fried et al., 2002; Hadziyannis et al., 2004; Furusyo et al., 2008). Recently, the non-structural 3/4A protease inhibitors telaprevir (TVR) and boceprevir were approved for administration in combination with PEG-IFN-Nα2b and RBV for the treatment of patients infected with HCV genotype 1. As a result, the rate of SVR by patients has risen to over 70% (Jacobson et al., 2011; Sherman et al., 2011; Kumada et al., 2012; Furusyo et al., 2013).

An on-treatment variable based on viral kinetics is now used for determining the best form of antiviral treatment management, as recommended by the American and European international consensus conferences (Ghany et al., 2009; European Association for the Study of the Liver, 2011). Currently, commercial real-time PCR platforms have become available: two different assay from Roche Molecular Systems (Pleasanton, CA, USA), a fully automated system named the first-generation Roche COBAS® AmpliPrep/COBAS® TaqMan® HCV (CAP/CTM) (Ogawa et al., 2010) and the COBAS® TaqMan® HCV, v2.0 for use with the high pure system (HPS/CTM) for manual sample presentation (Colucci et al., 2007), and the Abbott RealTime HCV assay (ART; Abbott Molecular, Des Plaines, IL, USA) (Ikezaki et al., 2011). Real-time PCR assays are

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generally preferred for quantification of HCV RNA because of their wide dynamic range and good sensitivity.

The objective of this prospective study was to assess the concordance between baseline and on-treatment samples using two real-time PCR assays (CAP/CTM and ART) and to evaluate the effectiveness of these assays for predicting the treatment outcome of a group of chronic hepatitis C patients infected with HCV genotype 1b who were being treated with TVR in combination with PEG-IFNα2b and RBV.

2. Materials and methods

2.1. Patients

The data of 65 Japanese patients infected with chronic HCV genotype 1b were analyzed, including 18 treatment-naïve (27.7%) and 47 treatment-experienced (72.3%) patients. All patients were recruited at Kyushu University Hospital and started TVR-based triple therapy between December 2011 and May 2012. The patient profile and laboratory data are summarized in Table 1. Exclusion criteria were: (1) positivity for antibody to human immunodeficiency virus or positivity for hepatitis B surface antigen; (2) clinical or biochemical evidence of hepatic decompensation (ascites, bleeding varices, or encephalopathy); (3) other causes of liver disease (autoimmune hepatitis, primary biliary cirrhosis, or steatohepatitis); (4) excessive active alcohol consumption (a daily intake of more than 40 g of ethanol) or drug abuse; (5) suspected HCC at entry; or (6) treatment with antiviral or immunosuppressive agents prior to enrollment. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki and was approved by the Ethics Committee of our hospital. Informed consent was obtained from all patients before enrollment.

2.2. Clinical and laboratory assessment

Clinical parameters included alanine aminotransferase, aspartate aminotransferase, γ-glutamyl-transpeptidase, α-fetoprotein, estimated glomerular filtration rate, white blood cell count, hemoglobin, platelet count, and HCV RNA. All patients had HCV RNA testing at baseline, on day 3 (the third day after the start of treatment), and every week from weeks 1 to 12. All were measured by standard laboratory techniques at our hospital. Body mass index was calculated as weight in kilograms/height in square meters.

Liver biopsy at entry for 61 (93.8%) of the 65 patients was done by experienced hepatologists. For each specimen, the stage of fibrosis (F0–4) and the grade of activity (A0–3) were established according to METAVIR score (The French METAVIR Cooperative Study Group, 1994).

Table 1
Demographic and clinical features of chronic hepatitis C patients.

Characteristic	Overall (n = 65)
Age (years)	62 (53–67)
Men, n (%)	33 (50.8)
Body mass index (kg/m^2)	22.5 (21.0–25.2)
Aspartate aminotransferase (IU/L)	43 (32–69)
Alanine aminotransferase (IU/L)	45 (29–73)
γ-glutamyl transpeptidase (IU/L)	39 (24–75)
Estimated glomerular filtration rate (mL/min/1.73 m^2)	83 (72–94)
α-fetoprotein (ng/mL)	4.7 (2.6–7.6)
White blood cell count (X10^9/L)	4.7 (4.0–5.8)
Hemoglobin (g/L)	139 (131–151)
Platelet count (X10^9/L)	151 (121–194)
Liver histology	
Fibrosis stage, F0–2/F3–4, n	43/18
Activity grade, A0–1/A2–3, n	35/26
Not determined, n	4
IL28B (rs8099917) genotype	
TT, n (%)	37 (56.9)
TG, n (%)	27 (41.5)
GG, n (%)	1 (1.5)
Previous treatment outcome*	
Naïve, n (%)	18 (27.7)
Relapse, n (%)	27 (41.5)
Partial or null response, n (%)	20 (30.8)

Data are expressed as number (%) or median (first-third quartile).
* Previous treatment was pegylated-interferon alpha and ribavirin.

2.3. Therapeutic protocol

All patients received a combination treatment of TVR (Telavic; Mitsubishi Tanabe Pharma, Osaka, Japan), PEG-IFNα2b (PEG-Intron; MSD, Tokyo, Japan), and RBV (Rebetol; MSD) for 12 weeks, followed by an additional 12 weeks of PEG-IFNα2b and RBV alone. TVR 750 mg was administrated three times a day at an 8-h interval after each meal and dose reduction of TVR was allowed when necessary. PEG-IFNα2b was injected subcutaneously once weekly at a dose of 1.5 μg/kg. RBV was given orally at a daily dose of 600–1000 mg based on body weight (600 mg for patients weighing <60 kg, 800 mg for those weighing 60–80 kg, and 1000 mg for those weighing >80 kg).

2.4. Efficacy of treatment

Treatment response was categorized as follows: rapid virological response (RVR), an undetectable HCV RNA at week 4; SVR, undetectable HCV RNA at week 24 after the end of treatment. These virological responses were analyzed on an intent-to-treat basis. Viral breakthrough was defined as a confirmed on-treatment increase in HCV RNA level of 1-$\log_{10}$ above nadir or greater than 100 IU/mL for patients who previously had undetectable HCV RNA or an HCV RNA level below 15 IU/mL by CAP/CTM or below 12 IU/mL by ART. The remaining patients whose HCV RNA never became undetectable were classified as non-responders.

2.5. Determination of HCV RNA level and HCV genotype

A total of 910 serum samples obtained from the 65 patients were tested in parallel with both the CAP/CTM and ART assays. Each of the specimens was frozen to −80 °C within 2 h of collection. HCV RNA was determined with the CAP/CTM and ART as per the manufacturers' recommendations. Sample preparation for the CAP/CTM was performed using the Cobas AmpliPrep and Cobas TaqMan instruments, and for the ART was performed using the Abbott m2000sp and m2000rt instruments. HCV genotype determination was by sequence determination in the 5′-nonstructual region of the HCV genome followed by phylogenetic analysis (Simmonds et al., 1994). HCV core amino acid substitution at position 70 (Core70) of HCV gene was determined before treatment for all patients. Amino acid substitution at HCV Core70 was analyzed by direct sequencing, as reported previously (Akuta et al., 2007).

CAP/CTM assay (version 1) results are reported as undetectable, detectable but <15 IU/mL, or detectable ($\geqslant$15 IU/mL). The range for linear quantification is between 43 and 6.9×10^7 IU/mL. The limit of detection differs for different HCV genotypes and is calculated by probit analysis (95% probability to detect HCV RNA), with HCV genotype 1 standards of 8.2 and 12.6 IU/mL in serum samples. ART assay results are reported as undetectable, detectable but <12 IU/mL, or detectable ($\geqslant$12 IU/mL). The range of linear quantification is between 12 and 10^8 IU/mL. The limit of detection differs for different HCV genotypes and is calculated by probit analysis (95% probability to detect HCV RNA), with HCV genotype 1 standards of 7.2 and 10.5 IU/mL in serum samples.

To minimize the possibility of contamination, the sample preparation, nucleic acid extraction, and PCR setup were performed in separate rooms from that in which amplification and detection were done. The CAP/CTM system incorporates uracil-*N*-glycosylase (UNG) 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. If the results were reported as undetectable, the samples were retested for confirmation.

2.6. Genetic testing

Human genomic DNA was extracted from peripheral blood. The allele of the single nucleoside polymorphism (SNP) rs8099917, near the IL28B gene, was determined using the ABI TaqMan allelic discrimination kit (7500 RealTime PCR System; Applied Biosystems, Carlsbad, CA, USA). Heterozygotes (TG) or homozygotes (GG) of the minor allele (A) are described as having the IL28B minor allele, whereas homozygotes for the major allele (TT) are described as having the IL28B major allele (Tanaka et al., 2009).

2.7. Statistical analysis

Statistical analyses were by SPSS Statistics 19.0 (IBM SPSS Inc, Chicago, IL, USA). Baseline continuous data are expressed as median (first-third quartile) and categorical variables as frequencies and percentages. The paired *t*-test and chi-square test were used for analysis and simple regression analysis was used to assess the correlation between the HCV RNA levels by the CAP/CTM and ART assays. The significance of trends in values was determined with the Cochran–Armitage trend test. A *P* value less than 0.05 was regarded as statistically significant in all analyses.

3. Results

3.1. Correlation between the HCV RNA measurements by the CAP/CTM and ART assays

Analysis of the correlation between the real-time PCR assays found the HCV RNA viral load measured by CAP/CTM to be highly correlated with that of the ART ($R^2 = 0.929$, $P < 0.0001$) (Fig. 1). However, the baseline HCV RNA level by CAP/CTM (6.5 [6.2–6.9] Log_{10} IU/mL) was significantly higher than that by ART (6.10 [5.57–6.38] Log_{10} IU/mL) ($P < 0.0001$).

3.2. Overall concordance between the CAP/CTM and ART assays

A categorical analysis of the overall concordance between CAP/CTM and ART is shown in Table 2. Of the 910 samples, HCV RNA was negative in 421 and positive in 190 by both assays. However, 168 (28.5%) of the 590 samples HCV RNA negative by CAP/CTM were positive by ART. Similarly, 17 (3.9%) of the 439 samples HCV RNA negative by ART were positive, but not quantifiable, by CAP/CTM.

When examined according to the week samples were obtained (Table 3), 82 (52.9%) of 155 samples HCV RNA negative by CAP/CTM were positive by ART in the 5 weeks after the initiation of triple therapy. However, the percentage of samples that were negative by CAP/CTM but positive by ART for HCV RNA decreased with time ($P < 0.001$ by Cochran–Armitage trend test).

3.3. The rate of HCV RNA negativity during treatment

The rate of HCV RNA negativity during treatment is shown in Fig. 2. Significant differences were found between the negativity rates by CAP/CTM and those by ART over the entire period ($P < 0.0001$, chi-square test). Notably, the rates by ART at weeks 3, 4, and 5 were significantly lower than those by CAP/CTM (21.5% vs. 50.8%, 36.9% vs. 70.8% and 44.6% vs. 81.5%; $P < 0.001$, $P < 0.0001$ and $P < 0.05$, respectively). For treatment-naïve patients and prior relapsers without liver cirrhosis ($n = 37$), the rate of RVR by CAP/CTM (83.8%) was significantly higher than that by ART (43.2%) ($P = 0.0056$).

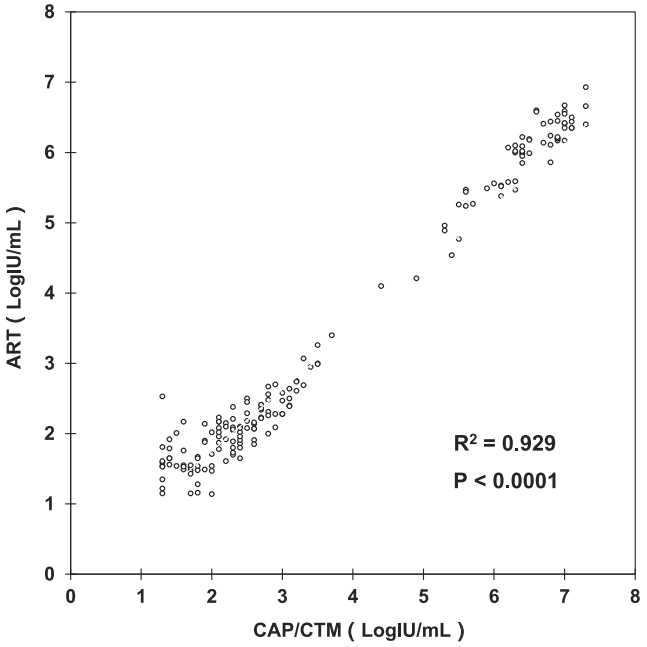

Fig. 1. Correlation of HCV RNA levels determined by CAP/CTM and ART. Results below the limit of quantification were excluded.

3.4. Correlation between CAP/CTM and ART assays by treatment outcome

The overall SVR rate was 86.2% (56 of 65). The SVR rate of IL28B TT (97.3%, 36 of 37) was significantly higher than that of IL28B TG/GG (71.4%, 20 of 28) ($P < 0.01$). All patients with RVR by ART ($n = 24$) achieved an SVR, however, 3 of 48 (6.2%) patients with RVR by CAP/CTM failed to achieve an SVR. The common criteria of these three patients are as follows: F4 stage of liver fibrosis, prior partial or null response, IL28B TG genotype, and mutant-type sequence at Core70. The time of first HCV RNA negativity by ART was at weeks 5 and 6 for two of these patients. The third patient never achieved negativity during treatment period.

The relationship between SVR and the time of first negativity is shown in Fig. 3. By CAP/CTM assay, HCV RNA negativity in the first 5 weeks was associated with a high rate of SVR compared to negativity after week five. In contrast, by ART assay, HCV RNA negativity in the first 7 weeks was associated with a high rate of SVR compared to negativity after week seven.

3.5. Positive predictive value (PPV) and negative predictive value (NPV) for SVR by CAP/CTM and ART assays

The PPV and NPV for SVR, calculated on the basis of undetectable HCV RNA as revealed by the CAP/CTM and ART assays at weeks 2, 4, 6, 8, and 12, are summarized in Table 4. The PPV were extremely high at weeks 2 and 4 in both assays. However, the NPVs of CAP/CTM were higher than those of ART at weeks 4, 6, and 8

Table 2
Overall concordance between the CAP/CTM and ART assays.

		ART assay			
		Not detected, *n*	<12 IU/mL, but detected, *n*	12–15 IU/mL, *n*	⩾15 IU/mL, *n*
CAP/CTM assay	Not detected, *n*	422	146	7	15
	<15 IU/mL, but detected, *n*	17	57	9	40
	⩾15 IU/mL, *n*	0	3	5	190

CAP/CTM, the Roche COBAS® AmpliPrep/COBAS® TaqMan® HCV assay; ART, the Abbott RealTime HCV assay.

Table 3
Comparison of HCV RNA detection by the CAP/CTM and ART assays.

Time from the start of treatment	HCV RNA positive by CAP/CTM		HCV RNA negative by CAP/CTM	
	n	Positive by ART *n* (%)	*n*	Positive by ART *n* (%)
Day 3	62	62 (100)	3	3 (100)
Week 1	59	59 (100)	6	3 (50.0)
Week 2	51	47 (92.2)	14	8 (57.1)
Week 3	32	31 (96.9)	33	20 (60.6)
Week 4	19	19 (100)	46	22 (47.8)
Week 5	12	10 (83.3)	53	26 (49.1)
Week 6	5	4 (80.0)	60	23 (38.3)
Week 7	4	2 (50.0)	61	20 (32.8)
Week 8	4	1 (25.0)	61	15 (24.6)
Week 9	2	1 (50.0)	63	7 (11.1)
Week 10	2	1 (50.0)	63	8 (12.7)
Week 11	3	1 (33.3)	62	8 (12.9)
Week 12	0	–	65	5 (7.7)
Total	255	238 (93.3)	590	168 (28.5)

CAP/CTM, the Roche COBAS® AmpliPrep/COBAS® TaqMan® HCV assay; ART; the Abbott RealTime HCV assay.

(week 4: 36.8% vs. 21.4%, week 6: 60.0% vs. 25.9%, and week 8: 75.0% vs. 25.0%).

3.6. Individual characteristics and viral kinetics of non-responders by ART assay

Only two patients (3.1%) never achieved negativity for HCV RNA by ART during the treatment period, and both failed to achieve an SVR. The common factors of two patients were IL28B TG genotype, mutant-type sequence at Core70, and prior null response. According to the viral kinetics, HCV RNA first became undetectable at weeks 3 and 8, respectively, by CAP/CTM, although a low-level of HCV RNA was detected at some time points.

3.7. Reappearance of residual HCV RNA during treatment

Of the 65 patients, 16 (24.6%) by CAP/CTM and 28 (43.1%) by ART experienced the reappearance of residual HCV RNA during the first 12 weeks of treatment after at one time having had undetectable HCV RNA by both assays, although none of the patients had viral breakthrough. Of these, 10 of 16 (62.5%) by CAP/CTM and 19 of 28 (67.9%) by ART required a reduced dosage of RBV or TVR because of adverse effects during the HCV RNA negative period.

The reappearance of residual HCV RNA rates of patients with RVR by CAP/CTM and ART were 27.1% (13 of 48) and 52.0% (13 of 25), respectively. However, almost all patients with RVR/reappearance of HCV by CAP/CTM (84.6%, 11 of 13) and ART (100%, 13 of 13) achieved SVR.

4. Discussion

This study presents real-time PCR assay (CAP/CTM and ART) assessment of the concordance between the results of on-treatment blood samples obtained from chronic hepatitis C patients who underwent treatment with TVR in combination with PEG-IFNα2b and RBV. Our results showed that both assays were highly correlated in quantitative analysis, but that ART had greater accuracy than CAP/CTM for detecting trace amounts of HCV RNA. Nevertheless, the CAP/CTM assay is clinically useful for HCV RNA testing for the prediction of non-SVR because its predictive values are higher than those of ART.

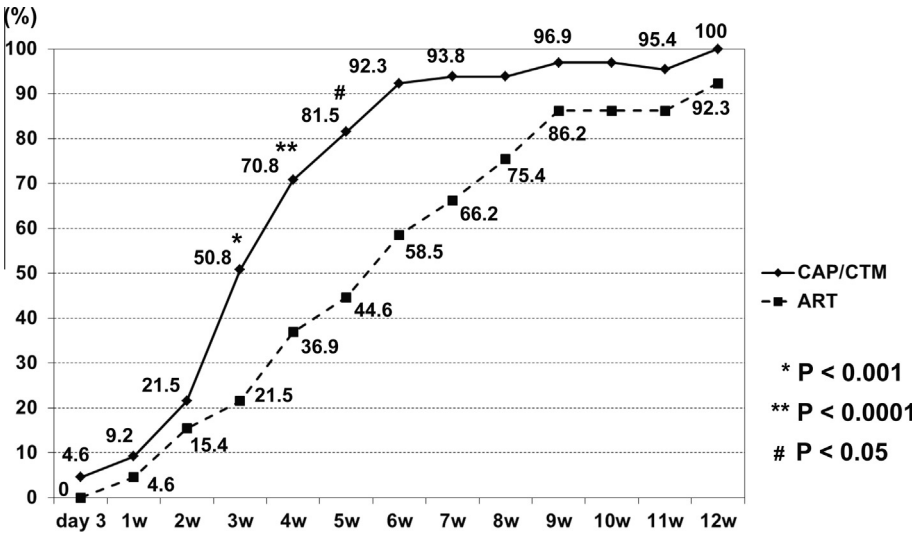

Fig. 2. The rate of HCV RNA negativity during telaprevir in combination with pegylated interferon α2b and ribavirin treatment.

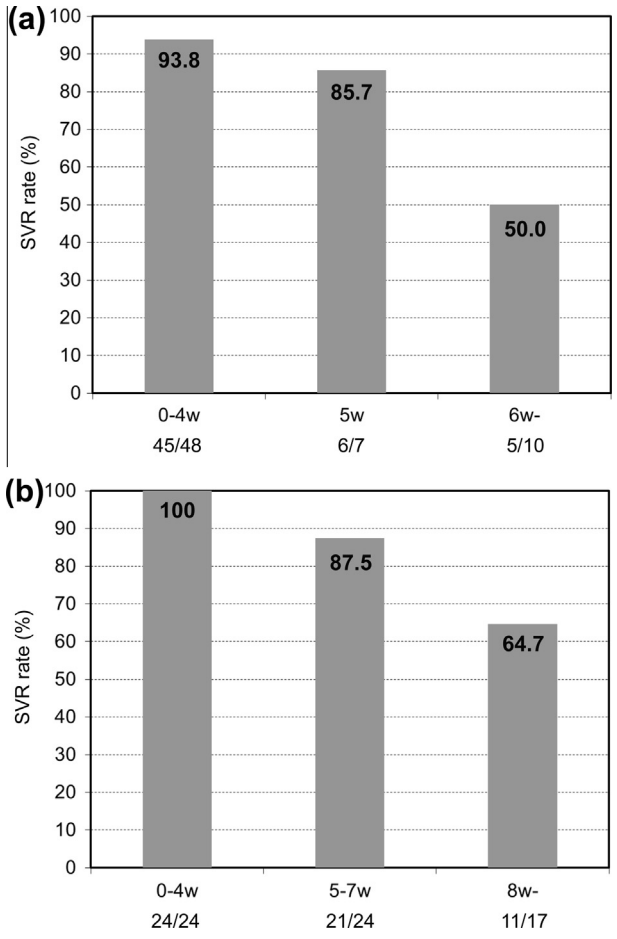

Fig. 3. The relationship between the time of first negativity of HCV RNA by (A) CAP/CTM or (B) ART and SVR (the Cochran–Armitage trend test; both $P < 0.001$).

According to previous studies, the prediction of SVR estimated on the basis of viral kinetics is clinically reliable for PEG-IFNα and RBV treatment (Davis et al., 2003; Berg et al., 2003; Furusyo et al., 2012; Ogawa et al., 2012). Positive predictive evidence early in the course of treatment could be used to reinforce the importance of compliance in ensuring a successful treatment outcome. The availability of protease inhibitors has profoundly changed the management of chronic hepatitis C. These new drugs require the highest HCV RNA sensitivity and dynamic range, because their success is mainly based on their ability to reduce the viral load in order to achieve the highest possible rate of SVR.

Table 4
PPV and NPV for SVR based on undetectable HCV RNA at week 2, 4, 6, 8, and 12 by CAP/CTM and ART.

	CAP/CTM		ART	
	PPV	NPV	PPV	NPV
Week 2	14/15 (93.3)	8/50 (16.0)	10/10 (100)	9/55 (16.4)
Week 4	44/46 (95.7)	7/19 (36.8)	23/23 (100)	9/42 (21.4)
Week 6	54/60 (90.0)	3/5 (60.0)	36/38 (94.7)	7/27 (25.9)
Week 8	53/61 (86.9)	3/4 (75.0)	44/49 (89.8)	4/16 (25.0)
Week 12	56/65 (86.2)	0	53/60 (88.3)	2/5 (40.0)

Data are expressed as number (%).
CAP/CTM, the Roche COBAS® AmpliPrep/COBAS® TaqMan® HCV assay; ART; the Abbott RealTime HCV assay; PPV, positive predictive value (The denominator is the number of patients with undetectable HCV RNA; the numerator is the number of patients who achieved SVR); NPV, negative predictive value (The denominator is the number of patients with detectable HCV RNA; the numerator is the number of patients who did not achieve SVR).

Surprisingly, 168 of 590 (28.5%) serum samples with undetectable HCV RNA by CAP/CTM tested positive by ART in this study. In contrast, the rate of serum samples with undetectable HCV RNA by ART but positive by CAP/CTM was significantly lower (3.9%, 17 of 439). Consequently, we showed that the timing of first negativity of HCV RNA by ART (mean 5.5 weeks) was significantly later than that by CAP/CTM (3.7 weeks) ($P < 0.001$). Moreover, in 2 of 65 (3.1%) patients, HCV RNA was detectable by ART throughout the treatment, even though HCV RNA was negative by CAP/CTM.

A recent randomized trial showed that 68% of the patients treated with TVR-based triple therapy achieved RVR by HPS/CTM (Jacobson et al., 2011). Of these, 84% in the RVR group achieved a SVR, which was significantly higher than the 56% with non-RVR. This shows the utility of HCV kinetics by week 4 for predicting treatment outcome. In our study, a high SVR rate (86.2%) was obtained for patients treated with TVR-based triple therapy. However, the RVR rate by ART (36.9%) was significantly lower than that by CAP/CTM (70.8%) ($P < 0.0001$) because the priority of low levels of HCV RNA detection by ART, although the RVR group achieved SVR at high rates by both assays (CAP/CTM: 93.8%, ART: 100%). Of note, we found that the NPVs for SVR of CAP/CTM were higher than those of ART at weeks 4, 6, and 8, but they did not reach significance. Simply, the predictive value of detectable HCV RNA for non-SVR by CAP/CTM is higher than by ART. This finding is of great importance to the management of direct-acting antiviral treatment in clinical practice. Although the detection of HCV RNA by ART is much more sensitive than by CAP/CTM, which makes ART superior for determining HCV RNA negativity, CAP/CTM would be preferable for HCV RNA testing to predict non-SVR.

Assessing RVR has the utility of guiding treatment duration in order to shorten treatment administration (Zeuzem et al., 2006; Moreno et al., 2010). Treatment-naïve patients and prior relapsers who have RVR without liver cirrhosis are suitable for shortened treatment duration. However, the interpretation of RVR is still difficult, as shown by the fact that the residual HCV RNA was detectable at some time points and undetectable at others by both assays, for 27.1% of the RVR patients by CAP/CTM and for 52.0% by ART. The main reason of the reappearance of HCV RNA was the TVR or RBV dose reduction because of adverse effects during the HCV RNA negative period. Of note, the reappearance of residual HCV RNA by both assays in patients who achieved RVR was not associated with non-SVR.

A limitation of this study is that we used the first-generation CAP/CTM. Recently, a second generation CAP/CTM was designed that has shown excellent performance and sensitivity (limit of detection equal to lower limit of quantitation at 15 IU/mL) across all HCV genotypes (Zitzer et al., 2013). Moreover, even though the limit of detection of the first-generation CAP/CTM is the same as version 2.0, HCV RNA of only 5 IU/mL is still detectable approximately 70% of the time in version 2.0. Further comparison studies of residual HCV RNA detection using CAP/CTM version 2.0 and ART are needed. Another limitation is that the study included only Japanese patients infected with HCV genotype 1b. HCV genotype 1b virus has a higher barrier to resistance than does genotype 1a, because it needs two nucleoside substitutions at position 155 in its protease to confer resistance to TVR, whereas genotype 1a needs only one substitution in the same position to become resistant (Sarrazin et al., 2007; Zhou et al., 2008).

In conclusion, HCV RNA levels measured by the CAP/CTM and ART assays were highly correlated in quantitative analysis of treatment with TVR in combination with PEG-IFNα2b and RBV. Although the ART assay is superior for the determination of HCV RNA negativity, the CAP/CTM assay may be preferable for HCV RNA testing to predict non-SVR in clinical practice. Moreover,

despite more frequent observation of the reappearance of residual HCV RNA during the TVR treatment period by ART than by CAP/CTM, there is no correlation with non-SVR.

5. Disclosures

The authors declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this paper.

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