Chronic hepatitis C: new diagnostic tools and therapeutic strategies
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SUSTAINED VIROLOGICAL RESPONSE IN CHRONIC HEPATITIS C PATIENTS AFTER A 6- AND A 36-MONTH IFN-ALPHA2B TREATMENT SCHEDULE. A MULTICENTRE, RANDOMIZED, CONTROLLED STUDY

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Submitted
SUMMARY

**Background** Several studies in patients with chronic hepatitis C (HCV) have shown that Interferon-alpha (IFN) treatment for 12 months is more effective than during 6 months in inducing a sustained virological response. Furthermore, IFN retreatment seemed to be effective in IFN relapsers and IFN non-responders. We compared the virological responses and kinetics in patients with chronic HCV during treatment with IFN for 6-36 months to a non-treated control group.

**Methods** In a multicentre, randomized, controlled trial, 88 patients with chronic HCV were enrolled (47 treated with IFN-alpha2b and 41 constituted an untreated control group). Treatment consisted of 5 million units (MU) IFN thrice a week (tiw) for 8 weeks and subsequently 2.5 MU IFN tiw for 16 weeks (‘standard treatment’). After week 24, in virological non-responders treatment was continued using 5 MU IFN tiw for up to week 156, whereas in virological responders IFN was discontinued. In case of a virological relapse, treatment with 5 MU IFN tiw was restarted and continued up to week 156. The follow-up period was for at least 6 months after the end of IFN treatment. Sustained virological response was determined 6 months after standard treatment and after long term treatment. In a subset of 8 responding, 16 non-responding and 19 control patients HCV-RNA load was measured at various time points using an experimental NASBA-QT assay.

**Findings** The sustained virological response rate was 6/47 (13%) after standard treatment and 19/47 (40%) after long-term treatment (McNemar’s Paired Test; P=0.0019). Of the 18 patients with a breakthrough or relapse during or after standard treatment, 14 (78%) became sustained virological responders upon long term treatment. Of the 4 patients who did not have a sustained virological response after long term treatment, 3 did not receive complete treatment due to side-effects and/or non-compliance. In patients who failed to respond to standard treatment, no virological response was observed during long term treatment. In the control group, no spontaneous clearance of HCV was observed. In the control patients, the HCV viral load remained stable during the observation period, but tended to increase after 3 years. In non-responders, the viral load decreased significantly during treatment, but tended to increase after cessation of treatment.

**Interpretation** Long term IFN (re)treatment enhanced the virological sustained response rate significantly and was particularly effective in patients with a breakthrough or relapse following standard treatment.
INTRODUCTION

Numerous studies have been performed to assess the efficacy of Interferon-alpha (IFN) for treatment of chronic hepatitis C virus (HCV) infection. In the majority of these studies, inclusion criteria were clinical non-A, non-B hepatitis and occasionally anti-HCV positivity [1, 2, 3, 4]. Retrospective testing of baseline HCV-antibodies and plasma HCV-RNA in these trials, revealed that up to 12% of the patients were anti-HCV negative while up to 25% did not have detectable HCV-RNA before the start of treatment. The aim of treatment in these studies was normalization of alanine aminotransferase (ALT) levels. Sustained ALT response rates, i.e. normalization of ALT persisting for at least 6 months after cessation of treatment, varied from 8% to 28% in different studies after treatment with IFN for 6 months at a dose of 3-6 MU 3 times a week (tiw). ALT response did not always correlate with a virological response [1, 5, 6].

In chronic HCV infection, HCV-RNA positivity correlated closely with the presence of inflammatory changes of the liver [7, 8, 9] and infectivity [10, 11]. It has been postulated that the aim of treatment of chronic HCV infection should be clearance of HCV-RNA. Recently, a number of studies have assessed the virological response to IFN treatment in chronic HCV infection [12, 13, 14, 15]. A sustained virological response rate, defined as non-detectable HCV-RNA in plasma at 6 months after cessation of treatment, was reported in up to 17% of patients after administration of IFN 3-6 MU tiw for 6 months [12, 13] respectively in 13-26% after 12 months of IFN treatment [14, 15]. Also, in IFN trials in which ALT response was assessed, prolonged treatment improved the response rate [1, 2, 4, 16].

The aim of the present study was to assess the efficacy of a 6-month course of IFN treatment (standard treatment), followed by prolonged treatment of virological non-responders and retreatment of virological relapers for a period of 2.5 years (long-term treatment). In IFN-treated patients, the virological responses after 3-years respectively 6 months treatment were compared. The results of the treated patients were also compared with those of untreated controls. Furthermore, to determine the kinetics of HCV in treated and untreated patients, viral load was measured using a sensitive quantitative assay. Factors predicting response to IFN were also assessed.

METHODS

Study design
This clinical trial was designed as a multicentre, randomized, controlled study. Five Dutch centers participated: Department of Gastrointestinal- and Liver Diseases, Academic Medical Center, Amsterdam; Van Creveldkliniek, National Hemophilia Center, University Hospital Utrecht; Department of Hematology, Radboud Hospital, Nijmegen; Department of Liver Disease, Dijkzigt Hospital, University Hospital Rotterdam; and Department of Hematology, University Hospital Leiden. The study was approved by the Medical Ethical Committee of each of the participating hospitals.

After stratification for hemophilia A, hemophilia B and no hemophilia, patients were randomized (1:1) to receive either treatment with IFN-alpha 2b or no treatment. Randomization was performed using a computer-generated randomization list, kept by the coordinating secretary only. Between June 1991 and December 1995, 103 patients were enrolled in the study (55 IFN treated and 48 non-treated control patients).
The treatment schedule consisted of ‘standard treatment’ of 5 million units (MU) IFN-alpha2b thrice a week (tiw) for 8 weeks and subsequently 2.5 MU tiw for 16 weeks. Between week 24 and 136 treatment of virological non-responders and patients with a break-through consisted of ‘prolonged treatment’ with 5 MU IFN tiw. In virological responders, defined as HCV-RNA negative on 2 consecutive visits, IFN was discontinued at week 24. In case of virological relapse 5 MU IFN tiw was restarted and continued up to week 156 (‘retreatment’). The follow-up period after the 3 year treatment period was at least 6 months.

At the time of analysis, information on 88 patients (41 controls and 47 treated patients) at the end of the 3.5 year study period was available. Of these patients, 26 controls and 34 treated patients had completed the protocol; 15 controls and 13 treated patients were lost to follow up (Figure 1a). An intention-to-treat analysis is presented on this group of 88 patients. Viral kinetics were studied in a subset of 19 control patients, 8 IFN non-responders and 16 IFN responders.

**Figure 1a:**
Trial profile of treated and non-treated patients.

![Trial profile of treated and non-treated patients.](image-url)
assays were performed. Furthermore, plasma samples for cDNA-PCR for HCV-RNA were taken at every visit and analyzed within 2 weeks. Additional plasma was collected for quantitative HCV-RNA measurements and HCV genotyping. When severe side-effects occurred (leukocytes <1.5x10^7/l; platelets <80x10^9/l; mental depression; severe subjective side-effects) the IFN dose was temporarily lowered to 2.5 MU tiw or discontinued.

**Definition of response**

The main outcome measure was sustained virological response after ‘standard treatment’ and after ‘long-term treatment’. Virological response was defined as non-detectable HCV-RNA by qualitative HCV-cDNA-PCR testing on at least two consecutive visits. A sustained virological response was defined as non-detectable HCV-RNA by qualitative HCV-cDNA-PCR testing at the end of treatment which continued for at least 6 months after the end of treatment. Breakthrough and relapse were defined as recurrence of detectable HCV-RNA in plasma after initial virological response, during treatment and after cessation of treatment respectively. All other patients were classified as virological non-responders.

**Virology**

Plasma samples for HCV-RNA detection were collected in the various participating hospitals and analyzed centrally (CLB) using cDNA-PCR. Up to June 1994, HCV-RNA was detected by cDNA-PCR as described previously [17]. After June 1994, a commercially available cDNA-PCR assay (HCV AMPLICOR assay, Roche Diagnostic Systems) was used. The qualitative cDNA-PCR assays detected 100% of EUROHEP standards containing 3800 genome equivalents (geq)/ml and 93% of EUROHEP standards containing 380 geq/ml [18, unpublished observations].

Quantitative HCV-RNA measurements using an experimental NASBA-QT assay for HCV-RNA detection [19] were performed of samples collected at baseline and, from a subset of the patients (see study design), at week 8, 16, 24, 36, 48, 60, 156 and 172. These quantitative measurements were performed with an experimental NASBA-QT assay for HCV-RNA detection [19]. This assay is an isothermal nucleic acid amplification method in which three calibrator RNAs are used as internal standards. Briefly, nucleic acids were isolated according to the method described by Boom et al [20]. Samples of 100 µl plasma in 900 µl lysis buffer were thawed and three calibrator RNAs were added together with 50 µl of silica suspension, to bind the released nucleic acids. After washing and drying, nucleic acids were dissolved in 50 µl elution buffer. Isolated RNA was amplified as described [19]. The quantitative detection limit of the assay was found to be 4 log/ml and the qualitative detection limit 3.3 log/ml (working with 100 µl input)[unpublished observations]. Using in vitro RNA standard preparations, the accuracy of the assay for the different genotypes was found to be: type la -3.4%, type lb +3.3%, type 2 -6.4%, type 3 +1.8%, type 4 +1.9%, and type 5 +2.9% of the log converted values [unpublished observations]. With each run of 10 samples a control sample was included. The control sample was analyzed 59 times and revealed an average value of 4.98 log/ml with a standard deviation (SD) of 0.17 log/ml. A difference in viral load of more than 2 times the SD (0.34 log/ml) was regarded as beyond the inter-assay variation.

HCV genotyping was performed on all baseline samples with restriction fragment length polymorphism (RFLP) as described previously by Davidson et al. [21].
Statistics

Intention-to-treat analysis was used to compare the sustained virological response frequencies after standard and long-term treatment. Patients which were lost to follow-up were classified as non-responders. For assessment of differences in response rate after standard and long-term treatment the McNemar’s Paired Test was used. Continuous variables were expressed as median, minimum and maximum values. The Mann-Whitney Test was applied to compare continuous variables between different groups or different time points. Categorial variables were expressed as frequency and percentages. The Fisher’s Exact Test was used to compare frequencies between different groups. All calculations were performed with GraphPad InStat, version 2.04a.

Table 1
Characteristics of treated patients and non-treated control patients.

<table>
<thead>
<tr>
<th>Hemophilia A or B</th>
<th>IFN treated (n=47)</th>
<th>Non-treated controls (n=41)</th>
<th>P (^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20 (43%)</td>
<td>21 (51%)</td>
<td>NS</td>
</tr>
<tr>
<td>Male</td>
<td>40 (85%)</td>
<td>34 (83%)</td>
<td>NS</td>
</tr>
<tr>
<td>Age [median (min-max)]</td>
<td>37 (19-65)</td>
<td>40 (17-72)</td>
<td>NS</td>
</tr>
<tr>
<td>Lost to follow-up</td>
<td>13 (28%)</td>
<td>15 (37%)</td>
<td>NS</td>
</tr>
<tr>
<td>HCV Genotype:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>14 (30%)</td>
<td>18 (44%)</td>
<td>NS</td>
</tr>
<tr>
<td>2</td>
<td>8 (17%)</td>
<td>1 (2%)</td>
<td>NS</td>
</tr>
<tr>
<td>3</td>
<td>15 (32%)</td>
<td>11 (27%)</td>
<td>NS</td>
</tr>
<tr>
<td>4</td>
<td>3 (6%)</td>
<td>1 (2%)</td>
<td>NS</td>
</tr>
<tr>
<td>5</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
<td>NS</td>
</tr>
<tr>
<td>nt(^2)</td>
<td>6 (13%)</td>
<td>9 (22%)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Pretreatment HCV-RNA log/ml [median (min-max)]

<table>
<thead>
<tr>
<th>IFN treated (n=47)</th>
<th>Non-treated controls (n=41)</th>
<th>P (^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.09 (0-6.92)</td>
<td>6.30 (3.33-7.42)</td>
<td>NS</td>
</tr>
</tbody>
</table>

\(^1\) The Fisher’s Exact Test was used for categorial variables and the Mann-Whitney Test for continuous variables; NS=non-significant.
\(^2\) nt = not tested, lost to follow-up.

RESULTS

Response to treatment

Patient characteristics in the treated and non-treated groups at baseline were not significantly different (Table 1). A sustained virological response was observed in 6/47 patients (13%; 95% CI 5-26%) upon standard treatment and in 19/47 (40%; 95% CI 26-56%) after long-term treatment (McNemar’s Paired Test P=0.0019) (Figure 1, Table 2). Of the 22 patients with a breakthrough or relapse during or after standard treatment, 18 completed the study (Figure 1b). Fourteen out of these 18 (78%; 95% CI 52-94%) became sustained virological responders after completing the long-term treatment schedule. Three of the 4 patients who were not successfully retreated after a relapse, did not receive the full course of treatment because of side-effects and non-compliance. One patient was a non-responder during retreatment. Prolonged IFN therapy was not effective in virological non-responders following standard treatment. All non-treated control patients remained HCV-RNA positive during the observation period.
Figure 1b
Trial profile of standard and long-term treatment:

- **47 patients treated with IFN:**
  - 8 weeks 5 MU tiw
  - 16 weeks 2.5 MU tiw
- **7 lost to follow-up**
- **40 completed standard treatment**
  - (4 on reduced dose)
- **6 SVR:**
  - no treatment
- **4 breakthrough:**
  - prolonged IFN 5 MU tiw til SVR
- **18 relapsers:**
  - IFN retreatment 5 MU tiw
- **12 non-responders:**
  - prolonged IFN 5 MU tiw til SVR
- **1x SVR**
  - 3x relapse: retreatment 5 MU tiw
- **1 lost to follow-up**
  - 5 completed study
- **47 patients treated with IFN:**
- **10 lost to follow-up**
- **40 completed standard treatment**
  - (4 on reduced dose)
- **1 SVR**
  - 3x relapse: retreatment 5 MU tiw
- **1 lost to follow-up**
  - 1 completed study

* the other patients who completed the study had a breakthrough (3x) or non-response

Side-effects and drop-outs

All treated patients experienced typical side-effects of IFN, such as flu-like syndrome, headache, myalgia, mild psychological effects (loss of concentration, irritability) and increased hair loss. During standard treatment, the IFN dose was adjusted in 4/40 (10%) of the patients who completed standard treatment because of mental depression (1x), low platelet counts (1x) or severe flu-like symptoms (2x). During long-term treatment, the dose was adjusted in 8/34 (24%) patients because of depression (3x), low platelet count (1x) or severe ‘subjective’ side effects (4x). During long-term treatment, therapy was discontinued in 7 patients because of mental depression (3x), personal circumstances (1x), malaise (2x) and low platelet count (1x) (Figure 1).

Table 2:
Sustained response rates after standard and long-term IFN treatment.

<table>
<thead>
<tr>
<th></th>
<th>Sustained response rate after standard treatment</th>
<th>Sustained response rate after long-term treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n/n (%) 95% CI</td>
<td>n/n (%) 95% CI</td>
</tr>
<tr>
<td>IFN treatment</td>
<td>6/47 (13%; 5-26%)</td>
<td>19/47 (40%; 26-56%)</td>
</tr>
<tr>
<td>Non-treated controls</td>
<td>0/41 (0%; 0-9%)</td>
<td>0/41 (0%; 0-9%)</td>
</tr>
</tbody>
</table>

1 McNemar’s Paired Test
Table 3:
Prognostic factors for sustained virological response versus non-(sustained)-response after standard and long-term treatment in 34 IFN treated patients who completed the study protocol.

<table>
<thead>
<tr>
<th></th>
<th>STANDARD TREATMENT:</th>
<th></th>
<th>LONG-TERM TREATMENT:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sustained response</td>
<td>Non-(sust.)response</td>
<td>P</td>
</tr>
<tr>
<td>Age [median (min-max)]</td>
<td>(25-41)</td>
<td>(19-62)</td>
<td>NS</td>
</tr>
<tr>
<td>Genotype: n (%)</td>
<td>1</td>
<td>0 (0%)</td>
<td>12 (41%)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0 (0%)</td>
<td>8 (28%)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>3 (60%)</td>
<td>7 (24%)</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>2 (40%)</td>
<td>1 (3%)</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0 (0%)</td>
<td>1 (3%)</td>
</tr>
<tr>
<td>Pretreatment HCV-RNA log/ml [median (min-max)]</td>
<td>4.34 (0-5.36)</td>
<td>6.23 (4.97-6.92) &lt;0.0001</td>
<td>6.02 (0-6.90)</td>
</tr>
</tbody>
</table>

1 The Fisher’s Exact Test was used for categorical variables and the Mann-Whitney Test was used for continuous variables; NS = not significant.
Predicting factors for sustained response
In Table 3, the age, HCV genotype and pretreatment HCV viral load of patients with sustained and non-(sustained) response after standard and prolonged treatment are presented. Only patients who completed the study (n=34) were included. The median pretreatment viral load was significantly lower in the group of sustained responders than in non-(sustained)-responders, after standard treatment as well as after prolonged treatment. Prevalence of different genotypes and age of the patients did not differ significantly between sustained responders and non-(sustained)-responders.

HCV-RNA load measurements
In Figure 2, HCV-RNA levels of non-treated control patients (Figure 2.a), non-responders to treatment (Figure 2.b) and patients who had a transient or sustained response to treatment (Figure 2.c) are shown at various time points. Furthermore, the corresponding results of the qualitative HCV-RNA measurements are presented. In thirteen samples discordant results were obtained between the cDNA-PCR and the NASBA-QT assays: 12 were positive using the cDNA-PCR and negative using the NASBA-QT assay and 1 was negative using the cDNA-PCR and positive using the NASBA-QT assay. These discordant results were all obtained in patients who probably had a low HCV-RNA load at the time of sampling. The median viral load in the control group tended to increase after 156 and 172 weeks relative to the baseline value (6.96 log/ml and 6.78 log/ml versus 6.3 log/ml; NS). The median viral load in the non-responder group decreased significantly by week 48 (P=0.021) and tended to increase after the treatment period (relative to the baseline level). The median viral load in the responder group decreased significantly both during and after treatment relative to the baseline value (P ≤0.003).

DISCUSSION
The long-term IFN treatment schedule used in this study, was associated with a higher sustained virological response rate than occurred upon standard IFN treatment (40% versus 13%; P=0.0019). In particular, retreatment of patients following virological breakthrough or relapse which occurred during or after standard treatment was highly successful: 14 out of 18 patients (78%) with a breakthrough (observed after dose adjustment during standard treatment) or relapse became sustained virological responders after retreatment. Three patients who received incomplete IFN retreatment due to side-effects and non-compliance had a breakthrough during retreatment, and 1 patient did not respond to retreatment for unknown reasons. For patients who did not respond to standard treatment, we observed no benefit from long-term prolonged IFN treatment.

The results of the present analysis are consistent with those of Toyoda et al [22], who found that patients which lost HCV-RNA from serum during IFN treatment, even for a short period of time, had a greater chance of undergoing a sustained response after retreatment than virological non-responders. They reported a sustained virological response rate of 73% after retreatment of patients with virological relapse [22]. Chow et al reported a 19% sustained virological response rate after 12 months retreatment of IFN in responders who had relapsed, while no sustained responses were observed in patients who had not responded to standard IFN treatment [23]. Rabinovitz et al found a 43% virological sustained response rate in patients who had relapsed after responding to IFN and 13%
Figure 2:
Course of HCV-RNA load in log/ml determined by the NASBA-QT assay (top) and the qualitative cDNA-PCR (bottom) in 19 control patients (fig. 2a), 8 virological non-responding patients (fig. 2b), and 16 virological responding patients (fig. 2c).

Assay limits: for quantitative detection 4 log/ml; for qualitative detection 3.3 log/ml (grey zone).

Statistics: the difference from the base line was tested using the Mann-Whitney Test: 2.a) At week 156 and 172 the median HCV-RNA load was significantly higher respectively tended to be higher than at baseline (P=0.03 and P=0.075 respectively); 2.b) At week 48 the median HCV-RNA load was significantly less than at baseline (P=0.021), and at week 172 (after cessation of treatment) the median HCV-RNA load tended to be higher than at baseline (P=0.09); 2.c) During the treatment course and follow-up period the median load was significantly less than at baseline (P 0.003).
virological sustained response in previous non-responders to IFN [24]. Heathcote et al reported 28% and 58% sustained virological response after 6 and 12 month courses of retreatment in relapsers and 5% and 13% sustained virological response in non-responders respectively [25]. In these 3 studies [23, 24, 25], the treatment strategy was however not based on virological response, but on ALT normalization, and HCV-RNA measurements were only performed at the end of the follow-up period. In contrast to these findings, Weiland et al. [26] found no virological sustained response after a 6-months retreatment course in 10 responders who had a virological relapse. However, in the latter study retreatment was started more than 12 months after the first course, a period that is probably long enough for the immune modulatory effect of the first course of IFN to be lost. The high (78%) sustained response rate after retreatment in our study can be explained by a combination of the long duration of retreatment (up to 29 months), the relatively high dose (5 MU tiw), and the short period between relapse and start of retreatment (1-3 months).

In the present study, age, HCV genotype and pretreatment viral titer were investigated as possible predictors of a sustained virological response. Patients with a sustained response had a significantly lower pretreatment HCV-RNA load than non-(sustained)-responders. However, within the group of sustained responders a wide range of HCV levels was found. No differences in response were found in relation to age or HCV genotype, however the number of patients was limited. In several IFN studies the pretreatment HCV-RNA load was found to be the most important factor in predicting response to IFN treatment [27-31]. In addition to viral load, in other studies HCV genotype 1 was associated with non-response [1, 12].

In treated patients a decrease of the HCV-RNA load was observed, followed by disappearance of HCV-RNA in responders. In non-responders, the viral load tended to exceed the pretreatment levels after cessation of treatment. In other studies the HCV-RNA load also decreased in non-responders during treatment, but to a lesser degree than in responders [32,33]. Non-responders appear to have a higher degree of quasispecies diversity than responders to IFN treatment [34-37]. In non-responders, part of the quasispecies population may not be sensitive to IFN or, during IFN treatment, IFN resistant variants may develop [38, 39].

In the group of untreated control patients we found a tendency for HCV-RNA levels to increase after a follow-up period of 3 years. This observation differs from those of Hollingsworth et al. [40] and Naito et al. [9], who found stable viral loads during follow-up periods of 11 months and 6 years respectively. These findings may be explained by the relative short follow-up period in the first study, and probably the less sensitive quantitative technique available at the time of the second study. Gretch et al found a correlation between high HCV-RNA load and progression of chronic liver disease, which may be in accordance with our finding [41].

The outcome of this study leads us to recommend an individualized IFN treatment schedule for patients with chronic hepatitis C. This includes standard IFN treatment for 6 months, followed by long-term (re)treatment in patients in which a virological breakthrough or relapse occurs during or after standard treatment. Combination treatment with Ribavirin may be an option for retreatment in IFN non-responders and for primary treatment in patients with a high HCV-RNA load [42, 43].
CONTRIBUTORS

M Damen co-ordinated the study and the central data collection. Data collection at the individual centres was performed by CJ Weegink, EP Mauser-Bunschoten, E Haan, HM vd Berg, D Bresters and RAFM Chamuleau. The protocol was designed by HW Reesink, HTM Cuypers, D Bresters, PN Lelie and EP Mauser-Bunschoten. HTM Cuypers, P Sillekens and MC Hermus did the virological studies. The data were analyzed by M Damen and the paper was written by M Damen and HW Reesink with comments of the other authors.

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REFERENCES


