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Can the Abbott RealTime hepatitis C virus assay be used to predict therapeutic outcomes in hepatitis C virus-infected patients undergoing triple therapy?

LIVER

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Original Article

ABSTRACT

Background/Aims: We compared the predictive abilities of the Abbott Real Time hepatitis C virus (HCV) assay (ART) with those of standard serum HCV ribonucleic acid (RNA) detection methods in patients undergoing triple therapy, which involves treatment with a protease inhibitor combined with pegylated interferon and ribavirin.

Materials and Methods: In this study, 28 patients underwent triple therapy. The hepatitis C virus ribonucleic acid (HCV RNA) level of each patient was measured at weeks 0, 4, 8, and 12 after the initiation of therapy using the Roche COBAS AmpliPrep/COBAS TaqMan HCV assay version 1.0 (CAP/CTM v1.0) and ART.

Results: At week 8 after the initiation of therapy, the sustained virological response (SVR) rate among patients who tested negative and positive for HCV RNA using CAP/CTM v1.0, was 80.0% (20/25) and 33.3% (1/3), and using ART, it was 91.3% (21/23) and 0.0% (0/5), respectively. Although at week 8, the predictive capability of CAP/CTM v1.0 was 78.5%, ART was found to be a more accurate predictor of future SVR status with a rate of 92.9%.

Conclusion: These results indicate that the presence or absence of serum HCV RNA, evaluated using ART at week 8 after the initiation of therapy, may be useful for predicting therapeutic outcomes in patients receiving triple therapy.

Keywords: Hepatitis C, real-time PCR, telaprevir, pegylated interferon, clinical predictor

INTRODUCTION

Worldwide, approximately 150 million people suffer from chronic hepatitis C (CHC). Approximately 50% of patients with chronic hepatic disease annually develop cirrhosis (1), and 7% tend to develop hepatocellular carcinoma (2). Interferon (IFN)-based antiviral therapy is particularly important for delaying disease progression. A high cure rate was recently achieved by combination therapy using a pegylated interferon (PegIFN)/ribavirin (RBV) (combination therapy) and therapy using a PegIFN/RBV/direct-acting antiviral agent (DAA) (triple therapy) (3-10). More recently, interferon-free DAA combination therapies involving DAAs against two or more viral targets present an attractive alternative for HCV therapy; however, because DAAs can be compromised by the development of drug resistance, the risk of resistance-associated variants remains a potential problem.

Single-nucleotide polymorphisms (SNPs) in interleukin 28B (IL28B) are associated with the therapeutic effects of IFN-based antiviral therapy and are often used as predictors of the patient's response to treatment (11,12). However, hepatitis C relapses were observed in some patients after the completion of therapy. Currently, despite the use of SNPs in IL28B as a predictor for the

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therapeutic outcome, it is difficult to predict which patients will achieve a sustained virological response (SVR) and which ones will most likely relapse.

Currently, there are several commercially available hepatitis C virus (HCV) viral load assays. Real-time polymerase chain reaction (PCR) assays are generally preferred because of their wide range of detection and sensitivity to low quantities of viral RNA (13, 14). Two commercial real-time PCR platforms are the COBAS AmpliPrep/COBAS TaqMan HCV assay version 1.0 (CAP/CTM v1.0) (Roche Molecular Systems, Inc., Pleasanton, CA, USA) and the Abbott RealTime HCV assay (ART) (15-17). The recently developed ART has an improved lower limit of detection of 1.08 Log international units per milliliter (IU/mL) of HCV RNA in human serum or plasma, allowing for a more sensitive quantitation of hepatitis C virus ribonucleic acid (HCV RNA) than that achieved by CAP/CTM v1.0. However, because the efficiency of ART in clinical practice has not been sufficiently studied, we examined the relationship between the efficacy of triple therapy and HCV RNA levels measured using ART in patients early after treatment. In this study, we also compared these results with those obtained using the traditional CAP/CTM v1.0 and a second version of the CAP/CTM assay (CAP/CTM v2.0) (18).

MATERIALS AND METHODS

Patients

Fifty-five patients with serogroup 1 (genotype 1b) CHC and high viral loads (HCV RNA concentration of ≥5.0 Log IU/mL) received triple therapy [PegIFN/RBV/telaprevir (TVR)] from February 2012 to May 2014. CHC diagnosis was defined as the patient having abnormal serum concentrations of alanine aminotransferase (ALT>37 IU/L) that were either continuously or intermittently associated with persistently positive test results for serum HCV RNA for more than six months. Patients were excluded from this study if they met one of the seven following conditions: First, they tested positive for the serum hepatitis B surface antigen or the human immunodeficiency virus antibody. Second, they had clinical symptoms of diseases such as ascites, encephalopathy, jaundice, and variceal hemorrhage or were diagnosed with hepatic failure [a prothrombin time (PT) of less than 40% and/or PT international normalized ratio of 1.5 or more]. Third, they had hepatocellular carcinoma confirmed by dynamic contrast-enhanced computed tomography (CT) and/or magnetic resonance imaging (MRI). Fourth, they had liver disease that was not caused by HCV infection such as autoimmune hepatitis, primary biliary cirrhosis, or a fatty liver. Fifth, they had alcohol-related liver diseases. Alcohol abuse was defined as the consumption of alcohol that exceeded 30g per day for females and 40 g per day for males over the last 6 months. Sixth, they underwent other antiviral therapies or immunosuppressive chemotherapies. Seventh, they were pregnant. Among 55 patients, 28 patients who met our inclusion criteria completed triple therapy, and serum samples were taken from them before the initiation of therapy and at 4, 8, and 12 weeks post treatment. Written informed consent was obtained from the 28 patients, and this study was approved by the Ethics Committee of St. Marianna University School of Medicine Hospital (approval number from Ethics Committee of St. Marianna University School of Medicine Hospital: No. 2037, UMIN000007704).

Treatment protocol

All patients were administered the triple therapy of PegIFN-α2b (PegIntron®, MSD K.K., Tokyo, Japan), RBV (Rebetol®, MSD K.K., Tokyo, Japan) and TVR (Telavic®, Mitsubishi Tanabe Pharma Corporation, Osaka, Japan) for 12 weeks, followed by an additional 12 weeks of combination therapy of PegIFN/RBV administration. The PegIFN-α2b and RBV doses, dose reduction, and discontinuation of administration were carried out according to the manufacturer's instructions. Typically, TVR is administered orally at a dose of 750 mg, three times a day after meals (total dose of 2,250 mg per day). However, in this study, it was administered orally at 500 mg, three times a day after meals (total dose of 1,500 mg per day) to patients who met two of the following three criteria: body weight of <60 kg, female, or hemoglobin concentration <13 g/dL for males, and <14 g/dL for females, at the start of drug administration.

Serum HCV RNA detection

Serum HCV RNA levels in patients receiving triple therapy were measured using CAP/CTM v1.0 (Roche Molecular Systems, Inc., Pleasanton, CA, USA) during routine clinical practice. The dynamic range of CAP/CTM v1.0 is 1.2–7.8 Log IU/mL. Serum HCV RNA levels were measured using ART (Abbott Molecular, Inc., Des Plaines, IL, USA), after completion of therapy using serum samples that had been collected at 0, 4, 8, and 12 weeks after initiation of triple therapy, and stored at -80°C. The dynamic range of ART is 1.08–8.0 Log IU/mL. HCV serotype was determined using Imcheck F-HCV Gr"Kokusai®" kit (Sysmex Corporation, Hyogo, Japan). The dynamic range of CAP/CTM v2.0 (Roche Molecular Systems, Inc., Pleasanton, CA, USA) was the same as that of CAP/CTM v1.0. Measurements were generated using commercial kits, according to the manufacturer's instructions.

To evaluate the sensitivity and reproducibility of the ART, CAP/CTM v1.0, and CAP/CTM v2.0, an HCV genotype 1b patient specimen was used to prepare 5 serial dilutions (0.49, 0.8, 1.4, 1.7, and 2.0 log10 IU/mL), each of which was tested 6 times over 3 days. The HCV RNA titer for this dilution panel was calculated by averaging all the serum HCV RNA concentration results from the undiluted specimen (7.8 log10 IU/mL with the CAP/CTM v1.0) and adjusting for the dilution factor.

Therapy evaluation

To evaluate the effects of triple therapy, patients who tested negative for HCV RNA in serum at 24 weeks post treatment, as determined by the CAP/CTM v1.0 assay were classified as having a SVR. Patients who tested negative for serum HCV RNA at the completion of triple therapy determined by the CAP/CTM

v1.0, but then relapsed within 24 weeks after treatment, were classified as relapsed patients. Patients who tested positive for serum HCV RNA at the completion of triple therapy determined by the CAP/CTM v1.0 were classified as showing no response (NR). Patients with serum HCV RNA levels that increased by ≥2 Log IU/mL from their baseline levels determined by the CAP/CTM v1.0 while receiving triple therapy were classified as having a viral breakthrough.

IL28B SNP analysis

Written informed consent to analyze SNP rs8099917 in IL28B (12) was obtained from all 28 subjects. SNP analysis was performed using the Invader Plus assay (SRL, Inc., Tokyo, Japan) with a commercial kit, according to manufacturer's instructions.

Statistical analysis

We calculated the positive predictive value, negative predictive value, and accuracy to reveal the predictive capability of ART and CAP/CTM v1.0 in determining SVR status on the basis of the timing of HCV RNA clearance in serum. All statistical analyses were performed using Prism 5 for Windows (Graphpad Software Inc.; La Jolla, CA, USA).

RESULTS

Patient characteristics

Table 1 shows a summary of the profiles of all 28 patients prior to triple therapy. There were 17 male and 11 female patients, with a median age of 60 years (range, 20–77 years). The median HCV RNA level in serum samples was 6.8 Log IU/mL (range, 5.4–7.6 Log IU/mL), and the genotype of *IL28B* was favorable (TT) in 19 patients, and unfavorable (TG/GG) in nine patients. The overall SVR rate in the subjects was 75.0% (21/28). Patients who did not achieve SVR were comprised of three relapse patients, three viral breakthrough patients, and one patient with an NR (the list of non-SVR patients were shown in supplementary table). The SVR rate among patients with a favorable *IL28B* (TT) genotype was 94.7% (18/19). On the other hand, the SVR rate among the patients with an unfavorable *IL28B* (TG/GG) genotype was 33.3% (3/9).

Relationship between timing of serum HCV RNA clearance and SVR rate

Figure 1 illustrates the relationship between the time at which HCV RNA levels disappear from serum samples, and the patients' prospective SVR rate, as determined by CAP/CTM v1.0 and ART. All patients who tested negative for serum HCV RNA using CAP/CTM v1.0 at week 4 (15/15) achieved SVR while 46.2% (6/13) of the patients who tested positive for serum HCV RNA at week 4 were later found to maintain SVR. The SVR rate among patients testing negative for serum HCV RNA using CAP/CTM v1.0 at week 8 was 80.0% (20/25), and patients who continued to test positive for serum HCV RNA at week 8 had an SVR rate of 33.3% (1/3). With one exception, all patients who achieved SVR status tested negative for serum HCV RNA at week 8. However, all three patients who later had a relapse were also shown to test negative for serum HCV RNA at week 8, by CAP/CTM v1.0 (data not shown). The SVR rate among patients

Table 1. Patient characteristics

	n=28
Gender	
Male/Female	17/11
Age (years)	60 (20–77)
Past Treatment	
Non response/Naive/Relapse	6/17/5
White blood cell (/mL)	4,800 (3,400–7,600)
Neutrophil cell (/mL)	2,689 (850–4,409)
Hemoglobin (g/dL)	14.0 (11.4–19.6)
Platelet (x10^4/mL)	17.2 (7.6–29.5)
ALT (IU/L)	43 (24–352)
HCV RNA (Log IU/mL)	6.8 (5.4–7.6)
IL28B SNPs	
Favorable (TT)/Unfavorable (TG·GG)	19/9
Initial dose of Telaprevir	
2,250 mg/1,500 mg	15/13
Treatment outcomes	
All cases; SVR/Relapse/Breakthrough/NR	21/3/1/3
IL28B favorable (TT); SVR/Relapse/Breakthrough/NR	18/1/0/0
IL28B unfavorable (TG/GG); SVR/Relapse/Breakthrough/NR	3/2/1/3

Quantitation was determined by CAP/CTM v1.0

ALT: alanine aminotransferase; HCV RNA: hepatitis C virus ribonucleic acid; CAP/CTM v1.0: Roche COBAS® AmpliPrep/COBAS® TaqMan® HCV assay version 1.0; *IL28B*: Interleukin 28B; n: number of patients; SVR: sustained virological response; NR: no response

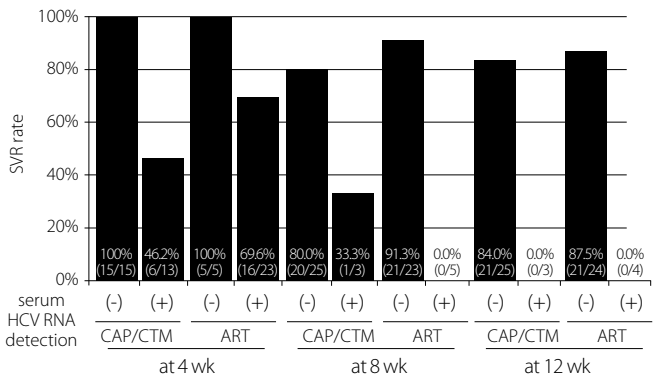

Figure 1. The relationship between the serum HCV RNA clearance time and SVR rate, as determined using CAP/CTM v1.0 and ART. The SVR rate is noted at the bottom of each column. The number of patients who achieved SVR, and the total number of patients in each group, is noted in parentheses. CAP/CTM v1.0, Roche COBAS® AmpliPrep/COBAS® TaqMan® HCV assay version 1.0; ART, Abbott RealTime HCV assay; (+), positive for HCV RNA; (–): negative for HCV RNA; wk: week

who tested negative for serum HCV RNA using ART at week 4 was 100% (5/5), which was the same as that determined by CAP/CTM v1.0. Interestingly, the SVR rate among patients who continued to test positive for serum HCV RNA at week 4 after the initiation of therapy was 69.6% (16/23) using ART. The SVR rate among the patients who tested negative for serum HCV RNA at week 8 using ART was 91.3% (21/23). Although all the three patients who later went on to have a relapse, they had tested negative for serum HCV RNA at week 8 using CAP/CTM v1.0; two of these patients had tested positive for HCV RNA at week 8 using ART (data not shown). Additionally, among five patients who tested positive for serum HCV RNA using ART at week 8, one patient tested negative for serum HCV RNA at week 12; however, none of the five patients who tested positive for serum HCV RNA at 8 weeks using ART achieved SVR status. On the other hand, one patient who tested positive for serum HCV RNA using CAP/CTM v1.0 at week 8 turned to test negative at week 12, resulting in SVR status at the conclusion of this study. In contrast, another patient who tested negative for serum HCV RNA at week 8 tested positive at week 12 using CAP/CTM v1.0, resulting in a viral breakthrough.

Table 2. Predictive ability of CAP/CTM v1.0 and ART in determining SVR status on the basis of HCV RNA clearance time in patient's serum samples

	Assay	PPV (%)	NPV (%)	Accuracy (%)
4 wk	CAP/CTM v1.0	100.0	53.8	78.6
	ART	100.0	30.4	42.9
8 wk	CAP/CTM v1.0	80.0	66.7	78.6
	ART	91.3	100.0	92.9
12 wk	CAP/CTM v1.0	84.0	100.0	85.7
	ART	87.5	100.0	89.3

SVR: sustained virological response; CAP/CTM v1.0: Roche COBAS® AmpliPrep/COBAS® TaqMan® HCV assay version 1.0; ART: Abbott RealTime HCV assay; PPV: positive predictive value; NPV: negative predictive value

Predicting patient's SVR status on the basis of serum HCV RNA clearance time, using CAP/CTM v1.0 and ART

Table 2 shows the predictive ability of CAP/CTM v1.0 and ART in determining SVR. The predictive capability of the CAP/CTM v1.0 assay was 78.6% at week 4, and 78.6% at week 8, after the initiation of therapy. The ART assay was found to be a more accurate predictor for future SVR status with a rate of 42.9% at week 4, and 92.9% at week 8. Moreover, the predictive capability of the CAP/CTM v1.0 and ART assays at week 12 were 85.7% and 89.3%, respectively. These results indicate that a positive test result with the ART assay for serum HCV RNA at week 8 after the initiation of therapy can be used to predict the improbability of achieving SVR (non-SVR) status in patients undergoing triple therapy.

Evaluation of serum HCV RNA positive status at week 8 after the initiation of therapy, using ART

Table 3 shows a summary of the five patients who continued to test positive for serum HCV RNA at week 8, as determined by ART. Of these patients, two later went on to relapse, one was found to be NR, and two showed viral breakthrough. In the two patients who later relapsed, HCV RNA was detected in their serum samples at week 8 using ART, although they had tested negative for serum HCV RNA at the same time using CAP/CTM v1.0 (case 1 and 2 in Table 3). Interestingly, the two patients with viral breakthrough continued to test positive for serum HCV RNA detected by ART throughout the course of treatment, despite having tested negative for serum HCV RNA using CAP/CTM v1.0 (case 3 and 4 in Table 3).

Reproducibility of ART, CAP/CTM v1.0, and CAP/CTM v2.0

To evaluate the reproducibility of the CAP/CTM and ART, diluted pools of HCV RNA were prepared (2.0, 1.7, 1.4, 0.8, and 0.49 Log IU/mL) using a serum sample obtained from a patient with a high serum concentration of HCV RNA.

Table 3. Summary of patients with detectable HCV RNA levels using ART at 8 weeks after the start of treatment

Case	Age	Gender	*IL28B*	Assay	Pre (Log IU/mL)	2w (Log IU/mL)	4w (Log IU/mL)	8w (Log IU/mL)	12w (Log IU/mL)	Outcome
1	61	F	TT	CAP/CTM v1.0	7.0	2.0	<1.2 (+)	ND	ND	Relapse
				ART	6.22	NC	<1.08 (+)	<1.08 (+)	ND	
2	39	M	TG	CAP/CTM v1.0	6.6	1.9	<1.2 (+)	ND	ND	Relapse
				ART	6.03	1.86	<1.08 (+)	<1.08 (+)	ND	
3	72	F	TG	CAP/CTM v1.0	7.2	2.7	1.7	<1.2 (+)	<1.2 (+)	NR
				ART	6.60	2.04	1.45	<1.08 (+)	<1.08 (+)	
4	67	M	TG	CAP/CTM v1.0	7.0	1.8	<1.2 (+)	<1.2 (+)	ND	BT
				ART	6.38	1.93	1.35	<1.08 (+)	<1.08 (+)	
5	66	M	TT	CAP/CTM v1.0	6.8	1.9	1.6	ND	1.2	BT
				ART	6.27	1.48	1.31	<1.08 (+)	<1.08 (+)	

HCV RNA: hepatitis C virus ribonucleic acid; M: male; F: female; TT: TT is the favorable genotype of IL28B; TG: TG is the unfavorable genotype of IL28B; w: week; ART: Abbott RealTime HCV assay; CAP/CTM v1.0: Roche COBAS® AmpliPrep/COBAS® TaqMan® HCV assay version 1.0; ND: not detected; NC: not complied; NR: no response; BT: breakthrough

Table 4. Reproducibility of ART, CAP/CTM v1.0, and CAP/CTM v2.0 assays

Diluted pools of a patient specimen with a high HCV RNA concentration															
Expected titer	**2 Log IU/mL**			**1.7 Log IU/mL**			**1.4 Log IU/mL**			**0.8 Log IU/mL**			**0.49 Log IU/mL**		
Assay Method	**v1.0**	**v2.0**	**ART**	**v1.0**	**v2.0**	**ART**	**v1.0**	**v2.0**	**ART**	**v1.0**	**v2.0**	**ART**	**v1.0**	**v2.0**	**ART**
1st assay	2.0	2.3	1.9	1.4	1.8	1.6	<1.2 (+)	1.5	1.4	<1.2 (+)	1.3	<1.1 (+)	<1.2 (+)	<1.2 (+)	<1.1 (+)
2nd assay	2.2	2.4	1.9	1.6	2.0	1.7	<1.2 (+)	1.4	1.5	<1.2 (+)	<1.2 (+)	<1.1 (+)	<1.2 (+)	<1.2 (+)	<1.1 (+)
3rd assay	2.1	2.3	2.0	1.5	1.9	1.7	<1.2 (+)	1.2	1.4	<1.2 (+)	<1.2 (+)	<1.1 (+)	<1.2 (+)	<1.2 (+)	<1.1 (+)
4th assay	2.0	2.2	1.9	1.3	2.0	1.7	<1.2 (+)	1.2	1.4	<1.2 (+)	<1.2 (+)	<1.1 (+)	<1.2 (+)	<1.2 (+)	<1.1 (+)
5th assay	2.0	2.2	2.0	1.6	1.9	1.6	<1.2 (+)	1.9	1.5	ND	ND	<1.1 (+)	ND	ND	ND
6th assay	1.9	2.1	1.9	1.5	1.9	1.6	<1.2 (+)	1.7	1.4	ND	ND	<1.1 (+)	ND	ND	ND
Mean±SD	2.0±0.09	2.3±0.10	1.9±0.05	1.5±0.11	1.9±0.07	1.7±0.05	-	1.5±0.25	1.4±0.05	-	-	-	-	-	-

HCV RNA: hepatitis C virus ribonucleic acid; ART: Abbott RealTime HCV assay; ND: not detected; SD: standard deviation
v1.0 (Roche COBAS® AmpliPrep/COBAS® TaqMan® HCV assay version 1.0)
v2.0 (Roche COBAS® AmpliPrep/COBAS® TaqMan® HCV assay version 2.0)

Six serum samples per concentration were analyzed using ART, CAP/CTM v1.0, and CAP/CTM v2.0 (Table 4). HCV RNA in serum was detectable using ART and CAP/CTM v2.0, at a concentration of 1.4 Log IU/mL; however, we could only detect a positive signal for serum HCV RNA using CAP/CTM v1.0. Furthermore, the previously prepared 1.4 Log IU/mL concentration was determined to be in the range of 1.4–1.5 Log IU/mL and 1.2–1.9 Log IU/mL using ART and CAP/CTM v2.0, respectively. Positive signals for serum HCV RNA were detected in all samples using ART (0.8 Log IU/mL), indicating that results obtained from ART were more reproducible than those from CAP/CTM v1.0 as well as CAP/CTM v2.0, since the former achieved the lowest standard deviation for any given concentration (Table 4).

DISCUSSION

The outcome of genotype 1 CHC has been markedly improved by the adoption of PegIFN/RBV/DAA triple therapy (3-10). It was previously shown that SNPs in *IL28B* are useful predictors of the effects of IFN-based therapy for CHC (11, 12); however, relapses can still occur in patients with the favorable *IL28B* allele. It is also known that the presence of HCV RNA in serum after the initiation of therapy correlates closely with the therapeutic outcome (19). Efforts have been made to improve the patient's SVR rate by introducing a response-guided therapy, whose duration is based on the time needed for the patient's serum to be cleared of HCV RNA (20,21). The protocol for a triple therapy clinical trial using simeprevir in Japan includes response-guided therapy (22-24), where the administration of combination therapy may be extended to clinical practice, according to the therapeutic outcomes predicted during the early stages of treatment. On the other hand, discontinuation of therapy is recommended if the patient continues to test positive for serum HCV RNA for 12 weeks after the initiation of therapy, as they are unlikely to achieve SVR status (25,26). Therefore, it is very important to accurately measure serum HCV RNA levels in patients undergoing IFN therapy for CHC, in order to determine the appropriate course of treatment.

CAP/CTM v1.0 has been the most frequently used method to detect HCV RNA levels in serum. However, the accuracy of the assay in detecting genotype 4 CHC was found to be problematic (27). Recently, this assay has been replaced with an improved testing method, CAP/CTM v2.0. Similar to worldwide trends, CAP/CTM v1.0 was replaced in Japan by CAP/CTM v2.0 in April 2014 because of the high failure rate of the former in detecting genotype 2 CHC (18). CAP/CTM v2.0 is more capable than CAP/CTM v1.0 at detecting lower HCV RNA concentrations in serum. However, test accuracy, which is dependent on viral genotype, is lower in CAP/CTM v2.0 compared to CAP/CTM v1.0 (27). ART was developed as an HCV RNA detection system that differs from both CAP/CTM v1.0 and CAP/CTM v2.0. Since ART has a lower limit of detection (1.08 Log IU/mL), this assay is a more sensitive method than both CAP/CTM v1.0 and CAP/CTM v2.0. Previous studies have shown a good correlation between the patient's HCV RNA status determined using ART, then when determined using CAP/CTM v2.0 (27).

Ogawa et al. (28) (2013) compared the results of serum HCV RNA detection obtained by CAP/CTM v1.0 with those obtained by ART, in patients infected with genotype 1b CHC that were undergoing triple therapy. Their results showed that 28.5% of the patients who tested negative for serum HCV RNA using CAP/CTM v1.0 tested positive for HCV RNA using ART. However, 3.9% of the patients who tested negative for HCV RNA, using ART, tested positive for serum HCV RNA using CAP/CTM v1.0. Additionally, the percentage of patients testing negative for serum HCV RNA using ART at weeks 3, 4, and 5 after the initiation of therapy, was statistically significantly lower than the percentage of those testing negative for serum HCV RNA using CAP/CTM v1.0. In our study, the percentage of patients testing negative for serum HCV RNA using ART (17.9%) was significantly lower than the percentage of those testing negative

Supplementary table. List of all Non-SVR patients with HCV RNA levels during treatment and 4 weeks after completion of therapy

	Age	Gender	IL28B	Method	Pre (LogIU/mL)	2w (LogIU/mL)	4w (LogIU/mL)	8w (LogIU/mL)	12w (LogIU/mL)	24w (LogIU/mL)	Post 4w (LogIU/mL)	Outcome
1	61	F	TT	CAP/CTM v1.0	7.0	2.0	<1.2 (+)	ND	ND	ND	5.4	Relapse
				ART	6.22	NC	<1.08 (+)	<1.08 (+)	ND	NC	NC	
2	39	M	TG	CAP/CTM v1.0	6.6	1.9	<1.2 (+)	ND	ND	ND	2.8	Relapse
				ART	6.03	1.86	<1.08 (+)	<1.08 (+)	ND	NC	NC	
3	20	M	TG	CAP/CTM v1.0	6.2	3.4	1.4	ND	ND	ND	5.6	Relapse
				ART	5.97	2.81	<1.08 (+)	ND	ND	NC	NC	
4	72	F	TG	CAP/CTM v1.0	7.2	2.7	1.7	<1.2 (+)	<1.2 (+)	-[5]	6.4	NR
				ART	6.60	2.04	1.45	<1.08 (+)	<1.08 (+)	-[5]	NC	
5	67	M	TG	CAP/CTM v1.0	7.0	1.8	<1.2 (+)	<1.2 (+)	ND	4.8	5.9	BT
				ART	6.38	1.93	1.35	<1.08 (+)	<1.08 (+)	NC	NC	
6	67	F	GG	CAP/CTM v1.0	7.4	2.1	<1.2 (+)	ND	<1.2 (+)	6.8	7.4	BT
				ART	6.78	2.29	1.27	ND	<1.08 (+)	NC	NC	
7	66	M	TT	CAP/CTM v1.0	6.8	1.9	1.6	ND	1.2	6.7	7.4	BT
				ART	6.27	1.48	1.31	<1.08 (+)	<1.08 (+)	NC	NC	

HCV RNA: hepatitis C virus ribonucleic acid; SVR: sustained virological response; M: male; F: female; w: week; ART: Abbott RealTime HCV assay; ND: not detected; NC: not complied; NR: no response; BT: breakthrough

CAP/CTM v1.0 (Roche COBAS® AmpliPrep/COBAS® TaqMan® HCV assay version 1.0)

-[5]According to response-guided therapy, discontinuation of therapy was selected because the patient continues to test positive for HCV RNA for 12 weeks after the initiation of therapy

for serum HCV RNA by CAP/CTM v1.0 (53.6%) (data not shown). These differences may be due to the improved lower limit of detection in the ART assay, as compared with that of CAP/CTM v1.0. In this study, HCV RNA was detected in serum samples that were subjected to multiple freeze/thaw cycles, which may have affected the results. Yet, the percentage of patients who tested negative for HCV RNA using ART was lower than the percentage of those who tested negative for HCV RNA using CAP/CTM v1.0. Additionally, results obtained from ART were more reproducible than those obtained from CAP/CTM v1.0, since the former showed the lowest standard deviation for each sample at any given concentration. Also, the ART assay efficiently detected HCV RNA levels at low concentrations. These results indicate that ART has better reproducibility, and allows for more sensitive detection of HCV RNA in serum than either CAP/CTM v1.0 or CAP/CTM v2.0.

In this study, patients who tested negative for HCV RNA early after the initiation of therapy, using CAP/CTM v1.0 or ART, showed a high SVR rate. On the other hand, those who continued to test positive for HCV RNA at week 8 after the initiation of therapy, as determined by ART, did not achieve SVR. Although CAP/CTM v1.0 is currently being replaced by CAP/CTM v2.0, the lower limit of detection of CAP/CTM v2.0 for HCV RNA is the same as that of CAP/CTM v1.0 (i.e., 1.2 Log IU/mL). The results from our reproducibility tests using diluted samples suggest that similar results would be obtained if these samples were to be tested by CAP/CTM v2.0, and compared to those obtained using ART. The results obtained in this study demonstrate that the detection of HCV RNA in serum samples using ART, at 8 weeks after the initiation of therapy, is an efficient predictor of the effects of IFN-based triple therapy in CHC patients.

A limitation of this study is that the number of cases is small. Therefore, additional studies using a larger number of patients would be desirable. Another limitation is that triple therapy using TVR is no longer the standard of care and has been supplanted by triple therapy using the second-wave, first-generation protease inhibitor simeprevir (29). Consequently, it is necessary to perform similar studies with triple therapy using simeprevir.

More recently, there has been a gradual shift to treating CHC patients with oral DAAs, rather than with IFN-based therapy (30,31). However, some patients are difficult to treat because of an increase in drug resistance (30,31), and in many developing countries where HCV is endemic, IFN-based therapy will remain the first choice because of the high cost of DAAs (32). In addition, since there are CHC patients that experience relapse or viral breakthrough after completion of oral therapy with DAAs (33,34), IFN still plays an important role in the treatment of CHC patients. IFN's broad antiviral activity could help clear resistant strains and improve the chance of successful re-treatment with DAA therapy; therefore, we should consider using sensitive detection methods such as ART in order to accurately monitor IFN-based therapeutic outcomes.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of St. Marianna University School of Medicine Hospital.

Informed Consent: Written informed consent was obtained from patients who participated in this study.

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