Abbott RealTime Hepatitis C Virus (HCV) and Roche Cobas AmpliPrep/Cobas TaqMan HCV Assays for Prediction of Sustained Virological Response to Pegylated Interferon and Ribavirin in Chronic Hepatitis C Patients

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Two commercial real-time PCR assays are currently available for sensitive hepatitis C virus (HCV) RNA quantification: the Abbott RealTime HCV assay (ART) and Roche Cobas AmpliPrep/Cobas TaqMan HCV assay (CAP/CTM). We assessed whether the two real-time PCR assays were more effective than Roche Cobas Amplicor HCV Monitor test, v.2.0 (CAM) for prediction of the sustained virological response (SVR) to pegylated interferon (PEG-IFN) plus ribavirin (RBV) in chronic hepatitis C. Sixty patients chronically infected with HCV genotype 1b (37 males and 23 females, 53 ± 12 years of age) were treated with PEG-IFNα2b plus RBV for 48 weeks. Stored specimens at nine time points for each patient (at baseline, on treatment, and 24 weeks after treatment) were tested by the two real-time PCR assays and CAM. Twenty-six (43.3%) patients reached SVR. The positive predictive values (PPVs) for SVR of undetectable HCV RNA at week 12 by CAM, ART, and CAP/CTM were 74.3%, 88.0%, and 95.2%, respectively. An undetectable HCV RNA level by CAM, ART, and CAP/CTM correctly predicted SVR at week 4 in 100%, 100%, and 100% of patients, at weeks 5 to 8 in 91.7%, 100%, and 100% of patients, at weeks 9 to 12 in 55.6%, 75%, and 87.5% of patients, and at weeks 13 to 24 in 0%, 26.7%, and 40% of patients, respectively. Of 16 patients who relapsed after treatment, HCV RNA was detectable in 2 patients at the end of treatment by CAP/CTM but undetectable by ART and CAM. HCV RNA tests using ART and CAP/CTM are considered to be more effective at predicting SVR than CAM, and the PPV for SVR was slightly higher in CAP/CTM than in ART.

The quantification of hepatitis C virus (HCV) RNA is essential for the management of chronic hepatitis C therapy based on the combination of pegylated interferon (PEG-IFN) and ribavirin (RBV). Based on pivotal trials in large multicenter studies, positive and negative predictions of sustained virological response (SVR) using viral load kinetics have been established and are now used for recommendations on antiviral therapy management by the American and European international consensus conferences (6, 9, 17). Initially, the effectiveness of antiviral therapy had been controlled by HCV RNA quantification: the Abbott RealTime HCV assay (ART; Abbott Molecular, Inc., Des Plaines, IL) and the Roche Cobas AmpliPrep/Cobas TaqMan HCV assay (CAP/CTM; Roche Molecular Systems, Inc., Pleasanton, CA). The reported sensitivity of these real-time PCR assays is higher than that of CAM, they are reportedly not prone to carryover contamination, and they have a consistently wider dynamic range of quantification, which makes them particularly useful for quantifying the full range of viral genome levels observed in treated and untreated patients. Real-time PCR is rapidly replacing other technologies for the routine quantification of HCV RNA.

In the present study using stored serum samples collected from patients chronically infected with HCV who were receiving PEG-IFNα2b and RBV, ART and CAP/CTM were clinically evaluated, with particular regard to predicting effectiveness of treatment. The serum samples were obtained at various time points during the therapy, and the viral load in each of the specimens was evaluated using CAM, ART, and CAP/CTM.

MATERIALS AND METHODS

Patients. A total of 60 patients were enrolled in this study from those who received therapy with PEG-IFNα2b plus RBV for chronic HCV infection at three hospitals (Naogoya City University Hospital, Social Insurance Chukyo Hospital, and Nagoya City Johoku Hospital). The mean age of the population was 53 years, and 37 (61.7%) were male. All patients had genotype 1b HCV. The mean alanine transaminase level was 63.4 ± 39.1 IU/liter, and the mean platelet count was (161.1 ± 6.0) × 104/mm3. The mean HCV RNA level was 2,311 ± 1,600 kIU/ml by CAM. Other causes of chronic hepatitis and human immunodeficiency virus infection were excluded by appropriate serological testing and/or liver histology. All patients were treated with PEG-IFNα2b (1.5 mg/kg of body weight by subcutaneous injection once a week) plus RBV (600 to 1,000 mg daily ac-
According to body weight) orally for 48 weeks. The doses of PEG-IFN-alpha-2b plus RBV were individually reduced during the treatment whenever needed to lessen side effects, and these dose reductions were done according to the labeling.

Written informed consent was obtained from each patient, and the study was approved by the local ethics committee in accordance with the 1975 Declaration of Helsinki.

Definitions of response. All patients had HCV RNA testing at weeks 4, 8, 12, 16, 20, 24, and 48 of the combination therapy. Follow-up testing was performed at week 72. On-treatment virological response, SVR, and relapse were defined on the basis of CAM results in accordance with standard definitions. On-treatment response was defined as undetectable serum HCV RNA determined during the antiviral therapy. SVR was defined as serum HCV RNA tested as undetectable 6 months after the end of therapy.

Detection of HCV RNA. A total of 486 serum samples were obtained from the 60 patients. Each of the specimens was frozen to −80°C within 2 h of collection (10). HCV RNA quantitation in the sera was performed by CAM, ART, and CAP/CTM according to the corresponding manufacturer's instructions. ART, which is provided with an automated sample preparation, was carried on the m2000sp and the m2000rt instruments, and CAP/CTM, which is also provided with an automated sample preparation, was carried on the Cobas AmpliPrep and Cobas TaqMan instruments. The lower limits of detection were 12 IU/ml as reported for ART (13), 15 IU/ml for CAP/CTM (7), and 50 IU/ml for CAM (14). Positive results (signals) below the quantitative HCV RNA concentrations are referred to as “low positive” when registered by ART and CAP/CTM.

In general, the HCV RNA levels were measured for each patient in 9 serum specimens obtained at baseline; at weeks 4, 8, 12, 16, 20, 24, and 48 of treatment; and finally 6 months following the discontinuation of therapy.

Statistical analysis. Descriptive statistics are shown as the mean ± standard deviation or the median and interquartile range as appropriate. Categorical variables were compared between groups by the χ² test or Fisher’s exact test, and noncategorical variables were compared by the Mann-Whitney U test. A P value of <0.05 was considered significant.

RESULTS

Virological response. Among the 60 patients, 26 (43.3%) had SVR, 16 (26.7%) relapsed, 15 (25%) did not respond, and 3 (5%) discontinued the treatment due to side effects.

Comparison of CAM, ART, and CAP/CTM testing. The proportion of cases with undetectable HCV RNA revealed by each of the three assays among the patients tested at different time points during the therapy is depicted in Fig. 1. At week 12, the difference between CAM and CAP/CTM was significant (P < 0.05), but at the other time points, the difference was not significant.

Of the 251 specimens with HCV RNA undetectable by CAM, ART and CAP/CTM revealed the presence of viral genome copies in 36 and 50 specimens, respectively. In addition, CAP/CTM allowed detection of HCV RNA in 25 specimens that were negative by ART: 190 specimens were undetectable by both CAP/CTM and ART. On the other hand, there were some 11 specimens with HCV RNA undetectable by CAP/CTM but positive by ART: 25 specimens were detectable by both CAP/CTM and ART.

Prediction of SVR based on each assay. The positive predictive value (PPV) and negative predictive value (NPV) for SVR was evaluated on the basis of undetectable HCV RNA as revealed by each of the three assays at weeks 4, 8, 12, 16, 20, and 24 of the start of treatment (Table 1). At week 12, the PPVs by CAM, ART, and CAP/CTM were 74.3%, 88.0%, and 95.2%, respectively. When we combined results of these two assays at week 12, the PPV and NPV of undetectable HCV RNA by both ART and CAP/CTM were 94.4% and 78.6%, respectively, and those by ART and/or CAP/CTM were 89.3% and 96.9%, respectively. Of note, all cases in which HCV RNA was undetectable by ART or CAP/CTM at week 8 reached the SVR. As shown in Fig. 2, HCV RNA at first undetectable by CAM, ART, and CAP/CTM could predict SVR at weeks 9 to 12 in 55.6%, 75%, and 96.9%, respectively, and at weeks 13 to 24 in 0%, 26.7%, and 40% of patients, respectively.

Prediction of relapse by CAP/CTM testing at the end of treatment. Of 16 patients who relapsed after treatment, very low levels of HCV RNA (positive results below the limit of quantification) were detectable in 2 patients at the end of treatment by CAP/CTM but undetectable by ART or CAM. On the other hand, of 26 patients who reached SVR, very low

**FIG. 1.** Proportion of HCV RNA samples negative by CAM, ART, and CAP/CTM at each period after the beginning of treatment.
levels of HCV RNA at the end of treatment were detectable in 2 patients by CAP/CTM and in 1 patient by ART but undetectable by CAM (Fig. 3). In these three cases, HCV RNA was undetectable afterward.

**DISCUSSION**

The two commercial real-time PCR assays currently available for highly sensitive HCV RNA quantification were comparatively evaluated in this study. These assays are reportedly more sensitive than previous assays, have a wider dynamic range of quantification than CAM, and are “specific,” “accurate,” “precise,” and “reproducible” (3, 7, 13, 15, 22, 23).

The proportion of HCV RNA-negative specimens as determined by CAP/CTM or ART was lower than that by CAM during the whole treatment period, especially at week 12 (CAP/CTM versus CAM; P < 0.05); implying higher sensitivity of these new real-time PCR assays, which makes them superior in their clinical value to CAM.

According to pivotal trials in large multicenter studies, positive and negative predictions of SVR estimated on basis of viral load kinetics are clinically reliable and are now used in antiviral therapy management as recommended by consensus of the American and European international conferences (6, 9, 17). The ability to predict either a positive or negative therapeutic response is of obvious benefit to clinicians and patients (12, 20). Positive predictive evidence early in the course of treatment could be used to reinforce the importance of compliance in ensuring a successful outcome. Conversely, negative predictive capability would allow clinicians to discontinue therapy early during the course of treatment, which would save health care resources and, more important, could prevent drug-related adverse events. A dynamic prediction model based on the early virological response showed that the outcome of combination therapy with PEG-IFN and RBV is dependent on the rapidity of the viral response (6, 18, 19). Therefore, early virologic response monitoring is now the standard of care for tailoring treatment to the individual patient’s response (2, 4, 5, 11). The data for the proposed algorithm for the management of antiviral therapy in patients chronically in-
fected with HCV were mainly based on measurement of viral load by CAM. We sought to determine whether testing by the two real-time PCR assays during therapy could further improve accuracy of predicting SVR to PEG-IFN and RBV therapy. Almost all patients whose HCV RNA went undetectable until week 8 as determined by ART, CAP/CTM, or CAM had reached SVR. The rates of SVR among patients who had HCV RNA that was at first undetectable during weeks 9 and 12 by CAM, ART, and CAP/CTM were 55.6%, 75.0%, and 87.5%, respectively (Fig. 2), suggesting that ART or CAP/CTM could be more specific than CAM in predicting SVR during the first 12 weeks of the therapy. In comparing ART to CAP/CTM, the PPVs for SVR of undetectable HCV RNA during the first 12 weeks were slightly higher in CAP/CTM than ART, but the difference did not reach significance. Recently, it has been reported that the sensitivity of CAP/CTM was slightly higher than that of ART on the basis of the limit of detection using international HCV WHO standard specimens (genotype 1a) (24). Further limit of detection studies using serum samples with other genotypes are needed to conclude the sensitivity between ART and CAP/CTM.

Recently, it has been reported that extension of treatment with PEG-IFN plus RBV from 48 to 72 weeks increases the rate of SVR in patients with late virological response defined as being HCV RNA positive at week 12 but negative at week 24 (1). In this study, some of the patients who at week 12 had HCV RNA undetectable by CAM were found to be positive by ART or CAP/CTM, and 60% (6/10, ART) or 57% (8/14, CAP/CTM) of these patients failed to achieve SVR. In such cases, extended treatment up to 72 weeks might provide a higher rate of SVR.

The rate of successful HCV eradication in chronically infected patients has significantly increased following the introduction of combination therapy with PEG-IFN plus RBV. Nevertheless, still from 15 to 25% of the patients who become HCV RNA negative by CAM during and at the end of therapy had viral relapse after treatment withdrawal. There are no identified on-treatment markers able to predict whether a patient will develop SVR or will relapse. Recently, it has been reported that minimal residual viremia detected by transcription-mediated amplification assay (the lower limit of detection is 5 to 10 IU/ml) (21) at the end of therapy in CAM-negative cases could reliably predict relapse after therapy withdrawal (8). In this study, 2 of 16 patients who had relapsed after treatment had HCV RNA detectable by CAP/CTM but undetectable by ART or CAM at the end of treatment. Minimal residual viremia detected by CAP/CTM might be predictive of posttreatment relapse. Whether relapse could have been prevented by continuing treatment for a longer duration remains to be confirmed in prospective controlled trials.

On the other hand, of 26 patients who reached SVR, 3 patients had HCV RNA detectable by ART or CAP/CTM at the end of treatment (Fig. 3). Similarly, it has been reported that minimal residual viremia was detectable by a transcrip-

![FIG. 3. Very low levels of HCV RNA three patients who reached SVR. Of 26 patients who reached SVR, very low levels of HCV RNA (positive results below the limit of quantification) were detectable in 3 patients by ART or CAP/CTM at the end of treatment. The outcome of HCV RNA testing by CAM is represented as positive (+) or negative (−). Positive results of HCV RNA below the limit of quantification represent <1.08 log IU/ml by ART and <1.2 log IU/ml by CAP/CTM. In case 1, HCV RNA was detectable at the end of therapy by CAP/CTM and at week 12 after the end of therapy by ART but undetectable afterward. In case 2, HCV RNA was detectable at the end of therapy by CAP/CTM, and in case 3, HCV RNA was detectable by ART but undetectable afterward. y.o., years old.

### Table 1

<table>
<thead>
<tr>
<th>Case</th>
<th>Age</th>
<th>Treatment</th>
<th>Outcome</th>
<th>Week 4</th>
<th>Week 8</th>
<th>Week 12</th>
<th>Week 20</th>
<th>Week 24</th>
<th>Week 48</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50 y.o. female</td>
<td>PEG-IFNα 2b + RBV</td>
<td>After the end of therapy</td>
<td>CAM</td>
<td>ART (log IU/mL)</td>
<td>CAP/CTM (log IU/mL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>40 y.o. male</td>
<td>PEG-IFNα 2b + RBV</td>
<td></td>
<td></td>
<td></td>
<td>1.2 &lt;1.08</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>52 y.o. male</td>
<td>PEG-IFNα 2b + RBV</td>
<td></td>
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tion-mediated amplification assay (the lower limit of detection is 5 to 10 IU/ml) at the end of treatment in the patients who reached SVR (16). One possibility is that in some patients with SVR, HCV RNA became undetectable even after discontinuation of therapy. However, it might be difficult to exclude contamination, false positivity, or detection of low levels of noninfectious fragments of the virus genome of little clinical significance. These explanations remain hypotheses; therefore, it will be necessary to examine minimal residual viremia by highly sensitive assays during or after treatment with PEG-IFN and RBV in large-scale prospective cohorts.

In conclusion, the results show that real-time PCR using either of the two newly available commercial assays instead of CAM can provide higher clinical value for the management of therapeutic responses to chronic hepatitis C. The ability to detect extremely low levels of serum HCV RNA may allow clinicians to predict outcome of treatment with higher confidence and develop more individualized protocols for treatment in terms of optimal duration.

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REFERENCES


