Comparison of the VERSANT™ HCV RNA qualitative assay (transcription-mediated amplification) and the COBAS AMPLICOR™ hepatitis C virus test, version 2.0, in patients undergoing interferon-ribavirin therapy

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Abstract

Hepatitis C virus (HCV)-infected patients were tested for the presence of HCV RNA using two qualitative assays at various time points during interferon-ribavirin therapy. Among patients treated for 48 weeks, transcription-mediated amplification and the COBAS AMPLICOR Hepatitis C Virus Test results at Week 24 predicted subsequent virologic non-response or virologic relapse in 12/15 (80%) and 8/15 (53%) patients, respectively. © 2003 Elsevier Inc. All rights reserved.

The VERSANT HCV RNA Qualitative Assay utilizing transcription-mediated amplification technology (TMA; Bayer HealthCare LLC, Berkeley, CA) can reportedly detect HCV RNA at concentrations <10 International Units (IU)/mL with >98% specificity (Sawyer et al., 2000; Ross et al., 2001; Krajden et al., 2002). Currently, the most widely used method of qualitative HCV RNA detection in the United States is the COBAS AMPLICOR Hepatitis C Virus Test, Version 2.0 (AMPLICOR; Roche Molecular Systems, Inc., Branchburg, NJ) utilizing reverse transcription-polymerase chain reaction (RT-PCR) technology. The AMPLICOR assay has a limit of detection ranging from 60 to 100 IU/mL (for plasma and serum, respectively) combined with a specificity approaching 100% (Lee et al., 2000; Roche Molecular Systems 2001). The performance characteristics of both TMA and AMPLICOR are well established, and both have received U.S. Food and Drug Administration (FDA) approval for the qualitative detection of HCV RNA in patient specimens.

Recent anti-HCV treatment algorithms suggest that tailoring antiviral therapy based on HCV genotype as well as viral titer can optimize therapeutic outcome in patients chronically infected with HCV (EASL 1999; NIH 2002). Patients chronically infected with HCV genotypes 2 and 3 are more likely to respond to interferon-ribavirin combination therapy than are those infected with HCV genotype 1 (NIH 2002). Furthermore, sustained virologic response, defined by the absence of detectable HCV RNA 24 weeks post-treatment, among patients infected with genotypes 1, 2, and 3 has been correlated with a low pre-treatment viral titer (NIH 2002). Clearance of HCV RNA, as determined by RT-PCR, at various time points during interferon-ribavirin combination therapy (including prior to the end-of-treatment) has also been suggested as a predictor of sustained virologic response following therapy (Brouwer et al., 1999; McHutchison et al., 1999; McHutchison et al., 2001). It has recently been shown that the increased sensitivity of TMA, as compared to RT-PCR, can improve the detection of extremely low levels of HCV RNA in end-of-treatment specimens and serve as a predictor of sustained virologic response following anti-HCV therapy (Sarrazin et al., 2000; Comanor et al., 2001; Sarrazin et al., 2001). However, there is currently a lack of data concerning the use of the more sensitive TMA with specimens obtained from patients while...
on therapy (i.e., prior to the end-of-treatment) to predict sustained virologic response following combination therapy. The current study was undertaken to determine if TMA could increase the sensitivity of residual HCV RNA detection in patient specimens obtained during interferon-ribavirin therapy. If possible, TMA might improve the prediction of virologic non-response or virologic relapse (defined by the presence of detectable HCV RNA at the end of treatment or at 24 weeks post-treatment, respectively) in these patients. In turn, the increased sensitivity of TMA may potentially enable early cessation of a therapeutic regimen unlikely to result in sustained virologic response (with the usual duration of therapy) or, alternatively, enable identification of patients for whom extended therapy may be of benefit.

We retrospectively performed parallel TMA and AMPLICOR HCV RNA detection on serum specimens obtained from a well-characterized cohort of 44 HCV-infected patients undergoing interferon-ribavirin therapy. All patients were treated for at least 24 weeks; some patients, including those without detectable HCV RNA at Week 24, were treated for an additional 24 weeks (48 weeks total, n = 33). A total of 111 serum specimens obtained 12, 24, and 48 weeks after the initiation of therapy, and stored at -70°C, were studied. Specimens collected more than four weeks from each of the previously stated time points were excluded from the analysis for the purpose of this study. The end-of-treatment specimen (24 or 48 weeks) obtained from all patients was collected within one week of the date treatment was discontinued in order to obtain a true end-of-treatment result free from potentially rebounding post-treatment HCV RNA levels in some patients. Concurrent TMA and AMPLICOR testing was performed to avoid loss of HCV RNA due to multiple freeze/thaw of patient specimens. Virologic response was assessed 24 weeks after the discontinuation of therapy in both the 24- and 48-week patient groups using RT-PCR; the study included 16 sustained virologic responders, 21 virologic non-responders, and seven virologic relapsers.

The TMA assay was performed following the manufacturer’s suggested procedure, and included three positive (reactive) and three negative (non-reactive) calibrators in addition to an internal amplification control that was added to each specimen prior to the amplification process. Three specimens (two from Week 24 and one from Week 48) yielded invalid results by TMA (non-reactive with a non-reactive internal control) and required repeat testing from a new frozen aliquot. All three specimens were determined to be non-reactive upon retesting.

The AMPLICOR assay was performed in accordance with the manufacturer’s instructions, including the use of the positive, negative, and internal amplification controls provided in the assay kit. A single 24-week specimen resulted in an “equivocal” test result requiring retesting. The specimen was repeated in duplicate (per manufacturer’s protocol) from another frozen serum aliquot and determined to be negative.

HCV genotyping was performed with pre-treatment specimens obtained from all patients using the VERSANT™ HCV Genotype Assay (LiPA; Bayer HealthCare LLC). HCV genotype distribution among the 44 patients included 26 patients with genotype 1, nine patients with genotype 2, five patients with genotype 3, and two patients with genotype 4. Additionally, two patient’s specimens yielded HCV that could not be genotyped using this method.

Pre-treatment HCV RNA titers were also determined using either the VERSANT™ HCV RNA 2.0 Assay (Bayer HealthCare LLC) or the SuperQuantr™ Assay (National Genetics Institute, Los Angeles, CA). HCV RNA titers were converted to IU/mL using previously established conversion factors of 6.3 equivalents/IU (Saldanha et al., 1999; Germer et al., 2002) and 3.4 copies/IU (NGI 1999; Comanor et al., 2001), respectively. Viral titers ranged from 9,118 to >19,047,620 IU/mL among these patients.

Agreement between the TMA and AMPLICOR assays was estimated at 12 and 24 weeks (24- and 48-week treatment groups) with kappa statistics and 95% confidence intervals for those kappa statistics. Agreement was also estimated at 48 weeks (48-week treatment group). The kappa statistics were evaluated based on a scale suggested by Landis and Koch (Landis et al., 1977). The ability of each assay to predict virologic non-response or relapse was assessed in the 48-week treatment group by calculating the proportion of non-responders and relapsers who had a positive assay result at Week 24. In addition, 95% confidence intervals were calculated for the estimated proportions.

At Week 12, there were 39 specimens available for testing; 38/39 TMA and AMPLICOR results were in concordance (kappa = 0.94; 95% CI, 0.83 to 1.0), with a single discordant result (AMPLICOR-positive, TMA-non-reactive). The results obtained from the 33 specimens tested at Week 48 were in complete concordance (kappa = 1.0). At Week 24, there were 39 specimens available for testing. Among them, 34/39 yielded concordant TMA and AMPLICOR results (kappa = 0.74; 95% CI, 0.54 to 0.95), while five yielded discordant results (all TMA-reactive, AMPLICOR-negative). All five discordant specimens were from patients in the 48-week treatment group, and four of the five (80%) were from patients who ultimately either failed to respond or relapsed virologically following 48 weeks of therapy (Table 1).

No significant differences in the performance of TMA and AMPLICOR were noted among the 12- and 48-week specimens included in this study and the overall results support a recent analytical comparison of these assays (Krajden et al., 2002). However, a single TMA-non-reactive, AMPLICOR-positive result was observed among the 12-week specimens. Review of the original TMA data revealed no evidence of specimen inhibition or technical errors which may have contributed to this discordance, and
Table 1
VERSANT HCV RNA qualitative assay (TMA) and COBAS AMPLICOR hepatitis C virus test, version 2.0 (AMPLICOR) discordant results at week 24 (48-week treatment group)

<table>
<thead>
<tr>
<th>HCV genotype (LiPA)</th>
<th>Pre-treatment viral titer (IU/mL)</th>
<th>TMA result</th>
<th>AMPLICOR Long-term virologic response</th>
</tr>
</thead>
<tbody>
<tr>
<td>2a</td>
<td>85,294 reactive negative*</td>
<td>sustained response</td>
<td></td>
</tr>
<tr>
<td>1a</td>
<td>2,111,111 reactive negative relapse</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1b</td>
<td>970,588 reactive negative relapse</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4c/4d</td>
<td>&gt;1,470,588 reactive negative relapse</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-typeable</td>
<td>1,441,176 reactive negative non-response</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Original test result was "equivocal". Repeat testing in duplicate (per manufacturer’s protocol) yielded a negative result.

Among the 24-week specimens, a total of 17 patients in the 48-week treatment group were ultimately identified as either virologic non-responders or virologic relapsers; 15 patients had specimens available for testing at this time point. TMA results obtained from these 24-week specimens predicted either subsequent virologic non-response or virologic relapse in 12/15 (80%; 95% CI, 52% to 96%) patients treated for 48 weeks (Table 2), while AMPLICOR predicted ultimate virologic non-response or virologic relapse in just 8/15 (53%; 95% CI, 27% to 79%) of these patients (Table 3). While neither assay was able to correctly identify all virologic non-responders and virologic relapsers, TMA yielded improved prediction of treatment failure among patients treated with a 48-week course of interferon-ribavirin therapy.

In summary, the TMA assay proved to be a reliable alternative to RT-PCR (AMPLICOR) in this study. The increased sensitivity of the TMA assay may make this assay more useful than AMPLICOR in ultimately predicting either virologic non-response or virologic relapse with current antiviral therapies. Additional studies of larger numbers of patients are indicated to further assess the clinical utility of the TMA assay for the qualitative detection of HCV RNA in patients undergoing antiviral therapy.

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References


