Original Paper: Liver

Digestion

Digestion 2000;61:90–97

Received: April 15, 1999
Accepted: June 25, 1999

# Retreatment with Interferon-Alpha and Ribavirin in Primary Interferon-Alpha Non-Responders with Chronic Hepatitis C

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**Key Words**
Interferon-α/ribavirin retreatment · Interferon non-responder · Chronic hepatitis C

**Abstract**
***Background/Aims:*** Combination therapy with interferon-α (IFN-α) plus ribavirin is more efficacious than IFN-α monotherapy in previously untreated patients with chronic hepatitis C and patients with IFN-α relapse. Only limited data are available in IFN-α non-responders. In a multicenter trial we therefore evaluated the efficacy of combination therapy in IFN-α-resistant chronic hepatitis C. ***Methods:*** Eighty-two patients (mean age 46.8 years, 54 males, 28 females) with chronic hepatitis C were treated with IFN-α-2a (3 × 6 MIU/week) and ribavirin (14 mg/kg daily) for 12 weeks. Thereafter, treatment was continued only in virological responders (undetectable serum HCV RNA at week 12) with an IFN-α dose of 3 × 3 MIU/week and without ribavirin for a further 9 months. The primary study endpoint was an undetectable HCV RNA by RT-PCR at the end of the 24-week follow-up period. ***Results:*** After 12 weeks of combination therapy, an initial virological response was observed in 29 of 82 (35.4%) patients. Due to a high breakthrough rate after IFN-α dose reduction and ribavirin discontinuation, an end-of-treatment response was only achieved in 12 of 82 (14.6%) patients. After the follow-up period, a sustained virological response was observed in 8 of 82 (9.8%) patients. Infection with HCV genotype 3 was the only pretreatment parameter, which could predict a sustained response (HCV-1, 5%; HCV-3, 57.1%; $p < 0.001$). ***Conclusions:*** Despite a high initial response rate of 35.4%, sustained viral clearance was achieved only in 9.8% of the retreated primary IFN-α non-responders. Higher IFN-α induction and maintenance dose, as well as prolonged ribavirin treatment may possibly increase the virological response rates in non-responders, particularly in those infected by HCV-1.



Dedicated to Prof. Dr. med. Dr. med. vet. Dr. h.c. K.H. Meyer zum Büschenfelde on the occasion of his 70th birthday.
Gerlinde Teuber, Thomas Berg and Robert M. Hoffmann contributed equally to this study.

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0012–2823/00/0612–0090$17.50/0

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## Introduction

Hepatitis C virus (HCV) infection is a common cause of chronic liver disease and may progress to liver cirrhosis and hepatocellular carcinoma in a substantial proportion of infected individuals [1–4]. At the end of interferon-α (IFN-α) monotherapy with 3 MIU three times weekly subcutaneously for 6–12 months, a virological response with apparent clearance of serum HCV RNA can be achieved in 30–40% of patients. However, during follow-up relapses occur in 50–80% of these patients. Therefore, IFN-α monotherapy with 3 MIU three times weekly subcutaneously for 6–12 months may induce a sustained virological response in only 10–20% of patients with chronic hepatitis C [5–8].

Recently published studies indicated a synergistic therapeutic effect of orally administered ribavirin added to standard IFN-α treatment in chronic hepatitis C [9–12]. Ribavirin (1-β-*D*-ribofuranosyl-1,2,4,-triazole-3-carboxamide) is a synthetic guanosine analogue with a broad spectrum of antiviral activities against several DNA and RNA viruses, including flaviviruses [13–15]. Furthermore, ribavirin exhibits immunomodulatory effects, e.g. shifting the balance of pro-inflammatory and anti-inflammatory cytokines towards a Th1 response by inhibiting the Th2 cytokine production [16].

Compared with IFN-α monotherapy, combination treatment with IFN-α and ribavirin for 6–12 months improves the long-term virological response rates to approximately 40–50% in patients with chronic hepatitis C [17–21]. The combination treatment is highly effective in the retreatment of patients with chronic hepatitis C relapsing after primary IFN-α monotherapy [22–24]. IFN-α/ribavirin combination therapy has recently been registered in the United States for treatment of chronic hepatitis C and is awaiting further approval in several European countries. However, the therapeutic value of combined IFN-α and ribavirin retreatment is controversially discussed in patients with chronic hepatitis C who did not respond to a primary IFN-α treatment [22, 23, 25–27].

Therefore, the aims of the present study were to evaluate the therapeutic efficacy and the tolerability of retreatment with combined IFN-α/ribavirin as an induction therapy for 12 weeks followed by maintenance therapy with IFN-α alone for 9 months in a cohort of patients with chronic hepatitis C who failed to respond to primary IFN-α monotreatment. In addition, potential parameters predicting a sustained response, such as age, sex, disease duration, ALT, AST, γ-GT, pretreatment viral load, HCV genotype, and the absence of cirrhosis, were analyzed.

## Patients and Methods

In this prospective, open and uncontrolled multicenter trial, 82 patients with chronic hepatitis C not responding to previous IFN-α monotherapy with a minimal total IFN-α dose of 108 MIU and a treatment duration of at least 12 weeks were enrolled during December 1995 and November 1996. Patients were eligible when they met all of the following inclusion criterias: (1) non-response to a previous IFN-α treatment with persistence of serum HCV RNA; (2) a treatment-free interval of at least 24 weeks; (3) elevated ALT levels; (4) a positive anti-HCV test; (5) detectable serum HCV RNA; (6) compensated liver disease; (7) baseline hemoglobin of at least 11.0 g/dl, and (8) age between 18 and 70 years. Demographic, biochemical and virological pretreatment characteristics are summarized in detail in table 1. Hepatitis B virus or human immunodeficiency virus type 1 and 2 coinfection as well as concomitant autoimmune disease and metabolic disorders were excluded by appropriate biochemical and serological tests. Further exclusion criteria were severe concurrent diseases, including malignancy, systemic immunosuppressive treatment within the last 24 weeks prior to study begin and a history of depression. Liver biopsy was performed in all patients before initiation of the primary IFN-α therapy. In 58 patients an additional biopsy was obtained within 12 months before retreatment. In the 24 patients, who refused a liver biopsy at this time, the result of the biopsy before the primary IFN-α treatment was considered for histological classification (table 1). No histological result in these 24 patients was older than 47 (mean 24; range 12–47) months. Inflammatory activity and extent of fibrosis were histologically assessed according to the 1994 revised classification of chronic hepatitis [28].

*Measurement of Serum HCV RNA*

Serum HCV RNA was assessed by a standardized qualitative reverse transcription-polymerase chain reaction (RT-PCR) assay (Amplicor HCV®, Hoffmann-La Roche, Germany) [29]. Individual pretreatment serum HCV RNA concentrations and HCV genotype (according to the classification of Simmonds et al. [30]) were determined by quantitative RT-PCR (Amplicor Monitor HCV 1.0®, Hoffmann-La Roche, Germany) and a reverse hybridization assay (Inno LiPA HCV II®, Innogenetics, Belgium), respectively [30–33].

*Study Design*

All patients were treated with 6 MIU IFN-α-2a thrice weekly subcutaneously and ribavirin (14 mg/kg of body weight daily, i.e. 600–1,200 mg) in two divided doses orally for 12 weeks (fig. 1). Thereafter, ribavirin therapy was discontinued in all patients, while IFN-α treatment was reduced to 3 MIU thrice weekly and continued for additional 9 months only in patients with undetectable serum HCV RNA at treatment week 12. The follow-up period was 24 weeks in all patients. Clinical examination and laboratory assessments, including blood count and aminotransferases, were performed between pretreatment weeks 4–12, at the initiation of treatment, every 2 weeks for the first 12 weeks of treatment and monthly thereafter until the end of treatment. Follow-up visits were scheduled 4, 12 and 24 weeks after discontinuation of treatment. Serum HCV RNA was quantitatively assessed before initiation of treatment, and qualitatively at 3 and 12 months during treatment as well as at the end of the follow-up period.

The study protocol was approved by the local ethics committees for medical research of the participating study centers according to the Declaration of Helsinki. Because all patients enrolled in the present study were previous IFN-α non-responders, a retreatment

**Table 1.** Demographic, clinical, biochemical, virological and histological parameters in 82 non-responders to previous IFN-α monotherapy with chronic hepatitis C

| | |
|---|---|
| *Demography* | |
| Age, years | 46.8 ± 10.3 (26–70) |
| Sex, males/females | 54/28 |
| Mode of transmission | |
| Posttransfusional | 32 (39.0%) |
| Intravenous drug abuse | 11 (13.4%) |
| Occupational | 2 (2.4%) |
| Sexual | 1 (1.2%) |
| Unknown | 36 (44.0%) |
| Disease duration, years[1] | 12.3 ± 8.7 (1–36) |
| Primary treatment | |
| Total IFN-α dose, MIU | 360 ± 184 (108–1,248) |
| Duration, months | 6.6 ± 3.0 (3–14) |
| *Biochemistry*[2] | |
| ALT, U/l | 80 ± 57 (25–338) |
| AST, U/l | 43 ± 34 (4–250) |
| γ-GT, U/l | 52 ± 41 (4–277) |
| Albumin, g/dl | 4.6 ± 0.5 (3.5–5.7) |
| Bilirubin, μmol/l | 13.7 ± 6.8 (5.1–35.9) |
| Prothrombin time, % | 101 ± 14 (65–133) |
| *Virology* | |
| HCV RNA positive | 82 |
| HCV RNA, $\times 10^6$ copies/ml[3] | 1.022 ± 0.894 (0.001–3.334) |
| HCV genotype | |
| HCV-1 | 60 (73.2%) |
| HCV-2 | 2 (2.4%) |
| HCV-3 | 7 (8.6%) |
| HCV-4 | 2 (2.4%) |
| Unclassifiable | 2 (2.4%) |
| Not assessed | 9 (11.0%) |
| *Histology* | |
| Inflammatory activity | |
| Minimal to mild | 44 |
| Moderate to severe | 38 |
| Fibrosis score | |
| 0–3 | 49 |
| 4 (cirrhosis) | 33 |

ALT = Alanine aminotransferase; AST = aspartate aminotransferase; γ-GT = γ-glutamyltranspeptidase. The values are given as the mean ± SD (range), or number of patients.

[1] Disease duration was defined as the time between the first diagnosis of hepatitis C or previous non-A, non-B hepatitis later on confirmed as hepatitis C.

[2] Normal reference ranges: 4–23 U/l for ALT, 6–18 for AST, 4–28 for γ-GT, 35–55 g/l for albumin, 3.4–23.9 μmol/l for bilirubin, 70–130% for prothrombin time.

[3] Pretreatment serum HCV RNA concentration was quantitatively assessed in 60 of 82 patients.

arm with IFN-α monotherapy as a control group was refused for ethical reasons [34–37]. Informed written consent was obtained from all patients.

*Definition of Response and Study Endpoints*

Virological response to treatment was defined as undetectable serum HCV RNA by qualitative RT-PCR (sensitivity 1,000 copies/ml) at treatment week 12 ('initial virological response'), at the end of treatment and at the end of follow-up ('sustained virological response'). At the same time points the biochemical response, defined as an ALT value within the normal range, was evaluated. The primary endpoint of this study was undetectable serum HCV RNA at the end of the follow-up period. Secondary efficacy parameters were the initial virological response and the virological end-of-treatment response. As additional secondary endpoints the biochemical responses were analyzed at the corresponding time points.

*Statistical Analysis*

Statistical analysis was based on an intention-to-treat methodology. Patients who discontinued treatment or were lost during follow-up were considered as virological and biochemical non-responders. Data are presented as mean ± standard deviation (range). Potential pretreatment response predicting parameters were identified by univariate analysis. Continuous and dichotomous variables were analyzed by Student's t test and the $\chi^2$ test, respectively.

## Results

*Biochemical and Virological Response*

After 12 weeks combined IFN-α and ribavirin retreatment an initial virological response with undetectable serum HCV RNA at week 12 was achieved in 29 of 82 (35.4%) patients with chronic hepatitis C not responding to a primary IFN-α monotherapy (table 2). The initial virological response was associated with normalization of ALT levels in 21 of 29 of these patients. An isolated initial biochemical response without clearance of serum HCV RNA was achieved in 15 of 82 (18.3%) patients. According to the protocol, however, treatment was discontinued in patients with normal ALT values at week 12, when serum HCV RNA was still detectable. After reduction of the IFN-α dose from 3 × 6 to 3 × 3 MIU/week and discontinuation of ribavirin treatment, a breakthrough with recurrence of serum HCV RNA was observed in 17 of 29 (58.6%) patients with an initial virological response. The breakthrough occurred in most patients within treatment months 4–6 (range 4–8 months). Due to this high breakthrough rate, a virological end-of-treatment response was observed in only 12 of 82 (14.6%) patients. Aminotransferase levels had normalized in 7 of 12 patients with a virological end-of-treatment response. During the follow-up period, 4 of 12 patients with a virological end-of-treatment response relapsed. Thus, a virological sustained

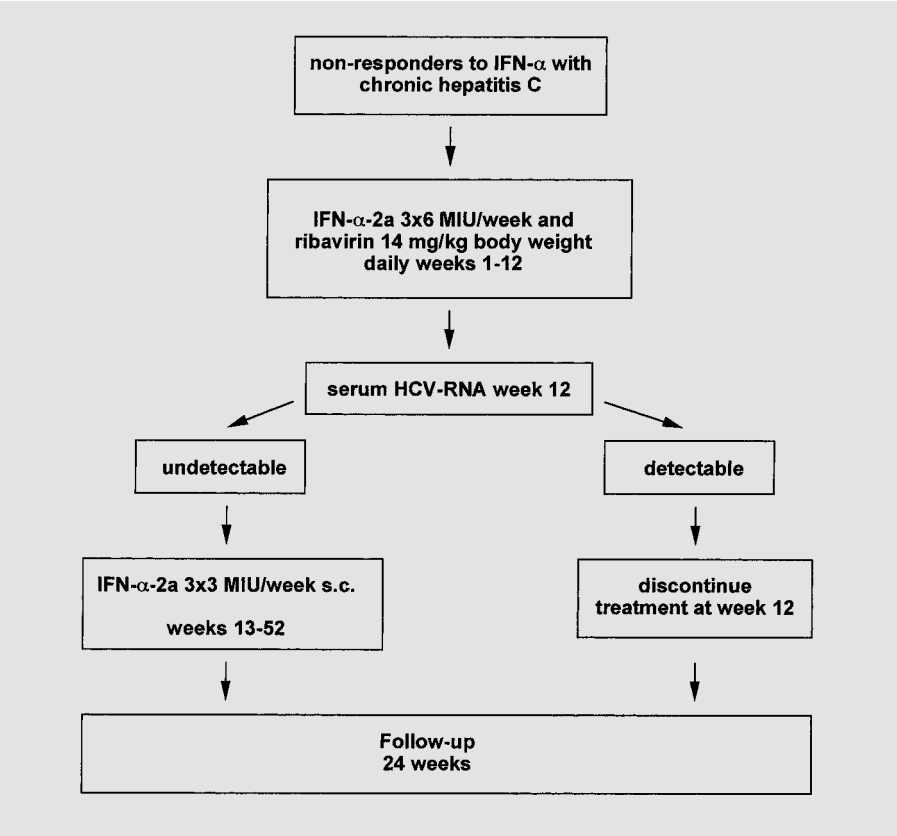


**Fig. 1.** Study design.

**Table 2.** Biochemical and virological response to IFN-α/ribavirin retreatment in 82 non-responders to previous monotherapy at treatment week 12, at end of treatment and end of follow-up

| | Initial response (treatment week 12) | End of treatment response (treatment week 52) | Sustained response (follow-up week 24) |
|---|---|---|---|
| Biochemical response | 15 (18.3%) | 9 (11.0%) | 11 (13.4%) |
| Virological response | 29 (35.4%) | 12 (14.6%) | 8 (9.8%) |
| Biochemical and virological response | 21 (25.6%) | 7 (8.5%) | 6 (7.3%) |

Treatment in patients with detectable serum HCV RNA at week 12 was discontinued regardless of the aminotransferase levels. Virological response: undetectable serum HCV RNA by qualitative RT-PCR (sensitivity 1,000 copies/ml). Biochemical response: ALT value within the normal range ($\leq$23 U/l).

response with undetectable serum HCV RNA after 24 weeks of follow-up was achieved only in 8 of 82 (9.8%) primary IFN-α non-responders with chronic hepatitis C. Seven of these patients also revealed normal ALT values. Furthermore, in 4 additional patients normalization of ALT levels was observed at the end of follow-up despite persisting hepatitis C viremia.

*Predictors of a Sustained Virological Response to Retreatment*

Potential clinical, biochemical, virological and histological pretreatment response predictors were analyzed in patients with a sustained virological response and compared with patients without a sustained virological response (table 3). Using a univariate model, only infection

**Table 3.** Univariate analysis of pretreatment parameters possibly predicting a sustained virological response to IFN-α/ribavirin retreatment in primary IFN-α non-responders with chronic hepatitis C

| | Sustained virological responders (n = 8) | Virological non-responders (n = 74) | p |
|---|---|---|---|
| Age, years | 46.1 ± 13.9 (28–63) | 46.9 ± 10.0 (26–70) | 0.8509 |
| Sex | | | 0.5658 |
| Male | 6 | 48 | |
| Female | 2 | 26 | |
| Disease duration, years[1] | 8.9 ± 6.1 (3–20) | 12.7 ± 8.9 (1–36) | 0.2401 |
| ALT, U/l | 82 ± 37 (32–141) | 80 ± 59 (25–338) | 0.9280 |
| AST, U/l | 43 ± 17 (21–68) | 43 ± 36 (2–250) | 0.9930 |
| γ-GT, U/l | 35 ± 28 (11–92) | 54 ± 44 (4–227) | 0.2679 |
| γ-GT/ALT | 0.48 ± 0.26 (0.18–0.73) | 0.80 ± 0.58 (0.09–3.3) | 0.1580 |
| Serum HCV RNA concentration, × $10^6$ copies/ml[2] | 0.149 ± 0.051 (0.103–0.204) | 1.068 ± 0.893 (0.001–3.334) | 0.1182 |
| HCV genotype[3] | | | <0.0001 |
| HCV-1 | 3 | 57 | |
| HCV-3 | 4 | 3 | |
| Histology | | | 0.1946 |
| Absence of cirrhosis | 7 | 42 | |
| Cirrhosis | 1 | 32 | |

ALT = Alanine aminotransferase; AST = aspartate aminotransferase; γ-GT = γ-glutamyltranspeptidase. Values are given as mean ± SD (range), or number of patients.

[1] Disease duration was defined as the time between first diagnosis of hepatitis C or previously non-A, non-B hepatitis later on confirmed as hepatitis C.

[2] Pretreatment serum HCV RNA concentration was assessed in 60 of 82 patients.

[3] HCV genotype was unclassifiable or not determined in 11 patients.

with HCV genotype 3 was associated with the development of a sustained virological response. Clearance of serum HCV RNA was achieved in 4 of 7 (57.1%) patients infected with HCV genotype 3 compared with 3 of 60 (5%) patients with HCV genotype 1 infection ($p < 0.001$). In our patients previously not responding to primary IFN-α treatment, no relation was found between a sustained response to combined antiviral retreatment and the patients' age, disease duration, ALT, AST, γ-GT, γ-GT/ALT, individual pretreatment serum HCV RNA concentration or the presence of cirrhosis.

*Side Effects*

Nearly all patients (n = 77; 93.9%) developed at least one adverse event during the treatment period. Most side effects (n = 284) occurred during the initial 12 weeks of IFN-α/ribavirin combination therapy (table 4). Mild to moderate flu-like symptoms were the most frequently observed adverse events. During ribavirin treatment 19.5% of the treated patients developed anemia with a serum hemoglobin level of <11.0 g/dl. The ribavirin dose was reduced in 14 patients to 7 mg/kg body weight daily or discontinued (n = 4 patients) due to hemoglobin levels of <10.0 g/dl, fatigue, weakness, dizziness, depression, nausea, and vomiting. A reduction of the IFN-α dose from 6 to 3 MIU thrice weekly before week 12 was necessary in 7 patients due to leukopenia, weakness, depression, myalgia, arthralgia, and headache, respectively. In 3 additional patients, both drugs were discontinued due to adverse events at treatment weeks 1 (nausea, diarrhea), 4 (depression), and 12 (anemia, nausea, dizziness), respectively. During IFN-α treatment with 3 × 3 MIU/week (weeks 13–52) most side effects improved or resolved. Further dose reductions were not required. After discontinuation of treatment, all reported side effects were fully reversible. Serious adverse events were not observed in any patient during the entire treatment period. The drop-out of 1 patient at month 4 of treatment was not related to side effects. After the end of treatment, 3 patients did not attend the scheduled follow-up visit at week 24.

**Table 4.** Side effects of combined IFN-α/ribavirin retreatment (weeks 1–12) followed by IFN-α monotherapy (weeks 13–52) in 82 primary IFN-α non-responders with chronic hepatitis C

| Adverse event | IFN-α/ribavirin weeks 1–12 (n = 82) | IFN-α weeks 13–52 (n = 29) |
|---|---|---|
| *Flu-like* | | |
| Chills and fever | 56 (68.3%) | 2 (6.9%) |
| Fatigue | 46 (56.1%) | 6 (20.7%) |
| Headache | 22 (26.8%) | 4 (13.8%) |
| Myalgia | 13 (15.9%) | 2 (6.9%) |
| Arthralgia | 8 (9.8%) | 2 (6.9%) |
| *Gastrointestinal* | | |
| Nausea | 21 (25.6%) | 1 (3.5%) |
| Loss of appetite | 9 (11.0%) | 0 |
| Diarrhea | 4 (4.9%) | 0 |
| *CNS* | | |
| Insomnia | 11 (13.4%) | 2 (6.9%) |
| Depression | 8 (9.8%) | 1 (3.5%) |
| Disturbances of concentration | 2 (2.4%) | 2 (6.9%) |
| Weakness | 7 (8.5%) | 0 |
| Dizziness | 9 (11.0%) | 0 |
| *Dermatological* | | |
| Hair loss | 7 (8.5%) | 4 (13.8%) |
| Pruritus | 6 (7.3%) | 0 |
| Rash | 2 (2.4%) | 0 |
| Sicca syndrome | 3 (3.7%) | 1 (3.5%) |
| *Respiratory* | | |
| Dry cough | 6 (7.3%) | 0 |
| Dyspnea | 1 (1.2%) | 0 |
| *Blood count* | | |
| Anemia[1] | 16 (19.5%) | 1 (3.5%) |
| Leucopenia[2] | 20 (24.4%) | 2 (6.9%) |
| Thrombocytopenia[3] | 7 (8.5%) | 3 (10.4%) |

[1] Hemoglobin < 10 g/dl.
[2] Leukocytes < 4.0/nl.
[3] Platelets < 100/nl.

## Discussion

In this multicenter trial, efficacy and tolerability of combined IFN-α and ribavirin treatment were prospectively assessed in patients with chronic hepatitis C not responding to a previous IFN-α treatment. This trial was designed in 1994 and it was originally anticipated that ribavirin may exhibit direct antiviral effects. Thus, the rational for the study design was an induction phase with IFN-α plus ribavirin to achieve optimal initial response rates followed by IFN-α monotherapy to maintain the virological response. With respect to the reported poor response rates in non-responders receiving IFN-α retreatment [34–37], a control group with patients receiving IFN-α alone was not justifiable for ethical considerations. As previously described, sustained loss of serum HCV RNA regularly leads to considerable histological improvement [38, 39]. Thus, a second liver biopsy after retreatment was not performed in our patients and serum HCV RNA 24 weeks after treatment was analyzed as the primary efficacy parameter in this study.

In the present study, including 82 primary IFN-α non-responders, a virological sustained response was achieved in only 9.8% of patients retreated with a combination of IFN-α and ribavirin. Despite an initially high virological response rate of 35.4% after 12 weeks of combination therapy, a virological end-of-treatment response was observed in only 14.6% of the retreated non-responders with chronic hepatitis C. Breakthrough with the recurrence of serum HCV RNA was observed in 15 of 17 patients with an initial response within 3 months after discontinuation of ribavirin therapy and IFN-α dose reduction. In the remaining 2 patients' breakthrough occurred at 7 and 8 months, respectively. Thus, low-dose IFN-α monotherapy is not able to maintain the high initial response rate induced by IFN-α/ribavirin induction therapy. However, as in untreated patients receiving combination therapy, the relapse rate in patients with a virological end-of-treatment response was lower than described for patients after IFN-α monotherapy [20, 21].

In previously published pilot studies concerning IFN-α/ribavirin retreatment in IFN-α non-responders, the reported sustained response rates varied considerably between 0 and 30% [22, 23, 25–27]. In contrast to the treatment schedule used in this trial, the duration of IFN-α/ribavirin retreatment was in general at least 24 weeks. In the present study, the sustained low virological response rate to IFN-α/ribavirin retreatment appears to be related to the short duration of ribavirin treatment and possibly to early IFN-α dose reduction. However, the majority of the previous studies revealed, in accordance with our results, low overall virological sustained response rates of approximately 10% in non-responders with chronic hepatitis C [22, 25, 27]. With respect to the HCV genotype distribution, the present study showed a beneficial therapeutic effect of IFN-α/ribavirin retreatment in non-responders infected by HCV genotypes 3 with a virological sustained response rate of 57.1%. The results of this trial indicate that the retreatment schedule with an IFN-α/ribavirin induction therapy followed by a low-dose

IFN-α monotherapy seems not to be effective in non-responders, infected with HCV genotype 1. Thus, the varying response rates, reported in the published pilot studies, may be explained by small study populations and the HCV genotype distribution.

In the present study, other host and viral factors associated with a response to antiviral therapy in untreated patients with chronic hepatitis C, including sex, presence of cirrhosis and pretreatment viral load [40, 41], showed no predictive role in the setting of IFN-α/ribavirin retreatment in non-responders. The lack of an association between the individual serum HCV RNA concentrations and the response to treatment in our patients may be explained by the limitations of the HCV RNA quantification assay used in this study. As previously described, amplification of this commercially available RT-PCR assay is not linear and not genotype-independent leading to an underestimation of the serum HCV RNA concentration in patients with high viremia ($>10^6$ copies/ml) and patients infected with genotype HCV-2 and 3 [30, 31].

During IFN-α/ribavirin retreatment, side effects occurred in most of our patients (93.9%) within the first 3 months of treatment. Most side effects were mild and transient. However, dose reduction or discontinuation of IFN-α and/or ribavirin therapy were required in 28 of 82 (34.1%) patients due to the reported adverse events. After discontinuation of treatment, all side effects were fully reversible.

In summary, retreatment with IFN-α and ribavirin in the applied dose regimen seems to have a beneficial therapeutic effect only in non-responders with chronic hepatitis C infected with HCV genotype 3, while it is of low efficacy in non-responders with HCV genotype 1 infection. Thus, similar to untreated patients with chronic hepatitis C, genotype-dependent therapeutic strategies should be evaluated in the retreatment of non-responders. Furthermore, the potential therapeutic value of a high-dose IFN-α induction and maintenance therapy as well as prolonged treatment with ribavirin, and the addition of amantadin has to be elucidated in future studies.

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