Towards optimal treatment for chronic hepatitis C infection
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Low level HCV viraemia after initial response during antiviral therapy: transcription-mediated amplification predicts treatment failure

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Short communication

Low-level HCV viraemia after initial response during antiviral therapy: transcription-mediated amplification predicts treatment failure

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Preliminary results of these studies were disclosed at the 11th International Symposium on Hepatitis C and Related Viruses, Heidelberg, Germany, 3–7 October 2004 (p43), and the 12th International Symposium on Viral Hepatitis and Liver Disease, Paris, France, 1–5 July 2006 (p249).

Background: In chronic hepatitis C patients with an initial virological response (IVR) during antiviral therapy (that is, HCV RNA becomes negative before week 16 of treatment) the significance of reappearance of viraemia below the detection limit of PCR is not known. We studied this phenomenon in subsets of patients.

Methods: We assessed HCV RNA at weeks 16 and 20 of therapy by PCR and by more sensitive transcription-mediated amplification (TMA) in 23 patients with breakthrough or relapse and in 34 patients with sustained virological response (SVR). All patients participated in a high-dose-interferon induction study for difficult-to-treat patients. Therapy consisted of amantadine hydrochloride and ribavirin, combined with interferon-α2b induction during the first 6 weeks and thereafter combined with weekly pegylated interferon-α2b.

Results: Among the 57 IVR patients, we detected transient or persistent reappearance of low levels of HCV RNA in 10 of the 23 (43%) patients with eventual breakthrough or relapse; but in none of the 34 SVR patients. In 5 of 10 patients reappearing HCV RNA was only detectable by TMA.

Conclusion: Reappearance of low levels of HCV RNA in patients with IVR predicts treatment failure.

Introduction

The current antiviral therapy for chronic hepatitis C virus (HCV) infection consists of administration of pegylated interferon-α and ribavirin for 12–24 weeks (genotype 2 and 3) [1–4], 24–28 weeks (genotype 1) [2–5], or 48 weeks (genotype 4) [3] and leads to a sustained virological response (SVR) in ~50% (genotype 1 and 4) to 80% (genotype 2 and 3) of patients.

The duration of interferon-α and ribavirin therapy is based on HCV genotype, baseline HCV RNA load [5] and the HCV RNA level after 4 weeks [1,5], 12 weeks [6,7] or 24 weeks of antiviral therapy [6]. Interferon-α- and ribavirin-based therapy is expensive and causes a wide range of side effects in the majority of patients. Therefore, the goals of HCV RNA detection and quantification during antiviral therapy are (i) to stop treatment in patients predicted to experience treatment failure [6,7], and (ii) to shorten treatment duration in rapid responders [1,5].

HCV RNA levels below the lower limits of detection (LLD) of quantitative assays (600–615 IU/ml) can be detected by the more sensitive, qualitative PCR (LLD: 50 IU/ml). The consensus rules for decisions on treatment duration at 4 and 24 weeks of treatment are based on qualitative PCR [1,5–7]. However, more sensitive
Low-level HCV viraemia during antiviral therapy

detection of HCV RNA is possible using transcription-mediated amplification (TMA; LLD: 5 IU/ml). The relevance of this 10-fold increased sensitivity of TMA to decisions regarding treatment duration is unknown. Detection of HCV RNA by TMA but not by PCR (PCR-negative, TMA-positive viraemia, HCV RNA <50 but >5 IU/ml) after 12 or 24 weeks of treatment has been observed in very few patients, and was followed by virological non-response in most cases [8–11].

One-hundred and three HCV-infected patients were included in a study on the effects of high-dose-induction treatment for difficult-to-treat patients. In a sub-study of this study we determined the significance for treatment outcome of low-level viraemia during antiviral therapy. We performed PCR and TMA at weeks 16 and 20 on samples from patients with an initial response, who later experienced breakthrough, relapse or sustained virological response.

Patients and methods

Patients and trial design

A trial was designed to study the influence of daily high-dose interferon induction on early viral kinetics and treatment outcome in difficult-to-treat hepatitis C patients (that is, patients with any HCV genotype who had not responded to previous interferon treatment, and treatment-naïve patients infected with HCV genotype 1 or 4). In total, 103 patients were recruited in the trial, 97 patients, with baseline characteristics as presented in Table 1, received one or more doses of treatment. A flow chart of the trial is depicted in Figure 1. Among the 97 patients who received treatment, 57 patients showed an initial virological response (IVR): HCV RNA was undetectable by TMA (see below) before 16 weeks. Subsequently they experienced breakthrough, relapse or sustained virological response. These 57 IVR patients are the subjects of the analysis presented here: they were studied for reappearance of low-level HCV RNA at week 16 and 20. The baseline characteristics of the 57 IVR patients are presented in Table 2.

All patients were treated with triple therapy consisting of: amantadine hydrochloride 200 mg/day (Symmetrel®; Novartis, Basel, Switzerland) and ribavirin (Rebetol®; Schering-Plough, Kenilworth, NJ, USA) 1,000 or 1,200 mg/day (dependent on body weight) for a total of 24 or 48 weeks, combined with interferon-α2b induction (IntronA®; Schering-Plough) during the first 6 weeks, and thereafter combined with weekly pegylated interferon-α2b (PegIntron®, Schering-Plough), 1.5 µg/kg for a total of 24 or 48 weeks. The interferon induction scheme during the first 6 weeks was as follows: weeks 1 and 2: 18 MU/day in 3 divided doses; weeks 3 and 4: 9 MU/day in 3 divided doses; week 5 and 6: 6 MU/day in 2 divided doses. Patients with a decrease of HCV RNA <3 log at week 4 were treated for 48 weeks, whereas patients with a decrease of HCV RNA ≥3 log at week 4 were randomized to stop treatment early at 24 weeks or continue to 48 weeks. Treatment was stopped in all patients who were positive for HCV RNA by PCR at week 24. All patients were followed for 24 weeks after completion of therapy. The study was approved by the institutional review board. Written informed consent was obtained from each patient. Data regarding early viral kinetics are not shown: these results will be presented in another publication.

Table 1. Baseline characteristics of patients who received one or more doses of treatment

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Treat-naive</th>
<th>Previous treatment failure</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>41</td>
<td>25</td>
<td>66</td>
</tr>
<tr>
<td>2</td>
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<tr>
<td>3</td>
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<td>7</td>
<td>7</td>
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<tr>
<td>4</td>
<td>12</td>
<td>9</td>
<td>21</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Figure 1. Flow chart of the trial

[Diagram showing the flow of patients through the trial, with outcomes such as 103 included, 6 did not show, 97 intention to treat, 13 dropped out, 27 non-responders (TMA remains positive during treatment), 57 initial viral responders (TMA-negative before 16 weeks), 34 SVR, 23 BT/REL.]

BT/REL, breakthrough or relapse; SVR, sustained virological response; TMA, transcription-mediated amplification.
Table 2. Baseline characteristics of the 57 patients with IVR (i.e. TMA-negative before week 16 of antiviral therapy)

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Sustained virological response</th>
<th>BT/REL</th>
<th>BT/REL and low-level viraemia at week 16 or 20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients, n</td>
<td>57</td>
<td>34</td>
<td>23</td>
<td>10</td>
</tr>
<tr>
<td>Male/Female, n/n</td>
<td>44/13</td>
<td>26/8</td>
<td>18/5</td>
<td>8/2</td>
</tr>
<tr>
<td>Mean age (range), years</td>
<td>43.5 (19–63)</td>
<td>44.1 (25–63)</td>
<td>42.7 (19–50)</td>
<td>39.9 (19–49)</td>
</tr>
<tr>
<td>Treatment naive/previous treatment failure, n/n</td>
<td>34/23</td>
<td>23/11</td>
<td>11/12</td>
<td>5/5</td>
</tr>
<tr>
<td>Genotype, n</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCV 1</td>
<td>39</td>
<td>21</td>
<td>18</td>
<td>9</td>
</tr>
<tr>
<td>HCV 2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>HCV 3</td>
<td>7</td>
<td>5</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>HCV 4</td>
<td>8</td>
<td>6</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>HCV 5</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Decrease of HCV RNA ≥3 log_{10} at week 4, n (%)</td>
<td>48 (84)</td>
<td>27 (79)</td>
<td>21 (91)</td>
<td>9 (90)</td>
</tr>
</tbody>
</table>

BT/REL, breakthrough or relapse; HCV, hepatitis C virus; IVR, initial virological response; TMA, transcription-mediated amplification.

Specimen collection and HCV RNA assessment
Blood samples were obtained in EDTA-containing Vacutainer tubes (Becton Dickinson, Alphen aan den Rijn, the Netherlands); the samples were centrifuged and plasma samples were frozen at -80°C within 24 h of collection. We collected and tested samples during antiviral therapy at standard time points at day 0, weeks 4 and 12, at end-of-treatment (week 24 or 48), and at end-of-follow-up (week 48 or 72) by TMA, PCR and bDNA (see below). To determine the presence of low-level viraemia, we tested additional samples drawn at weeks 16 and 20.

The presence and level of HCV RNA was determined and categorized as follows:

**HCV RNA levels ≥615 IU/ml.** HCV RNA levels were determined quantitatively using the bDNA VERSANT® HCV 3.0 assay (Bayer Diagnostics, Berkeley, CA, USA). The dynamic range of this assay is 615–7.7×10^6 IU/ml HCV RNA [12].

**HCV RNA ≥50 and <615 IU/ml.** The HCV RNA level was determined to be between 50 and 615 IU/ml if the sample tested negative in the bDNA assay, but positive in the qualitative PCR (COBAS® Amplicor HCV Test v2.0, Roche Molecular Systems, Branchburg, NJ, USA; LLID: 50 IU/ml HCV RNA) [13].

**HCV RNA ≥5 and <50 IU/ml.** The HCV RNA level was determined to be between 5 and 50 IU/ml if the sample tested negative in the PCR assay, but positive in the TMA (VERSANT® HCV qualitative assay, Bayer Diagnostics; LLID: 5 IU/ml) [14].

**HCV RNA <5 IU/ml.** The HCV RNA level was determined to be <5 IU/ml when the TMA tested negative.

HCV genotype.

HCV genotypes were determined using the TruGene® HCV genotyping assay (Bayer Diagnostics).

Statistical analysis.
Statistical analysis was performed using SPSS version 12.0.2 for Windows (SPSS Inc., Chicago, IL, USA). The association between the presence of HCV RNA at weeks 16 or 20 on the one hand and treatment failure on the other was determined using the McNemar test with a two-tailed P-value. Associations were assessed for each week separately (presence of HCV RNA at week 16 or at week 20) as well as for both time points in a row (presence of HCV RNA at weeks 16 and/or 20). The level of significance was reset to 0.025 to correct for multiple comparisons.

Results
We studied 57 patients with IVR (that is, TMA-negative before week 16 of antiviral therapy). Subsequently 9/57 patients showed a breakthrough during therapy, 14/57 patients relapsed after cessation of therapy, and 34/57 patients achieved an SVR. Sensitive testing for presence of HCV RNA by TMA at weeks 16 and 20 revealed that the TMA assay remained negative in all 34 patients who achieved an SVR, but 10 of the 23 patients with eventual breakthrough or relapse showed transient or persistent reappearance of low levels of HCV RNA. In eight patients HCV RNA reappeared at week 16, in two patients at week 20 (Table 3). The association between reappearance of HCV RNA at week 16 or 20 and subsequent treatment failure was significant (week 16: P<0.001; week 20: P<0.001; week 16 and/or 20: P=0.002). In five of these 10 patients the reappearing HCV RNA was also
Table 3. TMA and PCR results at weeks 16 and 20 for the 57 patients with IVR

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Week 16</th>
<th>Week 20</th>
</tr>
</thead>
<tbody>
<tr>
<td>TMA+</td>
<td>14*</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>TMA+ PCR-</td>
<td>9</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>TMA+ PCR+</td>
<td>5</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>TMA-</td>
<td>92</td>
<td>45</td>
<td>47</td>
</tr>
<tr>
<td>Total</td>
<td>106</td>
<td>53</td>
<td>53</td>
</tr>
</tbody>
</table>

*Four patients were hepatitis C virus (HCV)-positive by transcription-mediated amplification (TMA+) at both time points; samples from eight patients were available at only one of the two time points. NR, initial virological response; PCR+, HCV-positive by PCR; PCR-, HCV-negative by PCR; TMA+, HCV-negative by TMA.

detectable by PCR (Table 3). There was no correlation between the decrease in HCV RNA at week 4 and subsequent breakthrough, relapse or SVR.

The predictive value of HCV RNA detection by TMA at week 16 or 20 for treatment failure was 100%. The predictive value of a negative TMA test result at week 16 and 20 for SVR was 72%.

We categorized the low-level viraemia of the 10 patients into three patterns (Figure 2): (i) breakthrough, but with detection by TMA 4 weeks earlier than by PCR in 3/10 patients (Figure 2A); (ii) transient low-level viraemia preceding breakthrough by 16–30 weeks in 3/10 patients (Figure 2B); and (iii) transient low-level viraemia preceding relapse by 4–32 weeks in 4/10 patients (Figure 2C).

Discussion

In patients with an IVR, we detected transient or persistent reappearance of low-level viraemia after 16 and 20 weeks of antiviral therapy in 10/23 patients with eventual breakthrough or relapse, but not in 34 patients who achieved SVR. The reappearing HCV RNA was detected with TMA, and in some cases also with PCR. Low-level viraemia preceded breakthrough or relapse by 4–32 weeks. Thus, sensitive detection of reappearing HCV RNA enables early prediction of treatment failure, with a high predictive value.

Persistence of HCV RNA detectable by TMA but not by PCR during treatment in patients with eventual breakthrough, relapse or non-response has been described previously [8–10]. In three studies, these patients were studied at weeks 12 [15] and 24 [9,10,15] only, during 48 week treatment regimens. Our results confirm the significance of reappearance of HCV RNA detected only by TMA [8]. Two important differences between these studies and our study are that we sampled more frequently during treatment and we included patients with SVR in our analysis, thus enabling the calculation of the predictive value of reappearing viraemia by TMA for failure of treatment.

Our analysis revealed the presence of HCV RNA between the standard sampling points at week 12 and week 24. The fact that reappearance of viraemia during treatment seems limited to patients with eventual breakthrough or relapse makes it a clinically relevant phenomenon. In such patients treatment could be stopped earlier.

By contrast, results from the HALT-C trial have shown that 6/27 patients with reappearance of HCV detectable by TMA at week 24 did achieve SVR [16]. However, (i) the HALT-C trial deals with a different
patient population (previous non-responders with advanced liver disease), (ii) patients were treated with a different treatment regime, and (iii) patients were not evaluated at week 16.

The reappearance of low-level HCV viraemia we observed might be related to the therapeutic regimen that was applied to the patients. We treated our patients with high doses of interferon-α during the first 6 weeks of treatment (6 MU/day in two divided doses during weeks 5 and 6) and then switched to standard, lower, dose pegylated interferon-α. This may have provoked a viral rebound after the first 6 weeks. The rate of breakthrough/relapse after initial response in our study was 40% (52% in previous non-responders and 32% in naive patients). This is slightly higher than the 25% breakthrough/relapse rate after initial response in naive patients during standard treatment [7]. However, the reappearance of HCV RNA at week 16 or 20 in 43% of patients with eventual breakthrough or relapse suggests that transient or persistent reappearance of low-level viraemia after 16 and 20 weeks of antiviral therapy is also likely to occur during standard treatment. To verify this, samples taken between weeks 16 and 24 from patients treated with the standard of care (pegylated interferon/ribavirin) should be (re)analysed by TMA.

Our results indicate that reappearance of low levels of HCV RNA is a reliable predictor for treatment failure in patients with an IVR during therapy.

Acknowledgements

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Potential conflict of interest: Dr Reesink is a consultant for Schering-Plough and has received grants from Hoffmann-La Roche and Schering-Plough. Dr Beld is a consultant for Bayer Diagnostics.

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